Springer Series in Translational Stroke Research

Selva Baltan
S. Thomas Carmichael
Carlos Matute
Guohua Xi
John H Zhang *Editors*

White Matter Injury in Stroke and CNS Disease

Springer

Springer Series in Translational Stroke Research

Series Editor
John Zhang

For further volumes:
http://www.springer.com/series/10064

Selva Baltan • S. Thomas Carmichael
Carlos Matute • Guohua Xi • John H Zhang
Editors

White Matter Injury in Stroke and CNS Disease

Springer

Editors
Selva Baltan
Department of Neurosciences
Cleveland Clinic Lerner Research Institute
Cleveland, OH, USA

Carlos Matute
Departamento de Neurociencias, Achucarro Basque Center for Neuroscience,
Universidad del País Vasco (UPV/EHU), and Centro de, Investigación Biomédica en Red en
Enfermedades Neurodegenerativas (CIBERNED), Leioa, Spain

John H Zhang
Department of Physiology
Loma Linda University
Loma Linda, CA, USA

S. Thomas Carmichael
Department of Neurology
University of California, Los Angelos
Los Angeles, CA, USA

Guohua Xi
Department of Neurosurgery
University of Michigan
Ann Arbor, MI, USA

ISBN 978-1-4614-9122-4 ISBN 978-1-4614-9123-1 (eBook)
DOI 10.1007/978-1-4614-9123-1
Springer New York Heidelberg Dordrecht London

Library of Congress Control Number: 2013953313

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Preface

Studies of cerebral white matter are arguably the stepchild of the neurobiology of disease. Accounting for over half the brain volume of the adult human, it would be surprising if cerebral white matter structure was not intimately involved in disease progression for most neurological disorders of the brain. Yet the learned reader of the scientific literature might justifiably conclude that brain insults affect neurons, and that neuronal protection and repair should be the main targets for translational neuroscience.

The field of white matter biology and disease is emerging to fill this scientific void. Fueled by new imaging modalities, molecular tools and cellular and animal models, studies of white matter development, injury, and repair are beginning to generate new concepts of disease progression in Alzheimer's disease, new descriptions and biomarkers of axonal pathology in traumatic brain injury and stroke, and a more detailed understanding of white matter health in normal aging. The chapters in this book explore these new developments in each of these categories: normal white matter structure and imaging; developmental white matter injury and its unique anatomical and temporal profile; progressive white matter degeneration as a major component of Alzheimer's disease and, surprisingly, normal aging; white matter energy dynamics, axonal support, and ischemic injury; acute structural white matter insults in stroke and head trauma; and progressive white matter injury in toxic exposures.

Neuroscience is a field driven by technology. Our emerging understanding of white matter health, disease, and repair is no exception to this principle. Chapters in this book review the MRI sequences that have provided structural insight into white matter tracts, such as diffusion tensor imaging, high angular resolution MRI, NMR spectroscopy, and their combination in multimodal MRI in humans and preclinical models. These approaches clearly provide indices of white matter structure in ischemia, head injury, and neurodegenerative diseases and might provide biomarkers for clinical stratification of patients for treatment.

One of the most important areas of study in white matter disease is the field of white matter progenitor biology. The most abundant progenitor cell in the brain, the

oligodendrocyte progenitor cell (OPC), is a card-carrying member of the white matter club. Originally and literally described as a precursor to mature oligodendrocytes, these cells have in fact a far more complicated biology, with multipotent potential to differentiate into neurons, with an ability to form synapses, and a capacity to recognize injury, migrate, and participate in scar formation (the "reactive OPC"). These principles are reviewed and the tools necessary for detailed study of the OPC are discussed.

Though it is tempting in the Preface to this book to push the "white matter-centric" view far so as to draw out distinct and neglected biological principles of CNS disease, the study of white matter injury reaches its most important points when integrated back into a more whole neurobiology of disease. Several principles discussed in these chapters illustrate this point. At a system's level, in Alzheimer's disease the integrated function of white matter tracts and overlying neuronal areas are key to understanding disease progression. On a cellular level the myelinated axon interacts with its neighboring oligodendrocyte to form an axoglial unit. This unit interconnects metabolic shuttling, cell-adhesion signals, and triggers compartmentalization in both the axon and its nodes with the oligodendrocyte that is key to normal signal propagation. In traumatic brain injury, stroke, periventricular leukomalacia, and other brain injuries the axoglial unit is disturbed, and this can be progressive even after the initial insult is completed. These points are clearly made throughout this book.

If the Preface as a beginning is anything it leads into the chapters and then beyond, in this case to the more robust study of white matter structure, function, disorder and recovery. The latest tools for this journey are described, from genetic cellular fate-mapping to MRI imaging and network analysis. New principles are set, and these now need future examination, such as the cognitive dysfunction of aging, acute injury, and progression; white matter progenitor biology and tissue repair; and subcellular energy dynamics and signaling. The reader, like the editors and authors before him, is set on this journey. A more famous beginning lead into a journey and an examination of the principles beyond, "Somewhere in La Mancha, in a place whose name I do not care to remember, a gentleman lived not long ago, one of those who has a lance and ancient shield on a shelf and keeps a skinny nag and a greyhound for racing." With a relatively new conception of the importance and role of white matter disease it is time to lower the lance and charge.

Cleveland, OH, USA — Selva Baltan
Los Angeles, CA, USA — S. Thomas Carmichael
Leioa, Spain — Carlos Matute
Ann Arbor, MI, USA — Guohua Xi
Loma Linda, CA, USA — John H Zhang

Contents

Part I White Matter and Evaluation

1 White Matter: Basic Principles of Axonal Organization and Function 3
Alexander Velumian and Marina Samoilova

2 White Matter Injury and Potential Treatment in Ischemic Stroke 39
Mingke Song, Anna Woodbury, and Shan Ping Yu

3 CADASIL and Animal Models 53
Francesco Blasi, Anand Viswanathan, and Cenk Ayata

4 Neuroimaging of White Matter Injury: A Multimodal Approach to Vascular Disease 67
Gary A. Rosenberg, Branko Huisa, Fakhreya Y. Jalal, and Yi Yang

5 Diffusion MRI Biomarkers of White Matter Damage in Traumatic Brain Injury 91
Maria Ly, Samuel Ji, and Michael A. Yassa

Part II White Matter Injury in Stroke and Other CNS Disorders

6 Mechanisms Underlying the Selective Vulnerability of Developing Human White Matter 109
Paul A. Rosenberg

7 Neonatal Experimental White Matter Injury 143
Zhengwei Cai

8 Focal Ischemic White Matter Injury in Experimental Models 169
Robert Fern

9 White Matter Injury in Global Cerebral Ischemia 181
Shinichi Nakao and Yan Xu

10 Experimental Global Ischemia and White Matter Injury 197
Ji Hae Seo, Kazuhide Hayakawa, Nobukazu Miyamoto, Takakuni Maki, Loc-Duyen D. Pham, Eng H. Lo, and Ken Arai

11 White Matter Injury After Experimental Intracerebral Hemorrhage 219
Kenneth R. Wagner

12 White Matter Repair in Subcortical Stroke 257
Elif G. Sözmen and S. Thomas Carmichael

13 White Matter Injury in Subarachnoid Hemorrhage in Humans 271
Gian Marco De Marchis and Stephan A. Mayer

14 Degenerative Brain Diseases and White Matter Injury 281
George Bartzokis and Po H. Lu

15 Unmyelinated and Myelinated Axons Exhibit Differential Injury and Treatment Responses Following Traumatic Injury 321
Thomas M. Reeves, Adele E. Doperalski, and Linda L. Phillips

16 Age-Dependent Mechanisms of White Matter Injury After Stroke 373
Selva Baltan

17 White Matter Damage in Multiple Sclerosis 405
María Victoria Sánchez-Gómez, Fernando Pérez-Cerdá, and Carlos Matute

Part III Pathophysiology of White Matter Injury

18 Calcium Dyshomeostasis in White Matter Injury 433
Elena Alberdi, Asier Ruiz, and Carlos Matute

19 Inflammation and White Matter Injury in Animal Models of Ischemic Stroke 461
Lyanne C. Schlichter, Sarah Hutchings, and Starlee Lively

20 Oxidative Stress in White Matter Injury 505
Hideyuki Yoshioka, Takuma Wakai, Hiroyuki Kinouchi, and Pak H. Chan

21 Acute Axonal Injury in White Matter Stroke 521
Jason D. Hinman and S. Thomas Carmichael

Part IV Other White Matter Injuries

22 The Interplay Between White Matter, Mitochondria, and Neuroprotection 539
R. Anne Stetler, Rehana K. Leak, Zheng Jing, Xiaoming Hu, Yanqin Gao, Guodong Cao, and Jun Chen

23 Heavy Metals and White Matter Injury 555
Yang V. Li

24 Anesthesia and White Matter Injury 571
Phillip Vlisides and Zhongcong Xie

About the Editors 585

Index 589

Contributors

Elena Alberdi Departamento de Neurociencias, Achucarro Basque Center for Neuroscience, Universidad del País Vasco (UPV/EHU), Leioa, Spain

Centro de Investigación Biomédica en Red en Enfermedades Neurodegenerativas (CIBERNED), Leioa, Spain

Ken Arai Neuroprotection Research Laboratory, Departments of Radiology and Neurology, Massachusetts General Hospital/Harvard Medical School, Charlestown, MA, USA

Cenk Ayata, M.D. Neurovascular Research Laboratory, Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Stroke Service and Neuroscience Intensive Care Unit, Department of Neurology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Selva Baltan Department of Neurosciences, Cleveland Clinic Lerner Research Institute, Cleveland, OH, USA

George Bartzokis, M.D. Department of Psychiatry, Semel Institute for Neuroscience and Human Behavior, The David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

Greater Los Angeles VA Healthcare System, West Los Angeles, CA, USA

Francesco Blasi, Pharm.D., Ph.D. Neurovascular Research Laboratory, Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Zhengwei Cai, Ph.D. Department of Pediatrics, University of Mississippi Medical Center, Jackson, MS, USA

Guodong Cao, Ph.D. Department of Neurology and Center of Cerebrovascular Disease Research, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

State Key Laboratory of Medical Neurobiology and Institute of Brain Sciences, Fudan University Shanghai Medical College, Shanghai, China

Geriatric Research, Educational and Clinical Center, Veterans Affairs Pittsburgh Health Care System, Pittsburgh, PA, USA

S. Thomas Carmichael, M.D. Department of Neurology, University of California, Los Angeles, Los Angeles, CA, USA

Pak H. Chan, Ph.D. Department of Neurosurgery, Stanford University School of Medicine, Stanford, CA, USA

Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, CA, USA

Program in Neurosciences, Stanford University School of Medicine, Stanford, CA, USA

Jun Chen, M.D. Department of Neurology and Center of Cerebrovascular Disease Research, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

State Key Laboratory of Medical Neurobiology and Institute of Brain Sciences, Fudan University Shanghai Medical College, Shanghai, China

Geriatric Research, Educational and Clinical Center, Veterans Affairs Pittsburgh Health Care System, Pittsburgh, PA, USA

Adele E. Doperalski, Ph.D. Department of Anatomy and Neurobiology, Virginia Commonwealth University, Richmond, VA, USA

Robert Fern, Ph.D. Cell Physiology and Pharmacology, University of Leicester, Leicester, UK

Yanqin Gao, M.D. State Key Laboratory of Medical Neurobiology and Institute of Brain Sciences, Fudan University Shanghai Medical College, Shanghai, China

Kazuhide Hayakawa Neuroprotection Research Laboratory, Departments of Radiology and Neurology, Massachusetts General Hospital/Harvard Medical School, Charlestown, MA, USA

Jason D. Hinman Department of Neurology, University of California, Los Angeles, Los Angeles, CA, USA

Xiaoming Hu, Ph.D. Department of Neurology and Center of Cerebrovascular Disease Research, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

State Key Laboratory of Medical Neurobiology and Institute of Brain Sciences, Fudan University Shanghai Medical College, Shanghai, China

Geriatric Research, Educational and Clinical Center, Veterans Affairs Pittsburgh Health Care System, Pittsburgh, PA, USA

Branko Huisa, M.D. Department of Neurology, University of New Mexico Health Sciences Center, Albuquerque, NM, USA

Sarah Hutchings Genes & Development,Toronto Western Research Institute, University Health Network,Toronto, Canada

Department of Physiology, University of Toronto, Toronto, ON, Canada

Fakhreya Y. Jalal, Ph.D. Department of Neurology, University of New Mexico Health Sciences Center, Albuquerque, NM, USA

Samuel Ji Department of Psychological and Brain Sciences, Johns Hopkins University, Baltimore, MD, USA

Zheng Jing, Ph.D. Department of Neurology and Center of Cerebrovascular Disease Research, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

Geriatric Research, Educational and Clinical Center, Veterans Affairs Pittsburgh Health Care System, Pittsburgh, PA, USA

Hiroyuki Kinouchi, M.D., Ph.D. Department of Neurosurgery, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Yamanashi, Japan

Rehana K. Leak, Ph.D. Division of Pharmaceutical Sciences, Mylan School of Pharmacy, Duquesne University, Pittsburgh, PA, USA

Yang V. Li Department of Biomedical Sciences, Ohio University, Athens, OH, USA

Starlee Lively Genes & Development, Toronto Western Research Institute, University Health Network, Toronto, ON, Canada

Eng H. Lo Neuroprotection Research Laboratory, Departments of Radiology and Neurology, Massachusetts General Hospital/Harvard Medical School, Charlestown, MA, USA

Po H. Lu, Psy.D. Department of Neurology, The David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

Maria Ly Department of Psychological and Brain Sciences, Johns Hopkins University, Baltimore, MD, USA

Takakuni Maki Neuroprotection Research Laboratory, Departments of Radiology and Neurology, Massachusetts General Hospital/Harvard Medical School, Charlestown, MA, USA

Gian Marco De Marchis, M.D. Division of Neurocritical Care, Department of Neurology, New York Presbyterian Hospital/Columbia University Medical Center, New York, NY, USA

Carlos Matute Departamento de Neurociencias, Achucarro Basque Center for Neuroscience, Universidad del País Vasco (UPV/EHU), Leioa, Spain

Centro de Investigación Biomédica en Red en Enfermedades Neurodegenerativas (CIBERNED), Leioa, Spain

Stephan A. Mayer, M.D. Division of Neurocritical Care, Department of Neurology, New York Presbyterian Hospital/Columbia University Medical Center, New York, NY, USA

Nobukazu Miyamoto Neuroprotection Research Laboratory, Departments of Radiology and Neurology, Massachusetts General Hospital/Harvard Medical School, Charlestown, MA, USA

Shinichi Nakao, M.D., Ph.D. Department of Anesthesiology, Kinki University Faculty of Medicine, Osaka, Japan

Fernando Pérez-Cerdá Departamento de Neurociencias, Achucarro Basque Center for Neuroscience, Universidad del País Vasco (UPV/EHU), Leioa, Spain

Centro de Investigación Biomédica en Red en Enfermedades Neurodegenerativas (CIBERNED), Leioa, Spain

Loc-Duyen D. Pham Neuroprotection Research Laboratory, Departments of Radiology and Neurology, Massachusetts General Hospital/Harvard Medical School, Charlestown, MA, USA

Linda L. Phillips, Ph.D. Department of Anatomy and Neurobiology, Virginia Commonwealth University, Richmond, VA, USA

Thomas M. Reeves, Ph.D. Department of Anatomy and Neurobiology, Virginia Commonwealth University, Richmond, VA, USA

Gary A. Rosenberg, M.D. Department of Neurology, University of New Mexico Health Sciences Center, Albuquerque, NM, USA

Paul A. Rosenberg Neurology, Children's Hospital Boston, Boston, MA, USA

Asier Ruiz Departamento de Neurociencias, Achucarro Basque Center for Neuroscience, Universidad del País Vasco (UPV/EHU), Leioa, Spain

Centro de Investigación Biomédica en Red en Enfermedades Neurodegenerativas (CIBERNED), Leioa, Spain

Marina Samoilova Toronto Western Hospital, University Health Network, Toronto, Canada

María Victoria Sánchez-Gómez Departamento de Neurociencias, Achucarro Basque Center for Neuroscience, Universidad del País Vasco (UPV/EHU), Leioa, Spain

Centro de Investigación Biomédica en Red en Enfermedades Neurodegenerativas (CIBERNED), Leioa, Spain

Lyanne C. Schlichter Genes & Development,Toronto Western Research Institute, University Health Network,Toronto, Canada

Department of Physiology, University of Toronto, Toronto, ON, Canada

Ji Hae Seo Neuroprotection Research Laboratory, Departments of Radiology and Neurology, Massachusetts General Hospital/Harvard Medical School, Charlestown, MA, USA

Mingke Song Department of Anesthesiology, Emory University School of Medicine, Atlanta, GA, USA

Elif G. Sözmen Department of Neurology, University of California, Los Angeles, Los Angeles, CA, USA

R. Anne Stetler, Ph.D. Department of Neurology and Center of Cerebrovascular Disease Research, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

State Key Laboratory of Medical Neurobiology and Institute of Brain Sciences, Fudan University Shanghai Medical College, Shanghai, China

Geriatric Research, Educational and Clinical Center, Veterans Affairs Pittsburgh Health Care System, Pittsburgh, PA, USA

Alexander Velumian Division of Neurosurgery, Departments of Surgery and Physiology, Faculty of Medicine, Toronto Western Hospital, University of Toronto, Toronto, ON, Canada

Anand Viswanathan, M.D., Ph.D. Stroke Research Center, Massachusetts General Hospital, Boston, MA, USA

Phillip Vlisides, M.D. Department of Anesthesia, Critical Care, and Pain Medicine, Massachusetts General Hospital, Boston, MA, USA

Kenneth R. Wagner Research (151), Department of Veterans Affairs Medical Center, Cincinnati, OH, USA

Department Neurology, University of Cincinnati College of Medicine, Cincinnati, OH, USA

Takuma Wakai, M.D. Department of Neurosurgery, Stanford University School of Medicine, Stanford, CA, USA

Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, CA, USA

Program in Neurosciences, Stanford University School of Medicine, Stanford, CA, USA

Department of Neurosurgery, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Yamanashi, Japan

Anna Woodbury Department of Anesthesiology, Emory University School of Medicine, Atlanta, GA, USA

Zhongcong Xie, M.D., Ph.D. Department of Anesthesia, Critical Care, and Pain Medicine, Massachusetts General Hospital, Boston, MA, USA

Yan Xu, Ph.D. Department of Anesthesiology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

Yi Yang, M.D., Ph.D. Department of Neurology, University of New Mexico Health Sciences Center, Albuquerque, NM, USA

Michael A. Yassa Department of Psychological and Brain Sciences, Johns Hopkins University, Baltimore, MD, USA

Hideyuki Yoshioka, M.D., Ph.D. Department of Neurosurgery, Stanford University School of Medicine, Stanford, CA, USA

Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, CA, USA

Program in Neurosciences, Stanford University School of Medicine, Stanford, CA, USA

Department of Neurosurgery, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Yamanashi, Japan

Shan Ping Yu Department of Anesthesiology, Emory University School of Medicine, Atlanta, GA, USA

Part I
White Matter and Evaluation

Chapter 1
White Matter: Basic Principles of Axonal Organization and Function

Alexander Velumian and Marina Samoilova

1.1 White Matter: General Notes

White matter occupies nearly half of the human brain and is packed with predominantly myelinated axons interconnecting different parts of the brain or spinal cord. White matter is topographically segregated from the gray matter which contains cell bodies of neurons, some of which communicate within local networks through their short axons, while others, integrating the results of local activity, send their long axons through the white matter to other areas of the CNS.

The white matter is highly topographically organized, and axons of similar origin typically run to their destinations in bundles occupying distinct parts of the white matter, forming various groups of association, commissural or long projection (tract) fibers (Filley 2010; Schmahmann et al. 2008), and thus represents a complex network of "communication highways" of the CNS that avoid local traffic and serve for rapid undisturbed delivery of signals. Some of these "highways" also serve for communicating signals from peripheral sources to their central destinations.

White matter is macroscopically identifiable in brain and spinal cord sections due to the presence of myelin that enwraps individual axons and provides electrical insulation that facilitates axonal conduction of action potentials. The high density of myelinated axons affects the optical reflective/absorptive properties of white matter. Due to the anisotropic diffusional properties of axons and myelin for water, white matter is identifiable within the whole brain or spinal cord by noninvasive imaging techniques such as MRI, and more specifically the diffusion MRI implemented

A. Velumian (✉)
Division of Neurosurgery, Departments of Surgery and Physiology, Faculty of Medicine, Toronto Western Hospital, University of Toronto, 399 Bathurst St, Room McL 5-403, Toronto, ON, Canada M5T 2S8
e-mail: velumian@uhnres.utoronto.ca

M. Samoilova
Toronto Western Hospital, University Health Network, Toronto, Canada

S. Baltan et al. (eds.), *White Matter Injury in Stroke and CNS Disease*, Springer Series in Translational Stroke Research 4, DOI 10.1007/978-1-4614-9123-1_1,

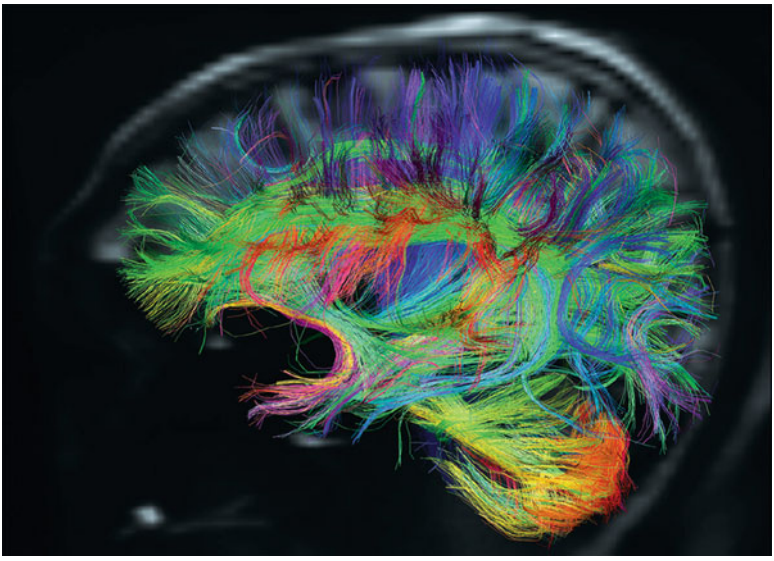


Fig. 1.1 MR tractography of cerebral white matter: The "nerve fibres" of the human (Jon Bardin's) brain traced by diffusion spectrum imaging, and colored to represent their direction. *Reproduced Bardin J* (2012) *Neuroscience*: *Making connections*. *Nature 483*:*394–396*

since mid-1980s, which is now a widely used clinical tool that allows for precise identification of white matter and areas of its damage. A further refinement of the MRI diffusion analysis has led to the development of MR tractography (Fig. 1.1), rapidly evolving since 1999 (Bardin 2012; Fujiyoshi et al. 2013; Le Bihan and Johansen-Berg 2012; Vargas et al. 2008).

1.1.1 White Matter: The "Nerves Within the Brain"?

The general organization of CNS white matter in terms of topographic organization of its axonal bundles and their source–target relationships resembles that of the peripheral nerves, which contain distinct bundles of motor and sensory axons running to or from specific body sites, and thus the white matter represents, in a sense, the "nerves within the brain." However, while the peripheral nerves are well designed to provide maximal protection of axons and independence of their operation from surrounding tissues on the way from source to target areas, the CNS white matter is less protected, and is exposed to possible "spillages" of neurotransmitters or products of metabolism from neighboring gray matter tissues. Specifically, the interface between the gray and white matter is not clearly defined, and the white matter does not have protective layers of connective tissue (*endo-*, *epi-*, and *perineurium*) that protect axonal bundles and whole nerves in the PNS. Furthermore, individual myelinated axons in the CNS white matter do not have a protective sleeve

of basal lamina that provides further microenvironment and mechanical protection to axons in PNS (Bunge et al. 1986).

The arrangement of nonmyelinated axons in CNS white matter also differs from that in PNS. In peripheral nerves, nonmyelinated axons are not loose, being tightly embedded into invaginations of a different, nonmyelinating type of Schwann cell, so that a single axon has its "niche," and a single Schwann cell may have tens of nonmyelinated axons embedded in individual niches (Peters et al. 1991). There are generally no such embeddings of nonmyelinated axons into any type of glial cell in the CNS white matter, although it has been noted that in areas where nonmyelinated axons predominate, they may be segregated into groups by "sheets or trabeculae" of astroglial processes (Peters et al. 1991).

In the CNS, there are no apparent physical barriers between the gray and white matter, and the homeostasis of extracellular environment around the axons in white matter is controlled by a specific type of glial cells, the fibrous astrocytes, whose long (up to 300 μm) processes run long the axons, while their other processes connect to blood vessels, similar to other types of astrocytes in the gray matter (Reichenbach and Wolburg 2005). There is no such cell type, and obviously no need for it, in the PNS. The dense packing of the axons in CNS white matter reduces the extracellular diffusion ("volume conduction") in the anisotropic and highly tortuous spaces around axon bundles (Sykova and Chvatal 2000), further protecting the extracellular environment of deeper located axons from diffusional influences from neighboring gray matter.

1.1.2 How "White" Is the Brain?

While some estimates suggest that white matter occupies nearly half of the volume of the human brain (Filley 1998, 2010), MRI voxel-based analysis in normal human subjects shows that the relative volume of the white matter in adult normal human brain is ~36 % (Perez-Duenas et al. 2006), apparently missing smaller white matter compartments. However, the brain may be "whiter" than we think because the majority of myelinated axons running through white matter originate from neurons located in the gray matter (others outside CNS, as in case of some sensory systems) and end in the gray matter to make synaptic contacts with other neurons, and are myelinated in their portions running in the gray matter, as detailed below, to maintain high conduction velocity. Furthermore, smaller bundles of myelinated axons may run within the gray matter (Filley 2010) and not be detectable by macroanatomical imaging approaches.

At the proximal end of the axon, the myelin coating typically starts within less than 100 μm from the cell body of the neuron (Coombs et al. 1957; Kole 2011; Peters et al. 1991; Stuart et al. 1997). An exception from this rule is the retinal ganglion cells that give rise to optic nerve axons. While these cells may be located millimeters (in rodents) or more (in case of a human eye) away from site of optic nerve origin, their axons remain unmyelinated up to the optic disc where they bundle up

together to form the optic nerve. Because the retinal part of these axons is located on the inner (vitreal) surface of the retina, this design (although not common for all species) improves the light passage through the retina to photoreceptors, which would otherwise be highly distorted by light scattering if myelin was present.

At their distal end, upon reentering the gray matter in the target area, the axons retain their myelin sheaths up to their synaptic terminals, i.e., within microns from synaptic endings. Thus the "white" (=myelinated) part of the brain extends, at a microscopic level, beyond the boundaries of white matter, but may not be visualizable macroscopically due to the less compact organization of myelinated axons at their origin and destination sites compared to bulk white matter.

1.1.3 What Makes the White Matter Unique?

The white matter does not have neuronal cell bodies or their dendrites, which are located in the gray matter. Accordingly, there are no synapses in the white matter. The lack of classical synapses in the white matter should not be confused with the proposed presence of "axo-myelinic synapses" that imply a vesicular transmitter release from the axons in the internodal area under the myelin sheath, acting on receptors on the inner (adaxonal) surface of the myelin sheath (Stys 2011), or a release of neurotransmitter from the trunk of unmyelinated axons in white matter, acting on surrounding glia (Alix and de Jesus Domingues 2011).

Although myelinated axons are also present within the gray matter, there are specific relationships between them in white matter such as highly compact packaging along with their surrounding glia and less blood vessels that create a unique microenvironment for maintaining high fidelity axonal transmission with minimal energy supply.

In PNS, individual myelin segments are formed by Schwann cells that, after forming a multi-turn compact wrap around the axon, have their nucleus embedded within the outer cytoplasmic layer of the myelin sheath (Peters et al. 1991; Spiegel and Peles 2002). The CNS myelin is formed by oligodendrocytes whose cell bodies, after forming the multi-layer wrap, remain positioned outside the myelin sheaths (Bunge et al. 1961; Peters et al. 1991) (Fig. 1.2), being connected to individual myelin segments with processes that are sometimes called oligodendrites (Kamasawa et al. 2005; Rash 2010).

The myelin sheaths of CNS white matter axons differ from those in peripheral nerves in several respects. The white matter myelin sheaths, formed by oligodendrocytes (Bunge et al. 1961; Peters et al. 1991), are generally thinner for the same size of axon (a space saver!), and have myelin sheath periods about 10–15 % thinner compared to peripheral myelin sheaths formed by Schwann cells (Peters et al. 1991).

In the nodal area, the CNS myelin does not have microvilli extending towards each other from opposing myelin sheaths that are characteristic of PNS myelin; however, in the CNS the nodal areas are not "nude" and are covered by astrocytic endfeet (Peters et al. 1991; Scherer and Arroyo 2009). The CNS myelin has distinct sets of proteins, compared to PNS myelin (Baumann and Pham-Dinh 2001).

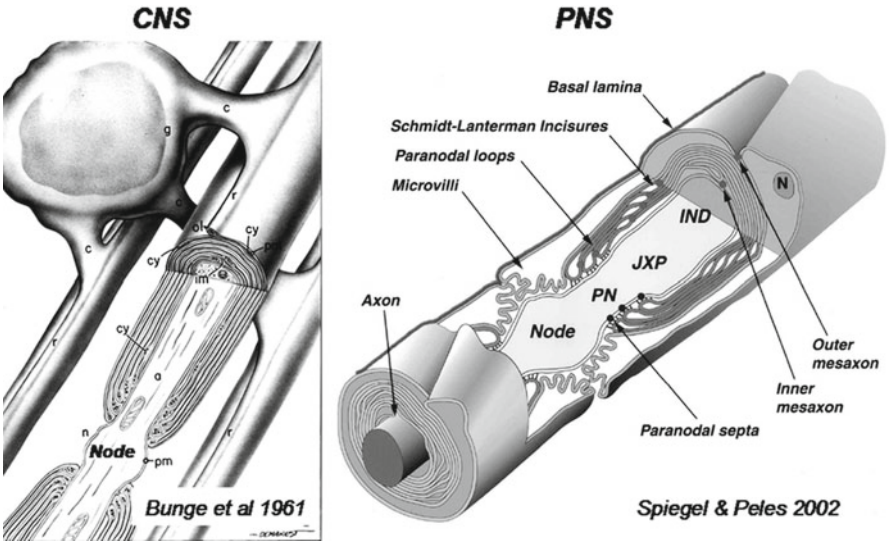


Fig. 1.2 Diagrammatic depiction of CNS and PNS myelin sheaths. White matter myelin sheaths (three shown in the diagram) are produced by an oligodendrocyte whose cell body remains outside the myelin sheath. The PNS myelin is formed by a Schwann cell whose nucleus (N) becomes embedded in the wide outer cytoplasmic layer of the sheath. Other details and differences are discussed in text. *Reproduced from Bunge MB, Bunge RP, Ris H* (1961) *Ultrastructural study of remyelination in an experimental lesion in adult cat spinal cord. J Cell Biol 10:67–94. and Spiegel I, Peles E* (2002) *Cellular junctions of myelinated nerves* (*Review*). *Mol Membr Biol 19:95–101*

1.2 The Axons of White Matter

1.2.1 Myelinated and Nonmyelinated (Unmyelinated) Axons

The majority of axons in mammalian CNS white matter are myelinated; however, some areas such as *corpus callosum*, the sacral segments of the spinal cord, and few others have large populations of nonmyelinated axons. Although nonmyelinated axons are generally believed to be smaller than myelinated axons, in the CNS white matter the upper range of diameters of nonmyelinated axons (0.3–0.5 μm, sometimes 0.8 μm) may overlap with the lower range of diameters of myelinated axons (reviewed in (Hildebrand et al. 1993)).

The basic functional differences between myelinated and nonmyelinated axons include the mode of conduction of action potentials (fast saltatory vs. slow continuous), and the actual speed (conduction velocity), which is 1 m/s or less in nonmyelinated axons of the CNS. In the CNS, the conduction velocity of myelinated axons increases sharply with axon diameter in a linear fashion, reaching nearly 6 m/s at axon diameters of 1 μm. In nonmyelinated axons, the conduction velocity increases

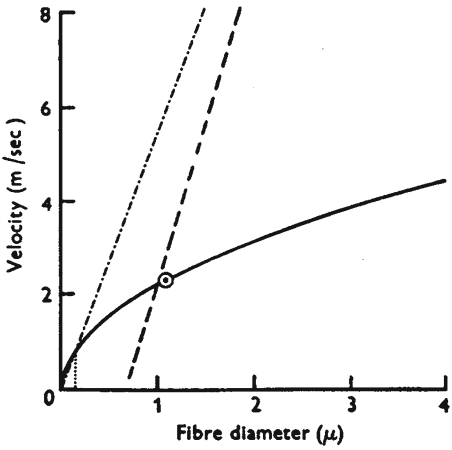


Fig. 1.3 Predicted relationships between conduction velocity and fiber diameter for small myelinated and nonmyelinated axons in CNS and PNS. The *solid line* represents the predicted conduction velocities for nonmyelinated axons regardless of location. The two *dashed steep lines* represent predicted relationships for myelinated fibers in PNS (intersecting with C-fiber curve at 1 mm diameter), and in CNS, intersecting the C-fiber curve at 0.2 mm axon diameter. These relationships show advantages of myelination over nonmyelination in terms of conduction velocities are apparent in CNS at nearly five times smaller axon diameters compared to PNS. *Reproduced from Waxman SG, Swadlow HA* (1977) *The conduction properties of axons in central white matter. Prog Neurobiol 8:297–324*

proportionally only to the square root of axon diameter, making these axons less competitive in terms of axonal conduction at diameters above 0.2 μm in CNS, and 1 μm in PNS (Waxman and Swadlow 1977) (Fig. 1.3).

1.2.2 Myelin and the Saltatory Propagation of Action Potentials

Myelin is a collective name for myelin sheaths that tightly enwrap the axons in a segmented fashion, creating conditions for fast saltatory ("jumping") propagation of action potentials (Huxley and Stampfli 1949; Stampfli 1954; Tasaki 1959; Waxman and Bangalore 2005; Waxman and Swadlow 1977).

Normally developed individual myelin segments have lengths along the axon ("internodal length") in the range of 100–200 times the diameter of the axon (Peters et al. 1991) and are separated from each other along the axons by narrow gaps known as nodes of Ranvier. The nodes of Ranvier, with densely clustered Na^+ channels (discussed below), are the key "relay stations" responsible for propagation of action potentials, and the length of the "jump" is defined by the internodal length. The nodes of Ranvier occupy only a minor portion of axonal membrane and their lengths are comparable with axon diameters. Thus, myelin covers nearly 99 % of the length of myelinated axons.

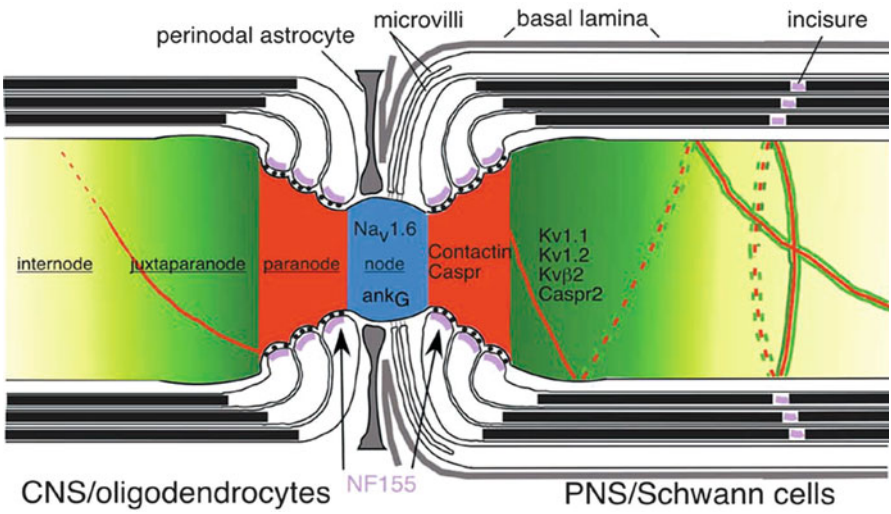


Fig. 1.4 Schematic drawing of a longitudinal aspect of nodal/paranodal/juxtaparanodal areas of myelinated axons in PNS and CNS, with relative locations of Na+channels and Kv1.1 and Kv1.2 K+channels. *Modified from Scherer SS, Arroyo EJ* (2002) *Recent progress on the molecular organization of myelinated axons. J Peripher Nerv Syst 7:1–12*

1.2.3 Topographic Segregation of Na⁺ and K⁺ Channels in Myelinated Axons

While Na^+ channels are clustered at the node, most of axonal K^+ channels are "hidden" under the myelin sheath in the juxtaparanodal area (Fig. 1.4). This unique spatial segregation of Na^+ and K^+ channels (Black et al. 1990; Rasband 2010, 2011; Salzer et al. 2008; Scherer and Arroyo 2002, 2009) defines the specific properties of action potentials in myelinated axons. While in many types of neurons the action potential is followed by an afterhyperpolarization shaped by K^+ channels (Barrett and Barrett 1976; Krnjevic et al. 1975; Storm 1990; Velumian and Carlen 1999), in myelinated axons the action potentials typically do not have an afterhyperpolarization and instead are followed by a prolonged depolarizing afterpotential (Barrett and Barrett 1982; Bowe et al. 1987) due to the restricted involvement of K^+ channels concealed under the myelin sheath (Kocsis and Waxman 1980).

Long-term changes in paranodal myelin–axon interactions in development (Girault and Peles 2002; Poliak and Peles 2003) and their reversal by disease, injury, or aging (Hinman et al. 2006; Waxman and Bangalore 2004) may result in altered molecular architecture and ion channel distribution around the node, with associated changes in axonal conduction properties and related neurological manifestations (Rasband 2010, 2011; Salzer et al. 2008; Scherer and Arroyo 2009).

The exposure of "hidden" K^+ channels after myelin retraction or breakdown is a critical factor in loss of axonal conduction, emphasized in both animal models

(Blight 1989; Nashmi and Fehlings 2001b; Targ and Kocsis 1985) and human spinal cord injury or in as multiple sclerosis patients (Cardenas et al. 2007; Hansebout et al. 1993; Hayes 2004). Targeting these channels with K^+ channel blockers helps restore lost axonal function after traumatic/ischemic CNS injury, as well as in demyelinating conditions such as MS. Trials of FDA-approved fampridine-SR, a clinical analog of fast K^+ channel blocker 4-aminopyridine, have shown safety and efficacy in MS patients (Kachuck 2009), and phase 3 trials showed improved leg strength and walking speed (Bever and Judge 2009).

1.2.4 Types of Na^+ and K^+ Channels in Myelinated Axons

1.2.4.1 Na^+ Channels

Myelinated and nonmyelinated axons use different subtypes of Na^+ channels for conducting action potentials. The nonmyelinated axons use $Na_v1.2$ channels that are diffusely distributed along these axons, while myelinated axons use $Na_v1.6$ channels clustered at the nodes of Ranvier (Waxman et al. 2004).

1.2.4.2 K^+ Channels

For the last three decades, it has been believed that K^+ channels of myelinated axons are located exclusively under the myelin sheath, based on the electrophysiological evidence (Chiu and Ritchie 1980, 1981, 1984) and insensitivity of action potentials of myelinated axons to K^+ channel blockers (Black et al. 1990; Kocsis and Waxman 1980; Waxman and Ritchie 1985), and the K^+ channels concealed under the myelin sheath were identified as containing $K_v1.1$ and $K_v1.2$ subunits of the Shaker family (Chiu et al. 1999; Rasband et al. 1998). While this basic principle remains unchallenged, more recent studies have shown the presence of other types of K^+ channels in myelinated axons, some of which are located in the nodal membrane. These channels include Kv3 (Shal-related) K^+ channels that are known to contribute to spike repolarization and rapid spiking behavior of many neurons (Rudy and McBain 2001) and Kv7 (KCNQ) channels known to underlie the so-called M-current in neurons (Brown and Passmore 2009) and the slow K^+ current at the nodes (Schwarz et al. 2006), playing roles in regulating neuronal and axonal excitability.

In the CNS, Kv3.1b, a unique splice variant of the Kv3.1 gene, was reported in a nearly all nodes in the lateral and ventral white matter containing many large myelinated axons and in relatively fewer nodes of the corticospinal tract or the optic nerve with smaller caliber axons. The function of Kv3.1b at the nodes of Ranvier of different types of axons remains largely unexplored. The ability of 4-aminopyridine to broaden CAPs recorded from adult rat optic nerve was preserved in Kv3.1-deficient mice, indicating that the effects of the drug were unrelated to Kv3.1 channels (Devaux et al. 2003).

KCNQ2 (Kv7.2) and KCNQ3 (Kv7.3) channels have been found at the initial segments of axons and the nodes of Ranvier (Devaux et al. 2004; Pan et al. 2006; Schwarz et al. 2006). Spinal cord and optic nerve axons express KCNQ2 at the nodes, while the KCNQ3 immunoreactivity detected at spinal cord nodes showed little overlap with KCNQ2 and may be localized in astrocytic end feet rather than the axon (Devaux et al. 2004). In cerebellar white matter, many nodes exhibit strong KCNQ3 immunoreactivity co-localized with KCNQ2 (Pan et al. 2006).

1.2.4.3 Other Ion Channels in Myelinated Axons

Myelinated axons in vertebrate CNS express a variety of other ion channels, with diverse time- and voltage-dependences of their gating properties (Bucher and Goaillard 2011; Debanne et al. 2011; Stirling and Stys 2010) that may contribute to the effects of ischemia: persistent Na^+ channels (Stys et al. 1993), voltage-gated Ca channels (Brown et al. 2001; Fern et al. 1995; Ouardouz et al. 2003), Ca-activated K^+ channels (K_{Ca}) channels of the BK (big conductance, Slo) type (Lev-Ram and Grinvald 1987; Ouardouz et al. 2006), and hyperpolarization-activated cyclic nucleotide-gated channels (Stys et al. 1998). In addition, recent reports indicate that glutamate receptors of the AMPA and kainate subtypes expressed on the internodal axolemma (Ouardouz et al. 2006, 2009a, b) and may play a central role in ischemic injury to spinal cord axons (Stirling and Stys 2010).

1.2.5 The Conduction Velocities of White Matter Myelinated Axons

The speed of propagation of action potentials along the axons (conduction velocity) may reach 120 m/s in largest diameter (20 μm) myelinated axons in PNS but rarely exceeds 50–70 m/s in the CNS (Debanne et al. 2011; Erlanger and Gasser 1937; Shapovalov 1975; Waxman and Swadlow 1977). In the CNS, which cannot accommodate large axons due space limitations, the diameters of myelinated axons typically range between 0.2 μm and slightly over 10 μm depending on the structure and species.

The conduction velocities of axons of specific type and location in the CNS or PNS are important electrophysiological parameters for characterizing a normal function or dysfunction of a specific peripheral nerve or a white matter tract. Because most CNS white matter axons cannot be recorded with intracellular microelectrodes due to their small size, a typical approach to record propagated action potentials is the use of various types of extracellular electrodes allowing to record at once the propagated action potentials from large bundles of axons, nerves or tracts (compound action potential, or CAP). This technique was established in 1920s–1930s in studies on peripheral nerves by Erlanger and Gasser (1944 Nobel Prize in Physiology or Medicine "for their discoveries relating to the highly differentiated functions of single nerve fibres"), who identified and characterized different types of myelinated axons in peripheral nerves (Erlanger and Gasser 1937).

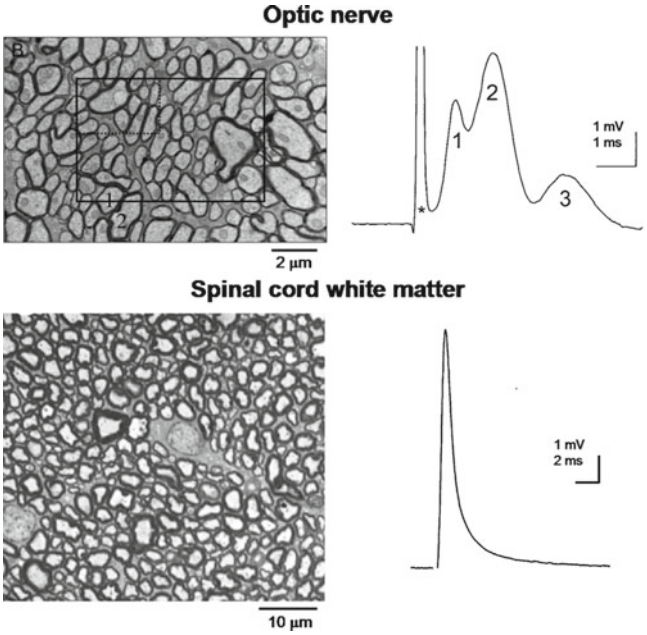


Fig. 1.5 Typical shapes of compound action potentials (CAPs) recorded from the rat optic nerve and the spinal cord white matter (WM). Note that while the optic nerve CAP has three peaks, the CAP of spinal cord WM exhibits only a single peak. A solution to this puzzle is shown in Fig. 1.6, discussed in detail in Velumian et al. 2011b. Note different scales of electron micrographs and hence larger calibers of spinal cord WM axons. *Modified from Allen L*, *Anderson S*, *Wender R*, *Meakin P*, *Ransom BR*, *Ray DE*, *Brown AM* (2006) *Fructose supports energy metabolism of some*, *but not all*, *axons in adult mouse optic nerve*. *J Neurophysiol 95*:*1917–1925*, *Fehlings MG*, *Nashmi R* (1997) *A new model of acute compressive spinal cord injury* in vitro. *J Neurosci Methods 71*:*215–224 and Nashmi R*, *Fehlings MG* (2001a) *Changes in axonal physiology and morphology after chronic compressive injury of the rat thoracic spinal cord*. *Neuroscience 104*:*235–251*

A specific advantage of CAP recording is the electrophysiological identification of distinct groups of axons by characteristic peaks, corresponding to differential arrival times of action potentials to recording site due to different conduction velocities (Erlanger and Gasser 1937). These advantages have been consistently realized in electrophysiological studies showing three peaks in optic nerve CAPs (Allen et al. 2006; Brown et al. 2003; Fern et al. 1998; Stys et al. 1998; Waxman et al. 1990) and two peaks in the *corpus callosum* CAPs (Reeves et al. 2005; Tekkok and Goldberg 2001; Tekkok et al. 2005), while other structures, such as the spinal cord white matter that has highly heterogeneous axonal content, have typically showed single-peak CAPs (Fehlings and Nashmi 1997; Fu et al. 2009; Kocsis 1985; Li et al. 1999; Li and Stys 2000; Nashmi et al. 2000; Nashmi and Fehlings 2001a; Ouardouz et al. 2006; Shi and Blight 1997; Utzschneider et al. 1991). Figure 1.5 shows a comparison of typical CAPs recorded from the optic nerve and the spinal cord white matter. The lack of distinct CAP peaks in spinal cord white matter could be due to an unfavorable combination of the higher conduction velocities and the conduction distances (5–20 mm)

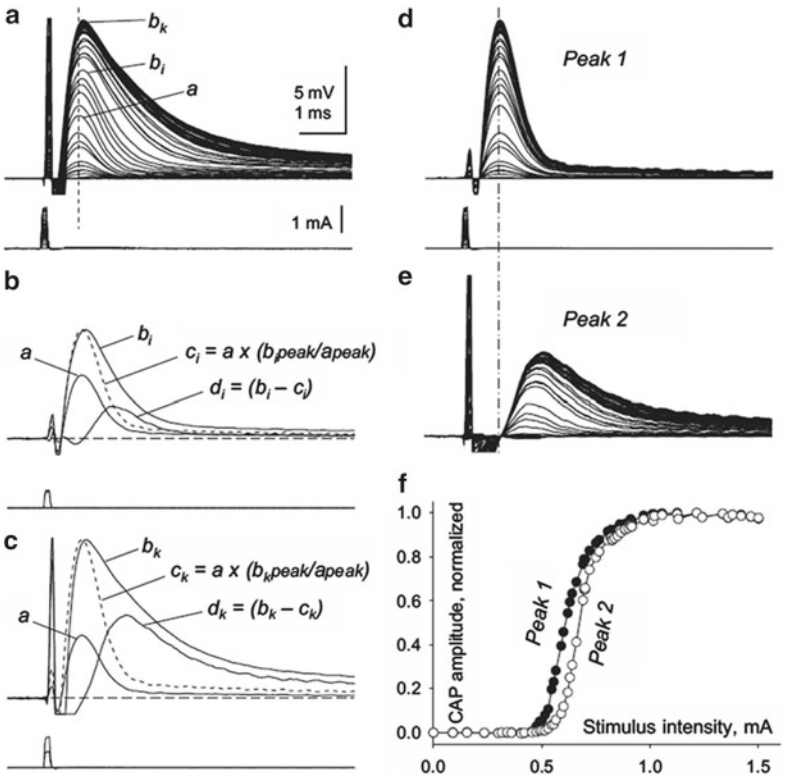


Fig. 1.6 Uncovering the hidden peaks of seemingly single-peak compound action potential (CAP) of spinal cord white matter recorded with double sucrose gap technique at conduction distance of 3.5 mm. (**a**) the increase of stimulation intensity causes not only increase of CAP amplitude but also a prolongation of CAP decay, suggesting a progressive contribution by slower conducting, higher threshold myelinated axons. (**b**, **c**) subtraction of normalized fast peak *a* from selected traces shown in (**a**) reveals hidden slow conducting component of the CAP (d_i and d_k). (**d**, **e**) the hidden fast and slow conducting components contributing to recorded CAP at different stimulation intensities traces, shown as families of traces corresponding to original traces shown in (**a**). (**f**) stimulus-response plots for *peaks 1* and *2* (normalized) showing differences in their activation thresholds. Further details in Velumian et al. 2011b. *Reproduced from Velumian AA*, *Wan Y*, *Samoilova M*, *Fehlings MG* (2011b) *Contribution of fast and slow conducting myelinated axons to single-peak compound action potentials in rat spinal cord white matter preparations. J Neurophysiol 105:929–941*

that did not allow for sufficient separation of the peaks. An improved double sucrose gap recording of spinal cord white matter CAPs and a detailed analysis of stimulus–response relationships combined with normalization–subtraction procedure has revealed a hidden slower conducting, higher threshold peak contributing to the decay phase of seemingly single-peak CAPs (Velumian et al. 2011b) (Fig. 1.6), and in some instances also an even slower conducting peak in dorsal white matter reflecting the conduction in nonmyelinated axons (Velumian et al. 2010).

One of the common sources of errors in many papers using CAP recording to characterize specific white matter pathways and measure their axonal conduction velocities

is the unjustified use of supramaximal stimulation intensities which are believed to activate all axons in the path but may cause artificial shortening of the CAP latency due to "current spread" effect, resulting in higher values of calculated conduction velocities (discussed in (Velumian et al. 2011b)). The problem is that with large currents, the stimulation may occur not only where the stimulating electrode is placed, but also at a distance which progressively increases with the increase of stimulating current pulse intensity. The extent of signal distortions caused by this effect can be assessed by analyzing the stimulus–response latency relationship. A shortening of the time interval between the stimulus artifact and the CAP peak with increasing CAP size, as it grows from submaximal to "supramaximal" size (reflecting the increase in stimulation intensity), is a clear indication of the "current spread effect" and resulting overestimation of calculated conduction velocity (discussed in (Velumian et al. 2011b)).

1.2.6 Why Are Axon Conduction Velocities Important: A Look Beyond the White Matter

The conduction velocities of distinct axonal populations are closely tied to their physiological roles. The end result of axonal conduction of action potential is a synaptic effect on the target neuron to excite or inhibit it. In case of long axonal connections such as the white matter axons, the direct effects on target cells are typically excitatory, while the inhibition, if present, occurs through local inhibitory interneurons. It is important to keep in mind that in the CNS, a single action potential of a single axon is typically insufficient to bring the postsynaptic (target) neuron to threshold to have a physiologically meaningful effect. To bring the target neuron to threshold, an action potential has to arrive to its synaptic terminal within a narrow time window of a few milliseconds with arrival of action potentials to other synapses on the same neuron in order to summate its effects with theirs (spatial summation); and alternative mechanism is repetitive action potentials in the same axon, arriving at a synapse at short intervals (temporal summation). While the spatial and temporal summation are now described in virtually every basic neuroscience textbook, somehow their role has not been so far linked to the mechanisms of neurological dysfunction after white matter injury. It is particularly important to realize that in the CNS, a minor decrease in conduction velocity of a particular axonal group may have profound neurological consequences due to changes in their ability to bring the target neuron to threshold of generating an action potential and sending it further down to other neurons.

1.3 The Myelin Sheaths of White Matter Axons

Some general features of myelin sheaths of CNS white matter were discussed in Sect. 1.1.3 of this chapter. The main features of myelin sheaths, as compact wraps, are mostly known from classical electron microscopic and X-ray diffraction studies

(Blaurock 1976; Fernandez-Moran and Finean 1957; Peters et al. 1991; Rosenbluth 1999). Some of the myelin membrane proteins that "zip" the membranes together on either intracellular on extracellular sides are common for PNS and CNS, such as the myelin basic protein (MBP), while others are specific for the CNS and include proteolipid protein (PLP), myelin–oligodendrocyte basic protein (MOBP), myelin–oligodendrocyte glycoprotein (MOG), and few others (Baumann and Pham-Dinh 2001; Scherer and Arroyo 2009).

1.3.1 The Thickness of Myelin Sheath

The thickness of myelin sheath is defined by the number of myelin membrane layers (turns) and their compactness. Larger axons have thicker myelin sheaths with more membrane layers in both the PNS and the CNS; however, the relative thickness of myelin sheaths is lower in the CNS, so that axons of same diameter have thinner myelin sheaths in the CNS compared to the PNS.

The relative thickness of the myelin sheath is quantified by the myelination ratio, or "g-ratio" (Chomiak and Hu 2009; ffrench-Constant et al. 2004; Hildebrand and Hahn 1978; Rushton 1951), which represents the ratio of the axon diameter to the diameter of axon with myelin ("fiber diameter").

In mature PNS axons, the myelination ratio is 0.6, while in the CNS it is 0.75 (Peters et al. 1991), indicating that axons of similar diameter have thinner myelin sheaths in the CNS compared to the PNS.

A practical use of the myelination ratio for determining the number of myelin layers is illustrated in Table 1.1 that shows calculations of the total membrane area of myelin segments made by oligodendrocytes on axons of different diameters.

1.3.2 The "Compactness" of Myelin Sheath

The compact organization of internodal myelin, known from many textbooks, is believed to be the main factor defining the efficient electrical insulation of the axons, accordingly, the efficient "jumping" of action potentials from node to node, insuring rapid propagation of action potentials. The compactness of myelin raises questions about how the cytoplasmic access to its membranes is achieved, which is critically important for myelin maintenance and repair.

The structural organization of the mature myelin sheath and its cytoplasmic compartments is known mostly from electron microscopic studies that used histologically processed, and hence dehydrated, tissue where the cytoplasmic compartments may have undergone significant shrinkage (discussed in (Peters et al. 1991); see also (Frotscher et al. 2007; Rostaing et al. 2006) with more recent discussions of the shrinkage problem). Shrinkage of myelin sheaths by histological processing was revealed by comparing the myelin sheath periods of fresh (X-ray diffraction) and

Table 1.1 Calculated membrane area of individual myelin sheaths made by a single oligodendrocyte on axons of different diameters

	Axon diameter (μm)			
	0.5	1	5	10
Myelin segment length (*l*), μm	50	100	500	1,000
Myelination ratio	0.75	0.75	0.75	0.75
Outer diameter of axon with myelin, μm	0.67	1.33	6.67	13.33
Myelin sheath thickness, μm	0.08	0.17	0.83	1.67
Myelin sheath period (layer thickness), Å	155	155	155	155
Myelin layer thickness, μm	0.0155	0.0155	0.0155	0.0155
Number of myelin layers (turns) (*n*)	5	11	54	108
Myelin sheath mid-thickness diameter (*D*), μm	0.58	1.17	5.83	11.67
Myelin segment total membrane area, μm^2	492	3,939	492,384	3,939,068
Double layer membrane area, μm^2	985	7,878	984,767	7,878,136
Equivalent sphere diameter, μm^2	17.71	50.09	560.02	1,583.97
Number of myelin segments by membrane area to correspond the sheath of a 10-μm axon	8,000	1,000	8	1

The total area of myelin sheath membrane is calculated as *p*D*n*l*, where *D* is average diameter of myelin sheath turn is measured at the mid-thickness of myelin sheath, *n* is the number of myelin layers (turns) and *l* is the length of myelin segment. The length of myelin segment is assumed to be 100× diameter of the axon (Peters et al. 1991). The thickness of myelin sheath is calculated from a myelination ratio (ratio of axon diameter to fiber (axon + myelin) diameter) of 0.75 that is characteristic of CNS myelin (Peters et al. 1991). The number of myelin membrane layers (turns) is calculated by dividing the thickness of myelin sheath by the myelin period (155 Å, taken from X-ray diffraction (Avila et al. 2005)). A double layer membrane area is assumed to be the two membranes of oligodendrocyte that come close together to form a single myelin layer. The bottom row shows how many myelin segments on smaller axons would correspond to the myelin sheath area made on a large (10 mm) diameter, suggesting that the membrane area 50–60 myelin segments made by an oligodendrocyte on small axons is far below the myelin sheath membrane area on a large axon

fixed (electron microscopy) tissue samples, showing reduction of periods from 17.5–18 nm to 11.5–14 nm (discussed in (Peters et al. 1991)). A more recent X-ray diffraction study of unfixed sciatic nerve and optic nerve in mice showed myelin sheath periods 17.1–17.4 nm and 15.3–15.5 nm, respectively (Avila et al. 2005).

The cytoplasmic compartments of myelin sheaths can be visualized by intra-myelin injections of hydrophilic fluorescent dyes such as Lucifer Yellow, widely used for visualizing the morphology of neurons in fixed or living tissue (Buhl 1993; Hanani 2012).

Injection of diffusible dyes into oligodendrocytes had been previously performed in living conditions (Butt 2005; Butt and Ransom 1989; Karadottir et al. 2005); however, the dye-filled spaces were analyzed only after histological processing and fixation of the tissues, which may have caused shrinkage or collapse of cytoplasmic spaces discussed above. Some inference about intra-myelin cytoplasmic spaces has been derived from fluorescent dye injection studies on PNS axons (Balice-Gordon et al. 1998; David et al. 1993; Jeng et al. 2006). A similar approach was recently used for studying the cytoplasmic spaces of living myelin sheaths of mature rat spinal cord white matter axons (Velumian et al. 2011a)

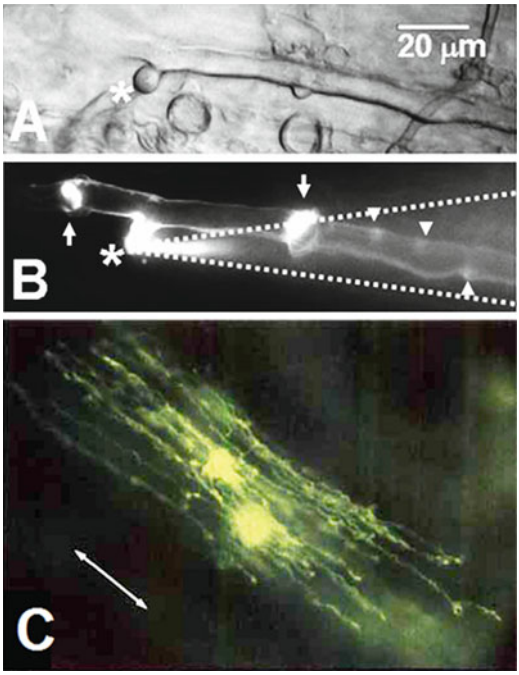


Fig. 1.7 Two extreme morphological types of oligodendrocytes, injected with fluorescent dye Lucifer Yellow to visualize their morphology. (**a**, **b**) type 4 oligodendrocyte (*asterisk*), myelinating a single large axon in rat spinal cord white matter (modified from Fig. 1 in Velumian et al. 2011a). (**c**) Two type 1 oligodendrocytes in optic nerve, with numerous parallel processes running along small axons (modified from Fig. 1 in Butt and Ransom 1989), brought to same scale with (**a**, **b**). Panels (**a**, **b**) represent the oligodendrocyte imaged with infrared optics (**a**) and with Lucifer yellow fluorescence (**b**). The patch pipette used injecting Lucifer yellow is seen in (**b**), outlined with *dashed lines*. (**b**) also shows two "open" Schmidt–Lanterman clefts (*arrows*) heavily filled with the diffusing dye, and several "partially open" Schmidt–Lanterman clefts (*arrowheads*). *Reproduced from Velumian AA, Samoilova M, Fehlings MG* (2011a) *Visualization of cytoplasmic diffusion within living myelin sheaths of CNS white matter axons using microinjection of the fluorescent dye Lucifer Yellow. NeuroImage 56:27–34. and Butt AM, Ransom BR* (1989) *Visualization of oligodendrocytes and astrocytes in the intact rat optic nerve by intracellular injection of lucifer yellow and horseradish peroxidase. Glia 2:470–475*

(Fig. 1.7b), which revealed that in living conditions, the sheaths are fenestrated by networks of diffusionally interconnected cytoplasmic spaces including Schmidt–Lanterman clefts that may exist in different open states. In addition, while it is widely believed that the outer cytoplasmic layer of CNS myelin sheaths has a longitudinal ridge-like appearance (also called "tongue") (Figs. 1.2 and 1.8), this may be a result of shrinking due to histological processing, and indeed fluorescent due injections into living CNS myelin with subsequent 3D reconstructions did not reveal any ridge or tongue, but rather an encuffing shape of the outer cytoplasm (Fig. 1.9) (Velumian et al. 2011a).

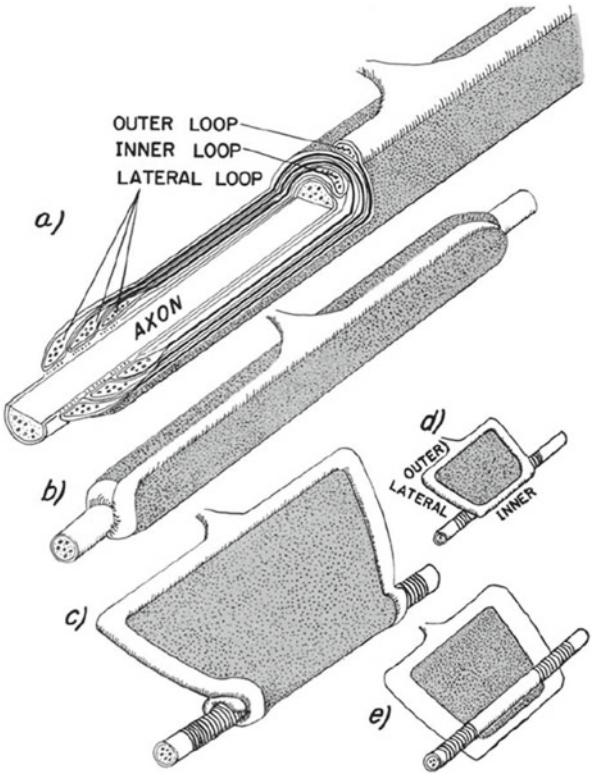


Fig. 1.8 A hypothetically rolled/unrolled myelin sheath, initially proposed for oligodendrocyte-made sheaths and explaining the nature of paranodal myelin loops and their relation to inner and outer myelin cytoplasm. The compact part of the myelin sheath is shown in *gray* and the cytoplasmic compartments are shown in *white*. *Reproduced from Hirano A*, *Dembitzer HM* (1967) *A structural analysis of the myelin sheath in the central nervous system*. *J Cell Biol 34*:*555–567*

1.3.3 Paranodal Specializations of Myelin Sheaths

The paranodal myelin loops, called so due their specific appearance on longitudinal electron microscopic images of nodal/paranodal area, in fact represent a spiraled continuum of lateral cytoplasmic ridge of the hypothetically "unrolled" myelin sheath (Fig. 1.8), first proposed in studies of CNS myelin (Hirano and Dembitzer 1967). As shown in Fig. 1.8 and also in Fig. 1.2, the cytoplasm of paranodal loops is connected to the perinuclear cytoplasm of myelin forming cell, either oligodendrocyte or the Schwann cell, through the outer cytoplasmic layer of the myelin sheath.

As shown in Fig. 1.10, the appearance of paranodal myelin loops in longitudinal sections depends on the level of cut, and may vary from true "loops" to transverse bridges, both reflecting the coiled lateral cytoplasmic ridge of the Hirano and Dembitzer (1967) rolled/unrolled model.

While the paranodal myelin loops are typical both in the CNS and the PNS, the CNS myelin does not have microvilli (see Sect. 1.1.3), but instead there is an astrocytic process over the nodal area. The outermost myelin "loop" of the paranodal area has gap junctional contact with the astrocytic process, believed to be involved in

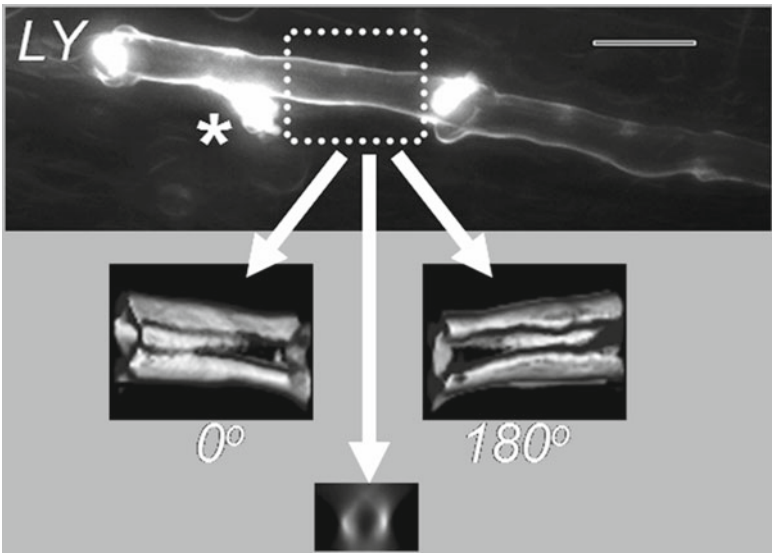


Fig. 1.9 3D reconstruction of Lucifer yellow-injected myelin sheath in spinal cord white matter, showing an encuffing rather than ridge-like shape of the outer cytoplasmic layer. The 3D reconstructed fragments are shown at two rotation angles, and also in a cross section mode. *Modified from Velumian AA, Samoilova M, Fehlings MG* (2011a) *Visualization of cytoplasmic diffusion within living myelin sheaths of CNS white matter axons using microinjection of the fluorescent dye Lucifer Yellow. NeuroImage 56:27–34*

"siphoning" of K^+ ions from juxtaparanodal sub-myelinic area (Kamasawa et al. 2005; Rash 2010) after its accumulation due to efflux from axons following action potential generation (Barrett and Barrett 1982; David et al. 1992, 1995). The "siphoning" hypothesis (Fig. 1.11), while being highly attractive, still needs experimental confirmation, as it also raises the question of how the axons replenish their lost K^+.

The molecular interactions at the myelin–axon interface in the paranodal region involve contactin, contactin-associated protein (caspr), and neurofascin (NF155), as well as few other proteins. The paranodal myelin "loops" are kept together on their lateral sides by tight junctions (Poliak et al. 2002; Scherer and Arroyo 2009), and have gap junctions connecting the neighboring loops and the outer loop to an astrocytic process (Arroyo and Scherer 2000; Kamasawa et al. 2005; Rash 2010).

The myelin sheath expresses a unique set of connexins: Cx47, Cx32, and Cx29 (Altevogt et al. 2002; Altevogt and Paul 2004; Kamasawa et al. 2005; Kleopa et al. 2004; Li et al. 1997; Nagy et al. 2003a, b; Scherer et al. 1995). Cx32 is the main connexin involved in making gap junctional shortcuts through areas of loose myelin, Schmidt–Lanterman clefts and paranodal myelin loops (Kamasawa et al. 2005; Li et al. 1997, 2004; Rash et al. 1997), while Cx47 is expressed predominantly in the outer myelin (Kamasawa et al. 2005). In addition to the possible role of myelin connexins to K^+ siphoning through myelin–astrocyte–blood capillary pathway

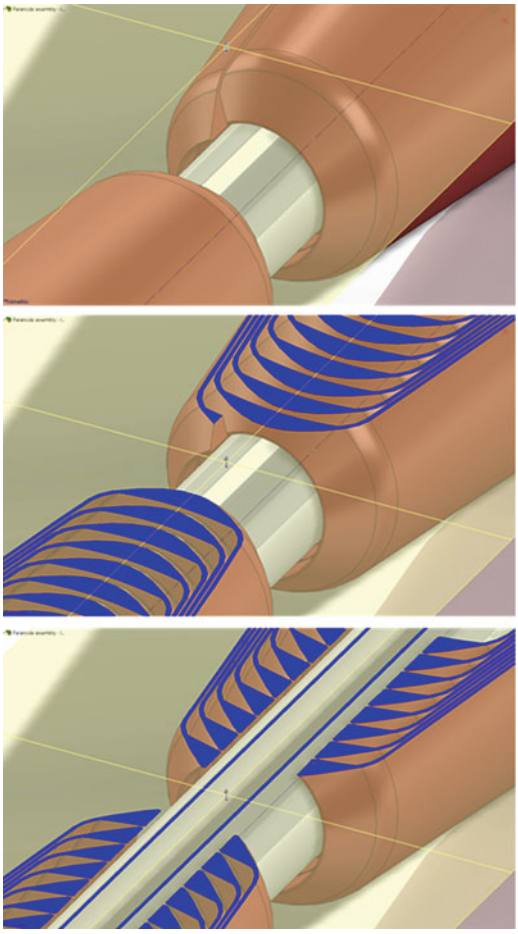


Fig. 1.10 3D model of paranodal myelin showing different appearances of paranodal myelin "loops" at two levels of longitudinal transections. Made using SolidWorks software (Dassault Systèmes). The intra-myelin cytoplasm in shown in *blue*

(Kamasawa et al. 2005; Rash 2010), it has been proposed (Nave 2010) that myelin–oligodendrocyte gap junctional contacts with astrocytes may be important for metabolic support.

1.3.4 Cytoplasmic Specializations of the Internodal Myelin

The internodal myelin has inner and outer cytoplasmic layers ("loops" in transverse sections, and in its "compact" part may have various cytoplasmic "pockets" as well as spiraled cytoplasmic tunnels known as Schmidt–Lanterman clefts (formerly called incisures), extending from outer towards inner cytoplasmic layer. The Schmidt–Lanterman clefts are well known in PNS myelin, while in CNS their presence had been debated (Baumann and Pham-Dinh 2001) until they were visualized ultrastructurally (Kamasawa et al. 2005) and by diffusion of injected fluorescent

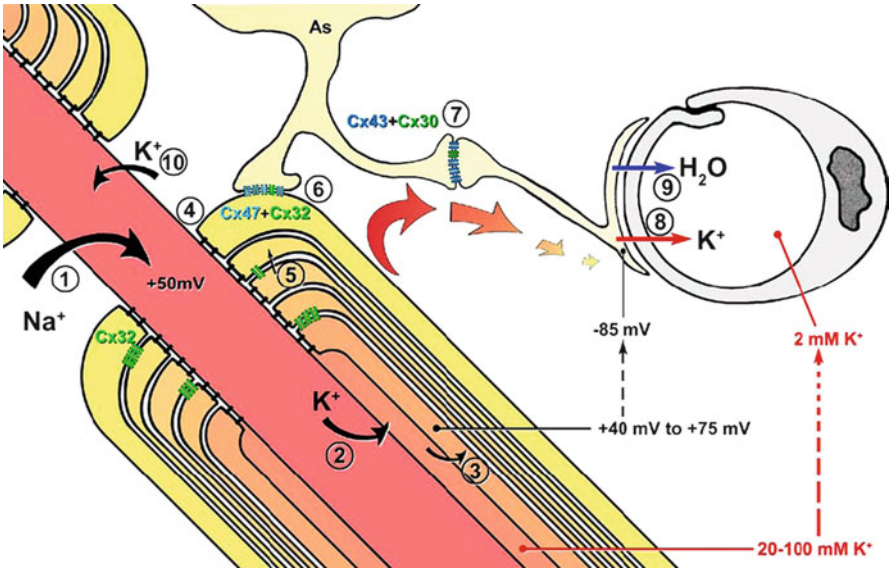


Fig. 1.11 Hypothetical pathway of potassium ion "siphoning" from under the myelin sheath following its juxtaparanodal accumulation after an action potential. The proposed pathway includes voltage-driven K^+ uptake by the inner cytoplasmic layer of myelin sheath (*3*), intracellular diffusion through paranodal myelin loops, facilitated by connexin32 (Cx32) autologous gap junctions (*5*), and movement from outermost paranodal loop to astrocyte via heterologous gap junction (*6*) and eventually to blood capillary. (Modified from Kamasawa et al. (2005). *Reproduced from Rash JE* (2010) *Molecular disruptions of the panglial syncytium block potassium siphoning and axonal saltatory conduction*: *pertinence to neuromyelitis optica and other demyelinating diseases of the central nervous system*. *Neuroscience 168*:*982–1008*

dye, which also revealed that they can exist in different "open states" within the same myelin segment (Velumian et al. 2011a) .

A diagram summarizing the current and emerging views of internodal myelin cytoplasm in the CNS is shown in Fig. 1.12. In the PNS myelin, the loops of the Schmidt–Lanterman clefts are interconnected via gap junctions that make diffusional shortcuts facilitating exchange between the outer and inner myelin layers (Balice-Gordon et al. 1998) and it is expected that same may occur in the CNS myelin due to the expression of Cx32 in Schmidt–Lanterman clefts (Kamasawa et al. 2005) (depicted in Fig. 1.12).

1.4 Periaxonal Glia and Glial–Axonal Networks of the White Matter

White matter macroglial cells, the astrocytes and oligodendrocytes, have been extensively studied immunohistochemically due to availability of a variety of specific immunomarkers (listed in (Ness et al. 2005) for oligodendrocytes and in

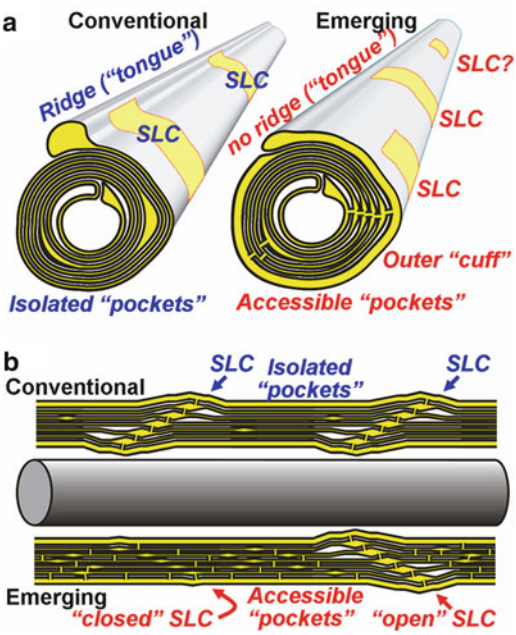


Fig. 1.12 Conventional and emerging views of spatial organization of cytoplasmic spaces of living CNS myelin sheaths. The new features, suggested by dye-injection study of living myelin, are shown with *red text*. The proposed models include intra-myelin gap junctions, which remain unexplored in CNS myelin in terms of dye diffusion. SLC: Schmidt–Lanterman cleft. *Reproduced from Velumian AA, Samoilova M, Fehlings MG* (2011a) *Visualization of cytoplasmic diffusion within living myelin sheaths of CNS white matter axons using microinjection of the fluorescent dye Lucifer Yellow. NeuroImage 56:27–34*

(Reichenbach and Wolburg 2005) for astrocytes). Direct electrophysiological or cellular-level imaging studies of these cells and their interactions with neuronal elements in the brain tissue have been limited for long time by the small size of their cell bodies and difficulties of their visual identification. Earlier studies (late 1980s–1990s) used fluorescent dye injection through intracellular microelectrodes or whole cell-recording patch pipettes with subsequent morphological identification of the cells (Berger et al. 1991; Butt and Ransom 1989, 1993; Chvatal et al. 1995; Giaume and McCarthy 1996; Pastor et al. 1998; Steinhauser et al. 1992; Weruaga-Prieto et al. 1996).

A development of transgenic animals with fluorescent protein expression restricted to specific glial cell types (Hirrlinger et al. 2005; Jabs et al. 2005; Matthias et al. 2003; Mulligan and MacVicar 2004; Schipke et al. 2002; Wallraff et al. 2004; Yuan et al. 2002) has boosted the studies of glial cells in acute or cultured brain tissue slices as well as in vivo; however, most of these studies have been done on the gray matter. Only a very limited number of studies using fluorescently tagged glial cells had been done so far in the white matter (Hamilton et al. 2008; Maglione et al. 2010; Shannon et al. 2007; Sun et al. 2010; Wasseff and Scherer 2011; Yuan et al. 2002).

1.4.1 The Four Morphological Types of Oligodendrocytes

The myelin-forming cells of the CNS, the oligodendrocytes, are richly present in white matter, along with their precursor cells. Unlike the Schwann cell, a single

oligodendrocyte may form myelin segments on multiple (up to 60) neighboring axons, and while this presents certain space-saving and unification advantages, in case of damage a death or dysfunction of a single oligodendrocyte will have a "multiplication effect" shutting down up to 60 axons at once.

Although the image of oligodendrocyte as a cell that makes tens of long parallel processes has become very popular in the literature and textbooks in the past two decades, this is only one of the four morphological types of myelinating oligodendrocytes. The reason why this multi-process type of oligodendrocytes has become popular may be due to the predominance of papers done on white matter structures with very small diameter axons where this type of oligodendrocytes is typical, such as the optic nerve and *corpus callosum*, and historically the first visualizations of the whole structure of oligodendrocytes with intracellular injection of fluorescent dye were performed in the optic nerve (Butt and Ransom 1989) (Fig. 1.7c). The name "oligodendrocytes," which historically implied "cells with very few processes," is in apparent discord with the popular image of these cells as myelinating tens of axons. In fact, there are other types of oligodendrocytes, present in white matter areas with larger axons, where they have only a few and even just one process.

The number of axons myelinated by individual oligodendrocytes depends on axon calibers (diameters), and oligodendrocyte with tens of processes myelinating the smallest caliber axons is in fact a "type 1" oligodendrocyte, one of the four main types of oligodendrocytes as described by Del Rio Hortega who discovered these cells in 1921 (cited from (Szuchet 1995; Szuchet and Seeger 2004)). The number of oligodendrocyte processes, and accordingly the number of myelinated axons, progressively decreases from type 1 to type 4 oligodendrocyte with increasing axon diameters, and the largest white matter axons have their myelin segments made by a single type 4 oligodendrocyte. Importantly, the cell body of even type 4 oligodendrocyte remains outside the myelin sheath (Fig. 1.7a, b) (Velumian et al. 2011a).

It might be expected that the total area of myelin membranes made by different types of oligodendrocytes is similar; however, calculations shown in Table 1.1 show that this is not the case. As shown in Table 1.1, it would take not 50–60 but 8,000 small myelin segments made by type 1 oligodendrocyte to be comparable with the area of a single segment made by type 4 oligodendrocyte.

1.4.2 The White Matter Astrocytes

The astrocytes of white matter are predominantly of a specific morphological type—fibrous, as opposed to protoplasmic astrocytes that are the main type of these cells in the gray matter (Reichenbach and Wolburg 2005; Shannon et al. 2007). The fibrous astrocytes have their long processes running parallel and along the axons for distances up to 300 μm (Mills et al. 2004; Reichenbach and Wolburg 2005; Shannon et al. 2007) (Fig. 1.13).

Many functional roles of astrocytes known from studies in gray matter, such as maintaining the integrity of blood–brain barrier, ammonium detoxification,

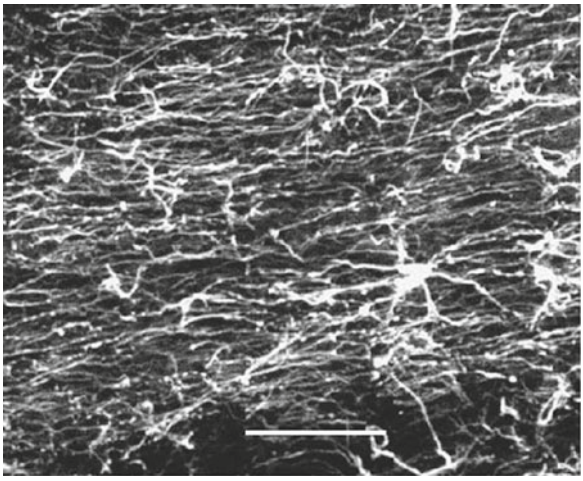


Fig. 1.13 Fibrous astrocytes immunohistochemically labelled astrocytes in rat spinal cord dorsal white matter with an astrocyte-specific marker GFAP. Note long astrocytic processes running between the axons parallel to rostro-caudal axis. The cell bodies of astrocytes are rarely seen with GFAP immunostaining due to specific localization of the glial fibrillary acidic protein (GFAP) to main processes of these cells. Scale bars: 50 mm. *Reproduced from Mills LR*, *Velumian AA*, *Agrawal SK*, *Theriault E*, *Fehlings MG* (2004) *Confocal imaging of changes in glial calcium dynamics and homeostasis after mechanical injury in rat spinal cord white matter*. *NeuroImage 21:1070–1083*

regulation of extracellular pH, free radical scavenging and others (Ransom et al. 2003) can be anticipated in the white matter as well, and some of them, such as the extracellular K^+ buffering, were initially proposed in studies on the optic nerve (Orkand et al. 1966, 1981), although more pronounced in some amphibian species that do not have myelinated axons in the optic nerve (Orkand et al. 1966).

White matter astrocytes are an important source of energy supply in white matter due to their deposits of glycogen which can be converted to lactate and shuttled to axons as oxidative fuel (Brown et al. 2003; Ransom and Fern 1997).

Astrocytic endfeet on brain capillaries are an important part of the blood–brain barrier (Abbott 2005), and studies in gray matter have shown the important role of astrocytes in controlling local cerebral microcirculation (Attwell et al. 2010; Gordon et al. 2008; Mulligan and MacVicar 2004).

An important new aspect of astrocytic K^+ buffering in white matter, relevant to axonal function, was proposed based on an immunoelectron microscopy study of the topography and co-associations of gap junction-forming proteins Cx47 and Cx32 (oligodendroglial) and connexin43 (astrocytic) (Kamasawa et al. 2005). This study identified autologous gap junctions between the loops of Schmidt–Lanterman clefts and between paranodal myelin loops, and heterologous gap junctions between astrocytes and oligodendrocytes, specifically in the paranodal myelin area. The authors proposed that astrocyte–myelin gap junctions are siphoning out elevated K^+

from sub-myelinic spaces in the juxtaparanodal area to the blood vessels via paranodal myelin loops myelin and astrocytes (Kamasawa et al. 2005; Rash 2010) (Fig. 1.11).

1.4.3 Glial–Axonal Networks of the White Matter

There is a clear gap between the accumulated knowledge about molecular expression and localization of white matter gap junction proteins and the limited functional data on their role in normal physiological conditions and after ischemic damage. The extent of astrocyte–oligodendrocyte gap junctional communications may be much broader (Orthmann-Murphy et al. 2008), and coupled with evidence for direct gap junctional communications between oligodendrocytes in some structures (*corpus callosum*: (Maglione et al. 2010; Wasseff and Scherer 2011)) of young animals but not in others of adult animals (spinal cord white matter: (Velumian et al. 2011a)), may require further studies to understand the organization and function of complex glial networks of white matter in relation to axonal function.

The astrocytic networks of the white matter are apparently not as extensive as in gray matter. Gray matter astrocytes are extensively interconnected via gap junctions, providing pathways for exchange with ions and low molecular weight substances and propagation of Ca^{2+} waves involved in above discussed extracellular K^+ buffering, control of microcirculation and many other functions. The gap junctional communications between astrocytes in gray matter had been well documented by dye coupling studies, where low molecular weight substances such as biocytin or fluorescent dyes injected into an individual astrocyte spread to other astrocytes (Ceelen et al. 2001; Konietzko and Muller 1994; Theis et al. 2005; Wallraff et al. 2004; Xu et al. 2010) or by Ca^{2+} waves spreading from a single activated astrocyte (Mulligan and MacVicar 2004). While it might be expected that similarly extensive gap junctional coupling exists between astrocytes within white matter as a part of "panglial syncytium" (Rash 2010), there is no direct evidence comparable to studies in gray matter to support this, with the exception of an early study done in hippocampal slices where a dye injected into an astrocyte in gray matter was also found a in a few astrocytes in the alveus (a white matter structure) while extensively filling numerous astrocytes in the gray matter (Konietzko and Muller 1994).

Our recent study on spinal cord white matter with Lucifer Yellow injections into oligodendrocytes or myelin sheaths did not reveal signs of dye transfer to astrocytes (Velumian et al. 2011a), and this could be due to either unidirectionality of dye coupling between astrocytes and oligodendrocytes, as shown in retina (Robinson et al. 1993), or a normally closed state of gap junctions between the myelin sheath and its contacting the astrocytic processes. Earlier studies with Lucifer Yellow also did not reveal dye coupling between glial cells in spinal cord white matter (Pastor et al. 1998). In *corpus callosum*, on the other hand,

injections of a smaller molecule, biocytin, into oligodendrocyte cell bodies, revealed a dye transfer to neighboring oligodendrocytes and, to less extent, astrocytes (Maglione et al. 2010). Further studies, with biocytin, are needed to test if spinal cord white matter glial cells are coupled to an extent similar to that in *corpus callosum*.

1.5 Concluding Remarks: From Function to Dysfunction

1.5.1 Relative Resistance of White Matter to Hypoxia/Ischemia

White matter is relatively more resistant to ischemia compared to gray matter. The metabolic rate of white matter is lower compared to gray matter; it receives less blood supply and has a higher infarction threshold (Arakawa et al. 2006; Bristow et al. 2005; Marcoux et al. 1982). Due to lower blood supply, the cellular elements of white matter have evolved for operating with lower levels of externally delivered oxygen and nutrients compared to gray matter (Harris and Attwell 2012; Hayashi et al. 1983; Ransom et al. 2004).

Although the ischemic injury of gray and white matter has a number of common characteristics, there are unique features of white matter damage (Bakiri et al. 2008; Baltan 2009; Matute 2010, 2011; Ransom et al. 2011; Stirling and Stys 2010; Stys and Waxman 2004), largely defined by the presence of different but interacting compartments: the axons, the oligodendrocyte–myelin units, and astrocyte–myelin interactions. The loss of axonal conduction therefore may be secondary to any or a combination of above compartments.

In the isolated rat optic nerve, the hypoxia/ischemia-induced axonal conduction failure and the recovery upon reperfusion correlated with developmental stage of myelination (Fern et al. 1998; Foster et al. 1982). On the other hand, optic nerves of myelin-deficient rats (Waxman et al. 1990) or the spinal cord following demyelination (Imaizumi et al. 1998) exhibit slower axonal conduction block during hypoxia and greater recovery upon reperfusion, suggesting that myelination, or changes associated with it, may confer susceptibility to energy deprivation.

Although ischemia may lead to irreversible functional and structural damage (i.e., in an in vitro *corpus callosum* (Tekkok et al. 2005)), in the isolated optic nerve the ultrastructural signs of axonal damage following anoxia/reperfusion are limited mainly to larger diameter axons, and the conduction is preserved in ~30 % of axons (Waxman et al. 1992, 1993). It has been proposed, among other mechanisms, that conduction block may be due to changes in the myelin or the paranodal axo-glial junctions. The ultrastructural changes in isolated optic nerve subjected to chemical ischemia (Micu et al. 2006) included, in addition to axonal damage, focal myelin sheath splitting and separation of the myelin lamellae.

1.5.2 Possible Mechanisms of White Matter Axonal Dysfunction in Acute Ischemia–Reperfusion

1.5.2.1 Ischemia-Induced Ca^{2+} Overload

Animal studies point to the central role of Ca^{2+} "dyshomeostasis" (an imbalance between transmembrane Ca^{2+} entry, intracellular Ca^{2+} sequestration and buffering, and membrane ion exchangers that would normally pump the excess Ca^{2+} out of the cells) in the pathophysiology of white matter (Bakiri et al. 2008; Baltan 2009; Matute 2010, 2011; Ransom et al. 2011; Stirling and Stys 2010; Stys and Waxman 2004), although the sites of Ca^{2+} accumulation (axon vs. myelin), the source of Ca^{2+} (extracellular vs. internal stores such as mitochondria and endoplasmic reticulum) and the routes of Ca^{2+} entry (reversed Na/Ca exchanger, voltage-dependent Ca channels and glutamate receptors of particular subtype and localization) may contribute differently to white matter damage in brain-region specific manner and vary depending on the degree of injury (i.e., ischemia as compared with hypoxia alone) and age. The complex cascade of events triggered by energy deprivation and Ca^{2+} influx, leading to irreversible white matter damage, is beyond the scope of this chapter.

The presence of Ca-permeable glutamatergic AMPA (reviewed in (Park et al. 2004)), purinergic $P2X_7$ (Domercq et al. 2010; James and Butt 2002; Matute et al. 2007), and the more recently found NMDA receptors in oligodendrocytes and the myelin sheath (Karadottir et al. 2005; Micu et al. 2006, 2007), suggest a role of Ca^{2+} influx into the myelin sheath as a factor possibly triggering Ca-mediated changes in myelin–axon interactions, reviewed in (Stys 2011).

1.5.2.2 Changes of Extracellular Levels of K^+

Extracellular K^+ ($[K^+]_o$) elevations that might contribute to the loss of axonal conduction in ischemia–reperfusion have received little attention, primarily due to the virtual absence of the effects of physiological/pathological increases of $[K^+]_o$ on myelinated axons (but see (Devaux and Gow 2008)). Anoxia produces less changes in $[K^+]_o$ in white matter compared to gray matter: 14 mM (Ransom et al. 1992) vs. 30 mM (Vyskocil et al. 1972) with bath $[K^+]_o$ 3 mM. Spreading depression-like events, known in gray matter (Somjen 2001), have not been reported in white matter. The K^+ released from myelinated axons likely accumulates in the periaxonal space under the myelin sheath to much higher concentrations (David et al. 1992, 1993), from where it may be dispersed by entering axons and oligodendrocytes via Na,K-ATPases (Ransom et al. 2000), leaking out via diffusional spaces in paranodal axo-glial junctions (Rosenbluth 2009) or siphoning out through myelin and its connexin-formed gap junctional network with astrocytes (Kamasawa et al. 2005; Rash 2010) that supposedly provides spatial K^+ buffering similar to that in astrocytic networks in gray matter (Kofuji and Newman 2004; Theis et al. 2005).

1.5.2.3 Role of Gap Junctions

Formed by connexins (Cxs), gap junctions allow for direct transfer of low molecular weight substances and ions, known between many cell types in the mammalian brain and other tissues. The importance of gap junctions in relation to myelinated axons is evident from human disorders, such as the X-linked form of Charcot–Marie–Tooth disease (PNS; mutations affecting Cx32) and Pelizaeus–Merzbacher-like diseases or hereditary spastic paraplegia (CNS; mutations affecting Cx47) (reviewed in (Kleopa et al. 2010; Rash 2010)) and in animal models (Magnotti et al. 2011; Menichella et al. 2003, 2006; Sargiannidou et al. 2009; Scherer et al. 2005). Deletion of astrocytic connexins Cx43 and Cx30 (Lutz et al. 2009) or astrocytic Cx43 and oligodendrocytic Cx32 (Magnotti et al. 2011) resulted in white matter pathology as well, further supporting the key functional role of gap junctional networks in white matter.

While the role of astroglial networks in injury (trauma/ischemia) is well studied in gray matter (Chew et al. 2010; Giaume et al. 2010), only a few studies have addressed this in white matter. Up-regulation of Cx43 was detected in traumatic spinal cord injury models (Cronin et al. 2008; Lee et al. 2005; O'Carroll et al. 2008; Theriault et al. 1997) and in optic nerve after ischemia (Danesh-Meyer et al. 2008). Suppression of Cx43 up-regulation using an Cx43-antisense oligodeoxynucleotide (Cronin et al. 2008; Danesh-Meyer et al. 2008) or blocking astrocyte gap junction communication with Cx43 mimetic peptide (O'Carroll et al. 2008) demonstrated beneficial effects after traumatic/ischemic injury. In addition, opening of pannexin hemichannels during ischemia may contribute to white matter damage (Domercq et al. 2010) similar to that in gray matter (Thompson et al. 2006).

1.5.3 Changes in Myelin and Myelin–Axon Interface

The quality of myelin insulation is the key factor defining the "leak-proof" propagation of action potentials. A leakage may occur at the paranodal myelin–axon interface, or through the internodal myelin if it decompacts, resulting in short-circuiting of the myelin insulation and slowing down or halting the axonal conduction of action potentials.

The tight contacts between paranodal myelin loops and the axonal membrane had been long believed to provide a diffusional (and hence electrical) barrier that "seals" around the axonal membrane (Rosenbluth 2009). Accumulating evidence suggests that this "seal" is not perfect and that there is a diffusional access from nodal to sub-myelinic space. Electron tomographic 3D analysis of the paranodal areas of the PNS (Perkins et al. 2008; Sosinsky et al. 2005) and the CNS (Nans et al. 2011) myelinated axons revealed a complex structure that may contain pathways for "lateral diffusion." The diffusional access to sub-myelinic space from the nodal area and around the Schmidt–Lanterman clefts of the internodal myelin had been directly demonstrated recently on the PNS axons with fluorescent tracers (Mierzwa et al. 2010;

Shroff et al. 2011). There is no comparable data on such diffusion in the CNS white matter axons, and it remains to be elucidated if these diffusional pathways could "open up" after injury or stroke. This, along with possible swelling and decompaction of the internodal myelin, could be the ultimate cause of axonal dysfunction in many pathological conditions.

References

Abbott NJ (2005) Dynamics of CNS barriers: evolution, differentiation, and modulation. Cell Mol Neurobiol 25:5–23

Alix JJP, de Jesus Domingues AM (2011) White matter synapses. Neurology 76:397–404

Allen L, Anderson S, Wender R, Meakin P, Ransom BR, Ray DE, Brown AM (2006) Fructose supports energy metabolism of some, but not all, axons in adult mouse optic nerve. J Neurophysiol 95:1917–1925

Altevogt BM, Paul DL (2004) Four classes of intercellular channels between glial cells in the CNS. J Neurosci 24:4313–4323

Altevogt BM, Kleopa KA, Postma FR, Scherer SS, Paul DL (2002) Connexin29 is uniquely distributed within myelinating glial cells of the central and peripheral nervous systems. J Neurosci 22:6458–6470

Arakawa S, Wright PM, Koga M, Phan TG, Reutens DC, Lim I, Gunawan MR, Ma H, Perera N, Ly J, Zavala J, Fitt G, Donnan GA (2006) Ischemic thresholds for gray and white matter. Stroke 37:1211–1216

Arroyo EJ, Scherer SS (2000) On the molecular architecture of myelinated fibers. Histochem Cell Biol 113:1–18

Attwell D, Buchan AM, Charpak S, Lauritzen M, MacVicar BA, Newman EA (2010) Glial and neuronal control of brain blood flow. Nature 468:232–243

Avila RL, Inouye H, Baek RC, Yin X, Trapp BD, Feltri ML, Wrabetz L, Kirschner DA (2005) Structure and stability of internodal myelin in mouse models of hereditary neuropathy. J Neuropathol Exp Neurol 64:976–990

Bakiri Y, Hamilton NB, Karadottir R, Attwell D (2008) Testing NMDA receptor block as a therapeutic strategy for reducing ischaemic damage to CNS white matter. Glia 56:233–240

Balice-Gordon RJ, Bone LJ, Scherer SS (1998) Functional gap junctions in the Schwann cell myelin sheath. J Cell Biol 142:1095–1104

Baltan S (2009) Ischemic injury to white matter: an age-dependent process. Neuroscientist 15: 126–133

Bardin J (2012) Neuroscience: making connections. Nature 483:394–396

Barrett EF, Barrett JN (1976) Separation of two voltage-sensitive potassium currents, and demonstration of a tetrodotoxin-resistant calcium current in frog motoneurones. J Physiol 255: 737–774

Barrett EF, Barrett JN (1982) Intracellular recording from vertebrate myelinated axons: mechanism of the depolarizing afterpotential. J Physiol 323:117–144

Baumann N, Pham-Dinh D (2001) Biology of oligodendrocyte and myelin in the mammalian central nervous system. Physiol Rev 81:871–927

Berger T, Schnitzer J, Kettenmann H (1991) Developmental changes in the membrane current pattern, K^+ buffer capacity, and morphology of glial cells in the corpus callosum slice. J Neurosci 11:3008–3024

Bever CT, Judge SI (2009) Sustained-release fampridine for multiple sclerosis. Expert Opin Investig Drugs 18:1013–1024

Black JA, Kocsis JD, Waxman SG (1990) Ion channel organization of the myelinated fiber. Trends Neurosci 13:48–54

Blaurock AE (1976) Myelin x-ray patterns reconciled. Biophys J 16:491–501
Blight AR (1989) Effect of 4-aminopyridine on axonal conduction-block in chronic spinal cord injury. Brain Res Bull 22:47–52
Bowe CM, Kocsis JD, Waxman SG (1987) The association of the supernormal period and the depolarizing afterpotential in myelinated frog and rat sciatic nerve. Neuroscience 21: 585–593
Bristow MS, Simon JE, Brown RA, Eliasziw M, Hill MD, Coutts SB, Frayne R, Demchuk AM, Mitchell JR (2005) MR perfusion and diffusion in acute ischemic stroke: human gray and white matter have different thresholds for infarction. J Cereb Blood Flow Metab 25: 1280–1287
Brown DA, Passmore GM (2009) Neural KCNQ (Kv7) channels. Br J Pharmacol 156:1185–1195
Brown AM, Westenbroek RE, Catterall WA, Ransom BR (2001) Axonal L-type Ca^{2+} channels and anoxic injury in rat CNS white matter. J Neurophysiol 85:900–911
Brown AM, Tekkok SB, Ransom BR (2003) Glycogen regulation and functional role in mouse white matter. J Physiol 549:501
Bucher D, Goaillard JM (2011) Beyond faithful conduction: short-term dynamics, neuromodulation, and long-term regulation of spike propagation in the axon. Prog Neurobiol 94: 307–346
Buhl EH (1993) Intracellular injection in fixed slices in combination with neuroanatomical tracing techniques and electron microscopy to determine multisynaptic pathways in the brain. Microsc Res Tech 24:15–30
Bunge MB, Bunge RP, Ris H (1961) Ultrastructural study of remyelination in an experimental lesion in adult cat spinal cord. J Cell Biol 10:67–94
Bunge RP, Bunge MB, Eldridge CF (1986) Linkage between axonal ensheathment and basal lamina production by Schwann cells. Annu Rev Neurosci 9:305–328
Butt AM (2005) Structure and function of oligodendrocytes. In: Kettenmann H, Ransom BR (eds) Neuroglia. Oxford University Press, Oxford, pp 36–47
Butt AM, Ransom BR (1989) Visualization of oligodendrocytes and astrocytes in the intact rat optic nerve by intracellular injection of lucifer yellow and horseradish peroxidase. Glia 2: 470–475
Butt AM, Ransom BR (1993) Morphology of astrocytes and oligodendrocytes during development in the intact rat optic nerve. J Comp Neurol 338:141–158
Cardenas DD, Ditunno J, Graziani V, Jackson AB, Lammertse D, Potter P, Sipski M, Cohen R, Blight AR (2007) Phase 2 trial of sustained-release fampridine in chronic spinal cord injury. Spinal Cord 45:158–168
Ceelen PW, Lockridge A, Newman EA (2001) Electrical coupling between glial cells in the rat retina. Glia 35:1–13
Chew SSL, Johnson CS, Green CR, nesh-Meyer HV (2010) Role of connexin43 in central nervous system injury. Exp Neurol 225:250–261
Chiu SY, Ritchie JM (1980) Potassium channels in nodal and internodal axonal membrane of mammalian myelinated fibres. Nature 284:170–171
Chiu SY, Ritchie JM (1981) Evidence for the presence of potassium channels in the paranodal region of acutely demyelinated mammalian single nerve fibres. J Physiol 313:415–437
Chiu SY, Ritchie JM (1984) On the physiological role of internodal potassium channels and the security of conduction in myelinated nerve fibres. Proc R Soc Lond B Biol Sci 220:415–422
Chiu SY, Zhou L, Zhang CL, Messing A (1999) Analysis of potassium channel functions in mammalian axons by gene knockouts. J Neurocytol 28:349–364
Chomiak T, Hu B (2009) What is the optimal value of the g-ratio for myelinated fibers in the rat CNS? A theoretical approach. PLoS One 4:e7754
Chvatal A, Pastor A, Mauch M, Sykova E, Kettenmann H (1995) Distinct populations of identified glial cells in the developing rat spinal cord slice: ion channel properties and cell morphology. Eur J Neurosci 7:129–142
Coombs JS, Curtis DR, Eccles JC (1957) The interpretation of spike potentials of motoneurones. J Physiol 139:198–231

Cronin M, Anderson PN, Cook JE, Green CR, Becker DL (2008) Blocking connexin43 expression reduces inflammation and improves functional recovery after spinal cord injury. Mol Cell Neurosci 39:152–160

Danesh-Meyer HV, Huang R, Nicholson LFB, Green CR (2008) Connexin43 antisense oligodeoxynucleotide treatment down-regulates the inflammatory response in an in vitro interphase organotypic culture model of optic nerve ischaemia. J Clin Neurosci 15:1253–1263

David G, Barrett JN, Barrett EF (1992) Evidence that action potentials activate an internodal potassium conductance in lizard myelinated axons. J Physiol 445:277–301

David G, Barrett JN, Barrett EF (1993) Activation of internodal potassium conductance in rat myelinated axons. J Physiol 472:177–202

David G, Modney B, Scappaticci KA, Barrett JN, Barrett EF (1995) Electrical and morphological factors influencing the depolarizing after-potential in rat and lizard myelinated axons. J Physiol 489(Pt 1):141–157

Debanne D, Campanac E, Bialowas A, Carlier E, Gl A (2011) Axon physiology. Physiol Rev 91:555–602

Devaux J, Gow A (2008) Tight junctions potentiate the insulative properties of small CNS myelinated axons. J Cell Biol 183:909–921

Devaux J, Alcaraz G, Grinspan J, Bennett V, Joho R, Crest M, Scherer SS (2003) Kv3.1b is a novel component of CNS nodes. J Neurosci 23:4509–4518

Devaux JJ, Kleopa KA, Cooper EC, Scherer SS (2004) KCNQ2 is a nodal K^+ channel. J Neurosci 24:1236–1244

Domercq M, Perez-Samartin A, Aparicio D, Alberdi E, Pampliega O, Matute C (2010) P2X7 receptors mediate ischemic damage to oligodendrocytes. Glia 58:730–740

Erlanger J, Gasser HS (1937) Electrical signs of nervous activity. University of Pennsylvania Press, Philadelphia

Fehlings MG, Nashmi R (1997) A new model of acute compressive spinal cord injury *in vitro*. J Neurosci Methods 71:215–224

Fern R, Ransom BR, Waxman SG (1995) Voltage-gated calcium channels in CNS white matter: role in anoxic injury. J Neurophysiol 74:369–377

Fern R, Davis P, Waxman SG, Ransom BR (1998) Axon conduction and survival in CNS white matter during energy deprivation: a developmental study. J Neurophysiol 79:95–105

Fernandez-Moran H, Finean JB (1957) Electron microscope and low-angle x-ray diffraction studies of the nerve myelin sheath. J Biophys Biochem Cytol 3:725–748

ffrench-Constant C, Colognato H, Franklin RJM (2004) The mysteries of myelin unwrapped. Science 304:688–689

Filley CM (1998) The behavioral neurology of cerebral white matter. Neurology 50:1535–1540

Filley CM (2010) White matter: organization and functional relevance. Neuropsychol Rev 20:158–173

Foster RE, Connors BW, Waxman SG (1982) Rat optic nerve: electrophysiological, pharmacological and anatomical studies during development. Brain Res 255:371–386

Frotscher M, Zhao S, Graber W, Drakew A, Studer D (2007) New ways of looking at synapses. Histochem Cell Biol 128:91–96

Fu Y, Sun W, Shi Y, Shi R, Cheng JX (2009) Glutamate excitotoxicity inflicts paranodal myelin splitting and retraction. PLoS One 4:e6705

Fujiyoshi K, Konomi T, Yamada M, Hikishima K, Tsuji O, Komaki Y, Momoshima S, Toyama Y, Nakamura M, Okano H (2013) Diffusion tensor imaging and tractography of the spinal cord: from experimental studies to clinical application. Exp Neurol 242:74–82

Giaume C, McCarthy KD (1996) Control of gap-junctional communication in astrocytic networks. Trends Neurosci 19:319–325

Giaume C, Koulakoff A, Roux L, Holcman D, Rouach N (2010) Astroglial networks: a step further in neuroglial and gliovascular interactions. Nat Rev Neurosci 11:87–99

Girault JA, Peles E (2002) Development of nodes of Ranvier. Curr Opin Neurobiol 12:476–485

Gordon GRJ, Choi HB, Rungta RL, Ellis-Davies GCR, MacVicar BA (2008) Brain metabolism dictates the polarity of astrocyte control over arterioles. Nature 456:745–749

Hamilton N, Vayro S, Kirchhoff F, Verkhratsky A, Robbins J, Gorecki DC, Butt AM (2008) Mechanisms of ATP- and glutamate-mediated calcium signaling in white matter astrocytes. Glia 56:734–749

Hanani M (2012) Lucifer yellow: an angel rather than the devil. J Cell Mol Med 16:22–31

Hansebout RR, Blight AR, Fawcett S, Reddy K (1993) 4-Aminopyridine in chronic spinal cord injury: a controlled, double-blind, crossover study in eight patients. J Neurotrauma 10:1–18

Harris JJ, Attwell D (2012) The energetics of CNS white matter. J Neurosci 32:356–371

Hayashi N, Green BA, Gonzalez-Carvajal M, Mora J, Veraa RP (1983) Local blood flow, oxygen tension, and oxygen consumption in the rat spinal cord. Part 1: Oxygen metabolism and neuronal function. J Neurosurg 58:516–525

Hayes KC (2004) The use of 4-aminopyridine (fampridine) in demyelinating disorders. CNS Drug Rev 10:295–316

Hildebrand C, Hahn R (1978) Relation between myelin sheath thickness and axon size in spinal cord white matter of some vertebrate species. J Neurol Sci 38:421–434

Hildebrand C, Remahl S, Persson H, Bjartmar C (1993) Myelinated nerve fibres in the CNS. Prog Neurobiol 40:319–384

Hinman JD, Peters A, Cabral H, Rosene DL, Hollander W, Rasband MN, Abraham CR (2006) Age-related molecular reorganization at the node of Ranvier. J Comp Neurol 495:351–362

Hirano A, Dembitzer HM (1967) A structural analysis of the myelin sheath in the central nervous system. J Cell Biol 34:555–567

Hirrlinger PG, Scheller A, Braun C, Quintela-Schneider M, Fuss B, Hirrlinger J, Kirchhoff F (2005) Expression of reef coral fluorescent proteins in the central nervous system of transgenic mice. Mol Cell Neurosci 30:291–303

Huxley AF, Stampfli R (1949) Evidence for saltatory conduction in peripheral myelinated nerve fibres. J Physiol 108:315–339

Imaizumi T, Kocsis JD, Waxman SG (1998) Resistance to anoxic injury in the dorsal columns of adult rat spinal cord following demyelination. Brain Res 779:292–296

Jabs R, Pivneva T, Huttmann K, Wyczynski A, Nolte C, Kettenmann H, Steinhauser C (2005) Synaptic transmission onto hippocampal glial cells with hGFAP promoter activity. J Cell Sci 118:3791–3803

James G, Butt AM (2002) P2Yand P2X purinoceptor mediated Ca2+ signalling in glial cell pathology in the central nervous system. Eur J Pharmacol 447:247–260

Jeng LJB, Balice-Gordon RJ, Messing A, Fischbeck KH, Scherer SS (2006) The effects of a dominant connexin32 mutant in myelinating Schwann cells. Mol Cell Neurosci 32:283–298

Kachuck NJ (2009) Sustained release oral fampridine in the treatment of multiple sclerosis. Expert Opin Pharmacother 10:2025–2035

Kamasawa N, Sik A, Morita M, Yasumura T, Davidson KG, Nagy JI, Rash JE (2005) Connexin-47 and connexin-32 in gap junctions of oligodendrocyte somata, myelin sheaths, paranodal loops and Schmidt-Lanterman incisures: implications for ionic homeostasis and potassium siphoning. Neuroscience 136:65–86

Karadottir R, Cavelier P, Bergersen LH, Attwell D (2005) NMDA receptors are expressed in oligodendrocytes and activated in ischaemia. Nature 438:1162–1166

Kleopa KA, Orthmann JL, Enriquez A, Paul DL, Scherer SS (2004) Unique distributions of the gap junction proteins connexin29, connexin32, and connexin47 in oligodendrocytes. Glia 47:346–357

Kleopa KA, Orthmann-Murphy JL, Sargiannidou I (2010) Gap junction disorders of myelinating cells. Rev Neurosci 21:397–419

Kocsis JD (1985) Aminopyridine-sensitivity of spinal cord white matter studied in vitro. Exp Brain Res 57:620–624

Kocsis JD, Waxman SG (1980) Absence of potassium conductance in central myelinated axons. Nature 287:348–349

Kofuji P, Newman EA (2004) Potassium buffering in the central nervous system. Neuroscience 129:1043–1054

Kole MH (2011) First node of Ranvier facilitates high-frequency burst encoding. Neuron 71: 671–682

Konietzko U, Muller CM (1994) Astrocytic dye coupling in rat hippocampus: topography, developmental onset, and modulation by protein kinase C. Hippocampus 4:297–306

Krnjevic K, Puil E, Werman R (1975) Evidence for Ca^{2+}-activated K^+ conductance in cat spinal motoneurons from intracellular EGTA injections. Can J Physiol Pharmacol 53:1214–1218

Le Bihan D, Johansen-Berg H (2012) Diffusion MRI at 25: exploring brain tissue structure and function. Neuroimage 61:324–341

Lee IH, Lindqvist E, Kiehn O, Widenfalk J, Olson L (2005) Glial and neuronal connexin expression patterns in the rat spinal cord during development and following injury. J Comp Neurol 489:1–10

Lev-Ram V, Grinvald A (1987) Activity-dependent calcium transients in central nervous system myelinated axons revealed by the calcium indicator Fura-2. Biophys J 52:571–576

Li S, Stys PK (2000) Mechanisms of ionotropic glutamate receptor-mediated excitotoxicity in isolated spinal cord white matter. J Neurosci 20:1190–1198

Li J, Hertzberg EL, Nagy JI (1997) Connexin32 in oligodendrocytes and association with myelinated fibers in mouse and rat brain. J Comp Neurol 379:571–591

Li S, Mealing GA, Morley P, Stys PK (1999) Novel injury mechanism in anoxia and trauma of spinal cord white matter: glutamate release via reverse Na+-dependent glutamate transport. J Neurosci 19:1–9

Li X, Ionescu AV, Lynn BD, Lu S, Kamasawa N, Morita M, Davidson KG, Yasumura T, Rash JE, Nagy JI (2004) Connexin47, connexin29 and connexin32 co-expression in oligodendrocytes and cx47 association with zonula occludens-1 (zo-1) in mouse brain. Neuroscience 126:611–630

Lutz SE, Zhao Y, Gulinello M, Lee SC, Raine CS, Brosnan CF (2009) Deletion of astrocyte connexins 43 and 30 leads to a dysmyelinating phenotype and hippocampal CA1 vacuolation. J Neurosci 29:7743–7752

Maglione M, Tress O, Haas B, Karram K, Trotter J, Willecke K, Kettenmann H (2010) Oligodendrocytes in mouse corpus callosum are coupled via gap junction channels formed by connexin47 and connexin32. Glia 58:1104–1117

Magnotti LM, Goodenough DA, Paul DL (2011) Deletion of oligodendrocyte Cx32 and astrocyte Cx43 causes white matter vacuolation, astrocyte loss and early mortality. Glia 59:1064–1074

Marcoux FW, Morawetz RB, Crowell RM, DeGirolami U, Halsey JH (1982) Differential regional vulnerability in transient focal cerebral ischemia. Stroke 13:339–346

Matthias K, Kirchhoff F, Seifert G, Huttmann K, Matyash M, Kettenmann H, Steinhauser C (2003) Segregated expression of AMPA-type glutamate receptors and glutamate transporters defines distinct astrocyte populations in the mouse hippocampus. J Neurosci 23:1750–1758

Matute C (2010) Calcium dyshomeostasis in white matter pathology. Cell Calcium 47:150–157

Matute C (2011) Glutamate and ATP signalling in white matter pathology. J Anat 219:53–64

Matute C, Torre I, Perez-Cerda F, Perez-Samartin A, Alberdi E, Etxebarria E, Arranz AM, Ravid R, Rodriguez-Antiguedad A, Sanchez-Gomez MV, Domercq M (2007) P2X7 Receptor blockade prevents ATP excitotoxicity in oligodendrocytes and ameliorates experimental autoimmune encephalomyelitis. J Neurosci 27:9525–9533

Menichella DM, Goodenough DA, Sirkowski E, Scherer SS, Paul DL (2003) Connexins are critical for normal myelination in the CNS. J Neurosci 23:5963

Menichella DM, Majdan M, Awatramani R, Goodenough DA, Sirkowski E, Scherer SS, Paul DL (2006) Genetic and physiological evidence that oligodendrocyte gap junctions contribute to spatial buffering of potassium released during neuronal activity. J Neurosci 26:10984–10991

Micu I, Jiang Q, Coderre E, Ridsdale A, Zhang L, Woulfe J, Yin X, Trapp BD, McRory JE, Rehak R, Zamponi GW, Wang W, Stys PK (2006) NMDA receptors mediate calcium accumulation in myelin during chemical ischaemia. Nature 439:988–992

Micu I, Ridsdale A, Zhang L, Woulfe J, McClintock J, Brantner CA, Andrews SB, Stys PK (2007) Real-time measurement of free Ca^{2+} changes in CNS myelin by two-photon microscopy. Nat Med 13:874–879

Mierzwa A, Shroff S, Rosenbluth J (2010) Permeability of the paranodal junction of myelinated nerve fibers. J Neurosci 30:15962–15968

Mills LR, Velumian AA, Agrawal SK, Theriault E, Fehlings MG (2004) Confocal imaging of changes in glial calcium dynamics and homeostasis after mechanical injury in rat spinal cord white matter. Neuroimage 21:1070–1083

Mulligan SJ, MacVicar BA (2004) Calcium transients in astrocyte endfeet cause cerebrovascular constrictions. Nature 431:195–199

Nagy JI, Ionescu AV, Lynn BD, Rash JE (2003a) Connexin29 and connexin32 at oligodendrocyte and astrocyte gap junctions and in myelin of the mouse central nervous system. J Comp Neurol 464:356–370

Nagy JI, Ionescu AV, Lynn BD, Rash JE (2003b) Coupling of astrocyte connexins Cx26, Cx30, Cx43 to oligodendrocyte Cx29, Cx32, Cx47: implications from normal and connexin32 knockout mice. Glia 44:205–218

Nans A, Einheber S, Salzer JL, Stokes DL (2011) Electron tomography of paranodal septate-like junctions and the associated axonal and glial cytoskeletons in the central nervous system. J Neurosci Res 89:310–319

Nashmi R, Fehlings MG (2001a) Changes in axonal physiology and morphology after chronic compressive injury of the rat thoracic spinal cord. Neuroscience 104:235–251

Nashmi R, Fehlings MG (2001b) Mechanisms of axonal dysfunction after spinal cord injury: with an emphasis on the role of voltage-gated potassium channels. Brain Res Brain Res Rev 38:165–191

Nashmi R, Jones OT, Fehlings MG (2000) Abnormal axonal physiology is associated with altered expression and distribution of Kv1.1 and Kv1.2 K^+ channels after chronic spinal cord injury. Eur J Neurosci 12:491–506

Nave KA (2010) Myelination and the trophic support of long axons. Nat Rev Neurosci 11:275–283

Ness JK, Valentino M, McIver SR, Goldberg MP (2005) Identification of oligodendrocytes in experimental disease models. Glia 50:321–328

O'Carroll SJ, Alkadhi M, Nicholson LFB, Green CR (2008) Connexin43 mimetic peptides reduce swelling, astrogliosis, and neuronal cell death after spinal cord injury. Cell Commun Adhes 15:27–42

Orkand RK, Nicholls JG, Kuffler SW (1966) Effect of nerve impulses on the membrane potential of glial cells in the central nervous system of amphibia. J Neurophysiol 29:788–806

Orkand RK, Orkand PM, Tang CM (1981) Membrane properties of neuroglia in the optic nerve of Necturus. J Exp Biol 95:49–59

Orthmann-Murphy JL, Abrams CK, Scherer SS (2008) Gap junctions couple astrocytes and oligodendrocytes. J Mol Neurosci 35:101–116

Ouardouz M, Nikolaeva MA, Coderre E, Zamponi GW, McRory JE, Trapp BD, Yin X, Wang W, Woulfe J, Stys PK (2003) Depolarization-induced Ca^{2+} release in ischemic spinal cord white matter involves L-type Ca^{2+} channel activation of ryanodine receptors. Neuron 40:53–63

Ouardouz M, Malek S, Coderre E, Stys PK (2006) Complex interplay between glutamate receptors and intracellular Ca^{2+} stores during ischaemia in rat spinal cord white matter. J Physiol 577:191–204

Ouardouz M, Coderre E, Basak A, Chen A, Zamponi GW, Hameed S, Rehak R, Yin X, Trapp BD, Stys PK (2009a) Glutamate receptors on myelinated spinal cord axons: I. GluR6 kainate receptors. Ann Neurol 65:151–159

Ouardouz M, Coderre E, Zamponi GW, Hameed S, Yin X, Trapp BD, Stys PK (2009b) Glutamate receptors on myelinated spinal cord axons: II. AMPA and GluR5 receptors. Ann Neurol 65:160–166

Pan Z, Kao T, Horvath Z, Lemos J, Sul JY, Cranstoun SD, Bennett V, Scherer SS, Cooper EC (2006) A common ankyrin-G-based mechanism retains KCNQ and Na_v channels at electrically active domains of the axon. J Neurosci 26:2599–2613

Park E, Velumian AA, Fehlings MG (2004) The role of excitotoxicity in secondary mechanisms of spinal cord injury: a review with an emphasis on the implications for white matter degeneration. J Neurotrauma 21:754–774

Pastor A, Kremer M, Moller T, Kettenmann H, Dermietzel R (1998) Dye coupling between spinal cord oligodendrocytes: differences in coupling efficiency between gray and white matter. Glia 24:108–120

Perez-Duenas B, Pujol J, Soriano-Mas C, Ortiz H, Artuch R, Vilaseca MA, Campistol J (2006) Global and regional volume changes in the brains of patients with phenylketonuria. Neurology 66:1074–1078

Perkins GA, Sosinsky GE, Ghassemzadeh S, Perez A, Jones Y, Ellisman MH (2008) Electron tomographic analysis of cytoskeletal cross-bridges in the paranodal region of the node of Ranvier in peripheral nerves. J Struct Biol 161:469–480

Peters A, Palay SL, Hd W (1991) The fine structure of the nervous system. Oxford University Press, New York

Poliak S, Peles E (2003) The local differentiation of myelinated axons at nodes of Ranvier. Nat Rev Neurosci 4:968–980

Poliak S, Matlis S, Ullmer C, Scherer SS, Peles E (2002) Distinct claudins and associated PDZ proteins form different autotypic tight junctions in myelinating Schwann cells. J Cell Biol 159:361–372

Ransom BR, Fern R (1997) Does astrocytic glycogen benefit axon function and survival in CNS white matter during glucose deprivation? Glia 21:134–141

Ransom BR, Walz W, Davis PK, Carlini WG (1992) Anoxia-induced changes in extracellular K^+ and pH in mammalian central white matter. J Cereb Blood Flow Metab 12:593–602

Ransom CB, Ransom BR, Sontheimer H (2000) Activity-dependent extracellular K^+ accumulation in rat optic nerve: the role of glial and axonal Na^+ pumps. J Physiol (Lond) 522:427–442

Ransom B, Behar T, Nedergaard M (2003) New roles for astrocytes (stars at last). Trends Neurosci 26:520–522

Ransom BR, Acharya AB, Goldberg MP (2004) Molecular pathophysiology of white matter anoxic-ischemic injury. In: Mohr JP, Dennis WC, James CG, Bryce W, FRCSEd H, Philip AW (eds) Stroke, 4th edn. Churchill Livingstone, Philadelphia, pp 867–881

Ransom BR, Goldberg MP, Baltan S (2011) Molecular pathophysiology of white matter anoxic-ischemic injury. In: Mohr JP, Wolf PA, Grotta JC, Moskowitz MA, Mayberg MR, von Kummer R (eds) Stroke: pathophysiology, diagnosis, and management. Elsevier, Philadelphia, pp 122–137

Rasband MN (2010) Clustered K^+ channel complexes in axons. Neurosci Lett 486:101–106

Rasband MN (2011) Composition, assembly, and maintenance of excitable membrane domains in myelinated axons. Semin Cell Dev Biol 22:178–184

Rasband MN, Trimmer JS, Schwarz TL, Levinson SR, Ellisman MH, Schachner M, Shrager P (1998) Potassium channel distribution, clustering, and function in remyelinating rat axons. J Neurosci 18:36–47

Rash JE (2010) Molecular disruptions of the panglial syncytium block potassium siphoning and axonal saltatory conduction: pertinence to neuromyelitis optica and other demyelinating diseases of the central nervous system. Neuroscience 168:982–1008

Rash JE, Duffy HS, Dudek FE, Bilhartz BL, Whalen LR, Yasumura T (1997) Grid-mapped freeze-fracture analysis of gap junctions in gray and white matter of adult rat central nervous system, with evidence for a “panglial syncytium” that is not coupled to neurons. J Comp Neurol 388:265–292

Reeves TM, Phillips LL, Povlishock JT (2005) Myelinated and unmyelinated axons of the corpus callosum differ in vulnerability and functional recovery following traumatic brain injury. Exp Neurol 196:126–137

Reichenbach A, Wolburg H (2005) Astrocytes and ependymal glia. In: Kettenmann H, Ransom BR (eds) Neuroglia. Oxford University Press, Oxford, pp 19–35

Robinson SR, Hampson EC, Munro MN, Vaney DI (1993) Unidirectional coupling of gap junctions between neuroglia. Science 262:1072–1074

Rosenbluth J (1999) A brief history of myelinated nerve fibers: one hundred and fifty years of controversy. J Neurocytol 28:251–262

Rosenbluth J (2009) Multiple functions of the paranodal junction of myelinated nerve fibers. J Neurosci Res 87:3250–3258

Rostaing P, Real E, Siksou L, Lechaire JP, Boudier T, Boeckers TM, Gertler F, Gundelfinger ED, Triller A, Marty S (2006) Analysis of synaptic ultrastructure without fixative using high-pressure freezing and tomography. Eur J Neurosci 24:3463–3474

Rudy B, McBain CJ (2001) Kv3 channels: voltage-gated K^+ channels designed for high-frequency repetitive firing. Trends Neurosci 24:517–526

Rushton WA (1951) A theory of the effects of fibre size in medullated nerve. J Physiol 115:101–122

Salzer JL, Brophy PJ, Peles E (2008) Molecular domains of myelinated axons in the peripheral nervous system. Glia 56:1532–1540

Sargiannidou I, Vavlitou N, Aristodemou S, Hadjisavvas A, Kyriacou K, Scherer SS, Kleopa KA (2009) Connexin32 Mutations cause loss of function in Schwann cells and oligodendrocytes leading to PNS and CNS myelination defects. J Neurosci 29:4736–4749

Scherer SS, Arroyo EJ (2002) Recent progress on the molecular organization of myelinated axons. J Peripher Nerv Syst 7:1–12

Scherer SS, Arroyo EJ (2009) Myelin: molecular architecture of CNS and PNS myelin sheath. In: Larry RS (ed) Encyclopedia of neuroscience. Academic, Oxford, pp 1169–1180

Scherer SS, Deschenes SM, Xu YT, Grinspan JB, Fischbeck KH, Paul DL (1995) Connexin32 is a myelin-related protein in the PNS and CNS. J Neurosci 15:8281–8294

Scherer SS, Xu YT, Messing A, Willecke K, Fischbeck KH, Jeng LJB (2005) Transgenic expression of human Connexin32 in myelinating Schwann cells prevents demyelination in Connexin32-null mice. J Neurosci 25:1550–1559

Schipke CG, Boucsein C, Ohlemeyer C, Kirchhoff F, Kettenmann H (2002) Astrocyte Ca2+ waves trigger responses in microglial cells in brain slices. FASEB J 16:255–257

Schmahmann JD, Smith EE, Eichler FS, Filley CM (2008) Cerebral white matter: neuroanatomy, clinical neurology, and neurobehavioral correlates. Ann N Y Acad Sci 1142:266–309

Schwarz JR, Glassmeier G, Cooper EC, Kao TC, Nodera H, Tabuena D, Kaji R, Bostock H (2006) KCNQ channels mediate I_{Ks}, a slow K^+ current regulating excitability in the rat node of Ranvier. J Physiol 573:17–34

Shannon C, Salter M, Fern R (2007) GFP imaging of live astrocytes: regional differences in the effects of ischaemia upon astrocytes. J Anat 210:684–692

Shapovalov AI (1975) Neuronal organization and synaptic mechanisms of supraspinal motor control in vertebrates. Rev Physiol Biochem Pharmacol 72:1–54

Shi R, Blight AR (1997) Differential effects of low and high concentrations of 4-aminopyridine on axonal conduction in normal and injured spinal cord. Neuroscience 77:553–562

Shroff S, Mierzwa A, Scherer SS, Peles E, Arevalo JC, Chao MV, Rosenbluth J (2011) Paranodal permeability in “myelin mutants”. Glia 59:1447–1457

Somjen GG (2001) Mechanisms of spreading depression and hypoxic spreading depression-like depolarization. Physiol Rev 81:1065–1096

Sosinsky GE, Deerinck TJ, Greco R, Buitenhuys CH, Bartol TM, Ellisman MH (2005) Development of a model for microphysiological simulations: small nodes of Ranvier from peripheral nerves of mice reconstructed by electron tomography. Neuroinformatics 3:133–162

Spiegel I, Peles E (2002) Cellular junctions of myelinated nerves (Review). Mol Membr Biol 19:95–101

Stampfli R (1954) Saltatory conduction in nerve. Physiol Rev 34:101–112

Steinhauser C, Berger T, Frotscher M, Kettenmann H (1992) Heterogeneity in the membrane current pattern of identified glial cells in the hippocampal slice. Eur J Neurosci 4:472–484

Stirling DP, Stys PK (2010) Mechanisms of axonal injury: internodal nanocomplexes and calcium deregulation. Trends Mol Med 16:160–170

Storm JF (1990) Potassium currents in hippocampal pyramidal cells. Prog Brain Res 83:161–187

Stuart G, Spruston N, Sakmann B, Hausser M (1997) Action potential initiation and backpropagation in neurons of the mammalian CNS. Trends Neurosci 20:125–131

Stys PK (2011) The axo-myelinic synapse. Trends Neurosci 34:393–400

Stys PK, Waxman SG (2004) Ischemic white matter damage. In: Robert AL, John WG, Hans L, Klaus-Armin N, Robert M, Bruce DT (eds) Myelin biology and disorders. Academic, San Diego, pp 985–1007

Stys PK, Sontheimer H, Ransom BR, Waxman SG (1993) Noninactivating, tetrodotoxin-sensitive Na^+ conductance in rat optic nerve axons. Proc Natl Acad Sci U S A 90:6976–6980

Stys PK, Hubatsch DA, Leppanen LL (1998) Effects of K^+ channel blockers on the anoxic response of CNS myelinated axons. Neuroreport 9:447–453

Sun D, Lye-Barthel M, Masland RH, Jakobs TC (2010) Structural remodeling of fibrous astrocytes after axonal injury. J Neurosci 30:14008–14019

Sykova E, Chvatal A (2000) Glial cells and volume transmission in the CNS. Neurochem Int 36:397–409

Szuchet S (1995) The morphology and ultrastructure of oligodendrocytes and their functional implications. In: Kettenmann H, Ransom BR (eds) Neuroglia. Oxford Univ. Press, New York, Oxford, pp 23–43

Szuchet S, Seeger MA (2004) Oligodendrocyte phenotypical and morphological heterogeneity: a reexamination of old concepts in view of new findings. In: Leif H (ed) Non-Neuronal cells of the nervous system: function and dysfunction. Advances in molecular and cell biology, vol 31. Elsevier, Amsterdam, pp 53–73

Targ EF, Kocsis JD (1985) 4-Aminopyridine leads to restoration of conduction in demyelinated rat sciatic nerve. Brain Res 328:358–361

Tasaki I (1959) Conduction of the nerve impulse. In: Field J, Magoun HW, Hall VE (eds) Handbook of physiology. Vol. 1. Neurophysiology. American Physiological Society, Washington, DC, pp 75–121

Tekkok SB, Goldberg MP (2001) AMPA/kainate receptor activation mediates hypoxic oligodendrocyte death and axonal injury in cerebral white matter. J Neurosci 21:4237–4248

Tekkok SB, Faddis BT, Goldberg MP (2005) AMPA/kainate receptors mediate axonal morphological disruption in hypoxic white matter. Neurosci Lett 382:275–279

Theis M, Sohl G, Eiberger J, Willecke K (2005) Emerging complexities in identity and function of glial connexins. Trends Neurosci 28:188–195

Theriault E, Frankenstein UN, Hertzberg EL, Nagy JI (1997) Connexin43 and astrocytic gap junctions in the rat spinal cord after acute compression injury. J Comp Neurol 382:199–214

Thompson RJ, Zhou N, MacVicar BA (2006) Ischemia opens neuronal gap junction hemichannels. Science 312:924–927

Utzschneider DA, Kocsis JD, Waxman SG (1991) Differential sensitivity to hypoxia of the peripheral versus central trajectory of primary afferent axons. Brain Res 551:136–141

Vargas MI, Delavelle J, Jlassi H, Rilliet B, Viallon M, Becker CD, Lovblad KO (2008) Clinical applications of diffusion tensor tractography of the spinal cord. Neuroradiology 50:25–29

Velumian AA, Carlen PL (1999) Differential control of three after-hyperpolarizations in rat hippocampal neurones by intracellular calcium buffering. J Physiol 517(Pt 1):201–216

Velumian AA, Wan Y, Samoilova M, Fehlings MG (2010) Modular double sucrose gap apparatus for improved recording of compound action potentials from rat and mouse spinal cord white matter preparations. J Neurosci Methods 187:33–40

Velumian AA, Samoilova M, Fehlings MG (2011a) Visualization of cytoplasmic diffusion within living myelin sheaths of CNS white matter axons using microinjection of the fluorescent dye Lucifer Yellow. Neuroimage 56:27–34

Velumian AA, Wan Y, Samoilova M, Fehlings MG (2011b) Contribution of fast and slow conducting myelinated axons to single-peak compound action potentials in rat spinal cord white matter preparations. J Neurophysiol 105:929–941

Vyskocil F, Kritz N, Bures J (1972) Potassium-selective microelectrodes used for measuring the extracellular brain potassium during spreading depression and anoxic depolarization in rats. Brain Res 39:255–259

Wallraff A, Odermatt B, Willecke K, Steinhauser C (2004) Distinct types of astroglial cells in the hippocampus differ in gap junction coupling. Glia 48:36–43

Wasseff SK, Scherer SS (2011) Cx32 and Cx47 mediate oligodendrocyte:astrocyte and oligodendrocyte:oligodendrocyte gap junction coupling. Neurobiol Dis 42:506–513

Waxman SG, Bangalore L (2004) Electrophysiologic consequences of myelination. In: Robert AL, John WG, Hans L, Klaus-Armin N, Robert M, Bruce DT (eds) Myelin biology and disorders. Academic, San Diego, pp 117–141

Waxman SG, Bangalore L (2005) Myelin function and saltatory conduction. In: Kettenmann H, Ransom BR (eds) Neuroglia. Oxford University Press, Oxford, pp 273–284

Waxman SG, Ritchie JM (1985) Organization of ion channels in the myelinated nerve fiber. Science 228:1502–1507

Waxman SG, Swadlow HA (1977) The conduction properties of axons in central white matter. Prog Neurobiol 8:297–324

Waxman SG, Davis PK, Black JA, Ransom BR (1990) Anoxic injury of mammalian central white matter: decreased susceptibility in myelin-deficient optic nerve. Ann Neurol 28:335–340

Waxman SG, Black JA, Stys PK, Ransom BR (1992) Ultrastructural concomitants of anoxic injury and early post-anoxic recovery in rat optic nerve. Brain Res 574:105–119

Waxman SG, Black JA, Ransom BR, Stys PK (1993) Protection of the axonal cytoskeleton in anoxic optic nerve by decreased extracellular calcium. Brain Res 614:137–145

Waxman SG, Craner MJ, Black JA (2004) Na^+ channel expression along axons in multiple sclerosis and its models. Trends Pharmacol Sci 25:584–591

Weruaga-Prieto E, Eggli P, Celio MR (1996) Topographic variations in rat brain oligodendrocyte morphology elucidated by injection of Lucifer Yellow in fixed tissue slices. J Neurocytol 25: 19–31

Xu G, Wang W, Kimelberg HK, Zhou M (2010) Electrical coupling of astrocytes in rat hippocampal slices under physiological and simulated ischemic conditions. Glia 58:481–493

Yuan X, Chittajallu R, Belachew S, Anderson S, McBain CJ, Gallo V (2002) Expression of the green fluorescent protein in the oligodendrocyte lineage: a transgenic mouse for developmental and physiological studies. J Neurosci Res 70:529–545

Chapter 2
White Matter Injury and Potential Treatment in Ischemic Stroke

Mingke Song, Anna Woodbury, and Shan Ping Yu

2.1 Introduction

According to the latest census, stroke has now fallen to the fourth leading cause of human death in the USA, yet remains a leading cause of long-term disability (Towfighi and Saver 2011). Multiple factors have contributed to successfully saving lives affected by stroke; meanwhile, there is real and urgent need for effective stroke therapy to minimize and treat the damaging repercussions of brain injury.

Ischemic stroke, occurring when blood supply to a region of the brain is occluded by blood clot, constitutes more than 80 % of stroke cases. The recombinant tissue plasminogen activator (tPA) is effective in treating acute ischemic stroke through dissolving the clot formed in blood vessels. Because of the narrow administration window (3–4.5 h) for tPA and its side effects (Lansberg et al. 2009; Del Zoppo et al. 2009), most patients with ischemic stroke cannot receive tPA treatment. Aside from tPA, a variety of neuroprotectants have been found effective in protecting gray matter and preventing neuronal death in rodent stroke models. However, all these drugs have failed to demonstrate beneficial outcomes in human stroke treatment (Cheng et al. 2004; Jeyaseelan et al. 2008). Although many factors may affect drug efficacy, underestimation of white matter injury is likely one important reason for the translational failures.

In the rodent brain used for stroke models, white matter constitutes a small part (~14 %) of the brain. Human brain differs from rodent brain in the proportion of white and gray matters. A human brain has almost equal proportional volumes of white matter and gray matter (Goldberg and Ransom 2003), and it has become a common view that human brain white matter is highly vulnerable to ischemia (Sozmen et al. 2012). The white matter is composed of astrocytes, oligodendrocytes,

M. Song • A. Woodbury • S.P. Yu, M.D., Ph.D. (✉)
Department of Anesthesiology, Emory University School of Medicine,
101 Woodruff Circle, WMR Building Suite 620, Atlanta, GA 30322, USA
e-mail: spyu@emory.edu

S. Baltan et al. (eds.), *White Matter Injury in Stroke and CNS Disease*, Springer Series in Translational Stroke Research 4, DOI 10.1007/978-1-4614-9123-1_2,

and myelinated axons responsible for transmitting input (afferent) and output (efferent) signals between neurons and neural networks. Oligodendrocyte processes produce the myelin sheath, which wraps around axons. The main function of the myelin sheath is to support axons and increase signal transduction speed along axonal fibers. Oligodendrocytes and axons, which compose the functional unit of the myelinated fiber, are vulnerable to many pathological conditions such as periventricular leukomalacia, brain trauma, vascular dementia, and cerebral ischemia (Volpe 2001; Kim et al. 2005; Matute et al. 2001; Dewar et al. 2003; Zaidi et al. 2004; Brown et al. 2002). Damaged axonal fibers and disrupted neuronal circuits contribute to functional deficits in these cerebrovascular diseases. In the current stroke research field, the pathological changes of ischemic white matter have become increasingly emphasized and investigated in mouse, rat, nonhuman primates, and human patients. Here, we summarize the current consensus and add some recent knowledge obtained from experimental stroke models for assessing white matter damage.

2.2 Morphological Assessment of Ischemic White Matter Damage

In histological sections of brain tissues, axonal fiber and oligodendrocytes can be identified under light and electron microscopes based upon their characteristic morphology, distribution, and ultrastructure. The corpus callosum, for example, is the largest white matter structure in the brain and relatively easy to identify. In a common stroke model, induced by occluding the middle cerebral artery (MCA) supplying the cerebral cortex, the corpus callosum, subcortical area, and striatum are the most suitable regions for assessing initial or secondary white matter injury. In a cerebral ischemia model, swollen oligodendrocytes and astrocytes in subcortical areas can be identified in the ischemic core by electron microscope 30 min after MCA occlusion (Pantoni et al. 1996). Three hours after ischemia, axonal swelling appears, and oligodendrocytes undergo pyknosis; 12–24 h later, pyknotic oligodendrocytes begin to necrose. Demyelination of myelin sheaths around axonal fiber occurs 30 min after ischemia and becomes a feature of white matter injury 12–24 h later (Pantoni et al. 1996). Evidence from a sciatic nerve ischemic model also suggests that swelling is the earliest morphological change in white matter injury (Nukada and Dyck 1987).

To obtain more detailed assessments of ischemic white matter damage, researchers employ more specific indicators and methodologies to detect cellular elements of interest in axons and oligodendrocytes. Neuronal markers, neurofilament (NF) and class III β-Tubulin (Tuj1), are frequently used to show the changes of axonal fiber in stroke and other neurological diseases. Reduction or loss in NF and Tuj1 positive processes can be detected by immunohistochemical staining 12–24 h after ischemia (Akpan et al. 2011). There are three major subunits of NF, termed: NF 68 (68 kDa), NF 150 (NF 150 kDa), and NF 200 (200 kDa). NF 68 is the most abundant of the three components, and has shown a more pronounced decrease in density by

immunohistochemistry 1–4 days after hippocampal ischemia in male Mongolian gerbils (Nakamura et al. 1992). Much earlier axonal damage can be detected by immunoblot analysis of brain sections to assess protein breakdown of neurofilament. It was reported that the proteolysis of NF 68 and NF 200 occurred 3 h after cerebral cortex ischemia in adult male rats (Aronowski et al. 1999). Earlier degradation of NF 150 and NF 200 at 15 min after cerebral ischemia was found in rats (Ogata et al. 1989). The three neurofilament components are all substrates of the calcium/calmodulin-dependent protease, calpain (Nixon and Lewis 1986; Zimmerman and Schlaepfer 1988). Thus, neurofilament protein degradation may be regarded as the consequence of calcium imbalance occurring soon after ischemic injury.

Compared with the above histopathologic staining, amyloid precursor protein (APP) is a more sensitive marker for assessment of ischemia-damaged axons. In healthy brain, APP is transported by fast axonal transport throughout axons and maintained at a low concentration that is hard to detect by immunohistochemistry. Once axons are damaged by ischemia, APP transportation slows, and accumulated APP becomes a useful injury reporter. Thus, APP staining has been used for quantitative assessment of axonal damage after cerebral ischemia and has shown advantages such as high sensitivity to ischemic insult and easy detection in assays (Imai et al. 2002).

For more specific identification of damage to oligodendrocytes, many markers have been developed, such as: myelin basic protein (MBP) for mature cells, lipid sulfatide (O4) for immature cells, and NG2 proteoglycan for precursor cells. Antiadenomatous polyposis coli (APC) and 2′, 3′-Cyclic-nucleotide 3′-phosphodiesterase (CNPase) are also used to label mature oligodendrocytes and detect damage. MBP protein is a major constituent of the myelin sheath, and myelin damage can be identified with antibody that recognizes degraded MBP (dMBP). In a rat focal cerebral ischemia model induced by endothelin-1 injection, the density of MBP progressively decreased in the ischemic core and penumbra region at 1, 3, and 7 days after stroke, while the density of dMBP progressively increased (Moxon-Emre and Schlichter 2010). Ischemia-damaged oligodendrocytes in the brain are also immunoreactive to the cytoskeletal protein, tau. The number of tau positive oligodendrocytes increased 6–8-folds at 40 min after the onset of cerebral ischemia (Irving et al. 1997).

Ischemia not only causes damage to oligodendrocytes but also induces oligodendrogenesis. This regenerative response from oligodendrocyte progenitor cells is usually assessed by BrdU incorporation with NG2 or O4 immuno-positive cell populations. The proliferation of NG2 oligodendrocyte progenitor cells takes place in a delayed fashion, showing increased maximal activity at 5–7 days after stroke induction, and then significant decline 14–28 days later (Iwai et al. 2010; Sozmen et al. 2009).

These morphological methods enable researchers to assess and quantify temporal and spatial changes in white matter after brain ischemia. Since white matter is vulnerable to ischemic damage, it is more and more recognized that the characteristic changes of white matter are of great importance when assessing extent of ischemic brain damage and evaluating therapeutic effect of treatment in animal and human stroke.

2.3 Ionic Mechanism of Ischemic Axonal Damage

A few minutes after occlusion of cerebral blood flow, ischemic brain tissue becomes deprived of oxygen and glucose, resulting in mitochondrial damage and failure of ATP synthesis. This energy depletion causes Na^+/K^+-ATPase dysfunction and cellular Na^+ and K^+ homeostasis disruption, leading to depolarization of the soma and axonal membrane. Membrane depolarization is one of the major early mechanisms contributing to excessive release of glutamate from neurons and glial cells, which overactivates glutamate receptors. This glutamate-mediated excitotoxicity causes rapid gray matter and white matter damage in acute ischemic stroke. Other mechanisms such as increased production of free radicals, inflammatory activity, apoptotic cascade activation and loss of trophic support have been implicated in ischemic injury. We here focus on the discussion of ionic mechanisms underlying ischemic white matter damage.

Myelinated axon fibers are sensitive to hypoxia and ischemia. The concentration gradient of Na^+ and K^+ and a resting membrane potential across the axonal membrane depend on a normal activity of Na^+/K^+-ATPase. Axoplasmic accumulation of Na^+ is mediated by failure of Na^+/K^+-ATPase. A persistent Na^+ influx and axonal swelling is a result of excessive Na^+ and Cl^- influx. Accumulation of cellular Na^+ may lead to activation of the reverse model of the Na^+/Ca^{2+} exchanger and axoplasmic Ca^{2+} increases (Philipson and Nicoll 2000). Membrane potential collapse will impair axonal conduction and suppress action potential propagation along the axonal fiber.

In a study of isolated rat optic nerve fibers (a white matter tract), energy deprivation depolarized neuronal resting membrane potential in a Na^+ influx dependent manner (Leppanen and Stys 1997). The evoked compound action potential (CAP) in adult optic nerves can be abolished by deprivation of either oxygen or glucose for 60 min (Fern et al. 1998). In an ischemic brain slice model, the corpus callosum injury was induced by deprivation of oxygen and glucose (OGD), and the function of corpus callosum was monitored by recording CAP. Blockade of Na^+ influx was found to partially protect corpus callosum function from OGD injury (Tekkok and Goldberg 2001). On the other hand, axoplasmic Ca^{2+} is also involved in damaged axonal function based on the observation that anoxia-induced suppression of CAP in rat optic nerve was restored close to control level by Ca^{2+}-free solution (Stys et al. 1990). Ca^{2+}-free solution preserved CAP in OGD-damaged corpus callosum and protected the axonal cytoskeleton against anoxia in optic nerve axons (Tekkok and Goldberg 2001; Waxman et al. 1993). These studies demonstrate that Na^+ and Ca^{2+} influx plays important roles in ischemia-induced axonal damage.

Glutamate-mediated excitotoxicity not only affects gray matter but is also involved in ischemic axonal injury. In axonal damage, activation of AMPA/kainate receptors but not NMDA receptors mediates the excitotoxic effect (Matute 1998; Li and Stys 2000; Domercq et al. 2005). The AMPA receptor blocker, NBQX, is able to preserve axonal structure and functional activity in brain slices treated with OGD conditions, and this effect has been explained as a secondary effect resulting from

protection of oligodendrocytes by NBQX (Tekkok and Goldberg 2001). Most recent studies have shown that rat dorsal column axons express glutamate receptor subunit 4 (GluR4) AMPA receptors, GluR5 and GluR6 containing kainate receptors. Application of AMPA/kainate receptor agonists induces progressive elevation of intra-axonal Ca^{2+} and impairs functional CAP in dorsal axons (Ouardouz et al. 2009a; Ouardouz et al. 2009b). These observations are consistent with the idea that glutamate excitotoxicity in axons is directly mediated by AMPA/kainate receptors. On the other hand, it has been suggested that the NMDA receptor is irrelevant in excitotoxic axonal death because these cells contains negligible levels of NMDA receptors (Jones and Baughman 1991). Although NMDA receptor blockers are effective in protection of gray matter, none of them were proven to be protective against axonal damage in focal cerebral ischemia and spinal cord injury (Yam et al. 2000; Agrawal and Fehlings 1997; Ouardouz et al. 2006). This might partially explain the failure of NMDA receptor antagonists in clinical stroke treatments.

2.4 Ionic Mechanism of Ischemic Oligodendrocyte Damage

The ionic mechanisms identified in ischemic axonal fibers are not completely applicable to oligodendrocytes. Blockade of Na^+ channels by TTX, while preventing axonal loss, does not prevent oligodendrocyte loss, so it can be deduced that Na^+ influx mediated by Na^+ channels is not involved in excitotoxic oligodendrocyte death (Tekkok and Goldberg 2001). Neurotoxic Ca^{2+} entry plays an important role in oligodendrocyte death, and the route of Ca^{2+} entry may include activation of AMPA/kainate receptors, NMDA receptors, voltage-gated Ca^{2+} channels, and possible reversal operation of Na^+/Ca^{2+} exchangers. Primarily cultured oligodendrocytes are vulnerable to AMPA/kainate receptor-mediated excitotoxicity and hypoxic–ischemic injury. It has been shown that direct activation of AMPA/kainate receptors by AMPA, kainate, glutamate, or by OGD shows dose-dependent toxicity to cultured oligodendrocytes. On the other hand, application of AMPA/kainate receptor antagonists or removal of Ca^{2+} from culture medium protects cultured oligodendrocytes from excitotoxic injury. Blockade of AMPA/Kainate receptors also suppresses OGD-induced Ca^{2+} entry (Yoshioka et al. 1995; Sanchez-Gomez and Matute 1999; McDonald et al. 1998).

Cultured oligodendrocytes can be divided into precursor and mature type cells based on their morphological and antigenic classification (Raff 1989). Oligodendrocyte precursor cells are much more vulnerable than mature cells to hypoxic–ischemic insults; this is associated with an enhanced activation of Ca^{2+} permeable AMPA/kainate receptors in these precursor cells (Deng et al. 2003). In mature oligodendrocytes, AMPA receptors rather than kainate receptors are suggested as the major mediator of excitotoxic cell death (Leuchtmann et al. 2003). Aside from this in vitro evidence, in situ and in vivo experiments also show that blockade of AMPA/kainate receptors reduces Ca^{2+}-dependent oligodendrocyte death in hypoxic–ischemic acute brain slices and hypoxic–ischemic injury in

developing white matter (Tekkok and Goldberg 2001; Follett et al. 2000). Although enhanced Ca^{2+} influx through Ca^{2+} permeable AMPA/kainate receptors alone is sufficient to initiate excitotoxicity in cultured oligodendrocytes, selective blockade of voltage-gated Ca^{2+} channels and the Na^{+}/Ca^{2+} exchangers still partially attenuates Ca^{2+} influx and reduces cell death induced by AMPA receptor activation (Alberdi et al. 2002; Chen et al. 2007). Therefore, activation of voltage-gated Ca^{2+} channels and the reversal Na^{+}/Ca^{2+} exchangers may contribute to neurotoxic Ca^{2+} entry following activation of AMPA/kainate receptors.

NMDA receptors are Ca^{2+} permeable receptors; it has long been thought that the NMDA receptor was not involved in oligodendrocyte death because these cells lack functional expression of NMDA receptors (Berger et al. 1992; Patneau et al. 1994; Liu and Almazan 1995). This concept, however, has been challenged by several reports showing the existence of NMDA receptor subunits and their functional expression in mature and immature oligodendrocytes of the cerebellum and corpus callosum. It was shown that activation of NMDA receptors contributes to ischemia-induced intracellular Ca^{2+} increase and oligodendrocyte damage (Karadottir et al. 2005; Salter and Fern 2005; Micu et al. 2006). Hence, it is likely that NMDA receptors also participate in hypoxic–ischemic injury of oligodendrocytes. The importance of this contribution in ischemic stroke remains to be further elucidated in animal experiments and human research.

In terms of glutamate-mediated excitotoxicity in ischemic white matter injury and oligodendrocyte loss, it must be acknowledged that this cell death mechanism was established on the simplistic and abstract models of in vitro and in vivo experiments that simulate acute ischemia and excitotoxic injury. In stroke patients, complex ischemic cascades are activated after the onset of stroke. Specifically, glutamate excitotoxicity is a dominant player in the acute phase of ischemic injury, but may not be responsible for all of the dynamic changes and pathological progression in the subacute and chronic stages. Cellular necrosis, apoptosis, and inflammation occur in succession and/or parallel from hours to days after stroke.

Recent evidence has shown that extracellular ATP may also act as an excitatory neurotransmitter, inducing Ca^{2+}-dependent ischemic damage to oligodendrocytes via activating P2X and P2Y receptors (Domercq et al. 2010; Arbeloa et al. 2012). It was shown that in addition to glutamate, enhanced ATP signaling during ischemia is also deleterious to oligodendrocytes and myelin, and impairs white matter function. Oligodendrocytes in culture under OGD condition display an inward current and cytosolic Ca^{2+} overload, which is partially mediated by P2X7 receptors. Oligodendrocytes release ATP after OGD through the opening of pannexin hemichannels. Consistently, oligodendrocyte death and optic nerve damage are partially reversed by P2X7 receptor antagonists, by the ATP degrading enzyme apyrase, and by blockers of pannexin hemichannels (Domercq et al. 2010). In primary cortical neuron cultures and in brain slices, OGD caused neuronal death that was reduced by Brilliant Blue G (BBG) at concentrations which specifically inhibit P2X7 receptors. In ischemic stroke rats, BBG produced a 60 % reduction in the extent of brain damage compared to treatment with vehicle alone (Arbeloa et al. 2012). These data indicate that ATP released during ischemia and the subsequent activation of

P2X7 receptor may contribute to white matter demise during stroke and point to this receptor type as a therapeutic target to limit tissue damage in cerebrovascular diseases.

2.4.1 Neuroprotection Targeting AMPA and NMDA Receptors

To date, there is no successful translation of glutamate receptor antagonism into efficient drugs for human stroke treatment. The underlying reasons may include, but are not limited to, severity and complexity of ischemic damage, narrowness of therapeutic window, unknown cell death mechanisms in humans, pathophysiological differences between animal and human stroke, lack of focus on ischemic white matter damage, and undesirable side effects of the experimental treatments in humans. Among these potential factors, white matter injury or axonal demyelination after stroke has remained less investigated compared to gray matter injury.

Despite failure in clinical trials, excessive activation of AMPA/NMDA receptors and consequent excitotoxicity are still a predominant theory for stroke pathology and a guide for the development of neuroprotective agents against acute ischemic damage. A novel AMPA receptor antagonist, SPD 502, is reported to reduce ischemic oligodendrocyte damage in a rat stroke model induced by MCA occlusion (McCracken et al. 2002). The well-known NMDA receptor antagonist memantine is clinically licensed by the FDA to treat moderate-to-severe Alzheimer's disease. Memantine is also found to protect corpus callosum oligodendrocytes and optic nerve fibers from ischemic damage at clinically relevant concentrations (Bakiri et al. 2008). Whether this approach will be effective or not in human patients, the idea that an effective stroke therapy will likely require a combinational approach that targets multiple receptors/channels and multiple signaling pathways is gaining in popularity.

For example, glutamate, released upon an ischemic insult, activates AMPA/kainate receptors, resulting in cell membrane depolarization and consequent activation of NMDA receptors. Thus, it is possible that combination therapy targeting AMPA and NMDA receptors will likely show more efficacy in preventing white matter injury. Glutamate also actives metabotropic receptors that indirectly affect cellular Ca^{2+}, cAMP, protein phosphorylation, and other signaling pathways (Mao and Wang 2002). However, the role of metabotropic glutamate receptors in oligodendrocyte toxicity is obscure and remains to be elucidated.

2.4.2 Neuroprotection Targeting Axonal Demyelination

An additional neuroprotective strategy is to target axonal demyelination (loss of myelin proteins). As discussed above, several pathways mediate intracellular Ca^{2+} accumulation in the acute phase of ischemic white matter damage. Neurotoxic Ca^{2+}

seems to be the common signal for axonal skeleton degeneration and oligodendrocyte myelin protein degradation. It is well known that excessive increases of cellular Ca^{2+} causes mitochondrial dysfunction and enhances generation of reactive oxygen species (ROS) and nitric oxide (NO) (LoPachin and Lehning 1997; Stys 1998; Coleman 2005), which all play important roles in axonal demyelination (Campbell and Mahad 2011; Linares et al. 2006; Smith et al. 1999). ROS inhibition and reduction of NO have been shown to be neuroprotective in experimental models of brain ischemia (O'Mahony and Kendall 1999; Tuttolomondo et al. 2009). Intracellular Ca^{2+} elevation activates Ca^{2+}-dependent protein kinases and neutral protease (calpain). Calpain activation has been identified as the trigger for axonal demyelination in stroke and multiple sclerosis (Lankiewicz et al. 2000; Shields et al. 1999). Calpain inhibition is effective to reduce neurofilament breakdown and attenuate axonal demyelination in ischemic axons and other injury models (Stys and Jiang 2002; Das et al. 2012). Thus, strategies targeting the downstream signaling behind neurotoxic Ca^{2+} are alternative neuroprotective approaches to prevent axonal demyelination.

Although axonal degradation and demyelination occur quickly in the ischemic core, a gradual restoration of oligodendrocytes and remyelination have been observed in the peri-infarct area (Gregersen et al. 2001; Tanaka et al. 2003). In the CNS of humans and animals, the capability of remyelination as well as regeneration is preserved for neurogenesis and repair activity following brain injury (Dubois-Dalcq et al. 2008; Duncan et al. 2009; Franklin and Ffrench-Constant 2008). Remyelinating activity has been shown effective in preventing axons from demyelination-associated degeneration (Irvine and Blakemore 2008). Revealing the mechanisms underlying remyelination will afford critical clues to develop novel regeneration strategies. In a mouse brain demyelination model, transplanted neural progenitor cells were found to enhance remyelination via secreting trophic factors: platelet-derived growth factor-AA, and fibroblast growth factor-2 (Einstein et al. 2009).

2.4.3 Pharmacological Hypothermia Therapy for Ischemic Stroke

Hypothermia, or cooling, is an established method to decrease metabolic activity and protect animal brain or isolated organs/tissues against a variety of injuries in the laboratory or operating room. The neuroprotective effect of therapeutic hypothermia has been consistently demonstrated in ischemic and traumatic brain injury (Tokutomi et al. 2007; Sahuquillo and Vilalta 2007; Kwon et al. 2008; Hemmen and Lyden 2009; Yenari and Hemmen 2010). Animal and human studies suggest that mild to moderate hypothermia (2–5 °C reduction) is generally safe and beneficial for functional recovery after cerebral ischemia. Early administration of physical cooling after cerebral ischemia reduces loss of immature oligodendrocytes in near-term fetal sheep (Roelfsema et al. 2004). Most recently, we have developed a novel

neurotensin receptor 1 (NTR1) agonist, ABS201, to effectively induce regulated hypothermia in a focal ischemic stroke model (Choi et al. 2012). We showed that pharmacologically induced hypothermia (PIH) reduces ischemic infarct volume, decreases cell death and improves recovery of sensorimotor function (Choi et al. 2012). We propose that PIH provides not only neuronal protection, but also protects against ischemia-induced axonal and neurovascular damage. The comprehensive effects of PIH are thus regarded as a brain protective therapy as compared to the conventional approach that targets only one individual cell type (e.g., neuron) or a single receptor/signaling pathway. Further investigations are necessary to explore brain protection strategies using hypothermia-inducing drugs or other approaches in protecting structure in the ischemic brain, including the white matter.

2.4.4 Stem Cell Therapy for Ischemic Stroke

A rapidly developing strategy for stroke therapy is the application of stem cells to repair ischemia-damaged brain tissue through either cell replacement or tropic action after transplantation. In a neonatal hypoxia–ischemia model, transplanted bone marrow stem cells reduced MBP loss and increased oligodendrogenesis in the damaged brain through adapting into the damaged tissue and stimulating several endogenous repair pathways (van Velthoven et al. 2010). Our recent study has shown that transplantation of bone marrow mesenchymal stem cells (BMSCs) into the peri-infarct region of adult stroke mice partially restored thalamocortical circuitry and enhanced functional recovery (Song et al. 2012). BMSCs were implanted 1 and 7 days after barrel cortex stroke. This treatment reduced infarct formation. The behavioral corner test showed better long-term recovery of sensorimotor activities in BMSC-treated mice. Six weeks post-stroke, extracellular recordings of field potentials in the BMSC-transplanted brain slices showed noticeable recovery of ischemia-disrupted intracortical activity from layer 4 to layers 2/3, and the thalamocortical circuit activity was also partially restored. Immunofluorescence showed that the density of neurons, axons and blood vessels in the peri-infarct area was significantly higher in BMSC-treated mice, accompanied with enhanced local blood flow. BMSC treatment increased the levels of SDF-1, VEGF, and BDNF in peri-infarct region. The expression of axonal growth associated protein-43 (GAP-43) was markedly increased, and the axonal growth inhibiting proteins, ROCK II and NG2, were suppressed in the BMSC-treated brain, suggesting a potential signaling pathway that mediates the BMSC effect on axon growth and regeneration. This study provides electrophysiological, morphological, and molecular evidence that BMSC transplantation has a clear potential to repair the ischemia-damaged neural networks involving gray and white matters. More efforts are needed to optimize the strategy by exploring the mechanisms underlying stem cell therapy and discerning the appropriate time window for stem cells to adapt to the environment of the ischemic brain.

2.5 Conclusion

Stroke therapy has come a long way in terms of improving survival and chronic treatment such as physical therapies, but current effective treatments for acute ischemic stroke patients are very limited. Recent studies have suggested that the key to improving recovery, restoring function, and reducing long-term disability lies in reducing white matter as well as gray matter injury. Multiple markers of axonal and oligodendrocyte injury have been identified and are being utilized. Therapy targeting AMPA and NMDA receptors, the ionic balance across membranes and a variety of second messengers may be promising. In addition, recent progresses in developing pharmacological hypothermia therapy and stem cell therapy have brought promising hopes for clinical applications of brain protective and regenerative medicine for stroke patients. Regardless, a more comprehensive approach to research involving multimodal treatments will likely result in a more effective overall stroke therapy in humans.

References

Agrawal SK, Fehlings MG (1997) Role of NMDA and non-NMDA ionotropic glutamate receptors in traumatic spinal cord axonal injury. J Neurosci 17:1055–1063

Akpan N, Serrano-Saiz E, Zacharia BE, Otten ML, Ducruet AF, Snipas SJ, Liu W, Velloza J, Cohen G, Sosunov SA, Frey WH 2nd, Salvesen GS, Connolly ES Jr, Troy CM (2011) Intranasal delivery of caspase-9 inhibitor reduces caspase-6-dependent axon/neuron loss and improves neurological function after stroke. J Neurosci 31:8894–8904

Alberdi E, Sanchez-Gomez MV, Marino A, Matute C (2002) Ca(2+) influx through AMPA or kainate receptors alone is sufficient to initiate excitotoxicity in cultured oligodendrocytes. Neurobiol Dis 9:234–243

Arbeloa J, Perez-Samartin A, Gottlieb M, Matute C (2012) P2x7 receptor blockade prevents ATP excitotoxicity in neurons and reduces brain damage after ischemia. Neurobiol Dis 45: 954–961

Aronowski J, Cho KH, Strong R, Grotta JC (1999) Neurofilament proteolysis after focal ischemia; when do cells die after experimental stroke? J Cereb Blood Flow Metab 19:652–660

Bakiri Y, Hamilton NB, Karadottir R, Attwell D (2008) Testing NMDA receptor block as a therapeutic strategy for reducing ischaemic damage to CNS white matter. Glia 56:233–240

Berger T, Walz W, Schnitzer J, Kettenmann H (1992) GABA- and glutamate-activated currents in glial cells of the mouse corpus callosum slice. J Neurosci Res 31:21–27

Brown AM, Tekkok SB, Ransom BR (2002) Hypoglycemia and white matter: pathophysiology of axon injury and role of glycogen. Diabetes Nutr Metab 15:290–293, discussion 293–294

Campbell GR, Mahad DJ (2011) Mitochondria as crucial players in demyelinated axons: lessons from neuropathology and experimental demyelination. Autoimmune Dis 2011:262847

Chen H, Kintner DB, Jones M, Matsuda T, Baba A, Kiedrowski L, Sun D (2007) AMPA-mediated excitotoxicity in oligodendrocytes: role for Na(+)-K(+)-CL(-) co-transport and reversal of Na(+)/CA(2+) exchanger. J Neurochem 102:1783–1795

Cheng YD, Al-Khoury L, Zivin JA (2004) Neuroprotection for ischemic stroke: two decades of success and failure. NeuroRx 1:36–45

Choi KE, Hall CL, Sun JM, Wei L, Mohamad O, Dix TA, Yu SP (2012) A novel stroke therapy of pharmacologically induced hypothermia after focal cerebral ischemia in mice. FASEB J 26:2799–2810

Coleman M (2005) Axon degeneration mechanisms: commonality amid diversity. Nat Rev Neurosci 6:889–898
Das A, Kelly Guyton M, Smith A, Iv GW, McDowell ML, Holmes C, Matzelle DD, Ray SK, Banik NL (2012) Calpain inhibitor attenuated optic nerve damage in acute optic neuritis in rats. J Neurochem 124(1):133–146
Del Zoppo GJ, Saver JL, Jauch EC, Adams HP Jr (2009) Expansion of the time window for treatment of acute ischemic stroke with intravenous tissue plasminogen activator: a science advisory from the American Heart Association/American Stroke Association. Stroke 40: 2945–2948
Deng W, Rosenberg PA, Volpe JJ, Jensen FE (2003) Calcium-permeable AMPA/kainate receptors mediate toxicity and preconditioning by oxygen-glucose deprivation in oligodendrocyte precursors. Proc Natl Acad Sci U S A 100:6801–6806
Dewar D, Underhill SM, Goldberg MP (2003) Oligodendrocytes and ischemic brain injury. J Cereb Blood Flow Metab 23:263–274
Domercq M, Etxebarria E, Perez-Samartin A, Matute C (2005) Excitotoxic oligodendrocyte death and axonal damage induced by glutamate transporter inhibition. Glia 52:36–46
Domercq M, Perez-Samartin A, Aparicio D, Alberdi E, Pampliega O, Matute C (2010) P2x7 receptors mediate ischemic damage to oligodendrocytes. Glia 58:730–740
Dubois-Dalcq M, Williams A, Stadelmann C, Stankoff B, Zalc B, Lubetzki C (2008) From fish to man: understanding endogenous remyelination in central nervous system demyelinating diseases. Brain 131:1686–1700
Duncan ID, Brower A, Kondo Y, Curlee JF Jr, Schultz RD (2009) Extensive remyelination of the CNS leads to functional recovery. Proc Natl Acad Sci U S A 106:6832–6836
Einstein O, Friedman-Levi Y, Grigoriadis N, Ben-Hur T (2009) Transplanted neural precursors enhance host brain-derived myelin regeneration. J Neurosci 29:15694–15702
Fern R, Davis P, Waxman SG, Ransom BR (1998) Axon conduction and survival in CNS white matter during energy deprivation: a developmental study. J Neurophysiol 79:95–105
Follett PL, Rosenberg PA, Volpe JJ, Jensen FE (2000) NBQX attenuates excitotoxic injury in developing white matter. J Neurosci 20:9235–9241
Franklin RJ, Ffrench-Constant C (2008) Remyelination in the CNS: from biology to therapy. Nat Rev Neurosci 9:839–855
Goldberg MP, Ransom BR (2003) New light on white matter. Stroke 34:330–332
Gregersen R, Christensen T, Lehrmann E, Diemer NH, Finsen B (2001) Focal cerebral ischemia induces increased myelin basic protein and growth-associated protein-43 gene transcription in peri-infarct areas in the rat brain. Exp Brain Res 138:384–392
Hemmen TM, Lyden PD (2009) Multimodal neuroprotective therapy with induced hypothermia after ischemic stroke. Stroke 40:S126–128
Imai H, McCulloch J, Graham DI, Masayasu H, Macrae IM (2002) New method for the quantitative assessment of axonal damage in focal cerebral ischemia. J Cereb Blood Flow Metab 22: 1080–1089
Irvine KA, Blakemore WF (2008) Remyelination protects axons from demyelination-associated axon degeneration. Brain 131:1464–1477
Irving EA, Yatsushiro K, McCulloch J, Dewar D (1997) Rapid alteration of tau in oligodendrocytes after focal ischemic injury in the rat: Involvement of free radicals. J Cereb Blood Flow Metab 17:612–622
Iwai M, Stetler RA, Xing J, Hu X, Gao Y, Zhang W, Chen J, Cao G (2010) Enhanced oligodendrogenesis and recovery of neurological function by erythropoietin after neonatal hypoxic/ischemic brain injury. Stroke 41:1032–1037
Jeyaseelan K, Lim KY, Armugam A (2008) Neuroprotectants in stroke therapy. Expert Opin Pharmacother 9:887–900
Jones KA, Baughman RW (1991) Both NMDA and non-NMDA subtypes of glutamate receptors are concentrated at synapses on cerebral cortical neurons in culture. Neuron 7:593–603
Karadottir R, Cavelier P, Bergersen LH, Attwell D (2005) NMDA receptors are expressed in oligodendrocytes and activated in ischaemia. Nature 438:1162–1166

Kim MJ, Lyoo IK, Kim SJ, Sim M, Kim N, Choi N, Jeong DU, Covell J, Renshaw PF (2005) Disrupted white matter tract integrity of anterior cingulate in trauma survivors. Neuroreport 16:1049–1053

Kwon BK, Mann C, Sohn HM, Hilibrand AS, Phillips FM, Wang JC, Fehlings MG (2008) Hypothermia for spinal cord injury. Spine J 8:859–874

Lankiewicz S, Marc Luetjens C, Truc Bui N, Krohn AJ, Poppe M, Cole GM, Saido TC, Prehn JH (2000) Activation of calpain I converts excitotoxic neuron death into a caspase-independent cell death. J Biol Chem 275:17064–17071

Lansberg MG, Bluhmki E, Thijs VN (2009) Efficacy and safety of tissue plasminogen activator 3 to 4.5 hours after acute ischemic stroke: a metaanalysis. Stroke 40:2438–2441

Leppanen L, Stys PK (1997) Ion transport and membrane potential in CNS myelinated axons. II. Effects of metabolic inhibition. J Neurophysiol 78:2095–2107

Leuchtmann EA, Ratner AE, Vijitruth R, Qu Y, McDonald JW (2003) AMPA receptors are the major mediators of excitotoxic death in mature oligodendrocytes. Neurobiol Dis 14:336–348

Li S, Stys PK (2000) Mechanisms of ionotropic glutamate receptor-mediated excitotoxicity in isolated spinal cord white matter. J Neurosci 20:1190–1198

Linares D, Taconis M, Mana P, Correcha M, Fordham S, Staykova M, Willenborg DO (2006) Neuronal nitric oxide synthase plays a key role in CNS demyelination. J Neurosci 26: 12672–12681

Liu HN, Almazan G (1995) Glutamate induces c-fos proto-oncogene expression and inhibits proliferation in oligodendrocyte progenitors: receptor characterization. Eur J Neurosci 7: 2355–2363

LoPachin RM, Lehning EJ (1997) Mechanism of calcium entry during axon injury and degeneration. Toxicol Appl Pharmacol 143:233–244

Mao L, Wang JQ (2002) Glutamate cascade to camp response element-binding protein phosphorylation in cultured striatal neurons through calcium-coupled group I metabotropic glutamate receptors. Mol Pharmacol 62:473–484

Matute C (1998) Characteristics of acute and chronic kainate excitotoxic damage to the optic nerve. Proc Natl Acad Sci U S A 95:10229–10234

Matute C, Alberdi E, Domercq M, Perez-Cerda F, Perez-Samartin A, Sanchez-Gomez MV (2001) The link between excitotoxic oligodendroglial death and demyelinating diseases. Trends Neurosci 24:224–230

McCracken E, Fowler JH, Dewar D, Morrison S, McCulloch J (2002) Grey matter and white matter ischemic damage is reduced by the competitive AMPA receptor antagonist, SPD 502. J Cereb Blood Flow Metab 22:1090–1097

McDonald JW, Althomsons SP, Hyrc KL, Choi DW, Goldberg MP (1998) Oligodendrocytes from forebrain are highly vulnerable to AMPA/kainate receptor-mediated excitotoxicity. Nat Med 4:291–297

Micu I, Jiang Q, Coderre E, Ridsdale A, Zhang L, Woulfe J, Yin X, Trapp BD, McRory JE, Rehak R, Zamponi GW, Wang W, Stys PK (2006) NMDA receptors mediate calcium accumulation in myelin during chemical ischaemia. Nature 439:988–992

Moxon-Emre I, Schlichter LC (2010) Evolution of inflammation and white matter injury in a model of transient focal ischemia. J Neuropathol Exp Neurol 69:1–15

Nakamura M, Araki M, Oguro K, Masuzawa T (1992) Differential distribution of 68 kd and 200 kd neurofilament proteins in the gerbil hippocampus and their early distributional changes following transient forebrain ischemia. Exp Brain Res 89:31–39

Nixon RA, Lewis SE (1986) Differential turnover of phosphate groups on neurofilament subunits in mammalian neurons in vivo. J Biol Chem 261:16298–16301

Nukada H, Dyck PJ (1987) Acute ischemia causes axonal stasis, swelling, attenuation, and secondary demyelination. Ann Neurol 22:311–318

O'Mahony D, Kendall MJ (1999) Nitric oxide in acute ischaemic stroke: a target for neuroprotection. J Neurol Neurosurg Psychiatry 67:1–3

Ogata N, Yonekawa Y, Taki W, Kannagi R, Murachi T, Hamakubo T, Kikuchi H (1989) Degradation of neurofilament protein in cerebral ischemia. J Neurosurg 70:103–107

Ouardouz M, Malek S, Coderre E, Stys PK (2006) Complex interplay between glutamate receptors and intracellular ca2+ stores during ischaemia in rat spinal cord white matter. J Physiol 577: 191–204

Ouardouz M, Coderre E, Basak A, Chen A, Zamponi GW, Hameed S, Rehak R, Yin X, Trapp BD, Stys PK (2009a) Glutamate receptors on myelinated spinal cord axons: I. Glur6 kainate receptors. Ann Neurol 65:151–159

Ouardouz M, Coderre E, Zamponi GW, Hameed S, Yin X, Trapp BD, Stys PK (2009b) Glutamate receptors on myelinated spinal cord axons: Ii. AMPA and glur5 receptors. Ann Neurol 65: 160–166

Pantoni L, Garcia JH, Gutierrez JA (1996) Cerebral white matter is highly vulnerable to ischemia. Stroke 27:1641–1646, discussion 1647

Patneau DK, Wright PW, Winters C, Mayer ML, Gallo V (1994) Glial cells of the oligodendrocyte lineage express both kainate- and AMPA-preferring subtypes of glutamate receptor. Neuron 12:357–371

Philipson KD, Nicoll DA (2000) Sodium-calcium exchange: a molecular perspective. Annu Rev Physiol 62:111–133

Raff MC (1989) Glial cell diversification in the rat optic nerve. Science 243:1450–1455

Roelfsema V, Bennet L, George S, Wu D, Guan J, Veerman M, Gunn AJ (2004) Window of opportunity of cerebral hypothermia for postischemic white matter injury in the near-term fetal sheep. J Cereb Blood Flow Metab 24:877–886

Sahuquillo J, Vilalta A (2007) Cooling the injured brain: How does moderate hypothermia influence the pathophysiology of traumatic brain injury. Curr Pharm Des 13:2310–2322

Salter MG, Fern R (2005) NMDA receptors are expressed in developing oligodendrocyte processes and mediate injury. Nature 438:1167–1171

Sanchez-Gomez MV, Matute C (1999) AMPA and kainate receptors each mediate excitotoxicity in oligodendroglial cultures. Neurobiol Dis 6:475–485

Shields DC, Schaecher KE, Saido TC, Banik NL (1999) A putative mechanism of demyelination in multiple sclerosis by a proteolytic enzyme, calpain. Proc Natl Acad Sci U S A 96: 11486–11491

Smith KJ, Kapoor R, Felts PA (1999) Demyelination: the role of reactive oxygen and nitrogen species. Brain Pathol 9:69–92

Song M, Mohamad O, Gu X, Wei L, Yu SP (2012) Restoration of intracortical and thalamocortical circuits after transplantation of bone marrow mesenchymal stem cells into the ischemic brain of mice. Cell Transplant (in press)

Sozmen EG, Kolekar A, Havton LA, Carmichael ST (2009) A white matter stroke model in the mouse: axonal damage, progenitor responses and MRI correlates. J Neurosci Methods 180: 261–272

Sozmen EG, Hinman JD, Carmichael ST (2012) Models that matter: white matter stroke models. Neurotherapeutics 9:349–358

Stys PK (1998) Anoxic and ischemic injury of myelinated axons in CNS white matter: from mechanistic concepts to therapeutics. J Cereb Blood Flow Metab 18:2–25

Stys PK, Jiang Q (2002) Calpain-dependent neurofilament breakdown in anoxic and ischemic rat central axons. Neurosci Lett 328:150–154

Stys PK, Ransom BR, Waxman SG, Davis PK (1990) Role of extracellular calcium in anoxic injury of mammalian central white matter. Proc Natl Acad Sci U S A 87:4212–4216

Tanaka K, Nogawa S, Suzuki S, Dembo T, Kosakai A (2003) Upregulation of oligodendrocyte progenitor cells associated with restoration of mature oligodendrocytes and myelination in peri-infarct area in the rat brain. Brain Res 989:172–179

Tekkok SB, Goldberg MP (2001) AMPA/kainate receptor activation mediates hypoxic oligodendrocyte death and axonal injury in cerebral white matter. J Neurosci 21:4237–4248

Tokutomi T, Morimoto K, Miyagi T, Yamaguchi S, Ishikawa K, Shigemori M (2007) Optimal temperature for the management of severe traumatic brain injury: effect of hypothermia on intracranial pressure, systemic and intracranial hemodynamics, and metabolism. Neurosurgery 61:256–265, discussion 265-256

Towfighi A, Saver JL (2011) Stroke declines from third to fourth leading cause of death in the United States: historical perspective and challenges ahead. Stroke 42:2351–2355

Tuttolomondo A, Di Sciacca R, Di Raimondo D, Arnao V, Renda C, Pinto A, Licata G (2009) Neuron protection as a therapeutic target in acute ischemic stroke. Curr Top Med Chem 9:1317–1334

van Velthoven CT, Kavelaars A, van Bel F, Heijnen CJ (2010) Repeated mesenchymal stem cell treatment after neonatal hypoxia-ischemia has distinct effects on formation and maturation of new neurons and oligodendrocytes leading to restoration of damage, corticospinal motor tract activity, and sensorimotor function. J Neurosci 30:9603–9611

Volpe JJ (2001) Neurobiology of periventricular leukomalacia in the premature infant. Pediatr Res 50:553–562

Waxman SG, Black JA, Ransom BR, Stys PK (1993) Protection of the axonal cytoskeleton in anoxic optic nerve by decreased extracellular calcium. Brain Res 614:137–145

Yam PS, Dunn LT, Graham DI, Dewar D, McCulloch J (2000) NMDA receptor blockade fails to alter axonal injury in focal cerebral ischemia. J Cereb Blood Flow Metab 20:772–779

Yenari MA, Hemmen TM (2010) Therapeutic hypothermia for brain ischemia: where have we come and where do we go? Stroke 41:S72–574

Yoshioka A, Hardy M, Younkin DP, Grinspan JB, Stern JL, Pleasure D (1995) Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (ampa) receptors mediate excitotoxicity in the oligodendroglial lineage. J Neurochem 64:2442–2448

Zaidi AU, Bessert DA, Ong JE, Xu H, Barks JD, Silverstein FS, Skoff RP (2004) New oligodendrocytes are generated after neonatal hypoxic-ischemic brain injury in rodents. Glia 46:380–390

Zimmerman UJ, Schlaepfer WW (1988) Calcium-activated neutral proteases (calpains) are carbohydrate binding proteins. J Biol Chem 263:11609–11612

Chapter 3
CADASIL and Animal Models

Francesco Blasi, Anand Viswanathan, and Cenk Ayata

Abbreviations

BBB	Blood–brain barrier
CADASIL	Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy
CBF	Cerebral blood flow
CVR	Cerebrovascular resistance
EGF	Epidermal growth factor
MCAo	Middle cerebral artery occlusion
N3KO	Notch3 knockout
$Notch3^{ECD}$	Notch3 extracellular domain
$NOTCH3^{ICD}$	Notch3 intracellular domain
PID	Peri-infarct depolarization
SD	Spreading depression
SVD	Small vessel disease
WT	Wild type

F. Blasi, Pharm. D., Ph.D.
Neurovascular Research Laboratory, Department of Radiology, Massachusetts General Hospital, Harvard Medical School, 149 13th Street, Room 6401, Charlestown, MA 02129, USA

A. Viswanathan, M.D., Ph.D.
Stroke Research Center, Massachusetts General Hospital, 175 Cambridge St., Suite 300, Boston, MA 02114, USA

C. Ayata, M.D. (✉)
Neurovascular Research Laboratory, Department of Radiology, Massachusetts General Hospital, 149 13th Street, Room 6408, Charlestown, MA 02129, USA

Stroke Service and Neuroscience Intensive Care Unit, Department of Neurology, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA
e-mail: cayata@partners.org

S. Baltan et al. (eds.), *White Matter Injury in Stroke and CNS Disease*, Springer Series in Translational Stroke Research 4, DOI 10.1007/978-1-4614-9123-1_3,

3.1 Genetics and Molecular Biology

CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy, MIM 125310) is an inherited neurovascular disorder caused by missense mutations in *NOTCH3*, a transmembrane receptor predominantly expressed in arterial smooth muscle cells in adult brain (Joutel et al. 1996). Linking CADASIL to chromosome 19q12 (Tournier-Lasserve et al. 1993) was a turning point in our understanding of its pathophysiology and the role of NOTCH3 in vascular function.

Majority of CADASIL mutations are located in *NOTCH3* exons 2-24, encoding for 34 epidermal growth factor (EGF) repeats domain, which constitutes the main extracellular arm of the NOTCH3 receptor (Joutel et al. 1997a, b). Mutation most commonly leads to an odd number of cysteine residues within the 34 EGF repeats, causing receptor homo/heterodimerization or multimerization (Dichgans et al. 2000; Donahue and Kosik 2004; Opherk et al. 2009). Other mutations unrelated to cysteine residues have also been reported (Coto et al. 2006; Liem et al. 2008; Roy et al. 2012). Although genetic testing represents the gold standard for CADASIL diagnosis (Markus et al. 2002), biopsy may be more definitive.

NOTCH3 receptor is part of an evolutionarily conserved transmembrane receptor family (Drosophila Notch homologues 1-4) involved in cell fate control, differentiation, proliferation, and apoptosis (Artavanis-Tsakonas et al. 1999; Iso et al. 2003a). Ligand binding (Delta-like 1–3 and Jagged 1, 2) triggers a conformational change leading to proteolytic cleavage and translocation of the intracellular domain ($NOTCH3^{ICD}$) to the nucleus (D'Souza et al. 2008). $NOTCH3^{ICD}$ then acts as a transcriptional co-activator of several genes implicated in development, including HES and HERP families (Iso et al. 2003b; Borggrefe and Oswald 2009).

3.2 Vascular and Brain Pathology in CADASIL Patients

The pivotal role of small vessels in disease pathogenesis has long been recognized (Van Bogaert 1955). The main pathological feature in CADASIL is a non-hypertensive, non-atherosclerotic, non-amyloid (i.e., pure) arteriopathy affecting primarily the small and medium size pial and penetrating vessels (Joutel 2011). Progressive degeneration of vascular smooth muscle cells and the pathognomonic accumulation of electron-dense granular osmiophilic material within the vascular basal lamina surrounding pericytes and smooth muscle cells are characteristic (Baudrimont et al. 1993; Miao et al. 2004). Although granular osmiophilic material accumulation is the hallmark, with specificity close to 100 % (Brulin et al. 2002; Ruchoux et al. 2002; Tikka et al. 2009), its constituents and role in vascular smooth muscle cell degeneration are just beginning to be explored. In particular, clusterin and collagen 18 alpha 1 (COL18A1) have recently been detected at high levels in the granular osmiophilic material in cerebral arteries and aorta of CADASIL patients

and transgenic mice (Arboleda-Velasquez et al. 2011). CADASIL patients also show an abnormal perivascular accumulation of the cleavage product Notch3 receptor extracellular domain (Notch3^{ECD}), but whether it is a constituent of the granular osmiophilic material is not yet clear (Joutel et al. 2000; Ishiko et al. 2006). Notch3^{ECD} and granular osmiophilic material accumulation distinguish CADASIL from other small vessel diseases, and may be pivotal to the disease pathogenesis.

Although histopathological and functional alterations are also present in systemic vessels (Ruchoux et al. 1994, 1995), neurological signs and symptoms dominate CADASIL. Vascular smooth muscle cell degeneration results in arterial fibrosis and thickening, enlargement of perivascular spaces, and narrowing of vascular lumen (Okeda et al. 2002; Yamamoto et al. 2009). These changes are believed to underlie the cerebral hypoperfusion detected in CADASIL patients even prior to the development of parenchymal lesions (Chabriat et al. 2000; Bruening et al. 2001; Tuominen et al. 2004).

The main clinical manifestations of the disease in patients include attacks of migraine with aura, mood disturbances (including apathy and depression), recurrent ischemic strokes, and progressive cognitive decline (Chabriat et al. 1995; Dichgans et al. 1998; Desmond et al. 1999; Reyes et al. 2009). Interestingly, migraine with aura is often the first clinical manifestation of CADASIL, present in 20–40 % of patients (Chabriat et al. 2009). Mood disturbances, including depression, are common (20 %). Apathy, defined as the absence of motivation, has been recently recognized as a major clinical manifestation that is present in about 40 % of patients with CADASIL, and is independent from depression (Reyes et al. 2009). Impairment in processing speed may be due to subcortical damage in dorsolateral prefrontal and cingulate circuits (Duering et al. 2012). Progressive cognitive decline leads to frank dementia in advanced stages of the disease (Buffon et al. 2006; Chabriat et al. 2009).

White matter hyperintensities (or leukoaraiosis) are thought to develop early in the disease course. These lesions may initially appear as discrete lesions predominating in periventricular areas and in the centrum semiovale and subsequently become more diffuse, affecting the external capsule and the anterior temporal lobes. Lesions in this location have been described as a characteristic pattern that is highly suggestive of the disease (Auer et al. 2001; O'Sullivan et al. 2001; Markus et al. 2002). It has been recently demonstrated that CADASIL patients with mutations in ligand binding domain of the Notch3 gene (EGFR 10-11) may have increased burden of white matter hyperintensities compared to patients with other common CADASIL mutations (Monet-Lepretre et al. 2009).

Ischemic stroke and transient ischemic attacks occur in large majority of patients and are the most frequent symptom in the disease (Chabriat et al. 1995, 1998; Dichgans et al. 1998; Bousser and Tournier-Lasserve 2001; Peters et al. 2004). Strokes are exclusively subcortical (Viswanathan et al. 2006a), involving the white matter as well as the grey matter nuclei, and occur in 60–85 % of patients ostensibly in the absence of comorbidities and conventional vascular risk factors (Desmond et al. 1999). Lacunar infarcts may be of variable shape, size, and number, and appear as hypointense lesions on T1-weighted imaging (Herve et al. 2005). Diffusion-weighted MRI may demonstrate areas of recent infarction (Gobron et al. 2006).

Other MRI lesions of subcortical vascular disease such as dilated perivascular spaces and cerebral microbleeds can also be seen in the disease (Dichgans et al. 2002; van den Boom et al. 2003; Cumurciuc et al. 2006). Cerebral microbleeds are detected on gradient echo sequences (T2*) in 25–69 % of patients. The frequency of cerebral microbleeds increases with age, blood pressure, hemoglobin A1c concentration, and extent of white matter damage (Dichgans et al. 2002; van den Boom et al. 2003; Viswanathan et al. 2006b).

Despite the extensive subcortical vascular lesions seen in CADASIL, recent studies have suggested that brain atrophy plays the most important role in disability and cognitive impairment in the disease (Viswanathan et al. 2010; Jouvent et al. 2012). The mechanism of brain atrophy in CADASIL remains unclear but may be partly related to apoptotic mechanisms in the cortex (Viswanathan et al. 2006a) that may be influenced by the burden of subcortical vascular disease, particularly lacunar infarctions (Jouvent et al. 2012). Although the impact of the subcortical lesions has been demonstrated in previous studies (Chabriat et al. 1999; Peters et al. 2004; Viswanathan et al. 2006b, 2007; Buffon et al. 2006), brain atrophy may represent a "final common pathway" in the pathophysiology of the disease.

No specific treatment is yet available for CADASIL although the use of the cholinesterase inhibitor donepezil may be useful in treating executive dysfunction in the disease as shown in one randomized trial (Dichgans et al. 2008). It remains to be tested whether specific stroke prevention measures (e.g., anti-thrombotics, statins) modify the disease course (Muqtadar and Testai 2012).

3.3 Mutant Mouse Models of CADASIL

Several mutant models linked to CADASIL-related genes and mutations have been developed (Ayata 2010a; Lee et al. 2012). Although Notch3 "knockout" mice provided some insight into the pathophysiology, they did not develop typical CADASIL neuropathology. More recent development of genetically engineered mice expressing human CADASIL mutations has further extended our knowledge. Some of the transgenic models overexpressing CADASIL mutant Notch3 genes (R90C, C428S, C455R, R1031C) did develop CADASIL-like vascular pathology such as arteriopathy and granular osmiophilic material deposits. However, the R169C (Joutel et al. 2010) and R170C (Wallays et al. 2011) mutants were the only models that developed neuropathological changes such as white matter degeneration and spontaneous infarcts. The genetic and phenotypic features of these and other models are summarized in Table 3.1.

3.3.1 Neurovascular Pathology

Although Notch3 knockout did not yield gross morphological abnormalities in cerebral vasculature or the parenchyma, histologically, cerebral vasculature was not normal: vascular smooth muscle cell layer was thinner, and arteries enlarged, with

Table 3.1 Notch3 mutant mice

Genetics	Mutant transgene	**Notch3 Null**	**R169C Rat Notch3**	**R170C Mouse Notch3**	**R90C Human Notch3**	**C428S Human Notch3**	**R142C Human Notch3**	**C455R Human Notch3**	**R1031C Human Notch3**
	Promoter	KO	PAC	KI	SM22α	SM22α	KI	Cond. KI	Cond. KI
	Background	C57Bl	FVB/N	50:50 129:SW	C57Bl	C57Bl	C57Bl	C57Bl	C57Bl
	Expression distribution	None	Arteries, capillaries	N.D.	Arteries	Arteries	Endogenous pattern	VSMC	VSMC
Cerebrovascular pathology	GOM accumulation	Absent	5 months	20 months	12 months	8-18 months	Absent	6 months	12 months
	$Notch3^{ECD}$ deposit	Absent	5 months	Absent	12 months	8-18 months	Absent	N.D.	N.D.
	VSMC abnormalities	Present	Present	Present	Present	Present	Absent	Present	Present
	Capillary density	N.D.	Reduced	Normal	N.D.	N.D.	N.D.	N.D.	N.D.
	Astrogliosis	Absent	Present	Present	Absent	N.D.	N.D.	N.D.	N.D.
	Leukoaraiosis	N.D.	Present	Absent	N.D.	N.D.	N.D.	N.D.	N.D.
	Lacunar strokes	N.D.	Absent	Present	N.D.	N.D.	N.D.	N.D.	N.D.
Cerebrovascular function	Resting CBF	Normal	Reduced	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	Functional hyperemia	N.D.	Reduced	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	Autoregulation (BP challenge)	Impaired	Impaired	N.D.	Impaired	N.D.	N.D.	N.D.	N.D.
	Hypercapnic/Acetazolamide-induced hyperemia	N.D.	Normal	N.D.	Reduced	N.D.	N.D.	N.D.	N.D.
	Infarct volume and CBF deficit after focal ischemia	Increased	N.D.	N.D.	N.D.	N.D.	N.D.	Increased	Normal

SM22α smooth muscle cell specific promoter, *Cond. KI* conditional knock-in, *PAC* P-1 derived artificial chromosome, *N.D.* not detected, *129:SW* sv-129:Swiss Webster, *BP* blood pressure

disorganized tunica media and less festooned tunica elastica interna, suggesting abnormal smooth muscle cell postnatal maturation (Domenga et al. 2004). These changes predicted loss of functional integrity of the arteries. Importantly, Notch3 gene knockout did not alter the endothelial morphology (Domenga et al. 2004). Transgenic models expressing R90C (Monet et al. 2007), C428S (Monet-Lepretre et al. 2009), C455R, and R1031C (Arboleda-Velasquez et al. 2011) mutations showed both smooth muscle cell degeneration and granular osmiophilic material and Notch3^{ECD} accumulation with aging (12 months, 8–18 months, 6 and 12 months, respectively); however, vascular pathology did not lead to any parenchymal degenerative changes. More recently, transgenic mice expressing the R169C or R170 mutations have been developed as models of the relatively more prevalent human CADASIL mutation R169C. The R169C mutant showed vascular granular osmiophilic material and Notch3^{ECD} accumulation already at 5–6 months of age, particularly within the basement membrane of smooth muscle cells (Joutel et al. 2010) similar to CADASIL patients. In contrast, R170C mutant showed no vascular abnormalities at 5 months of age, and granular osmiophilic material accumulation became detectable only after 20 months of age and in only six out of nine mice; Notch3^{ECD} accumulation was not detected at all. The discordance extended to the neuropathology as well. R169C mutant developed widespread astrogliosis, demyelination, and vacuolization in major white matter bundles including the corpus callosum, internal capsule, fimbria, anterior commissure, and white matter bundles in striatum, progressively between 12 and 20 months; cortex was spared, and no spontaneous ischemic infarcts were observed in any brain region. Providing a potential explanation for the white matter changes, resting CBF was reduced in the R169C mutant, detected initially in cortex, striatum, pallidum, amygdala and thalamus at 12 months, and later involving the white matter, where it reached 16 % below wild type at 18 months. Whether this mild degree of hypoperfusion, even if chronic, might explain the white matter injury remains to be determined. Nevertheless, capillary density was progressively reduced in corpus callosum, but not in cortex, between 6 and 20 months. Indeed, spatiotemporally, the complex neuropathological changes in the R169C mutant appeared to be most closely associated with capillary loss, although the direction of causation has been difficult to determine. In contrast to the R169C mutant, R170C mutant showed heterogeneous and variable neuropathology in 23 % of mice when assessed at 20 months of age (Wallays et al. 2011). These included microbleeds in 12 % (striatum, claustrum, somatosensory cortex, internal capsule), hemosiderin deposits in 5 % (external capsule, striatum, amygdala), perivascular inflammatory infiltrates in 8 % (striatum, cortex, external capsule, amygdala, claustrum), gliosis in 3 % (somatosensory cortex, external capsule), thrombosis in 7 % (striatum, external capsule), and lacunar strokes in 7 % (motor cortex, curiously limited to what appears to be the anterior and middle cerebral artery watershed). Despite these extensive and widespread lesions in the R170C mutant, there was no evidence for specific white matter pathology, such as demyelination and vacuolization. In summary, neuropathological incongruences notwithstanding, these mutant models together recapitulated critical CADASIL features. The discordance may be related to the transgenic strategies employed, or the genetic background (FVB/N for R169C and 129EvSv/Swiss for R170C).

3.3.2 Cerebrovascular Function

Altered cerebrovascular resistance and autoregulation has been a common theme in Notch3 mutant mouse models. For example, knockout mice were unable to autoregulate cerebral blood flow (CBF) by adjusting their cerebrovascular resistance (CVR) during hypertensive transients induced by Angiotensin II infusion, despite normal resting arterial blood pressures (Domenga et al. 2004). R90C transgenic mice also developed impaired vascular reactivity both in vitro and in vivo. For example, isolated tail arteries from 10-month-old R90C mutants showed an increase in pressure-induced myogenic tone, decrease in flow-induced dilatation, but no functional deficit after phenylephrine and acetylcholine challenge (Dubroca et al. 2005), suggesting a smooth muscle transduction defect. In vivo, 10- and 18-month-old R90C mutants showed reduced vasodilation to hypercapnic and acetazolamide challenges, and abnormal CBF autoregulation characterized by higher cerebrovascular resistance compared to wild type in response to hypertensive and hypotensive challenges (Lacombe et al. 2005). Lastly, the R169C mutant also showed impaired autoregulation and functional hyperemia, in vivo, as well as reduced myogenic tone, in vitro, when assessed at 5–6 months of age, suggesting that vascular dysfunction precedes the onset neuropathological changes at 12 months. Of note, hypercapnic hyperemia was preserved (Joutel et al. 2010).

3.3.3 Neurological Function

Neurological assessments have been carried out just in few CADASIL mutant mice. R142C mutants have been tested for locomotion (open field) and learning memory (water maze), but besides a subtle difference in explorative behavior, CADASIL mutants did not differ from wild type mice (Lundkvist et al. 2005). Behavioral analysis on R170C mutants revealed motor deficit in a small subset of mice at 20–22 months (12 %; ataxia, limb weakness, poorly correlated with neuropathology). However, none of the animals showed any cognitive impairment (Wallays et al. 2011).

3.3.4 Ischemic Challenge

Modeling leukoaraiosis and lacunar strokes of CADASIL experimentally has been problematic (Ayata 2010a). Nevertheless, challenging Notch3 mutant mice with ischemic stroke models and assessing the neurological and tissue outcome have revealed interesting genotype–phenotype associations. Middle cerebral artery occlusion was tested in Notch3 knockout and in R1031C and C455R CADASIL mutant mice (Arboleda-Velasquez et al. 2008, 2011). Homozygous Notch3 knockout mice showed a twofold increase in both the perfusion defect and the infarct size following middle cerebral artery occlusion compared to wild type and heterozygous

knockout (Arboleda-Velasquez et al. 2008). As expected, stroke phenotype was rescued by conditional transgenic expression of *NOTCH3* on a knockout background. Of note, Notch3 knockout mice showed a twofold increase in the frequency of spontaneous peri-infarct depolarization waves akin to spreading depression (see below), suggesting that the mechanism of ischemic vulnerability in CADASIL mutants is not limited to vascular dysfunction (Arboleda-Velasquez et al. 2008). Results in CADASIL transgenic mice expressing R1031C or C455R mutations on a knockout background have been more interesting (Arboleda-Velasquez et al. 2011). In this study, transgenic expression of not only the wild type gene but also the R1031C mutant *NOTCH3* rescued the stroke phenotype in 3–6-month-old knockout mice. In contrast, expression of C455R mutant *NOTCH3*, clinically associated with early stroke onset, appeared to worsen the outcome in the knockout, although this difference did not quite reach statistical significance. Interestingly, the ability of R1031C mutant *NOTCH3* to rescue the knockout phenotype was lost at 12 months, hinting towards an age-dependent loss of the mutant gene function (Arboleda-Velasquez et al. 2011). Altogether, these data suggested distinct genotype–phenotype associations in CADASIL, the shortcomings of middle cerebral artery occlusion models in mimicking the stroke pathophysiology in CADASIL notwithstanding.

3.3.5 Spreading Depression

Migraine with aura is often the first clinical symptom in CADASIL, preceding the strokes and cognitive impairment by many years (Liem et al. 2010). Recent data have suggested that CADASIL mutations enhance the susceptibility of cortex to spreading depression, and intense depolarization wave forming the electrophysiological substrate of migraine aura and a potential trigger for headache (Ayata 2010b). The R90C mutant mice displayed a decreased electrical threshold to trigger spreading depression and a higher frequency of spreading depressions in response to topical application of a depolarizing agent (Eikermann-Haerter et al. 2011). The data were surprisingly congruent with the increased susceptibility to peri-infarct depolarizations in the Notch3 knockout during focal ischemia, as outlined above (Arboleda-Velasquez et al. 2008). The cerebrovascular response to spreading depression was not altered in the mutants, clearly pointing towards an enhanced cortical excitability phenotype in CADASIL, also consistent with higher incidence of seizures in CADASIL patients. More work is needed to elucidate the mechanisms of hyperexcitability and determine whether it reflects a neuronal or astrocytic phenotype. Regardless, however, these data raise intriguing possibilities on a causative link between the hyperexcitability and spreading depression phenotype, and the progressive neuropathology of CADASIL. It remains to be determined whether suppression of spreading depression susceptibility modifies the disease progression in CADASIL.

3.3.6 Blood–Brain Barrier

Blood–brain barrier (BBB) disruption has been implicated in leukoaraiosis, lacunar strokes, and cognitive decline (Wardlaw et al. 2003; Taheri et al. 2011); however, its role in CADASIL is not known. The R169C mutant had intact BBB, assessed both ultrastructurally and using fluorescent tracer injection, at 12 months of age, at a time when astrogliosis was present without white matter loss (Joutel et al. 2010). Notably, pericytes, important regulators of BBB integrity (Armulik et al. 2010) do not appear to degenerate in CADASIL mutants. Of course, further studies testing BBB integrity to tracers of various molecular weights and multiple time points will be more definitive.

3.4 Future Directions

Despite the established role of Notch3 signaling during development and that *NOTCH3* mutations lead to vascular defects (Domenga et al. 2004), CADASIL pathogenesis is still debated (Spinner 2000; Lee et al. 2012). A hypomorphic phenotype (loss of function) is not supported by the observation that Notch3 deficiency (i.e., null mutant) does not recapitulate pathognomonic features of CADASIL in experimental animals, such as ultrastructural abnormalities and lacunar strokes, although Notch3 null mice are more susceptible to experimental stroke, as well as to spreading depression as a model for migraine with aura (Arboleda-Velasquez et al. 2008; Eikermann-Haerter et al. 2011). More plausible is a neomorphic phenotype (gain of a novel, pathological function), such as perivascular accumulation of $Notch3^{ECD}$ causing vascular dysfunction. As yet, there is no evidence for a hypermorphic phenotype (pathologically increased function of Notch3 receptor). Additional mutant models on knockout or wild type genetic background will undoubtedly be informative. In the future, it will be important to test experimental models of targeted ischemic challenges in CADASIL mutants. These include chronic low-grade hypoperfusion (e.g., bilateral carotid stenosis) to induce leukoaraiosis, or stereotaxic ischemic lesions in subcortical white matter (e.g., endothelin-1 infarcts) to mimic lacunar strokes (Shibata et al. 2007; Yoshizaki et al. 2008; Sozmen et al. 2009). Such models may recapitulate in mutant mice the cognitive dysfunction typical in CADASIL patients. Moreover, it is clear that the sensitivity of CADASIL mutant brains to ischemia is not limited to vascular dysfunction (see above). Further attention to individual cell types will provide new insight into how a smooth muscle-specific mutation can disrupt cell–cell interactions in the neurovascular unit.

Acknowledgements This work was supported by the National Institutes of Health (NS055104, NS061505, NS33335), Leducq Foundation (2012D000293), Neuroendovascular Research Fund from the Andrew David Heitman Foundation, and The Ellison Foundation.

References

Arboleda-Velasquez JF, Manent J, Lee JH, Tikka S, Ospina C, Vanderburg CR, Frosch MP, Rodriguez-Falcon M, Villen J, Gygi S, Lopera F, Kalimo H, Moskowitz MA, Ayata C, Louvi A, Artavanis-Tsakonas S (2011) Hypomorphic Notch 3 alleles link Notch signaling to ischemic cerebral small-vessel disease. Proc Natl Acad Sci U S A 108(21):E128–E135

Arboleda-Velasquez JF, Zhou Z, Shin HK, Louvi A, Kim HH, Savitz SI, Liao JK, Salomone S, Ayata C, Moskowitz MA, Artavanis-Tsakonas S (2008) Linking Notch signaling to ischemic stroke. Proc Natl Acad Sci U S A 105(12):4856–4861

Armulik A, Genove G, Mae M, Nisancioglu MH, Wallgard E, Niaudet C, He L, Norlin J, Lindblom P, Strittmatter K, Johansson BR, Betsholtz C (2010) Pericytes regulate the blood–brain barrier. Nature 468(7323):557–561. doi:10.1038/nature09522

Artavanis-Tsakonas S, Rand MD, Lake RJ (1999) Notch signaling: cell fate control and signal integration in development. Science 284(5415):770–776

Auer DP, Putz B, Gossl C, Elbel G, Gasser T, Dichgans M (2001) Differential lesion patterns in CADASIL and sporadic subcortical arteriosclerotic encephalopathy: MR imaging study with statistical parametric group comparison. Radiology 218(2):443–451

Ayata C (2010a) CADASIL: experimental insights from animal models. Stroke 41(10 Suppl): S129–S134

Ayata C (2010b) Cortical spreading depression triggers migraine attack: pro. Headache 50(4): 725–730. doi:10.1111/j.1526-4610.2010.01647.x

Baudrimont M, Dubas F, Joutel A, Tournier-Lasserve E, Bousser MG (1993) Autosomal dominant leukoencephalopathy and subcortical ischemic stroke. A clinicopathological study. Stroke 24(1):122–125

Borggrefe T, Oswald F (2009) The Notch signaling pathway: transcriptional regulation at Notch target genes. Cell Mol Life Sci 66(10):1631–1646. doi:10.1007/s00018-009-8668-7

Bousser M, Tournier-Lasserve E (2001) Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy: from stroke to vessel wall physiology. J Neurol Neurosurg Psychiatry 70(3):285–287

Bruening R, Dichgans M, Berchtenbreiter C, Yousry T, Seelos KC, Wu RH, Mayer M, Brix G, Reiser M (2001) Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy: decrease in regional cerebral blood volume in hyperintense subcortical lesions inversely correlates with disability and cognitive performance. AJNR Am J Neuroradiol 22(7):1268–1274

Brulin P, Godfraind C, Leteurtre E, Ruchoux MM (2002) Morphometric analysis of ultrastructural vascular changes in CADASIL: analysis of 50 skin biopsy specimens and pathogenic implications. Acta Neuropathol 104(3):241–248. doi:10.1007/s00401-002-0530-z

Buffon F, Porcher R, Hernandez K, Kurtz A, Pointeau S, Vahedi K, Bousser MG, Chabriat H (2006) Cognitive profile in CADASIL. J Neurol Neurosurg Psychiatry 77(2):175–180. doi:10.1136/jnnp.2005.068726, 77/2/175 [pii]

Chabriat H, Joutel A, Dichgans M, Tournier-Lasserve E, Bousser MG (2009) Cadasil. Lancet Neurol 8(7):643–653

Chabriat H, Levy C, Taillia H, Iba-Zizen MT, Vahedi K, Joutel A, Tournier-Lasserve E, Bousser MG (1998) Patterns of MRI lesions in CADASIL. Neurology 51(2):452–457

Chabriat H, Pappata S, Ostergaard L, Clark CA, Pachot-Clouard M, Vahedi K, Jobert A, Le Bihan D, Bousser MG (2000) Cerebral hemodynamics in CADASIL before and after acetazolamide challenge assessed with MRI bolus tracking. Stroke 31(8):1904–1912

Chabriat H, Pappata S, Poupon C, Clark CA, Vahedi K, Poupon F, Mangin JF, Pachot-Clouard M, Jobert A, Le Bihan D, Bousser MG (1999) Clinical severity in CADASIL related to ultrastructural damage in white matter: in vivo study with diffusion tensor MRI. Stroke 30(12): 2637–2643

Chabriat H, Vahedi K, Iba-Zizen MT, Joutel A, Nibbio A, Nagy TG, Krebs MO, Julien J, Dubois B, Ducrocq X et al (1995) Clinical spectrum of CADASIL: a study of 7 families.

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. Lancet 346(8980):934–939
Coto E, Menendez M, Navarro R, Garcia-Castro M, Alvarez V (2006) A new de novo Notch3 mutation causing CADASIL. Eur J Neurol 13(6):628–631. doi:10.1111/j.1468-1331.2006.01337.x, ENE1337 [pii]
Cumurciuc R, Guichard JP, Reizine D, Gray F, Bousser MG, Chabriat H (2006) Dilation of Virchow–Robin spaces in CADASIL. Eur J Neurol 13(2):187–190. doi:10.1111/j.1468-1331.2006.01113.x
D'Souza B, Miyamoto A, Weinmaster G (2008) The many facets of Notch ligands. Oncogene 27(38):5148–5167. doi:10.1038/onc.2008.229, onc2008229 [pii]
Desmond DW, Moroney JT, Lynch T, Chan S, Chin SS, Mohr JP (1999) The natural history of CADASIL: a pooled analysis of previously published cases. Stroke 30(6):1230–1233
Dichgans M, Holtmannspotter M, Herzog J, Peters N, Bergmann M, Yousry TA (2002) Cerebral microbleeds in CADASIL: a gradient-echo magnetic resonance imaging and autopsy study. Stroke 33(1):67–71
Dichgans M, Ludwig H, Muller-Hocker J, Messerschmidt A, Gasser T (2000) Small in-frame deletions and missense mutations in CADASIL: 3D models predict misfolding of Notch3 EGF-like repeat domains. Eur J Hum Genet 8(4):280–285. doi:10.1038/sj.ejhg.5200460
Dichgans M, Markus HS, Salloway S, Verkkoniemi A, Moline M, Wang Q, Posner H, Chabriat HS (2008) Donepezil in patients with subcortical vascular cognitive impairment: a randomised double-blind trial in CADASIL. Lancet Neurol 7(4):310–318. doi:10.1016/S1474-4422(08)70046-2
Dichgans M, Mayer M, Uttner I, Bruning R, Muller-Hocker J, Rungger G, Ebke M, Klockgether T, Gasser T (1998) The phenotypic spectrum of CADASIL: clinical findings in 102 cases. Ann Neurol 44(5):731–739. doi:10.1002/ana.410440506
Domenga V, Fardoux P, Lacombe P, Monet M, Maciazek J, Krebs LT, Klonjkowski B, Berrou E, Mericskay M, Li Z, Tournier-Lasserve E, Gridley T, Joutel A (2004) Notch3 is required for arterial identity and maturation of vascular smooth muscle cells. Genes Dev 18(22):2730–2735. doi:10.1101/gad.308904, 18/22/2730 [pii]
Donahue CP, Kosik KS (2004) Distribution pattern of Notch3 mutations suggests a gain-of-function mechanism for CADASIL. Genomics 83(1):59–65. doi:S0888754303002064 [pii]
Dubroca C, Lacombe P, Domenga V, Maciazek J, Levy B, Tournier-Lasserve E, Joutel A, Henrion D (2005) Impaired vascular mechanotransduction in a transgenic mouse model of CADASIL arteriopathy. Stroke 36(1):113–117
Duering M, Gonik M, Malik R, Zieren N, Reyes S, Jouvent E, Herve D, Gschwendtner A, Opherk C, Chabriat H, Dichgans M (2012) Identification of a strategic brain network underlying processing speed deficits in vascular cognitive impairment. Neuroimage 66C:177–183. doi:10.1016/j.neuroimage.2012.10.084
Eikermann-Haerter K, Yuzawa I, Dilekoz E, Joutel A, Moskowitz MA, Ayata C (2011) Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy syndrome mutations increase susceptibility to spreading depression. Ann Neurol 69(2):413–418
Gobron C, Viswanathan A, Bousser MG, Chabriat H (2006) Multiple simultaneous cerebral infarctions in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. Cerebrovasc Dis 22(5–6):445–446. doi:10.1159/000095287
Herve D, Mangin JF, Molko N, Bousser MG, Chabriat H (2005) Shape and volume of lacunar infarcts: a 3D MRI study in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. Stroke 36(11):2384–2388. doi:10.1161/01.STR.0000185678.26296.38
Ishiko A, Shimizu A, Nagata E, Takahashi K, Tabira T, Suzuki N (2006) Notch3 ectodomain is a major component of granular osmiophilic material (GOM) in CADASIL. Acta Neuropathol 112(3):333–339. doi:10.1007/s00401-006-0116-2
Iso T, Hamamori Y, Kedes L (2003a) Notch signaling in vascular development. Arterioscler Thromb Vasc Biol 23(4):543–553. doi:10.1161/01.ATV.0000060892.81529.8F, 01.ATV.0000060892.81529.8F [pii]
Iso T, Kedes L, Hamamori Y (2003b) HES and HERP families: multiple effectors of the Notch signaling pathway. J Cell Physiol 194(3):237–255. doi:10.1002/jcp.10208

Joutel A (2011) Pathogenesis of CADASIL: transgenic and knock-out mice to probe function and dysfunction of the mutated gene, Notch3, in the cerebrovasculature. Bioessays 33(1):73–80. doi:10.1002/bies.201000093

Joutel A, Andreux F, Gaulis S, Domenga V, Cecillon M, Battail N, Piga N, Chapon F, Godfrain C, Tournier-Lasserve E (2000) The ectodomain of the Notch3 receptor accumulates within the cerebrovasculature of CADASIL patients. J Clin Invest 105(5):597–605. doi:10.1172/JCI8047

Joutel A, Corpechot C, Ducros A, Vahedi K, Chabriat H, Mouton P, Alamowitch S, Domenga V, Cecillion M, Marechal E, Maciazek J, Vayssiere C, Cruaud C, Cabanis EA, Ruchoux MM, Weissenbach J, Bach JF, Bousser MG, Tournier-Lasserve E (1996) Notch3 mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia. Nature 383(6602):707–710. doi:10.1038/383707a0

Joutel A, Corpechot C, Ducros A, Vahedi K, Chabriat H, Mouton P, Alamowitch S, Domenga V, Cecillion M, Marechal E, Maciazek J, Vayssiere C, Cruaud C, Cabanis EA, Ruchoux MM, Weissenbach J, Bach JF, Bousser MG, Tournier-Lasserve E (1997a) Notch3 mutations in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), a mendelian condition causing stroke and vascular dementia. Ann N Y Acad Sci 826:213–217

Joutel A, Monet-Lepretre M, Gosele C, Baron-Menguy C, Hammes A, Schmidt S, Lemaire-Carrette B, Domenga V, Schedl A, Lacombe P, Hubner N (2010) Cerebrovascular dysfunction and microcirculation rarefaction precede white matter lesions in a mouse genetic model of cerebral ischemic small vessel disease. J Clin Invest 120(2):433–445

Joutel A, Vahedi K, Corpechot C, Troesch A, Chabriat H, Vayssiere C, Cruaud C, Maciazek J, Weissenbach J, Bousser MG, Bach JF, Tournier-Lasserve E (1997b) Strong clustering and stereotyped nature of Notch3 mutations in CADASIL patients. Lancet 350(9090):1511–1515. doi:10.1016/S0140-6736(97)08083-5, S0140-6736(97)08083-5 [pii]

Jouvent E, Mangin JF, Duchesnay E, Porcher R, During M, Mewald Y, Guichard JP, Herve D, Reyes S, Zieren N, Dichgans M, Chabriat H (2012) Longitudinal changes of cortical morphology in CADASIL. Neurobiol Aging 33(5):1002.e29–e36. doi:10.1016/j.neurobiolaging.2011.09.013

Lacombe P, Oligo C, Domenga V, Tournier-Lasserve E, Joutel A (2005) Impaired cerebral vasoreactivity in a transgenic mouse model of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy arteriopathy. Stroke 36(5):1053–1058. doi:10.1161/01.STR.0000163080.82766.eb

Lee JH, Bacskai BJ, Ayata C (2012) Genetic animal models of cerebral vasculopathies. Prog Mol Biol Transl Sci 105:25–55. doi:10.1016/B978-0-12-394596-9.00002-0

Liem MK, Lesnik Oberstein SA, Vollebregt MJ, Middelkoop HA, van der Grond J, Helderman-van den Enden AT (2008) Homozygosity for a NOTCH3 mutation in a 65-year-old CADASIL patient with mild symptoms: a family report. J Neurol 255(12):1978–1980. doi:10.1007/s00415-009-0036-x

Liem MK, Oberstein SA, van der Grond J, Ferrari MD, Haan J (2010) CADASIL and migraine: a narrative review. Cephalalgia 30(11):1284–1289

Lundkvist J, Zhu S, Hansson EM, Schweinhardt P, Miao Q, Beatus P, Dannaeus K, Karlstrom H, Johansson CB, Viitanen M, Rozell B, Spenger C, Mohammed A, Kalimo H, Lendahl U (2005) Mice carrying a R142C Notch 3 knock-in mutation do not develop a CADASIL-like phenotype. Genesis 41(1):13–22. doi:10.1002/gene.20091

Markus HS, Martin RJ, Simpson MA, Dong YB, Ali N, Crosby AH, Powell JF (2002) Diagnostic strategies in CADASIL. Neurology 59(8):1134–1138

Miao Q, Paloneva T, Tuominen S, Poyhonen M, Tuisku S, Viitanen M, Kalimo H (2004) Fibrosis and stenosis of the long penetrating cerebral arteries: the cause of the white matter pathology in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. Brain Pathol 14(4):358–364

Monet-Lepretre M, Bardot B, Lemaire B, Domenga V, Godin O, Dichgans M, Tournier-Lasserve E, Cohen-Tannoudji M, Chabriat H, Joutel A (2009) Distinct phenotypic and functional

features of CADASIL mutations in the Notch3 ligand binding domain. Brain 132(Pt 6): 1601–1612

Monet M, Domenga V, Lemaire B, Souilhol C, Langa F, Babinet C, Gridley T, Tournier-Lasserve E, Cohen-Tannoudji M, Joutel A (2007) The archetypal R90C CADASIL-NOTCH3 mutation retains NOTCH3 function in vivo. Hum Mol Genet 16(8):982–992. doi:10.1093/hmg/ddm042

Muqtadar H, Testai FD (2012) Single gene disorders associated with stroke: a review and update on treatment options. Curr Treat Options Cardiovasc Med 14(3):288–297. doi:10.1007/s11936-012-0179-4

O'Sullivan M, Jarosz JM, Martin RJ, Deasy N, Powell JF, Markus HS (2001) MRI hyperintensities of the temporal lobe and external capsule in patients with CADASIL. Neurology 56(5): 628–634

Okeda R, Arima K, Kawai M (2002) Arterial changes in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) in relation to pathogenesis of diffuse myelin loss of cerebral white matter: examination of cerebral medullary arteries by reconstruction of serial sections of an autopsy case. Stroke 33(11):2565–2569

Opherk C, Duering M, Peters N, Karpinska A, Rosner S, Schneider E, Bader B, Giese A, Dichgans M (2009) CADASIL mutations enhance spontaneous multimerization of NOTCH3. Hum Mol Genet 18(15):2761–2767. doi:10.1093/hmg/ddp211, ddp211 [pii]

Peters N, Herzog J, Opherk C, Dichgans M (2004) A two-year clinical follow-up study in 80 CADASIL subjects: progression patterns and implications for clinical trials. Stroke 35(7): 1603–1608. doi:10.1161/01.STR.0000131546.71733.f1, 01.STR.0000131546.71733.f1 [pii]

Reyes S, Viswanathan A, Godin O, Dufouil C, Benisty S, Hernandez K, Kurtz A, Jouvent E, O'Sullivan M, Czernecki V, Bousser MG, Dichgans M, Chabriat H (2009) Apathy: a major symptom in CADASIL. Neurology 72(10):905–910. doi:10.1212/01.wnl.0000344166.03470.f8

Roy B, Maksemous N, Smith RA, Menon S, Davies G, Griffiths LR (2012) Two novel mutations and a previously unreported intronic polymorphism in the NOTCH3 gene. Mutat Res 732(1–2): 3–8. doi:10.1016/j.mrfmmm.2012.02.004, S0027-5107(12)00044-9 [pii]

Ruchoux MM, Brulin P, Brillault J, Dehouck MP, Cecchelli R, Bataillard M (2002) Lessons from CADASIL. Ann N Y Acad Sci 977:224–231

Ruchoux MM, Chabriat H, Bousser MG, Baudrimont M, Tournier-Lasserve E (1994) Presence of ultrastructural arterial lesions in muscle and skin vessels of patients with CADASIL. Stroke 25(11):2291–2292

Ruchoux MM, Guerouaou D, Vandenhaute B, Pruvo JP, Vermersch P, Leys D (1995) Systemic vascular smooth muscle cell impairment in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. Acta Neuropathol 89(6):500–512

Shibata M, Yamasaki N, Miyakawa T, Kalaria RN, Fujita Y, Ohtani R, Ihara M, Takahashi R, Tomimoto H (2007) Selective impairment of working memory in a mouse model of chronic cerebral hypoperfusion. Stroke 38(10):2826–2832. doi:10.1161/STROKEAHA.107.490151

Sozmen EG, Kolekar A, Havton LA, Carmichael ST (2009) A white matter stroke model in the mouse: axonal damage, progenitor responses and MRI correlates. J Neurosci Methods 180(2): 261–272. doi:10.1016/j.jneumeth.2009.03.017

Spinner NB (2000) CADASIL: Notch signaling defect or protein accumulation problem? J Clin Invest 105(5):561–562. doi:10.1172/JCI9511

Taheri S, Gasparovic C, Huisa BN, Adair JC, Edmonds E, Prestopnik J, Grossetete M, Shah NJ, Wills J, Qualls C, Rosenberg GA (2011) Blood–brain barrier permeability abnormalities in vascular cognitive impairment. Stroke 42(8):2158–2163. doi:10.1161/STROKEAHA.110.611731

Tikka S, Mykkanen K, Ruchoux MM, Bergholm R, Junna M, Poyhonen M, Yki-Jarvinen H, Joutel A, Viitanen M, Baumann M, Kalimo H (2009) Congruence between NOTCH3 mutations and GOM in 131 CADASIL patients. Brain 132(Pt 4):933–939

Tournier-Lasserve E, Joutel A, Melki J, Weissenbach J, Lathrop GM, Chabriat H, Mas JL, Cabanis EA, Baudrimont M, Maciazek J et al (1993) Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy maps to chromosome 19q12. Nat Genet 3(3):256–259

Tuominen S, Miao Q, Kurki T, Tuisku S, Poyhonen M, Kalimo H, Viitanen M, Sipila HT, Bergman J, Rinne JO (2004) Positron emission tomography examination of cerebral blood flow and glucose metabolism in young CADASIL patients. Stroke 35(5):1063–1067. doi:10.1161/01.STR.0000124124.69842.2d, 01.STR.0000124124.69842.2d [pii]

Van Bogaert L (1955) Encephalopathie sous-corticale progressive (Binswanger) a evolution rapide chez deux soeurs. Med Hellen 24:961–972

van den Boom R, Lesnik Oberstein SA, Ferrari MD, Haan J, van Buchem MA (2003) Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy: MR imaging findings at different ages–3rd-6th decades. Radiology 229(3):683–690. doi:10.1148/radiol.2293021354

Viswanathan A, Godin O, Jouvent E, O'Sullivan M, Gschwendtner A, Peters N, Duering M, Guichard JP, Holtmannspotter M, Dufouil C, Pachai C, Bousser MG, Dichgans M, Chabriat H (2010) Impact of MRI markers in subcortical vascular dementia: a multi-modal analysis in CADASIL. Neurobiol Aging 31(9):1629–1636. doi:10.1016/j.neurobiolaging.2008.09.001

Viswanathan A, Gray F, Bousser MG, Baudrimont M, Chabriat H (2006a) Cortical neuronal apoptosis in CADASIL. Stroke 37(11):2690–2695

Viswanathan A, Gschwendtner A, Guichard JP, Buffon F, Cumurciuc R, O'Sullivan M, Holtmannspotter M, Pachai C, Bousser MG, Dichgans M, Chabriat H (2007) Lacunar lesions are independently associated with disability and cognitive impairment in CADASIL. Neurology 69(2):172–179. doi:10.1212/01.wnl.0000265221.05610.70, 69/2/172 [pii]

Viswanathan A, Guichard JP, Gschwendtner A, Buffon F, Cumurcuic R, Boutron C, Vicaut E, Holtmannspotter M, Pachai C, Bousser MG, Dichgans M, Chabriat H (2006b) Blood pressure and haemoglobin A1c are associated with microhaemorrhage in CADASIL: a two-centre cohort study. Brain 129(Pt 9):2375–2383. doi:10.1093/brain/awl177

Wallays G, Nuyens D, Silasi-Mansat R, Souffreau J, Callaerts-Vegh Z, Van Nuffelen A, Moons L, D'Hooge R, Lupu F, Carmeliet P, Collen D, Dewerchin M (2011) Notch3 Arg170Cys knock-in mice display pathologic and clinical features of the neurovascular disorder cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. Arterioscler Thromb Vasc Biol 31(12):2881–2888. doi:10.1161/ATVBAHA.111.237859, ATVBAHA.111.237859 [pii]

Wardlaw JM, Sandercock PA, Dennis MS, Starr J (2003) Is breakdown of the blood-brain barrier responsible for lacunar stroke, leukoaraiosis, and dementia? Stroke 34(3):806–812. doi:10.1161/01.STR.0000058480.77236.B3

Yamamoto Y, Ihara M, Tham C, Low RW, Slade JY, Moss T, Oakley AE, Polvikoski T, Kalaria RN (2009) Neuropathological correlates of temporal pole white matter hyperintensities in CADASIL. Stroke 40(6):2004–2011. doi:10.1161/STROKEAHA.108.528299, STROKEAHA.108.528299 [pii]

Yoshizaki K, Adachi K, Kataoka S, Watanabe A, Tabira T, Takahashi K, Wakita H (2008) Chronic cerebral hypoperfusion induced by right unilateral common carotid artery occlusion causes delayed white matter lesions and cognitive impairment in adult mice. Exp Neurol 210(2):585–591. doi:10.1016/j.expneurol.2007.12.005

Chapter 4
Neuroimaging of White Matter Injury: A Multimodal Approach to Vascular Disease

Gary A. Rosenberg, Branko Huisa, Fakhreya Y. Jalal, and Yi Yang

4.1 Introduction

The deep white matter of the brain is essential for communication between brain regions that control motor, sensory, and cognitive functions. In spite of the vulnerability of white matter myelinated tracts to a large number of pathological conditions, little was understood about the underlying disease processes without autopsy. This situation changed rapidly with the development of methods to obtain brain images tomographically, using arrays of X-rays. These new methods, which required large computers for image reconstruction, greatly advanced the understanding of the role of white matter in health and disease. Computed tomography (CT) was the first of the new imaging methods to use computers to form tomographic images of the brain by solving large numbers of simultaneous differential equations, revealing subtle differences between gray and white matter linked to pathological conditions (Hounsfield 1980). However, the major breakthrough in imaging occurred when the tomographic methods were applied to the proton signals from water to form magnetic resonance images (MRI) (Ordidge et al. 1981; Lauterbur 1982). Over the past three decades, steady advances in imaging techniques have taken place culminating in a wide variety of imaging methods, mainly based on magnetic resonance, to show pathological changes in the white matter. The major diseases with involvement of the white matter in the adult are multiple sclerosis, stroke, and dementia. This chapter focuses on the role of imaging of the white matter in acute and chronic vascular diseases since there are many excellent reviews on the role of MRI in multiple sclerosis (Filippi and Rocca 2008; Lucchinetti et al. 2008; Filippi et al. 2012).

G.A. Rosenberg, M.D. (✉) • B. Huisa, M.D. • F.Y. Jalal, Ph.D. • Y. Yang, M.D., Ph.D.
Department of Neurology, HSC Neurology, MSC 10 5620, University of New Mexico Health Sciences Center, Albuquerque, NM, USA
e-mail: grosenberg@salud.unm.edu

S. Baltan et al. (eds.), *White Matter Injury in Stroke and CNS Disease*, Springer Series in Translational Stroke Research 4, DOI 10.1007/978-1-4614-9123-1_4,

Table 4.1 Imaging modalities used in the diagnosis of white matter disease

Modality	Pros	Cons
Computed tomography	Shows leukoaraiosis due to pathological changes	Relatively insensitive
Magnetic resonance imaging (MRI)	Abnormalities of the white matter easily seen in pathological conditions	Nonspecific white matter findings include changes of normal aging
Magnetic resonance spectroscopic imaging (^{1}H-MRSI)	Biochemical changes seen with NAA fall in ischemia	Low resolution results in large areas of signal collection
Diffusion tensor imaging (DTI)	Indicates disrupted fiber tracts. Location correlates with neuropsychological testing	Relative images need template to show abnormalities
Dynamic contrast-enhanced MRI (DCEMRI)	Best method to quantitate subtle changes in blood–brain barrier (BBB)	Mainly useful in white matter

Magnetic resonance imaging (MRI) is ideal for showing pathological changes in the white matter because of the strong contrast between the signal from the gray and white matter (Table 4.1). Another advantage of MRI is the ability to use a relatively safe contrast agent, gadolinium diethylenetriaminepentacetate (Gd-DTPA), which allows visualization of contrast agent leakage across the blood–brain barrier (BBB). Newer, high-resolution MRI systems, operating from 3.0 to 7.0 T field strength, provide detailed information of lesion site and permeability characteristics. In addition to the MRI, there are other magnetic resonance methods that provide unique information. Most remain experimental and are only available at specialized centers. Since these may enter into clinical care, they are discussed in this review. They include nuclear magnetic resonance spectroscopy (NMRS), diffusion tensor imaging (DTI), and dynamic contrast-enhanced MRI (DCEMRI). NMRS uses the NMR as a spectrometer to measure a group of metabolites in brain tissue. DTI shows paths of water diffusion, which can be mathematically computed to link white matter fiber tracts. DCEMRI uses Gd-DTPA and provides a measure of BBB permeability. When several of these modalities are combined, a complex picture emerges of the integrity of the white matter that greatly enhances the diagnostic strength of MRI alone.

White matter diseases cover a wide range of pediatric and adult nervous system pathology. Many of the childhood diseases involve dysmyelination due to genetic defects. In the adult, white matter injury involves demyelination and ischemic/hypoxic damage. Acute ischemic injury results in necrosis of the myelinated fibers in specific vascular territories with damage crossing boundaries of the white and gray matter. Some illnesses involve an inflammatory process that disrupts the BBB and leads to proteolysis of the myelinated fibers. T-cells are implicated in this process in multiple sclerosis, where the inflammatory response related to T-cells that disrupt the BBB leaves gliotic scars in sites of old injury (Noseworthy et al. 2000). More recently, hypoxic injury with activation of macrophages/microglia has been advocated as an important mechanism of injury in multiple sclerosis

(Henderson et al. 2009). Another form of demyelination related to vascular diseases that involves mainly macrophages/microglia results in slowly progressive damage to the white matter characterized by disruption of the BBB. Often the cause of the progressive inflammatory process involves a reaction to cellular damage, particularly involving the endothelial cells, which are damaged by hypertension, diabetes, and hyperlipidemia. This is the type of injury involved in the more progressive white matter damage found in chronic vascular disease, while acute hypoxia/ischemia leads to generalized damage by necrosis and apoptosis.

4.2 Imaging in Acute Stroke

Acute ischemic injury causes damage to the white matter when loss of oxygen and glucose, results in necrosis and apoptosis in the core and surrounding penumbral tissues (Lo et al. 2003). The region demarcated as the penumbra has the potential for reversibility of the damage. Neurons and oligodendrocytes undergo cell death when the supply of oxygen drops below a critical threshold. In the early stages of an ischemic injury, neurons lose membrane potential, mitochondria cease to produce adenosine triphosphate (ATP), and in the extreme situations, necrosis leads to membrane rupture with cell death. Neurons and oligodendrocytes are highly vulnerable to loss of energy sources (Pantoni et al. 1996). Similar to the gray matter, white matter requires a constant supply of oxygen, which is threatened when the level of oxygen in the blood falls, such as during anesthetic accidents where nitrogen is inadvertently substituted for oxygen. Both glucose and oxygen are lost when the white matter is hypoperfused, such as during a cardiorespiratory arrest. Vulnerability of the white matter is related to patterns of development of cerebral blood vessels, which originate on the surface of the brain, penetrating into brain tissue to supply deeper structures (Duvernoy et al. 1983). Because the white matter is beneath the cortex in the central regions of the brain, it is a "watershed" that is the last site of the blood flow (De Reuck et al. 1980; Moody et al. 2004). Thus, with hypoperfusion the white matter is severely affected.

Imaging with CT shows areas of infarction in vascular territories by several hours after the infarct. These early changes in the CT are often subtle, consisting of loss of the normal distinction between the gray and white matter. If the region of infarction is extensive, brain edema appears around the infarct that suggests a threat of herniation. Until the introduction of CT into clinical practice, infarction with herniation was only evident at autopsy. With CT most infarcts could be seen by 24–72 h. In some patients with occlusion of the middle cerebral artery, cerebral edema can be seen in the insula region along with a hyper-dense middle cerebral artery sign, indicating clotted blood in the artery. Since the vascular territory generally involved larger areas than the white matter, the damage to the white matter often is obscured by the more extensive injury to the basal ganglia and cortex. Old regions of infarction that have formed cysts can be readily seen on CT since they have the same density as the water in the ventricles. Small regions of infarction in

Table 4.2 MRI findings in cerebrovascular disease

1. Acute stroke	Infarcts of large vessels cause necrosis/apoptosis in vascular territories including white matter. Lacunar strokes in basal ganglia and white matter cause discreet lesions due to small vessel disease
2. Chronic vascular disease in dementia	
A. Multiple strokes	Large territory strokes involve both gray and white matter
B. Single strategic stroke	Mainly thalamic region or splenium of corpus callous
C. Small vessel disease	Lacunar infarcts alone or accompanied with diffuse white matter demyelination with arteriolosclerosis
D. Hypoxic hypoperfusion	White matter damage due to cardiopulmonary arrest

the deep white matter can be seen on CT as lacunar (holes) in the white matter tracts. These produce symptoms of pure motor or pure sensory loss depending on the location in the internal capsule (Pantoni 2010).

MRI is much more sensitive to damage to the white matter than CT, making it the optimal method of imaging in diseases of the white matter (Table 4.2). In practice, the need to administer tissue plasminogen activator (tPA) within 4.5 h after the onset of stroke, and the requirement to obtain a CT to rule out bleeding, means that MRI is rarely done in the acute situation except at large research centers with continuous access to the scanner (Wintermark et al. 2008). It is common for a patient to have both an acute CT in the emergency room and a MRI several hours after admission, which increases the cost of care. Obtaining MRI in the acute situation is difficult in most hospitals, but the CT could be avoided in patients that are not candidates for tPA (Chalela et al. 2007).

Recent advances in CT instrumentation have made it possible to determine the site of occlusion and the tissue perfusion using contrast-enhanced angiography. In addition, perfusion CT shows the regions of decreased cerebral blood flow, blood volume and regions of increased permeability (Hom et al. 2011). Thus, CT provides important information about acute intracerebral hemorrhage and with the use of contrast-enhanced images and fast CT scanners, it can add additional information about vessel occlusions, tissue perfusion, and BBB permeability. However, there is a small risk of an allergic reaction and occasional deaths with the iodine-containing CT contrast agents, but adverse reactions are extremely rare with MRI contrast material.

The optimal imaging modality to determine white matter injury in acute stroke is MRI, which has the added advantage of showing the site of infarction within minutes after the onset. Using rapid scanning methods, the diffusive movement of water can be used as an indicator of cerebral edema (Moseley et al. 1990; Cvoro et al. 2009). Typically in an infarct, energy failure causes cellular swelling or cytotoxic edema. When cells swell the extracellular space is compressed and the diffusion of water is slowed. This is seen as an area of decreased signal on the apparent diffusion coefficient (ADC) scan, which appears black on MRI. The diffusion-weighted image (DWI) has a white signal, indicating a region of ischemic injury.

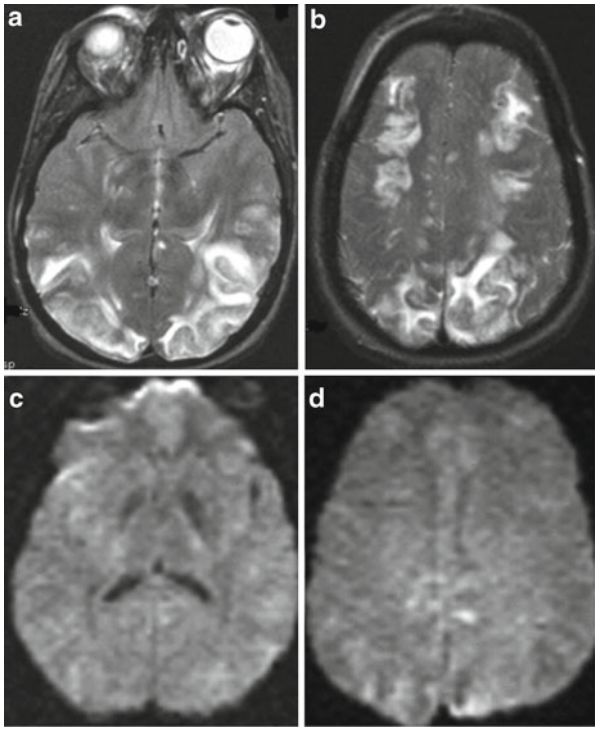


Fig. 4.1 Patient with hypertensive encephalopathy secondary to eclampsia with the HELLP (hemolysis, elevated liver enzymes, and low platelets) syndrome. (**a**) A T2-weighted MRI showing the extensive cerebral edema in the posterior white matter regions with less involvement of the gray matter. (**b**) A higher level shows the involvement in the frontal region. (**c**) Diffusion-weighted images (DWI) with only one small area of involvement. (**d**) Level similar to that seen in (**b**) above. The lack of DWI changes is consistent with this being a vasogenic type of edema, and the patient had a good recovery without residual symptoms

Conversely, vascular leakage or vasogenic edema without cytotoxic edema results in widening of the extracellular space. Water diffuses more rapidly through the distended space and the ADC is enhanced, producing an area with either no decrease in signal or of increased signal. An example of increased ADC is a patient with a hypertensive crisis in which there is vasogenic edema that spreads through the white matter sparing the gray matter. Such a pattern is seen with acute increased blood pressure in patients with hypertensive crisis due to eclampsia or acute renal disease (Fig. 4.1). Since the edema is vasogenic and the white matter fibers are initially widened, the FLAIR image clearly shows the extent of the edema in the white matter, while DWI does not show an area of ischemia. Since the vessels are leaking, but the cell membranes are intact, full recovery is possible.

4.3 Chronic White Matter Changes in Ischemia/Hypoxia

Many pathological processes damage the white matter (Table 4.3). The major cause of white matter injury in the elderly is vascular cognitive impairment (VCI) (Gorelick et al. 2011). This new term replaces several older ones, including multi-infarct dementia and vascular dementia, and encompasses all forms of cognitive loss due to vascular disease (Moorhouse and Rockwood 2008). It recognizes that

Table 4.3 Diagnostic classification of chronic white matter changes in the adult

Large vessel disease (multiple strokes or single strategic stroke)
Small vessel disease
Lacunar state
Basal ganglia (gray matter)
Subcortical white matter
White matter disease
Subcortical vascular disease (Binswanger's disease)
Chronic hypoperfusion of white matter border zone regions
Anoxic/hypoxic white matter damage (delayed post-anoxic encephalopathy)
Mixed AD/VCI
Cerebral amyloid angiopathy (CAA)
CADASIL
CNS vasculitis (infectious and inflammatory)
Adult onset leukodystrophies
Other causes of punctate lesions in white matter
CNS vasculitis (infectious and inflammatory)
Migraine
Head trauma (remote)
Infection (remote)

vascular disease can lead to types of intellectual loss that are different than seen with Alzheimer's disease (AD) (Hachinski et al. 2006). In AD the dominant impairments are in memory and language, which are affected early in the course, while timed tests that involve motor coordination occur late. In contrast, patients with VCI have relatively intact memory and language function, but are impaired in timed tests and executive function. While AD patients avoid motor problems until late in the course, VCI patients show impairments in gait and balance along with focal neurological findings at a relatively early stage.

There are several types of VCI, which is a heterogeneous group of diseases, including multiple large vessel strokes, lacunar infarcts in the basal ganglia, and subcortical vascular disease (SVD) with extensive involvement of the deep white matter (Table 4.3). Multiple lacunar strokes in the basal ganglia lead to Parkinsonism and impaired cognition. A strategically located lacunar stroke in the basal ganglia, generally in the left posterior thalamus, can cause intellectual loss. When there is extensive injury to the deep white matter due to vascular demyelination, the term Binswanger's disease is used. Patients with Binswanger's disease have apathy, gait imbalance, hyperreflexia, executive dysfunction on neuropsychological testing

Table 4.4 Animal models used for the study of vascular cognitive impairment

Animal model	Drug	Outcome	Reference
Rat BCAO	MMP inhibitor	Reduction of the severity of the WM lesions and the number of activated astroglia and microglia	(Nakaji et al. 2006)
mmp-2 knockout mice	MMP inhibitor	Reduction of the severity of the WM lesions and the number of activated astroglia and microglia	(Nakaji et al. 2006)
SHR/SP/JPD	Rosuvastatin	Delay appearance of T2 lesions compared with controls	(Sironi et al. 2005)
SHR/SP/JPD/UCAO	Minocycline	Reduction/elimination of T2 lesions compared with controls. Prolonged survival	(Jalal et al. 2012)

BCAO bilateral carotid artery occlusion, *SHR/SP* spontaneously hypertensive stroke-prone rat, *UCAO* unilateral carotid artery occlusion, *JPD* Japanese permissive diet

(Bennett et al. 1990). Often there are lacunar strokes in the basal ganglia and the white matter in Binswanger's disease. Pathologically, there are fibrosed arterioles that are surrounded by inflammatory cells of the macrophage/microglia type (Tomimoto et al. 1999; Rosenberg et al. 2001). Lacunar strokes and Binswanger's disease are associated with microvessel disease that is mainly considered due to hypertension and diabetes (Table 4.4).

Sporadic and hereditary forms of cerebral amyloid angiopathy (CAA), which affects small and medium size cortical vessels, is associated with substantial white matter hyperintensities (WMHs) (Smith et al. 2004). There is evidence to suggest that the white matter lesions in CAA are caused by impairment of blood flow within the white matter microvasculature (Thal et al. 2009). The WMHs in CAA are predominantly located in the occiput, which correlates with the predominance of amyloid deposition in the posterior regions (Vinters and Gilbert 1983). The use of echo gradient and more recently susceptibility-weighted image (SWI) MR have improved the diagnosis of CAA by showing the presence of multiple small regions of loss of signal due to the paramagnetic properties of blood. These microbleeds can be detected by MRI sequences that has been shown to be highly specific for CAA in patients older than 55, with no other identified cause for ICH (Knudsen et al. 2001).

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a genetically defined small vessel disease due to a mutation in the NOTCH3 gene. The prevalence of CADASIL is estimated to be of 1 in 25–50,000 in the general population (Narayan et al. 2012). Symptoms typically begin with migraine headache followed by ischemic stroke in the subcortical gray and white matter, and cognitive decline, resulting later in dementia. Changes in brain MRI, especially within the white matter, can be seen before clinical manifestations occur (Bousser and Tournier-Lasserve 1994). Areas of increased white matter signal occur in periventricular areas and in the centrum semiovale. Locations of WMHs highly suggestive of CADASIL include the external capsule and the anterior

part of the temporal lobes. The basal ganglia and thalamus are also affected with lacunar infarcts, microbleeds, and increase of dilated periventricular spaces (Auer et al. 2001). The progression of the WMHs leads to subsequent cortical atrophy (Peters et al. 2006).

Both infective and inflammatory CNS vasculitis are other causes of middle and small vessel disease leading to white matter lesions in the adults. These lesions occur in the setting of a more rapid progressive clinical course expanding also more rapidly than other causes of chronic microvascular brain disease.

The large vessel multiple stroke form of VCI generally involves the cortex, while small vessel disease has a predilection for the basal ganglia and deep white matter. CT provides evidence of extensive white matter damage, which has been termed "leukoaraiosis" or white matter changes (Hachinski et al. 1987). Leukoaraiosis describes the nonspecific changes in the cerebral white matter, but it can also frequently be seen on CT and MRI in normal elderly individuals. Leukoaraiosis is a descriptive term for rarefaction ("araiosis") of the white ("leuko") matter. These white matter changes are also commonly referred to as periventricular or subcortical white matter disease, and have been graded as to severity (Wahlund et al. 2001).

With the advent of CT scanning it was possible to see the attenuation of the white matter, which was initially interpreted as "ischemic" lesions and given the designation of Binswanger's disease. In 1981, *Lancet* in an editorial, described an "epidemic" of Binswanger's disease, which was thought prior to CT to be an extremely rare disease (Olszewski 1962). They cautioned against using CT for diagnosis since many normal individuals had similar findings. MRI, which is much more sensitive in detecting white matter changes than CT, further confounded the diagnosis of white matter lesions. Early MRI reports described asymptomatic elderly individuals with Binswanger's disease solely on the basis of the MRI findings (Kinkel et al. 1985). Subsequently, a number of studies have documented the high incidence of WMHs in normal individuals (Awad et al. 1986, Hunt et al. 1989). Several large surveys have demonstrated subtle neuropsychological findings in randomly selected elderly patients many of whom have vascular risk factors (Boone et al. 1992, Vermeer et al. 2003). An estimated 30 % of healthy elderly over the age of 65 have moderate WMHs, and about 7 % have severe changes in the white matter (Fig. 4.2) (Hunt et al. 1989). These studies have raised the important question of what is the cause of the WMHs in otherwise healthy individuals. Some investigators have attributed these WMHs to "silent strokes", and find an association with cognitive decline and dementia (Vermeer et al. 2003).

Large surveys of elderly patients that are collected by random sampling reveal an association of gait imbalance and urinary symptoms with WMHs (Wakefield et al. 2010). These subjects generally have a high incidence of vascular risk factors, including hypertension and diabetes. Gait is particularly sensitive to changes in the deep white matter, and falls are a risk in patients with white matter pathology. Hypertension is the main cause of the changes in the white matter in normal individuals, and growth of the WMHs is associated with increased blood pressure (White et al. 2011). White matter injury in elderly patients with apparent cognitive impairment show a diverse group of findings, ranging from large symmetric lesions associated with clinical findings to white matter lesions in their absence (Fig. 4.3).

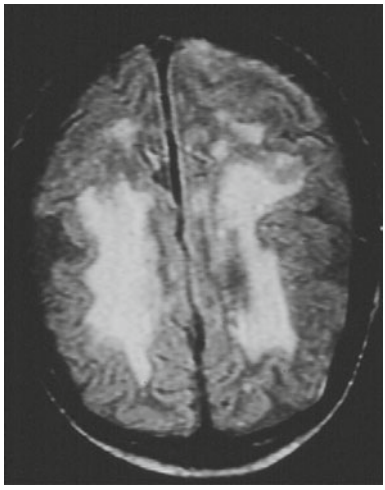


Fig. 4.2 White matter changes of aging shown in FLAIR MRI of the brain in a 92-year-old woman. Neurological and neuropsychological examinations were normal. There was extensive hyperintensive signal in the white matter. Proton NMR spectroscopy showed a normal signal in the white matter

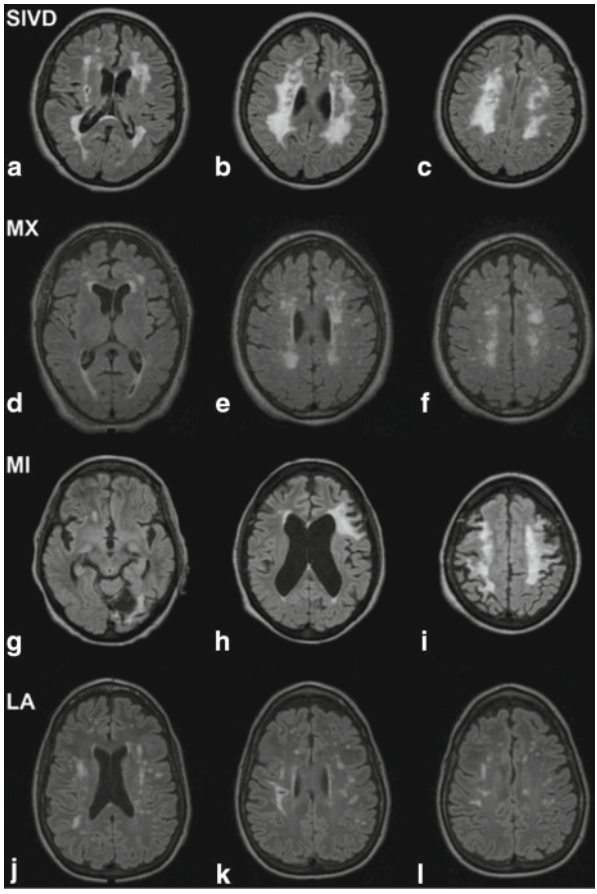


Fig. 4.3 FLAIR MRI scans from representative patients in the different subgroups. (**a**)–(**c**) Patients in the subcortical ischemic vascular disease (SIVD) group show extensive white matter hyperintensities (WMHs) in a relatively symmetric distribution. (**d**)–(**f**) Mixed VCI and AD (MX) patients have WMHs that are also symmetric. (**g**)–(**i**) Multiple infarct (MI) patients have asymmetric lesions consistent with strokes. (**j**)–(**l**) Leukoaraiosis (LA) patients have different patterns of WMHs that are difficult to characterize (Candelario-Jalil et al. 2011.)

4.4 Proton NMR Spectroscopy in Chronic Disease of the White Matter

Nuclear magnetic resonance was originally used to detect signals from atoms within molecules that resonated at specific frequencies (Mansfield and Maudsley 1976). Protons attached to a carbon backbone produce characteristic signals depending on the position in the structure and the other atoms in the vicinity. When the large signal from water is suppressed by specially designed pulse sequences, it is possible to obtain signals from protons of other molecules that are present in much smaller amounts. The main signals obtained with water-suppressed proton NMR spectroscopy (^{1}H-MRS) are *N*-acetylaspartate (NAA), creatine (Cr) and lactate. *N*-acetylaspartate is present at high concentrations in the brain particularly in axons, but in spite of its abundance its role in brain remains uncertain (Moffett et al. 2007). Because ischemic lesions have low NAA and Cr it is possible to use them as biomarkers for injury in the white matter.

Early ^{1}H-MRS studies collected signal from a single voxel, but more recently it is possible to use chemical shift imaging, which allows reliable measurements to be obtained simultaneously from multiple voxels in large regions of the brain (Gasparovic et al. 2011). The sites most amenable for studies of injury in the white matter are above the ventricles in the centrum semiovale. Measurements of NAA in normal individuals with extensive white matter damage show normal values that are similar to age-appropriate individuals with no white matter injury. However, in the patients with SVD the NAA is reduced, making it possible to identify those individuals with ischemic white matter disease and separate them from those with normal white matter (Fig. 4.4) (Sappey Marinier et al. 1992; Brooks et al. 1997).

Some investigators have proposed using the volume of WMHs as a surrogate biomarker for cognitive impairment, which would make the size of WMHs an indicator of progressive pathological change (Schmidt et al. 2004). The size of the white matter lesions increases over time in elderly patients. In some it is an indication of advancing vascular disease, but in others it may be a sign of aging. One possible way to separate the pathological changes in WMHs from those of normal aging is to use changes in the NAA signal since that presumably changes when the tissue is hypoxic.

4.5 Diffusion Tensor Imaging

Diffusion tensor imaging (DTI), a relatively new MR method, reveals disruption of the white matter fiber tracts. Fractional anisotropy (FA) is a value between zero and one that indicates the degree of anisotropy of a diffusion process; isotropic diffusion has a value of zero, which means it is equally restricted in all directions. When diffusion occurs only along one axis and is fully restricted in all other directions, it has a value of one. DTI is useful in tissues that have an internal fibrous structure

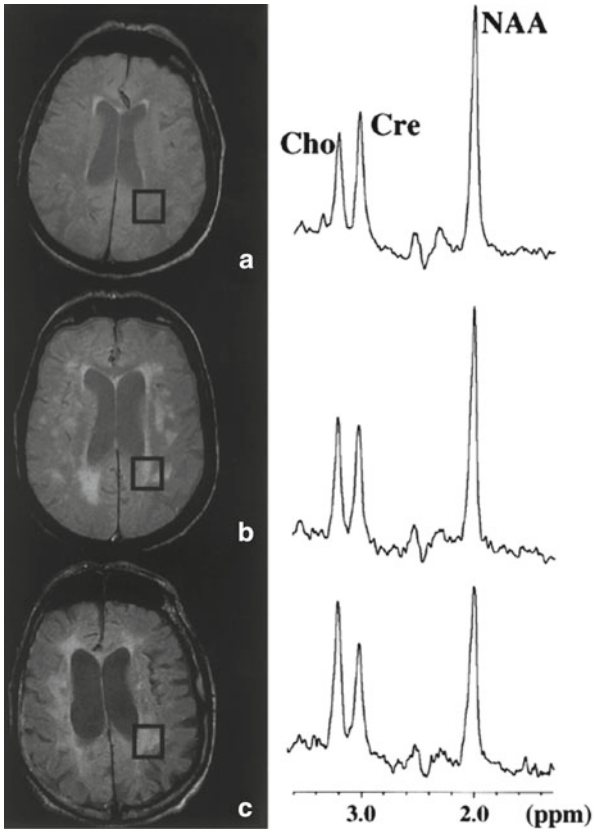


Fig. 4.4 MRI and spectra from representative patients in the three study groups: elderly asymptomatic normal control (**a**), elderly asymptomatic subject with severe white matter hyperintensities (**b**), and a subject with SAE with large confluent periventricular WMH regions. (**c**) (Brooks et al. 1997.)

analogous to crystals; water will then diffuse more rapidly in the direction aligned with the internal structure, and more slowly as it moves perpendicular to the preferred direction. This also means that the measured rate of diffusion will differ depending on the direction from which an observer is looking.

Damaged white matter shows low FA (axon damage) in ischemic tissue. DTI has been combined with hypothesis-free, voxel-based lesion–symptom mapping in patients with CADASIL. Lacunar lesions and white matter lesions were segmented on three-dimensional T1 and fluid-attenuated inversion recovery (FLAIR) sequences, respectively. Significant clusters for cognitive performance were detected for both lacunar lesions and WMHs with greatest effect seen in tests of processing speed, which is the predominantly affected cognitive domain in patients with lesions in the white matter. Combining lesion–symptom mapping data with information from a probabilistic white matter atlas (Montreal Neurological Institute), they found that the major sites of damage were the anterior thalamic radiation and the forceps minor. In multivariate models regional volumes of lacunar lesions and WMHs in the anterior thalamic radiation predicted performance in processing speed tasks, whereas there was no independent contribution of the

global volume of ischemic lesions. The authors conclude that lesion location for both lacunar and ischemic white matter lesions is critical, and that the anterior thalamic radiation is a major anatomical structure impacting on processing speed. Together these findings provide strong support for a central role of frontal–subcortical circuits in cerebral small vessel disease and vascular cognitive impairment (Duering et al. 2011).

Early studies with CT showed an association of frontal leukoaraiosis and gait instability, which has been confirmed with MRI (Masdeu et al. 1989). One study based on MRI voxels correlated gait difficulty with frontal white matter tracts. The tracts were obtained from an atlas of DTI-based white matter tracts. Lesions mainly involving the anterior and superior corona radiate which corresponded to projection fibers of the anterior thalamic radiations and long corticofugal motor tracts. There is growing evidence that white matter lesions affect gait through damage to the thalamic radiations, which are projection tracts connecting the frontal motor cortical areas with the basal ganglia, pontine, and bulbar subcortical nuclei (Srikanth et al. 2010). DTI from patients with VCI can be compared to normal controls to show the regions of decreased FA.

4.6 BBB Permeability in Chronic White Matter Damage

Disruption of the BBB occurs in patients with vascular dementia. Elevated CSF albumin was the first biomarker to show opening of the BBB, and the CSF albumin index, which is the ratio of the albumin in the CSF to that in the blood, is elevated in VCI (Wallin et al. 1990). The breakdown of the BBB is more often seen in the patients with vascular disease leading to dementia than in the patients with AD, where the role of the BBB is controversial. MRI can be used to visualize and quantitate leakage of Gd-DTPA across a disrupted BBB. Patients with VCI have increased BBB permeability (Wardlaw et al. 2009). Diabetics have abnormal BBB permeability, which is not surprising since the hallmark of diabetes is damage to the blood vessels (Starr et al. 2003). These studies were done with a semiquantitative method, which is useful for comparisons, but does not provide absolute numbers for transfer constant calculations.

In animals, many methods are available to quantify disruption of the BBB (Bradbury 1993). Patlak and colleagues measured permeability in rats using an autoradiographic method (Patlak et al. 1983). They used a one-compartment transfer model to calculate the rate of transfer of a radiolabeled tracer from the blood to the brain. Blood samples were removed for isotope measurement at frequent intervals after tracer injection. The radiolabeled tracer, ^{14}C-aminoisobutyric acid, is taken across the BBB and retained in the cells, allowing it to be visualized and quantitated by autoradiography. Using the time curve of the isotope in the blood and the final value of the isotope in the brain, transfer constants are formed graphically. The Patlak plot method has been adapted to MRI and used in rats (Ewing et al. 2003). Gd-DTPA is injected intravenously and measurements are made in the brain

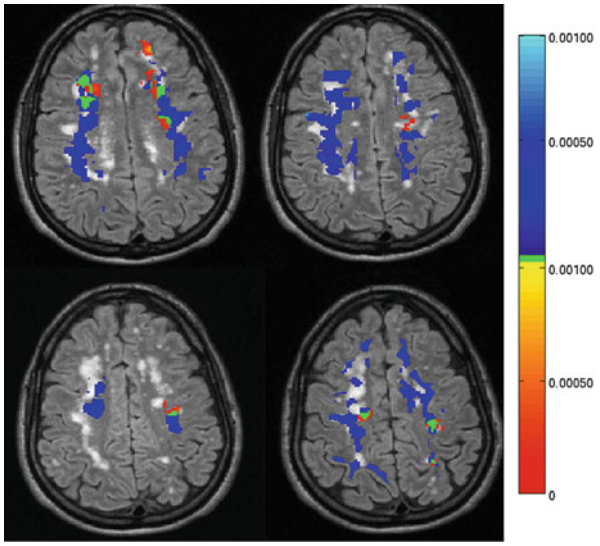


Fig. 4.5 Permeability maps constructed from Patlak plots in a patient with VCI. The images are color-coded to indicate different times for the scans, which were approximately 1 year apart. The *red* color indicates the map from the first visit, and the *blue* is for the second visit. The *green* is areas that overlap. There is very little overlap and the permeability map for the second visit is much more extensive than that seen for the first visit. (Courtesy of Dr. Arvind Caprihan, MRN, Albuquerque, NM.)

and blood over 30 min to extract the blood-to-brain transfer constants (Sood et al. 2008). Dynamic contrast-enhanced MRI can be used to quantify BBB transport in patients (Taheri et al. 2011b). Measurements can be more readily made in the white matter than in the gray matter because of contribution of the Gd-DTPA in the sulci is difficult to separate from the tissues. Patients with VCI had increased leakage of Gd-DTPA compared to a group of healthy controls (Taheri et al. 2011a). Quantification of the permeability was done over the entire white matter by calculating a mean intensity, which represents the sum of all the permeability constants, k_i, divided by the number of pixels. Individual voxel permeability constants are high in regions of high permeability and low in ones with less permeability, providing a topographic measure. Another possible representation is to calculate the volume of permeability, using the number of voxels above a given threshold, which is determined from a group of controls. When one number is desired that could be used to determine the effectiveness of a treatment in reducing BBB damage, either the mean intensity summed over the regions measured by the MRI or the volume of the voxels with increased permeability could be used. Ideally, both measures will be needed since they convey different information. An example of a patient measured twice over a one year period showed the growth of the permeability area from scan one to scan two (Fig. 4.5). Relatively little overlap was seen between the two scans, indicating that the old lesions had resolved, but new ones had formed. Further studies will be needed to optimize these measures and select one or both for use in clinical trials of agents that reduce the permeability.

None of the currently available MR methods by themselves provides sufficient diagnostic information to be useful in patient care decisions. Each of the methods provides information on a specific aspect of brain structure/function. However, it is possible to combine multiple measures and to identify the regions with damaged white matter fiber tracts related to leakage of the BBB (Fig. 4.6).

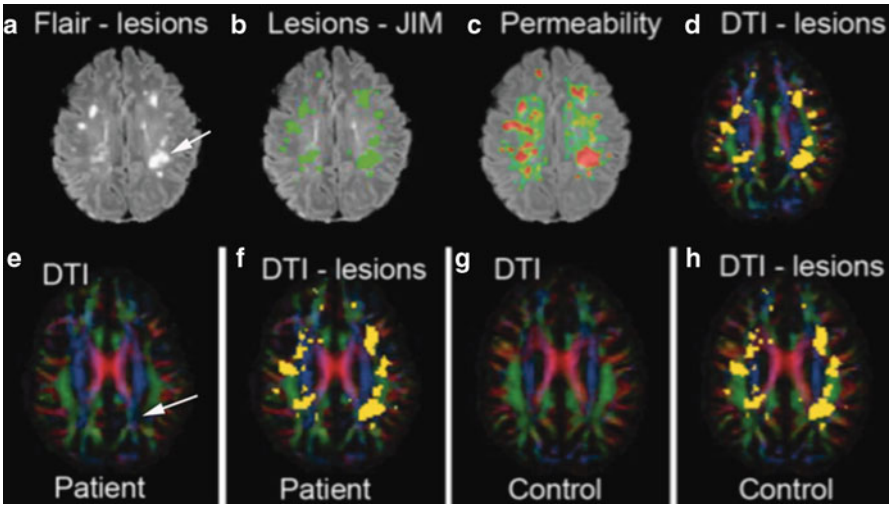


Fig. 4.6 Example of multimodal juxtaposition of FLAIR, DCEMRI, and DTI in a patient with VCI and a control. The *top row* shows the data aligned to the T2 image so that the data from different modalities can be compared on a voxel-by-voxel basis. (**b**) The white-matter intensity regions are segmented by JIM. (**c**) Permeability can be compared to FA (**d**) at the same locations. The *bottom row* shows data normalized to the Montreal Neurological Institute space. The FA values in lesions (**f**) can be compared to the mean FA values of one control subject (**h**). (Courtesy of Dr. Arvind Caprihan, MRN, Albuquerque, NM.)

4.7 Delayed Post-anoxic Leukoencephalopathy

Several acute forms of ischemic injury preferentially involve the white matter. A rare cause of white matter damage occurs with prolonged hypoxia or hypotension. An early report of this condition was made in drug addicts that had taken an overdose of heroin (Protass 1971). Severe hypoxia produces coma. After a week or two some patients regain consciousness, and remain awake for several days before lapsing again into coma. Occasionally they recover, but usually, if they do recover, there are major deficits. The MRI demonstrates the large lesions of the white matter, which are accompanied by cognitive deficits and neurological signs. In addition to the opiates, agents used to treat opioid addiction may also cause the syndrome. For example, methadone, when taken by naïve individuals, causes a respiratory arrest and hypotension. Typically, the patient has a period of anoxia prior to the cardiac arrest. The DWI shows injury to the white matter with sparing the gray matter.

A recent report described two patients who had delayed postanoxic leukoencephalopathy after taking one dose of "diverted" methadone given to them by friends to treat abdominal pain (Huisa et al. 2013). They had a prolonged respiratory arrest, and after resuscitation were found to have extensive damage to the deep white matter (Fig. 4.7). Magnetic resonance spectroscopy showed reduced signal from NAA, and there was increased vascular permeability (Fig. 4.8). One of the

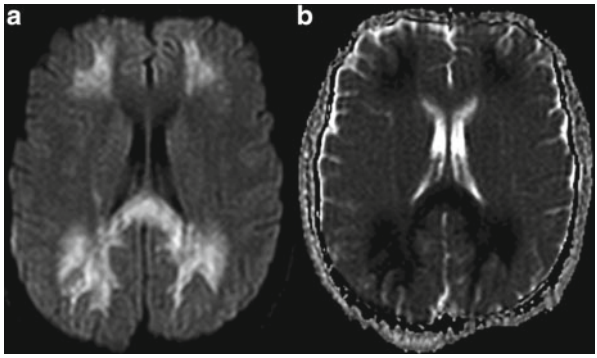


Fig. 4.7 Initial brain MRI of case 1 depicts: (**a**) An abnormal hyperintense Diffusion Weighted Imaging (DWI). (**b**) Low abnormal signal in Apparent Diffusion Coefficient (ADC) map. Initial T2 sequences were normal (not shown). Note mostly involvement of the white matter with sparing of cortex. (Huisa et al. 2013.)

patients was followed for several years, and had a good recovery with return to work. The other patient was lost to follow-up. The patient that recovered had a reduction in the size of the white matter lesions on FLAIR, an improvement in the NAA levels, and a reduction of the area of increased BBB permeability.

4.8 Animal Models of Chronic White Matter Damage

Animal models provide insights into the pathophysiology of chronic ischemia. MRI provides unique information about the timing and extent of injury in the animal models. Bilateral carotid artery occlusion (BCAO) in normotensive Wistar–Kyoto (WKY) rats produces white matter damage through hypoxic hypoperfusion (Ihara et al. 2001; Ueno et al. 2002; Wakita et al. 2002). Occluding both carotid arteries in the WKY causes damage to the white matter, oligodendrocyte death, BBB disruption, and behavioral changes (Fig. 4.9). Hypoxia induces expression of the matrix metalloproteinases (MMPs), which are the major proteases involved in the opening of the BBB (Nakaji et al. 2006, Rosenberg 2012). Tomimoto and colleagues used small coils to constrict the common carotid arteries of mice. Bilateral carotid artery stenosis was used to demonstrate the protection afforded to the mouse with the gene for *mmp-2* knocked out; they were found to have less white matter damage, supporting a crucial role for the MMPs in the damage to the white matter (Nakaji et al. 2006).

Damage to the white matter could be seen on MRI in the spontaneously hypertensive stroke prone rats (SHR/SP) given the Japanese Permissive Diet (JPD) that has low protein and high salt (Sironi et al. 2004b). They had increased permeability of the BBB as shown on MRI (Sironi et al. 2004a). In another study the same group showed that treatment with rosuvastatin, but not with simvastatin, exerts a beneficial effect by modulating the inflammatory condition that precedes the development of cerebral damage in these animals (Sironi et al. 2005).

When SHR/SP reach 12 weeks of age, there is markedly elevated blood pressure. If they are given the JPD and have one carotid artery occluded, they develop

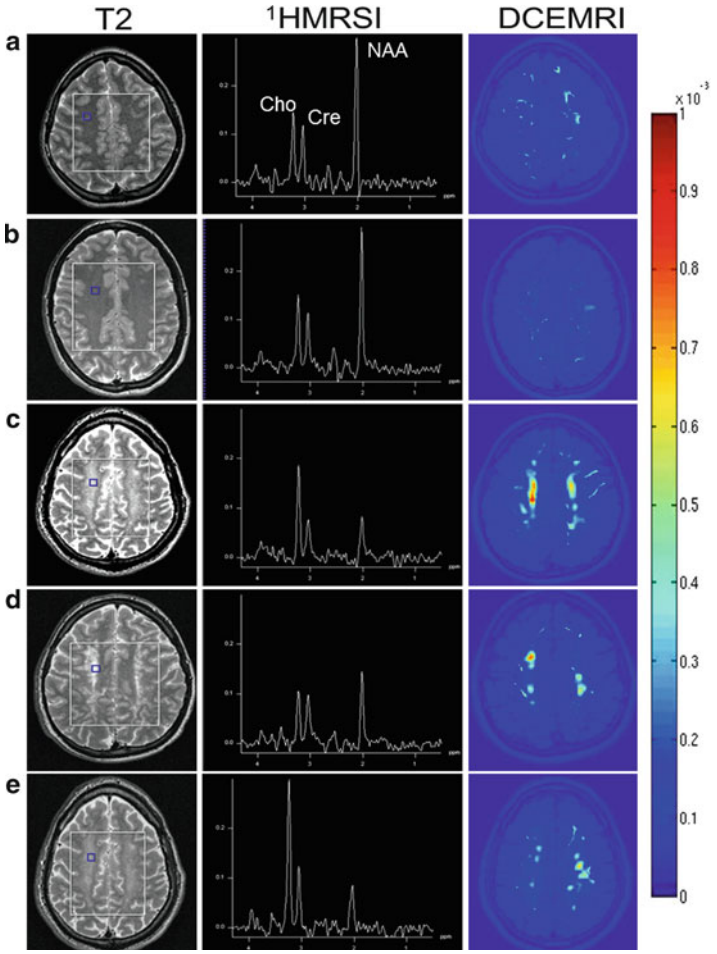


Fig. 4.8 T2-weighted images (*Left column*), representative spectra analysis (*second column*), and parametric images—color coded—representing BBB permeability map in the WM (*right column*). Age-matched control subjects, on rows A and B, case 1 at row C (2nd MRI) and row D (3rd MRI), and case 2 at row E. *Small square* in each T2 image indicates WM spectroscopic image voxel from which the spectrum shown was obtained. *Large square* represents the spectroscopic regions of interest. Note lower NAA and higher choline levels in patients relative to controls and partial normalization in case 1 at second visit. (Huisa et al. 2013)

damage to the white matter that can be seen on an MRI scan (Fig. 4.10). Death occurs around the 4th week (16th week of life); the brains show extensive injury to the white matter with opening of the BBB, breakdown of myelin, death of oligodendrocytes, and abnormal performance on the Morris water maze test (Fig. 4.11) (Jalal et al. 2012).

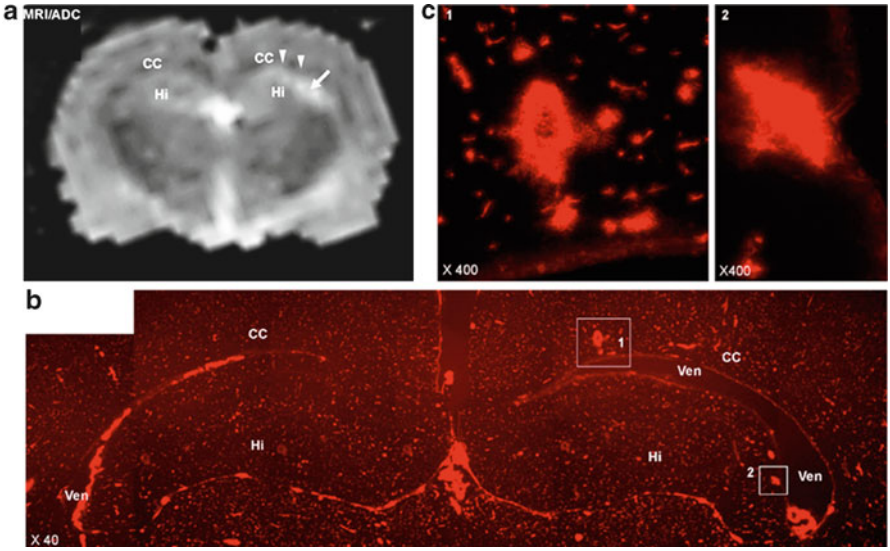


Fig. 4.9 (**a**) MRI was used to quantify ADC (map shown) and BBB permeability in rat WM and hippocampus at 3 days after BCAO. ADC showed damage in CC (*arrowheads*) and in hippocampus (Hi; *arrow*). (B) EB was injected intravenously 2 h before rat was killed to show BBB leakage. With the guidance of the ADC image, regions of EB leakage (*red*) were observed in the CC (see *inset 1*) and in the hippocampus (see *inset 2*). (**c**) Higher magnification images of *inset 1* and 2. (Sood et al. 2009.)

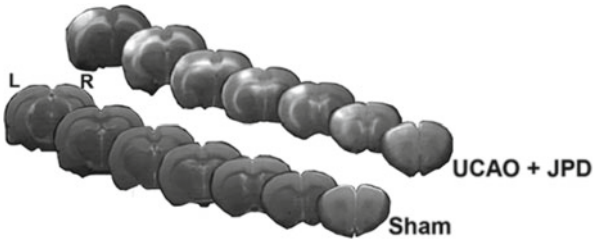


Fig. 4.10 T2-weighted images obtained from UCAO/JPD and sham-operated groups demonstrate hyperintense areas on both occluded (*right*; R) and nonoccluded (*left*; L) sides. An infarct is seen in nonoccluded cortex

4.9 Mechanisms of Ischemia-Induced Oligodendrocyte Death

Oligodendrocytes undergo a complex pattern of death in cerebral ischemia with an early loss from glutamate excitotoxicity and a slower death due to apoptosis (Masumura et al. 2001; Dewar et al. 2003). The vulnerability of oligodendrocytes to ischemia was shown morphologically (Pantoni et al. 1996), and the molecular mechanism was related to excitotoxicity via the glutamate AMPA receptors (Tekkok and Goldberg 2001). Tumor necrosis factor-α (TNF-α) and TNF death receptors play an important role in oligodendrocytes death as shown in vitro and in vivo

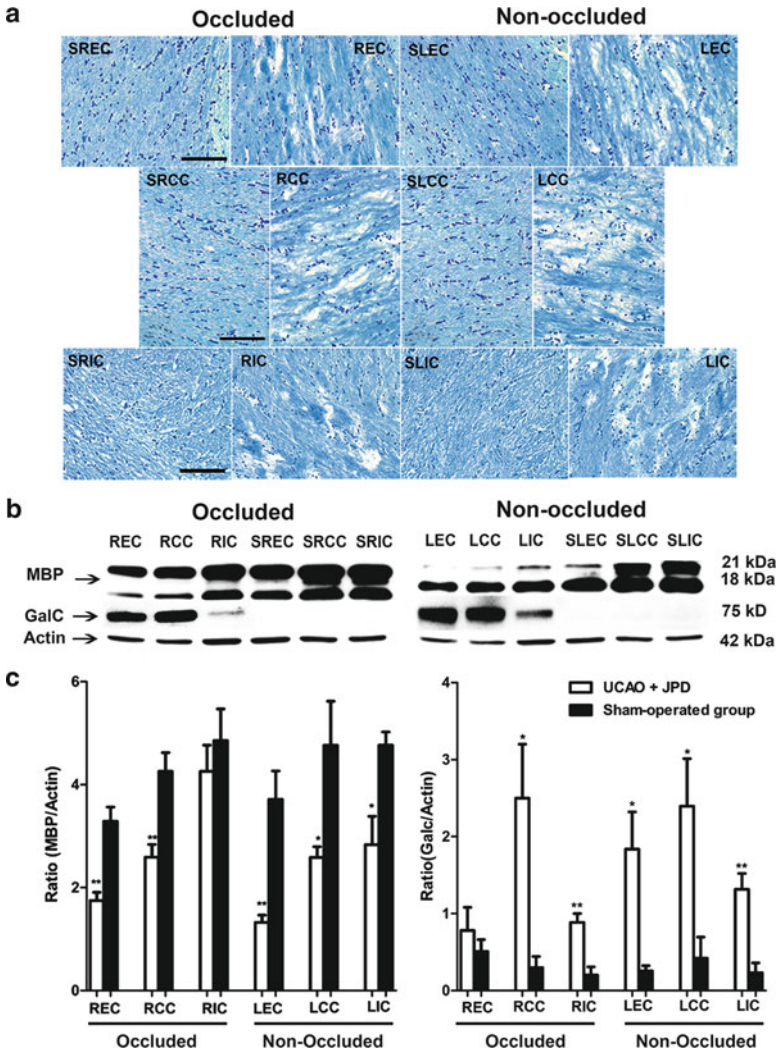


Fig. 4.11 Myelin loss and upregulation of immature Ols seen at 4–5 weeks following UCAO and JPD. (**a**) Klüver–Barrera staining showed no damage in sham-operated group external capsule (SEC), corpus callosum (SCC), and internal capsule (SIC) in both hemispheres. UCAO/JPD rats had myelin and cell loss, as well as vacuolation, on both occluded and nonoccluded sides of external capsule (EC), corpus callosum (CC), and internal capsule (IC). (**b**) Representative blots of MBP and GalC for occluded and nonoccluded sides of different areas of white matter. Actin was used as a loading control. (**c**) Western blot densitometric analysis of MBP and GalC was normalized to actin. Scale bar = 100 μm. $*P < 0.05$, $**P < 0.01$, significantly different compared with corresponding sham-operated control (S) ($n = 5$/group). (Jalal et al. 2012).

(Selmaj and Raine 1988). Cell surface sheddases, including TNF-α converting enzyme (TACE) and stromelysin-1 (MMP-3), regulate the TNF superfamily of death receptors by activating the ligands and removing the death receptors from the cell surface. Tissue inhibitor of metalloproteinases-3 (TIMP-3) plays a role in this process by inhibiting TACE and MMP-3. *Timp-3* knockout (KO) mice had less oligodendrocyte death than wild type (WT) after a middle cerebral artery occlusion with 24 and 72 h of reperfusion (Yang et al. 2011). The presence of *Timp-3* in the wild type led to an exaggerated inflammatory response with an increase in both infiltration of microglia/macrophages at 72 h and expression of MMP-3 and -9 in reactive astrocytes around blood vessels in the white matter.

The importance of animal models is that the development of white matter damage can be observed as it evolves over time. The multiple types of pathological changes in the white matter secondary to vascular disease cannot be mimicked in one model. Small vessel disease induces white matter that can be progressive with contributions from inflammatory processes. Understanding the progressive subcortical form of vascular cognitive impairment is an important goal of current research because that is the form that will be most amenable to clinical trials. A possible mechanism leading to oligodendrocyte death secondary to hypertension, diabetes, CADASIL, etc. is that hypoxic hypoperfusion leads to incomplete infarction. Hypoxia inducible factors induce a large number of genes in response to the impairment in tissue oxygenation. A molecular cascade is activated that releases paroteases that attack the blood vessels causing them to leak, and in conjunction with free radicals, cause bystander demyelination. Central to this process is the release of MMPs by intrinsic and recruited inflammatory cells (Fig. 4.12) (Rosenberg 2009).

4.10 Conclusions

Magnetic resonance methods have provided invaluable tools to study the white matter that have impacted the diagnosis and treatment of acute and chronic vascular diseases. Measurements of volume of WMH from the FLAIR or T2-weighted MRI correlate in large population studies with gait and cognition. However, individuals can have a high incidence WMH, particularly in those over the age of 75, which limits the diagnostic information in the individual patient. Adding other modalities improves the diagnostic accuracy. Proton MRS is an excellent method to determine the extent of ischemia in the regions with hyperintense signal. Diffusion tensor imaging shows regions of disrupted white matter fiber tracts, and has shown that the anterior white matter tracts radiating from the thalamus through the anterior limb of the internal capsule to the frontal cortex are abnormal in elderly individuals with gait disturbance. Finally, the demonstration of increased permeability in the white matter is an indication of an ongoing inflammatory process either due to damaged blood vessels (hypertension/diabetes/arteriolosclerosis) or due to a macrophage/

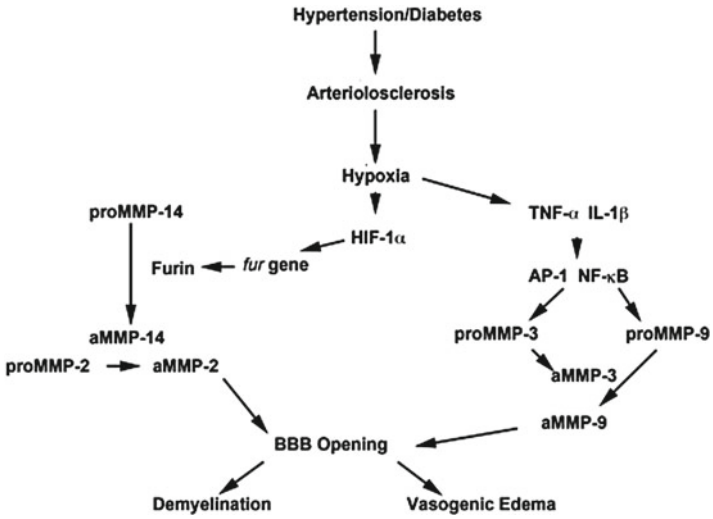


Fig. 4.12 A schematic of a possible mechanism for vascular demyelination in the Binswanger's type of VCI. Hypertension, diabetes mellitus, and other diseases that damage blood vessels can initiate the process by leading to thrombosis with hypoxia/ischemia or by inducing inflammation. Hypoxia secondary to hypoperfusion may be a cause. There is an increase in hypoxia inducing factor-1α (HIF-1α), which turns on cassettes of genes involved in both injury, such as the fur gene and VEGF and TGF-β, which are important in repair. Furin leads to the activation of MMP-2 through MT-MMP. MMP-2 can disrupt the tight junction proteins and open the BBB, creating edema, but it also can directly attack myelin. In addition, MMP-2 activates endothelin-1, which acts through calcium metabolism in the smooth muscle to cause vasoconstriction. The latter effect aggravates the hypoxia. On the repair side, the VEGF and ang2 act through the secretion of MMPs to cause angiogenesis and neurogenesis

microglia mediated proteolytic reaction. Currently, use of such a multimodal MR approach is only possible in a few research centers. However, once the optimal methods are determined, individualizing the diagnosis and formulating mechanism-specific treatments should be possible.

References

Auer DP, Putz B, Gossl C et al (2001) Differential lesion patterns in CADASIL and sporadic subcortical arteriosclerotic encephalopathy: MR imaging study with statistical parametric group comparison. Radiology 218:443–451

Awad IA, Johnson PC, Spetzler RF et al (1986) Incidental subcortical lesions identified on magnetic resonance imaging in the elderly. II. Postmortem pathological correlations. Stroke 17: 1090–1097

Bennett DA, Wilson RS, Gilley DW et al (1990) Clinical diagnosis of Binswanger's disease. J Neurol Neurosurg Psychiatry 53:961–965, see comments

Boone KB, Miller BL, Lesser IM et al (1992) Neuropsychological correlates of white-matter lesions in healthy elderly subjects. A threshold effect. Arch Neurol 49:549–554
Bousser MG, Tournier-Lasserve E (1994) Summary of the proceedings of the First International Workshop on CADASIL. Paris, May 19–21, 1993. Stroke 25:704–707
Bradbury MW (1993) The blood–brain barrier. Exp Physiol 78:453–472, Review
Brooks WM, Wesley MH, Kodituwakku PW et al (1997) 1H-MRS differentiates white matter hyperintensities in subcortical arteriosclerotic encephalopathy from those in normal elderly. Stroke 28:1940–1943
Candelario-Jalil E, Thompson J, Taheri S et al (2011) Matrix metalloproteinases are associated with increased blood–brain barrier opening in vascular cognitive impairment. Stroke 42: 1345–1350
Chalela JA, Kidwell CS, Nentwich LM et al (2007) Magnetic resonance imaging and computed tomography in emergency assessment of patients with suspected acute stroke: a prospective comparison. Lancet 369:293–298
Cvoro V, Wardlaw JM, Marshall I et al (2009) Associations between diffusion and perfusion parameters, N-acetyl aspartate, and lactate in acute ischemic stroke. Stroke 40:767–772
De Reuck J, Crevits L, De Coster W et al (1980) Pathogenesis of Binswanger chronic progressive subcortical encephalopathy. Neurology 30:920–928
Dewar D, Underhill SM, Goldberg MP (2003) Oligodendrocytes and ischemic brain injury. J Cereb Blood Flow Metab 23:263–274
Duering M, Zieren N, Herve D et al (2011) Strategic role of frontal white matter tracts in vascular cognitive impairment: a voxel-based lesion-symptom mapping study in CADASIL. Brain 134: 2366–2375
Duvernoy H, Delon S, Vannson JL (1983) The vascularization of the human cerebellar cortex. Brain Res Bull 11:419–480
Ewing JR, Knight RA, Nagaraja TN et al (2003) Patlak plots of Gd-DTPA MRI data yield blood–brain transfer constants concordant with those of 14C-sucrose in areas of blood–brain opening. Magn Reson Med 50:283–292
Filippi M, Rocca MA (2008) Multiple sclerosis and allied white matter diseases. Neurol Sci 29(Suppl 3):319–322
Filippi M, Rocca MA, Barkhof F et al (2012) Association between pathological and MRI findings in multiple sclerosis. Lancet Neurol 11:349–360
Gasparovic C, Bedrick EJ, Mayer AR et al (2011) Test-retest reliability and reproducibility of short-echo-time spectroscopic imaging of human brain at 3T. Magn Reson Med 66:324–332
Gorelick PB, Scuteri A, Black SE et al (2011) Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the American Heart Association/ American Stroke Association. Stroke 42:2672–2713
Hachinski V, Iadecola C, Petersen RC et al (2006) National Institute of Neurological Disorders and Stroke-Canadian Stroke Network vascular cognitive impairment harmonization standards. Stroke 37:2220–2241
Hachinski VC, Potter P, Merskey H (1987) Leuko-araiosis. Arch Neurol 44:21–23
Henderson AP, Barnett MH, Parratt JD et al (2009) Multiple sclerosis: distribution of inflammatory cells in newly forming lesions. Ann Neurol 66:739–753
Hom J, Dankbaar JW, Soares BP et al (2011) Blood–brain barrier permeability assessed by perfusion CT predicts symptomatic hemorrhagic transformation and malignant edema in acute ischemic stroke. AJNR Am J Neuroradiol 32:41–48
Hounsfield GN (1980) Computed medical imaging. Science 210:22–28
Huisa BN, Gasparovic C, Taheri S et al (2013) Imaging of subacute blood–brain barrier disruption after methadone overdose. J Neuroimaging 23(3):441–444
Hunt AL, Orrison WW, Yeo RA et al (1989) Clinical significance of MRI white matter lesions in the elderly. Neurology 39:1470–1474
Ihara M, Tomimoto H, Kinoshita M et al (2001) Chronic cerebral hypoperfusion induces MMP-2 but not MMP-9 expression in the microglia and vascular endothelium of white matter. J Cereb Blood Flow Metab 21:828–834

Jalal FY, Yang Y, Thompson J et al (2012) Myelin loss associated with neuroinflammation in hypertensive rats. Stroke 43:1115–1122
Kinkel WR, Jacobs L, Polachini I et al (1985) Subcortical arteriosclerotic encephalopathy (Binswanger's disease). Computed tomographic, nuclear magnetic resonance, and clinical correlations. Arch Neurol 42:951–959
Knudsen KA, Rosand J, Karluk D et al (2001) Clinical diagnosis of cerebral amyloid angiopathy: validation of the Boston criteria. Neurology 56:537–539
Lauterbur PC (1982) NMR zeugmatographic imaging in medicine. J Med Syst 6:591–597
Lo EH, Dalkara T, Moskowitz MA (2003) Mechanisms, challenges and opportunities in stroke. Nat Rev Neurosci 4:399–415
Lucchinetti CF, Gavrilova RH, Metz I et al (2008) Clinical and radiographic spectrum of pathologically confirmed tumefactive multiple sclerosis. Brain 131:1759–1775
Mansfield P, Maudsley AA (1976) Line scan proton spin imaging in biological structures by NMR. Phys Med Biol 21:847–852
Masdeu JC, Wolfson L, Lantos G et al (1989) Brain white-matter changes in the elderly prone to falling. Arch Neurol 46:1292–1296, see comments
Masumura M, Hata R, Nagai Y et al (2001) Oligodendroglial cell death with DNA fragmentation in the white matter under chronic cerebral hypoperfusion: comparison between normotensive and spontaneously hypertensive rats. Neurosci Res 39:401–412
Moffett JR, Ross B, Arun P et al (2007) N-Acetylaspartate in the CNS: from neurodiagnostics to neurobiology. Prog Neurobiol 81:89–131
Moody DM, Thore CR, Anstrom JA et al (2004) Quantification of afferent vessels shows reduced brain vascular density in subjects with leukoaraiosis. Radiology 233:883–890
Moorhouse P, Rockwood K (2008) Vascular cognitive impairment: current concepts and clinical developments. Lancet Neurol 7:246–255
Moseley ME, Cohen Y, Kucharczyk J et al (1990) Diffusion-weighted MR imaging of anisotropic water diffusion in cat central nervous system. Radiology 176:439–445
Nakaji K, Ihara M, Takahashi C et al (2006) Matrix metalloproteinase-2 plays a critical role in the pathogenesis of white matter lesions after chronic cerebral hypoperfusion in rodents. Stroke 37:2816–2823
Narayan SK, Gorman G, Kalaria RN et al (2012) The minimum prevalence of CADASIL in northeast England. Neurology 78:1025–1027
Noseworthy JH, Lucchinetti C, Rodriguez M et al (2000) Multiple sclerosis. N Engl J Med 343:938–952
Olszewski J (1962) Subcortical arteriosclerotic encephalopathy: review of the literature on the so-called Binswanger's disease and presentation of 2 cases. World Neurol 3:359–375
Ordidge RJ, Mansfield P, Coupland RE (1981) Rapid biomedical imaging by NMR. Br J Radiol 54:850–855
Pantoni L (2010) Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. Lancet Neurol 9:689–701
Pantoni L, Garcia JH, Gutierrez JA (1996) Cerebral white matter is highly vulnerable to ischemia. Stroke 27:1641–1646
Patlak CS, Blasberg RG, Fenstermacher JD (1983) Graphical evaluation of blood-to-brain transfer constants from multiple-time uptake data. J Cereb Blood Flow Metab 3:1–7
Peters N, Holtmannspotter M, Opherk C et al (2006) Brain volume changes in CADASIL: a serial MRI study in pure subcortical ischemic vascular disease. Neurology 66:1517–1522
Protass LM (1971) Delayed postanoxic encephalopathy after heroin use. Ann Intern Med 74:738–739
Rosenberg GA (2009) Matrix metalloproteinases and their multiple roles in neurodegenerative diseases. Lancet Neurol 8:205–216
Rosenberg GA (2012) Neurological diseases in relation to the blood–brain barrier. J Cereb Blood Flow Metab 32:1139–1151
Rosenberg GA, Sullivan N, Esiri MM (2001) White matter damage is associated with matrix metalloproteinases in vascular dementia. Stroke 32:1162–1168

Sappey Marinier D, Calabrese G, Hetherington HP et al (1992) Proton magnetic resonance spectroscopy of human brain: applications to normal white matter, chronic infarction, and MRI white matter signal hyperintensities. Magn Reson Med 26:313–327

Schmidt R, Scheltens P, Erkinjuntti T et al (2004) White matter lesion progression: a surrogate endpoint for trials in cerebral small-vessel disease. Neurology 63:139–144

Selmaj KW, Raine CS (1988) Tumor necrosis factor mediates myelin and oligodendrocyte damage in vitro. Ann Neurol 23:339–346

Sironi L, Gelosa P, Guerrini U et al (2004a) Anti-inflammatory effects of AT1 receptor blockade provide end-organ protection in stroke-prone rats independently from blood pressure fall. J Pharmacol Exp Ther 311:989–995

Sironi L, Gianazza E, Gelosa P et al (2005) Rosuvastatin, but not simvastatin, provides end-organ protection in stroke-prone rats by antiinflammatory effects. Arterioscler Thromb Vasc Biol 25:598–603

Sironi L, Guerrini U, Tremoli E et al (2004b) Analysis of pathological events at the onset of brain damage in stroke-prone rats: a proteomics and magnetic resonance imaging approach. J Neurosci Res 78:115–122

Smith EE, Gurol ME, Eng JA et al (2004) White matter lesions, cognition, and recurrent hemorrhage in lobar intracerebral hemorrhage. Neurology 63:1606–1612

Sood R, Yang Y, Taheri S et al (2009) Increased apparent diffusion coefficients on MRI linked with matrix metalloproteinases and edema in white matter after bilateral carotid artery occlusion in rats. J Cereb Blood Flow Metab 29(2):308–316

Sood RR, Taheri S, Candelario-Jalil E et al (2008) Early beneficial effect of matrix metalloproteinase inhibition on blood–brain barrier permeability as measured by magnetic resonance imaging countered by impaired long-term recovery after stroke in rat brain. J Cereb Blood Flow Metab 28:431–438

Srikanth V, Phan TG, Chen J et al (2010) The location of white matter lesions and gait–a voxel-based study. Ann Neurol 67:265–269

Starr JM, Wardlaw J, Ferguson K et al (2003) Increased blood–brain barrier permeability in type II diabetes demonstrated by gadolinium magnetic resonance imaging. J Neurol Neurosurg Psychiatry 74:70–76

Taheri S, Gasparovic C, Huisa BN et al (2011a) Blood-brain barrier permeability abnormalities in vascular cognitive impairment. Stroke 42:2158–2163

Taheri S, Gasparovic C, Shah NJ et al (2011b) Quantitative measurement of blood–brain barrier permeability in human using dynamic contrast-enhanced MRI with fast T(1) mapping. Magn Reson Med 65(4):1036–1042

Tekkok SB, Goldberg MP (2001) Ampa/kainate receptor activation mediates hypoxic oligodendrocyte death and axonal injury in cerebral white matter. J Neurosci 21:4237–4248

Thal DR, Capetillo-Zarate E, Larionov S et al (2009) Capillary cerebral amyloid angiopathy is associated with vessel occlusion and cerebral blood flow disturbances. Neurobiol Aging 30:1936–1948

Tomimoto H, Akiguchi I, Akiyama H et al (1999) Vascular changes in white matter lesions of Alzheimer's disease. Acta Neuropathol 97:629–634

Ueno M, Tomimoto H, Akiguchi I et al (2002) Blood–brain barrier disruption in white matter lesions in a rat model of chronic cerebral hypoperfusion. J Cereb Blood Flow Metab 22:97–104

Vermeer SE, Prins ND, Den Heijer T et al (2003) Silent brain infarcts and the risk of dementia and cognitive decline. N Engl J Med 348:1215–1222

Vinters HV, Gilbert JJ (1983) Cerebral amyloid angiopathy: incidence and complications in the aging brain. II. The distribution of amyloid vascular changes. Stroke 14:924–928

Wahlund LO, Barkhof F, Fazekas F et al (2001) A new rating scale for age-related white matter changes applicable to MRI and CT. Stroke 32:1318–1322

Wakefield DB, Moscufo N, Guttmann CR et al (2010) White matter hyperintensities predict functional decline in voiding, mobility, and cognition in older adults. J Am Geriatr Soc 58:275–281

Wakita H, Tomimoto H, Akiguchi I et al (2002) Axonal damage and demyelination in the white matter after chronic cerebral hypoperfusion in the rat. Brain Res 924:63–70

Wallin A, Blennow K, Fredman P et al (1990) Blood brain barrier function in vascular dementia. Acta Neurol Scand 81:318–322

Wardlaw JM, Doubal F, Armitage P et al (2009) Lacunar stroke is associated with diffuse blood–brain barrier dysfunction. Ann Neurol 65:194–202

White WB, Wolfson L, Wakefield DB et al (2011) Average daily blood pressure, not office blood pressure, is associated with progression of cerebrovascular disease and cognitive decline in older people. Circulation 124:2312–2319

Wintermark M, Albers GW, Alexandrov AV et al (2008) Acute stroke imaging research roadmap. AJNR Am J Neuroradiol 29:e23–e30

Yang Y, Jalal FY, Thompson JF et al (2011) Tissue inhibitor of metalloproteinases-3 mediates the death of immature oligodendrocytes via TNF-alpha/TACE in focal cerebral ischemia in mice. J Neuroinflammation 8:108

Chapter 5
Diffusion MRI Biomarkers of White Matter Damage in Traumatic Brain Injury

Maria Ly, Samuel Ji, and Michael A. Yassa

Abbreviations

AD	Axial diffusivity
ADC	Apparent diffusion coefficient
AOC	Alteration of consciousness
CT	Computed tomography
CTE	Chronic traumatic encephalopathy
DAI	Diffuse axonal injury
DSI	Diffusion spectrum imaging
DTI	Diffusion tensor imaging
FA	Fractional anisotropy
GCS	Glasgow coma scale
GFA	Generalized fractional anisotropy
GOS	Glasgow outcome scale
HARDI	High angular resolution diffusion imaging
HDFT	High definition fiber tracking
LOC	Loss of consciousness
MD	Mean diffusivity
MR	Magnetic resonance
NFT	Neurofibrillary tangles
ODF	Orientation distribution function
PTA	Post-traumatic amnesia
RD	Radial diffusivity

M. Ly • S. Ji • M.A. Yassa, Ph.D. (✉)
Department of Psychological and Brain Sciences, Johns Hopkins University,
3400 N. Charles St., Ames 216A, Baltimore, MD 21218, USA
e-mail: yassa@jhu.edu

S. Baltan et al. (eds.), *White Matter Injury in Stroke and CNS Disease*, Springer Series in Translational Stroke Research 4, DOI 10.1007/978-1-4614-9123-1_5,

ROI Region of interest
TAI Traumatic axonal injury
TBI Traumatic brain injury
TBSS Tract based spatial statistics

5.1 Overview of Traumatic Brain Injury

5.1.1 Incidence and Public Health Burden

Traumatic brain injury (TBI) is a leading cause of mortality, morbidity, and disability worldwide. In the USA alone, over 1.7 million people sustain a TBI, with 52,000 being fatal and 275,000 leading to hospitalization. The overall cost is more than US$56 billion annually (Faul et al. 2010). As these estimates exclude those receiving care in outpatient settings as well as those not receiving care, these numbers underestimate the size of the public health burden. The lifetime cost resulting from an instance of severe TBI is estimated to be between US$600,000 and $1.875 million, which is approximately 5.75 times the cost of healthcare for those without TBI (Rockhill et al. 2010). Other TBI-related sequelae, such as psychiatric illness, further contribute to a heavy public health burden (Rockhill et al. 2010). Rates of TBI are even higher in developing countries (Thurman et al. 2007), and the incidence of TBI in the USA is only rising; for between 2002 and 2006, TBI-related emergency room visits have increased by 14.4 %, while TBI-related hospitalizations have risen 19.5 % (Faul et al. 2010).

5.1.2 Demographics

Populations most susceptible to TBI include young children between the ages of 0 and 5, adolescents and young adults between the ages of 15 and 24, older adults over the age of 60, and athletes who participate in contact sports such as boxing, rugby, wrestling, and American football, and military veterans. Falls are the leading cause of TBI, contributing to over five hundred thousand emergency department visits and over sixty thousand hospitalizations, largely occurring in older adults and young children, while motor vehicle collisions are the leading cause of TBI-related deaths (Faul et al. 2010).

5.1.3 Definition and Categorization of TBIs

Traumatic brain injury, also referred to as intracranial injury, is defined by the Brain Injury Association of America (BIAA) as an alteration in brain function, or other

evidence of brain pathology, caused by an external force. Due to rapid deformations of the brain resulting from traumatic forces, cascades of pathological events are induced, resulting in the disruption of cerebral networks responsible for the regulation of cognitive, autonomic, emotional, and behavioral functions.

TBIs can be categorized by the mechanism and type of force by which it was induced or by its severity, due to differential predilection sites for injury and thus outcomes. Due to the breadth of some categories and the fact that most TBIs are sustained through a combination of mechanisms, the following categories are not exhaustive and usually overlap:

1. Open or penetrating injuries involve the exposure of the skull and dura mater, whether by means of penetration by an object or through extreme mechanical forces.
2. Closed, non-penetrating or blunt injuries do not involve exposure of the brain and can be sustained by biomechanical forces impacting the head.

Physical and mechanical forces responsible for TBI may cause injury with or without direct contact, at the site of the exertion of force (*coup*) and/or at a region contralateral to the exertion of the force (*contrecoup*). In cases where a collision occurs between an object in motion and a stationary head, focal injuries are more likely to occur at the collision site (Morrison et al. 1998), whereas cases where a collision occurs between a stationary object and a head in motion, focal injuries are more likely to occur on the *contrecoup* side (Poirier 2003). Injury without contact is possible when impulsive or inertial forces cause sudden motion of the head (e.g., whiplash) in cases of translational or rotational acceleration, usually resulting in *contrecoup* damage due to differential motion of the brain in the skull (Hardman and Manoukian 2002; Gennarelli 1993; Pang 1985). If the mechanical forces involved are significant, both coup and *contrecoup* injuries can occur (*coup–contrecoup*). Typical focal injuries resulting from *coup*, *contrecoup*, and *coup–contrecoup* blows are compressions of the skull at the site of the blow, localized contusions, as well as hematomas and damaged vessels in the frontal, temporal, and occipital areas (Ryan et al. 1994). Damage from *coup–contrecoup* injuries is usually greater in closed injuries than open injuries, since the lack of skull fracture in closed injuries allows pressure waves to reflect inwards toward brain tissue (Zappala et al. 2012).

Rotational acceleration forces can induce the twisting of the brain against the skull, resulting in shearing and stretching forces (Bigler 1990; Pang 1985) often causing diffuse axonal injuries (DAI) or traumatic axonal injury (TAI), petechial hemorrhages, and deep white matter degeneration, most prevalent in areas in proximity to grey and white matter junctions due to their differing densities (Li and Feng 2009). Additionally, angular and rotational forces due to centrifugal acceleration can cause laceration of the mid-sagittal anterior and ventral cortical areas, along with their deep white matter connections to the striatum and the brainstem (Zappala et al. 2012).

The Glasgow Coma Scale (GCS), most commonly utilized to assess the initial severity of TBI, measures neurological function by means of observing and scoring visual, verbal, and motor responses. A TBI is considered mild with scores between

13 and 15, moderate with scores between 9 and 12, and severe with scores between 3 and 8. Mild TBI is associated with an alteration of consciousness (AOC) of less than or equal to 24 h, a loss of consciousness (LOC) between 0 and 30 min, and post-traumatic amnesia (PTA) of less than or equal to 24 h, moderate TBI with an AOC greater than 24 h, a LOC between 30 min and 24 h, and PTA between 24 h and 7 days, and severe TBI with an AOC greater than 24 h, a LOC greater than or equal to 24 h, and PTA of 7 or more days.

In recent literature, mild TBI has been further differentiated into two categories: mild complicated TBI and mild uncomplicated TBI (Hulkower et al. 2013). Both types yield GCS scores between 13 and 15, but cases of complicated mild TBI have clinical manifestations unexplained by conventional imaging techniques, such as computed tomography (CT) and magnetic resonance (MR) imaging, while cases of uncomplicated mild TBI do not. As it is, the clinical features of mild complicated TBI are more similar to those of moderate TBI than those of mild uncomplicated TBI (Kashluba et al. 2008).

5.1.4 Outcomes and Comorbidities

In mild and moderate forms of TBI, predilection sites for damage include the orbitofrontal regions and temporal regions, including the amygdala and anterior hippocampus, making a combination of cognitive, behavioral, and affective disorders likely. Cognitive dysfunction may include impairment of short and long-term memory, executive function, attentional capacity, and impulse control, while depression and anxiety are common comorbid affective disorders (Warriner and Velikonja 2006). In severe forms of TBI, damage extends to the dorsolateral and medial frontal regions, the anterior cingulate cortex, the striatum, and their associated connections (Kraus et al. 2007; Langen et al. 2012; Cubillo et al. 2012), resulting in lethargy, reduced motivation and drive, impaired executive and linguistic function, impaired working memory, as well as visuospatial deficits. Additionally, in all severities of TBI, progressive brain atrophy occurs, although it is not clear how long the atrophy occurs after the incident of injury (Ross et al. 2012). In cases of moderate and severe TBI sustained in early to midlife, the Institute of Medicine Committee on Gulf War and Health reported a significant, 2–4 times risk of developing dementia (Institute of Medicine Committee on Gulf War and Health 2009). However, recent studies have demonstrated that for all types of TBI diagnoses the hazard ratio for incident dementia is around 2.6 (Barnes et al. 2011).

Severe TBIs occurring early in childhood can disrupt ongoing brain and social development, resulting in lowered intellectual capacity, decreased attention and memory, as well as slowed processing (Anderson et al. 2012). TBI occurring in older adults tend to yield poorer outcomes and higher mortality rates than in younger adults, with falls usually resulting in hematomas with mass effect (Ferrell and Tanev 2002). Moreover, the daily living activities of older adults are impacted even in mild TBI (Marquez et al. 2008; Ratcliff et al. 2005; Hukkelhoven et al. 2003).

Athletes who have sustained multiple TBIs and veterans who have sustained blast-related TBIs are also susceptible to developing chronic traumatic encephalopathy (CTE), a neurodegenerative disease characterized by the global atrophy of the brain, degeneration of the corpus callosum, widening of the ventricles, aggregation of neurofibrillary tangles (NFT) in the medial temporal lobe, hippocampus, parahippocampal gyrus, thalamus, mammillary bodies, amygdala, hypothalamus, and substantia nigra, resulting in uncharacteristic aggression, depression, memory impairment, parkinsonism, and dementia (Shively et al. 2012).

However, factors unique to the individual, such as premorbid personality, education, and gender, have been found to greatly influence the occurrence of the typical clinical manifestations of TBI-related lesions, and thus predictions of outcome merely based on severity and mechanism are unreliable (Henry et al. 2006; Mateer and Sira 2006; Gouick and Gentleman 2004; McDonald and Flanagan 2004; Lucas 1999). Likewise in pediatric TBI, premorbid skills, socioeconomic status, and family dysfunction were significant factors in whether significant cognitive dysfunction manifested (Anderson et al. 2012). Moreover, with the inconsistent and incorrect utilization of the GCS by medical personnel and researchers alike, the predictive validity of TBI by means of GCS is lowered (Zuercher et al. 2009; Balestreri et al. 2004; Rowley and Fielding 1991). Even more limiting is the fact that GCS scores the initial severity of the TBI, thus usually taking only into account primary injuries, such as focal contusions, skull fractures, subdural hematomas, and working memory impairments, while ignoring secondary injuries that may take days to surface, such as hypoxia, increased intracranial pressure, cerebral edema, decreased perfusion and thus focal ischemic injury to brain regions, hematomas with mass effect, or damage to the brain–blood barrier. As secondary injury is the leading cause of inpatient deaths subsequent to TBI (Marshall et al. 1991), the GCS alone cannot be a reliable predictor of outcome.

Alternatively, the Glasgow Outcome Scale (GOS) can be used to assess rehabilitative outcome (Jennett and Bond 1975). Typically utilized 6 months after the incident of injury, the GOS consists of six questions regarding physical, neurocognitive, and social disability. Scores range from 1 to 6, with scores between 1 and 3 being unfavorable (1 = death, 2 = vegetative state, 3 = severe disability with dependence on caregivers) and scores of 4 or 5 being favorable (4 = moderate disability but independent, 5 = good recovery). Although the GOS has high predictive validity in outcome (Jennett et al. 1976), the scale is too crude to yield specific outcomes or indications for specific therapies.

A potential direction for effective, individualized prediction of outcomes is possible in identifying loci of diffuse axonal injury (DAI), as it is the predominant mechanism of injury, morbidity and mortality in most cases of TBI (Meythaler et al. 2001; Gentry 1996; Murray et al. 1996; Gean 1994). We discuss these mechanisms of white matter injury below, and discuss the utility of noninvasive neuroimaging techniques focused on white matter (principally, diffusion tensor imaging) for localization of DAI, as well as for TBI assessment and prediction of outcomes.

5.1.5 *Mechanisms of White Matter Injury*

Diffuse axonal injury (DAI) is defined as widespread lesions of white matter tracts. Predilection sites of DAI include the parasagittal white matter of the cerebral cortex, the corpus callosum, the basal ganglia, the thalamus, the superior cerebral peduncles, and the pontine–mesencephalic junction due to their difference in density compared to the rest of the brain (Singh and Stock 2006; Meythaler et al. 2001). DAI can be categorized by severity using the Adams classification (Adams et al. 1989; Adams et al. 1977). Grade 1 (mild) DAIs are associated with usually microscopic, histological evidence of the degradation of white matter integrity in the cerebral cortex, corpus callosum, brainstem, and occasionally the cerebellum. Grade 2 (moderate) DAIs additionally include focal lesions of the corpus callosum, whereas Grade 3 (severe) DAIs include one or more lesions of the dorsolateral quadrant(s) of the rostral brainstem, especially in the superior cerebellar peduncle.

White matter injury in DAI is generally not caused by axons tearing upon impact, but rather by secondary, pathological cascades in response to physical or mechanical primary injury (Vik et al. 2006; Arundine et al. 2004; Wolf et al. 2001). Shear and strain forces from rotational acceleration can cause axonal stretch, which disrupts the integrity of the cytoskeleton and cytoplasm (Povlishock 1993), thus opening Na^+ channels in the axolemma. Elevated intracellular Na^+ levels result in the opening of voltage-gated Na^+ channels which in turn open Ca^{2+} channels (Pike et al. 2000; Young 1996), and also induce the reverse exchange of Ca^{2+} for Na^+, further elevating the levels of Ca^{2+} (Pike et al. 2000; Young 1996). High intracellular Ca^{2+} levels is the major cause of post-injury cellular damage, due to the activation of cysteine proteolytic enzymes inducing apoptosis, an upregulation of N-methyl-D-aspartate (NMDA) receptors resulting in cell excitotoxicity due to the excessive glutamatergic activity, a shortage of ATP due to mitochondrial death resulting in further elevated Ca^{2+} levels due to Ca-ATPase malfunctions, and the activation of phospholipases which increase production of arachidonic acid resulting in oxidative stress (Young 1996; Radi et al. 1994). These destructive pathways ultimately degenerate and misalign cytoplasmic elements. Axonal transport ceases due to degeneration and subsequent Wallerian degeneration ensues (Maxwell et al. 1997).

Detecting DAI in vivo is not always possible, as diffuse injury does not necessarily present with gross abnormalities on CT and MRI scans. Especially poignant are cases of mild complicated TBI, where patients suffer from post-concussive symptoms that may lead to long term disability, yet have normal appearing CT and MRI scans (Bazarian et al. 2007; Inglese et al. 2005; Hughes et al. 2004; Scheid et al. 2003). However, in recent years, with the advent of specialized MRI techniques, such as diffusion tensor imaging (DTI), a clearer picture of white matter tracts and potential insults to white matter has emerged (Kraus et al. 2007; Kumar et al. 2009). This type of scan, which we discuss in detail in the next section, allowed investigators for the first time to characterize DAI and assess its extent in TBI.

5.2 Diffusion Imaging Biomarkers for Traumatic Brain Injury

5.2.1 Diffusion Tensor Imaging

Diffusion tensor imaging (DTI) is a noninvasive diffusion MRI technique. It can map the brain's white matter fibers for the better understanding of brain functions by summarizing the movement of water in brain tissue (Mori 2007). Water molecules typically exhibit Brownian motion (the random translational motion of molecules); in the brain however, the diffusion of water molecules is constricted and directed by components like cell membranes and macromolecules, also known as anisotropic diffusion. Axons form homogenous bundles that create an internal structure where diffusion is anisotropic. This is due to the more rapid diffusion of water molecular in the direction aligned with the internal structure, and more slowly in the perpendicular direction. This also means that the measured rate of diffusion will differ depending on the direction from which an observer is looking. Thus, measuring the diffusion signal from multiple directions is essential to capture the full extent of anisotropy.

DTI makes some assumptions about the underlying diffusion signal. While each voxel (three-dimensional pixel) on an MRI image may contain information about thousands of axons and cells, the overall direction of diffusion within that voxel is summarized using a single mathematical description, the diffusion tensor. The diffusion tensor is simply a generalized multidimensional vector that is calculated based on six or more gradient directions, although early Monte Carlo simulations of different gradient sampling schemes suggested that at least 30 directions are required for robust estimation of both anisotropy and diffusivity (Jones 2004). Most DTI sequences today estimate diffusion using ~30 directions (with several additional scans that are not diffusion weighted, i.e., B_0 for reference). This rather simplistic model of the diffusion process assumes that the diffusion signal is linear and homogeneous within each voxel and cannot capture intra-voxel fiber complexities such as bending, twisting, crossing, and kissing fibers. However, in principal white matter tracts in the brain (corpus callosum for example), DTI is a reasonable first approximation of white matter directionality.

From the diffusion tensor, one can calculate scalar values that can be used to compare individuals or groups and assess white matter abnormalities. These measures include fractional anisotropy (FA), mean diffusivity (MD), axial and radial diffusivities (AD and RD, respectively), and the apparent diffusion coefficient (ADC). In the addition the principal eigenvector (largest directional component of diffusion within a voxel) can be used to conduct tractography, which offers insight into how different regions of the brain are interconnected (Mori 2007).

5.2.2 DTI Studies of TBI

Microstructural damage undetected by traditional imaging can be reliably evaluated by DTI, making it a very valuable tool for determining biomarkers in TBI. Animal studies have correlated abnormal DTI measures, such as lowered fractional anisotropy (FA), with TBI-related pathology even in cases of subtle white matter injuries (Li et al. 2011). Likewise, numerous studies in the past decade have successfully utilized abnormal DTI measures to differentiate between TBI patients and healthy controls and correlate DTI measures with TBI outcomes across varying severities and time after incident (Hulkower et al. 2013).

As DTI measures are sensitive to the flow of water as defined by structural features, the stage of diffuse axonal injury will determine the predictability of those measures in affected regions. In the acute stages of TBI, DTI values are less predictive due to the variability caused by cellular edema occurring prior to Wallerian degeneration of the affected axons. For example, if edema occurs intracellularly, axonal swelling will structurally confine the flow of water along the axon, and thus ADC tends to decrease while FA tends to increase (Edlow and Wu 2012; Wilde et al. 2008; Bazarian et al. 2007). If edema occurs extracellularly, water molecules will diffuse isotropically in the extracellular compartment, and thus ADC tends to increase, and FA tends to decrease (Edlow and Wu 2012; Wang et al. 2008; Huisman et al. 2004).

In contrast to acute TBI, the subacute or chronic stages of TBI yield more predictable patterns of DTI measures, since the loss of white matter structure in the later stages of TBI tends to increase the ADC and decrease FA. With greater predictability and thus outcome prediction, most DTI studies focus on the subacute/chronic stages of TBI rather than on acute stages (Edlow and Wu 2012).

Generally, three types of methods are commonly used in the analyses of DTI studies of TBI: a priori determined region of interest (ROI) analyses, whole-brain voxel-based analyses, and in-vivo tractography. ROI analyses are based on an a priori determined hypothesis, where a specific region is defined for analysis and extraction of DTI measures, which can be compared across individuals for group differences. A limitation of ROI analyses is that due to high intra- and inter-subject variability in anatomy and baseline FA, there may not be sufficient statistical power (Catani 2006). Accuracy of the results is also dependent on the consistency and reproducibility of the placement of the ROI across subjects or on the normalization of ROIs based on a standard template. Moreover, some areas may be overrepresented in literature, because they may be more commonly chosen as ROIs, rather than being a biomarker of TBI (or vice versa). The most common areas of decreased FA as found in ROI analyses include the corpus callosum, internal capsule, corona radiata, cingulum bundle, brainstem, and cerebral peduncles (Hulkower et al. 2013).

On the other hand, whole-brain analyses do not require a priori hypotheses, and analysis can be performed by a variety of methods, such as voxel-wise analysis, tract based spatial statistics (TBSS), and histogram analysis of white matter voxels. However, due to the large scope of whole-brain analysis, it is very difficult to

confidently ascribe a significant cluster to a specific tract in the brain (Catani 2006). The most common areas of decreased FA as found in whole-brain analyses are the superior and inferior longitudinal fasciculi, the corpus callosum, the internal capsule, corona radiata, and the cingulum bundle (summarized in Hulkower et al. 2013). This pattern demonstrates that ROI analyses and whole-brain analyses are largely consistent and show that larger tracts in the brain are susceptible to TBI damage. However, there is a potential bias here in that larger tracts are more likely to show positive group differences as detection is easier. More subtle tracts or crossing fibers are less likely to do so because DTI is not as sensitive to those structures.

In vivo tractography is a technique that allows reconstruction of white matter tracts based on tensor values. With tractography, white matter fiber connectivity can be visualized from remote regions, and virtual dissections of fibers in ROIs are possible through utilization of tractography algorithms within those ROIs. As this method takes into account individual variations in brain structure and can ascribe individual tracts, it circumvents some of the drawbacks of ROI and whole-brain analyses (Thiebaut de Schotten et al. 2011; Catani 2006). However, tractography is still limited in that it is a largely inferential technique and is especially sensitive to the DTI parameters utilized (i.e., b-values, voxel size, termination criteria), so results must be treated with caution and said parameters have to be taken into consideration (Edlow and Wu 2012). Nevertheless, the most common areas of decreased FA identified in tractography are largely consistent with ROI and whole-brain analyses and include the corpus callosum, cingulum bundle, fornix, fronto-occipital fasciculus, inferior longitudinal fasciculus, uncinate fasciculus, and the hippocampus (Hulkower et al. 2013).

Several studies have found that decreased FA in particular brain regions is correlated with deficits in performance on neuropsychological evaluation (Perlbarg et al. 2009; Tollard et al. 2009; Sidaros et al. 2008; Niogi et al. 2008; Kraus et al. 2007). For example, the utilization of DTI across the full GOS spectrum of TBI severity (vegetative state to good recovery) revealed that poor outcome is associated with increased FA and decreased ADC in the supratentorial white matter, corpus callosum, and the thalamus (Newcombe et al. 2011). Moreover, decreases in FA in the pons and midbrain regions are significantly different from healthy controls only in those with the worst outcomes (GOS = 2 or 3), possibly suggesting that those areas indicate permanent alterations in consciousness (Newcombe et al. 2011). Additionally, GCS scores have been found to correlate with FA in the corpus callosum, posterior limb of the internal capsule, superior longitudinal fasciculus, corticospinal tract, and superior and inferior fronto-occipital fasciculus (Yuan et al. 2007). Wang et al. (2008) have also suggested that the extent of decreased FA in the splenium of the corpus callosum may serve as a valuable biomarker for long term outcomes in TBI patients.

Post-injury memory deficits have been associated with lowered FA in the uncinate fasciculus, superior longitudinal fasciculus, and fornix (Palacios et al. 2011; Niogi et al. 2008). Decreased attentional control has been associated with lowered FA in the anterior left corona radiata (Niogi et al. 2008). Impaired executive function has been correlated with lowered FA in dorsolateral prefrontal regions

(Lipton et al. 2009). Motor weakness has been correlated with lowered FA in corticospinal tracts (Jang et al. 2009). Severity of post-concussive symptoms, such as memory deficits, depression, irritability, headaches, dizziness, have also been correlated with lowered FA in the corpus callosum (Wilde et al. 2008). Duration of coma is also correlated with significantly lowered ADC in the left anterior internal capsule and heightened FA in the splenium of the corpus callosum (Bazarian et al. 2007). Individualized patterns of axonal damage, as well as demonstration of recovery, can also be accounted for with DTI measures. For example, Lee and Newberg (2005) demonstrated with DTI tractography that a patient's left hemialexia was due to injury of the splenium, which disconnected the right visual cortex from left hemisphere language centers. Additionally, a longitudinal study following the rehabilitation of a severe TBI patient showed that increases in white matter FA correlated with neurocognitive improvement (Sidaros et al. 2008).

Overall, DTI has been an informative tool to investigate the functional neuroanatomy of TBI and shows reasonable consistency across studies and analytical approaches. Some of the most vulnerable white matter pathways in the brain include the corpus callosum, the internal capsule, thalamic radiations, cingulum, uncinate fasciculus, superior and inferior longitudinal fasciculi, as well as corticospinal tracts. These are larger tracts that form a large proportion of the long-range white matter architecture in the brain, which is potentially due to a bias in DTI. These macroscopic features are easily detected and examined by DTI, while other smaller pathways with more microscopic features may be less detectable by DTI techniques. Also, DTI is incapable of detecting changes in anisotropy in voxels where there are crossing fibers, which reduces its utility for examining other white matter pathways. Other diffusion-based techniques may be used to resolve these issues.

5.2.3 High Angular Resolution Diffusion Imaging

As mentioned in the previous section, DTI suffers from a critical limitation, which is the explicit assumption that there is only one fiber orientation that can be estimated at any given voxel. Since white matter architecture is complex with fibers crossing, bending and twisting often, and due to the coarse sampling resolution of our imaging techniques, this assumption is almost always known to be incorrect. More recently, techniques that model diffusion across a larger number of directions have been used to address this limitation. High Angular Resolution Diffusion Imaging (HARDI) samples the diffusion signal along many more directions (>100) modeling diffusion with an orientation distribution function (ODF) that can capture multiple orientations per voxel (Tuch et al. 2002, 2004; Wedeen et al. 2008).

Analytical techniques (e.g., Q-ball analysis) are used to reconstruct ODFs and generate scalar maps like generalized FA (GFA) (Descoteaux et al. 2009). Deterministic and probabilistic tractography can be conducted on ODFs as well

much like tensors. The resulting representation may be more informative as far as the true architecture of brain white matter, and may be more sensitive to subtle deficits. Recent work has also demonstrated that novel rotation-invariant scalar values can be extracted based on the ODFs and can summarize bidirectional diffusion better than simpler metrics like generalized FA. For example, rotation-invariant metrics can map the crossing and kissing fibers in the optic chiasm in HARDI scans (Schwab et al. 2013).

5.2.4 HARDI Studies of TBI

Since HARDI techniques have only been available for a short time, their applications to TBI are only in their infancy. For example, Morey et al. (2012) utilized HARDI to calculate partial volume fractions of crossing fibers in veterans with mild TBI. TBI patients were differentiated from healthy controls on the basis of having decreased partial volume fraction in the primary fiber (f1) in several white matter tracts including the corpus callosum, the posterior thalamic radiations, and the internal capsule. The duration of loss of consciousness experienced by the veterans was significantly correlated with decreased f1 in several other tracts including the anterior thalamic radiation, uncinate fasciculus, and the external capsule. Moreover, the feeling of being dazed and confused was significantly correlated with distributed, more peripheral decreased f1 in the posterior occipital cortex, posterior corona radiata, fornix, superior longitudinal fasciculus, cerebral peduncle, and posterior thalamic radiations. In general, FA and f1 values were largely consistent, but f1 was more specific, while FA was more sensitive. The study demonstrates the heterogeneity of white matter abnormalities but also suggests that HARDI is capable of detecting TBI-induced anomalies.

Shin et al. (2012) developed a novel imaging technique, high definition fiber tracking (HDFT), that utilizes HARDI to illustrate that a severe TBI patient's upper muscle weakness directly corresponded with volumetric loss in the corona radiata pathway through the internal capsules and with a loss of projecting fibers from the ventrolateral side of the motor cortex (responsible for motor control in upper extremities) in the corticospinal pathway. Critically, the authors showed that such specificity was only possible by using high angular resolution scanning and fiber tracking, since DTI measures in the same patient could not elucidate which pathway was injured, the extent of the injury, nor the projection field of the pathway (Shin et al. 2012).

While only preliminary, the above studies suggest that HARDI techniques may offer higher specificity and sensitivity than traditional DTI approaches in assessing white matter abnormalities in TBI. Future work streamlining these methods and improving acquisition and analysis techniques will be of critical value to improving our understanding of the neural basis of TBI-related white matter injury.

5.2.5 Summary

TBI is a leading cause of disability, morbidity, and mortality worldwide. However, due to the wide range of causal mechanisms possible, the variability of brain structure and premorbid conditions in patients, the inconsistent use of crude severity and outcome scales, and the insufficiency of current clinical neuroimaging tools (CT and MRI), the heterogeneity of TBI is often ill characterized, thus making outcome predictions and plans for treatment difficult to formulate. As diffuse axonal injury is a major cause of most TBI-related sequelae, diffusion tensor imaging can be a valuable tool for the evaluation of functional neuroanatomy following injury, since DTI is sensitive to axonal damage that is undetectable in CT and MRI. Thus far, the use of DTI across studies has been informative in the detection of axonal damage correlating with functional behaviors and physical outcomes. However, DTI suffers from two critical limitations—that results may be biased towards larger tracts due to ease in detection, and that DTI can only evaluate a single direction in each voxel, making structural changes in crossing fibers difficult to assess. High angular resolution diffusion imaging, although still in its infancy, can overcome these limitations and offer valuable insights. Few recent studies have suggested that HARDI techniques detect fiber damage with more specificity and sensitivity than that of DTI. Future directions for studying and evaluating biomarkers for TBI include streamlining diagnoses of TBI patients, utilizing consistent imaging parameters across studies, and further development of HARDI and DTI techniques for use in future clinical examinations.

References

Adams JH, Doyle D, Ford I, Gennarelli TA, Graham DI, McLellan DR (1989) Diffuse axonal injury in head injury: definition, diagnosis and grading. Histopathology 15:49–59

Adams JH, Mitchell DE, Graham DI, Doyle D (1977) Diffuse brain damage of immediate impact type: its relationship to "primary brain-stem damage" in head injury. Brain 100:489–502

Anderson V, Catroppa C, Godfrey C, Rosenfield JV (2012) Intellectual ability 10 years after traumatic brain injury in infancy and childhood: what predicts outcome? J Neurotrauma 29:143–153

Arundine M, Aarts M, Lau A, Tymianski M (2004) Vulnerability of central neurons to secondary insults after in vitro mechanical stretch. J Neurosci 24(37):8106–8123

Balestreri M, Czosnyka M, Chatfield DA, Steiner LA, Schmidt EA, Smielewski P, Matta B, Pickard JD (2004) Predictive value of Glasgow coma scale after brain trauma: change in trend over the past ten years. J Neurol Neurosurg Psychiatry 75:161–162

Barnes D, Krueger K, Byers A, Diaz-Arrastia, Yaffe K (2011) Traumatic brain injury and risk of dementia in older veterans. Poster presented at the annual meeting of Alzheimer's association international conference, Paris, France, 2011

Bazarian JJ, Zhong J, Blyth B, Zhu T, Kavcic V, Peterson D (2007) DTI detects clinically important axonal damage after mild TBI: a pilot study. J Neurotrauma 24(9):1447–1459

Bigler ED (1990) Neuropathology of traumatic brain injury. In: Bigler ED (ed) Traumatic brain injury: mechanisms of damage, assessment, intervention and outcome. PRO-ED, Austin, TX

Catani M (2006) Diffusion tensor magnetic resonance imaging tractography in cognitive disorders. Curr Opin Neurol 19(6):599–606

Cubillo A, Halari R, Smith A, Taylor E, Rubia K (2012) A review of fronto-striatal and fronto-cortical brain abnormalities in children and adults with attention deficit hyperactivity disorder (ADHD) and new evidence for dysfunction in adults with ADHD during motivation and attention. Cortex 48(2):177–198

Descoteaux M, Angelino E, Fitzgibbons S, Deriche R (2007) Regularized, fast, and robust analytical Q-ball imaging. Magn Reson Med 58(3):497–510

Edlow BL, Wu O (2012) Advanced neuroimaging in traumatic brain injury. Semin Neurol 32:374–400

Faul M, Xu L, Wald MM, Coronado VG (2010) Traumatic brain injury in the United States: emergency department visits, hospitalizations and deaths 2002–2006. Centers for Disease Control and Prevention, National Center for Injury Prevention and Control, Atlanta, GA

Ferrell RB, Tanev KS (2002) Traumatic brain injury in older adults. Curr Psychiatry Rep 4:354–362

Gean AD (1994) White matter shearing injury and brainstem injury. In: Imaging of head trauma. Raven, New York, pp 207–248

Gennarelli TA (1993) Mechanisms of brain injury. J Emerg Med 11:5–11

Gentry LR (1996) Head trauma. In: Atlas SW (ed) Magnetic resonance imaging of the brain and spine. Raven, New York, pp 611–647

Gouick J, Gentleman D (2004) The emotional and behavioural consequences of traumatic brain injury. Trauma 6(4):285–292

Hardman JM, Manoukian A (2002) Pathology of head trauma. Neuroimaging Clin N Am 12(2): 175–187

Henry JD, Philips LH, Crawford JR, Ietswaart M, Summers F (2006) Theory of mind following traumatic brain injury. J Neurotrauma 44(10):1623–1628

Hughes DG, Jackson J, Mason DL, Berry E, Hollis S, Yates DW (2004) Abnormalities on magnetic resonance imaging seen acutely following brain injury: correlation with neuropsychological tests and delayed recovery. Neuroradiology 46:550–558

Huisman TAGM, Schwamm LH, Schaefer PW, Koroshetz WJ, Shetty-Alva N, Ozsunar Y, Wu O, Sorensen AG (2004) Diffusion tensor imaging as potential biomarker of white matter injury in diffuse axonal injury. AJNR Am J Neuroradiol 25(3):370–376

Hukkelhoven CW, Steyerberg EW, Rampen AJ, Farace E, Habbema JD, Marshall LF, Murray GD, Maas AI (2003) Patient age and outcome following severe traumatic brain injury: an analysis of 5600 patients. J Neurosurg 99:666–673

Hulkower MB, Poliak DB, Rosenbaum SB, Zimmerman ME, Lipton ML (2013) A decade of DTI in traumatic brain injury: 10 years and 100 articles later. Am J Neuroradiol. Epub ahead of print

Inglese M, Makani S, Johnson G, Cohen B, Silver JA, Gonen O, Grossman RI (2005) Diffuse axonal injury in mild TBI: a diffusion tensor imaging study. J Neurosurg 103(2):298–303

Institute of Medicine Committee on Gulf War and Health (2009) Long-term consequences of traumatic brain injury, 7th edn, Gulf war and health. National Academies Press, Washington, DC

Jang SH, Kim DS, Son SM, Cho YW, Kim SH, Kim OL, Ahn SH (2009) Clinical application of tensor tractography for elucidation of the causes of motor weakness in patients with traumatic brain injury. NeuroRehabilitation 24(3):273–278

Jennett B, Bond M (1975) Assessment of outcome after severe brain damage. Lancet 1:480–484

Jennett B, Teasdale G, Braakman R, Minderhoud J, Knill-Jones R (1976) Predicting outcome in individual patients after severe head injury. Lancet 1:1031–1034

Jones DK (2004) The effect of gradient sampling schemes on measures derived from diffusion tensor MRI: a Monte Carlo study. Magn Reson Med 51(4):807–815

Kashluba S, Hanks RA, Casey JE et al (2008) Neuropsychologic and functional outcome after complicated mild traumatic brain injury. Arch Phys Med Rehabil 89:904–911

Kraus MF, Susmaras T, Caughlin BP, Walker CJ, Sweeney JA, Little DM (2007) White matter integrity and cognition in chronic traumatic brain injury: a diffusion tensor imaging study. Brain 130(10):2508–2519

Kumar R, Husain M, Gupta RK, Hasan KM, Haris M, Agarwal AK, Pandey CM, Narayana PA (2009) Serial changes in the white matter diffusion tensor imaging metrics in moderate traumatic brain injury and correlation with neuro-cognitive function. J Neurotrauma 26(4):481–495

Langen M, Leemans A, Johnston P, Ecker C, Daly E, Murphy CM, Dell' Acqua F, Durston S, Murphy DGM (2012) Fronto-striatal circuitry and inhibitory control in autism: Findings from diffusion tensor imaging tractography. Cortex 48(2):166–176

Lee B, Newberg A (2005) Neuroimaging in traumatic brain injury. NeuroRx 2(2):372–383

Li J, Li XY, Feng DF, Gu L (2011) Quantitative evaluation of microscopic injury with diffusion tensor imaging in a rat model of diffuse axonal injury. Eur J Neurosci 33(5):933–945

Li XY, Feng DF (2009) Diffuse axonal injury: novel insights into detection and treatment. J Clin Neurosci 16(5):614–619

Lipton ML, Gulko E, Zimmermann ME, Friedman BW, Kim M, Gellella E, Gold T, Shifteh K, Ardekani BA, Branch CA (2009) Diffusion-tensor imaging implicates prefrontal axonal injury in executive function impairment following very mild traumatic brain injury. Radiology 252(3): 816–824

Lucas J (1999) Traumatic brain injury and post-concussive syndrome. In: Snyder PJ, Nussbaum PD (eds) Clinical neuropsychology. APA, Washington DC, pp 243–265

Marquez de la Plata CD, Hart T, Hammond FM, Frol AB, Hudak A, Harper CR, O'Neil-Pirozzi TM, White J, Carlile M, Diaz-Arrastia R (2008) Impact of age on long-term recovery from traumatic brain injury. Arch Phys Med Rehabil 89:896–903

Marshall LF, Gautille T, Klauber MR, Eisenberg HM, Jane JA, Luerssen TG, Marmarou A, Foulkes MA (1991) The outcome of severe closed head injury. J Neurosurg 75:S28–S36

Mateer C, Sira C (2006) Cognitive and emotional consequences of TBI: Intervention strategies for vocational rehabilitation. NeuroRehabilitation 21(4):315–326

Maxwell WL, Povlishock JT, Graham DL (1997) A mechanistic analysis of nondisruptive axonal injury: a review. J Neurotrauma 14:419–440

McDonald S, Flanagan S (2004) Social perception deficits after traumatic brain injury: Interaction between emotion recognition, mentalizing ability, and social communication. Neuropsychology 18(3):572–579

Meythaler JM, Peduzzi JD, Eleftheriou E, Novack TA (2001) Current concepts: diffuse axonal injury-associated traumatic brain injury. Arch Phys Med Rehabil 82:1461–1471

Morey RA, Haswell CC, Selgrade ES, Massoglia D, Liu C, Weiner J, Marx CE, Cernak I, McCarthy G, MIRECC Work Group (2012) Effects of chronic mild traumatic brain injury on white matter integrity in Iraq and Afghanistan war veterans. Hum Brain Mapp 34:2986–2999. doi: 10.1002/hbm.22117

Mori S (2007) Introduction to diffusion tensor imaging. Elsevier Science, Amsterdam

Morrison AL, King TM, Korell MA, Smialek JE, Troncoso JC (1998) Acceleration-deceleration injuries to the brain in blunt force trauma. Am J Forensic Med Pathol 19(2):109–112

Murray JG, Gean AD, Evans SJ (1996) Imaging of acute head injury. Semin Ultrasound CT MR 17:185–205

Newcombe V, Chatfield D, Outtrim J, Vowler S, Manktelow A, Cross J, Scoffings D, Coleman M, Hutchinson P, Coles J, Carpenter TA, Pickard J, Williams G, Menon D (2011) Mapping traumatic axonal injury using diffusion tensor imaging: correlations with functional outcome. PLoS One 6(5):e19214

Niogi SN, Mukherjee P, Ghajar J, Johnson C, Kolster RA, Sarkar R, Lee H, Meeker M, Zimmerman RD, Manley GT, McCandliss BD (2008) Extent of microstructural white matter injury in post-concussive syndrome correlates with impaired cognitive reaction time: a 3T diffusion tensor imaging study of mild traumatic brain injury. AJNR Am J Neuroradiol 29(5):967–973

Palacios EM, Fernandez-Espejo D, Junque C, Sanchez-Carrion R, Roig T, Tormos JM, Bargallo N, Vendrell P (2011) Diffusion tensor imaging differences relate to memory deficits in diffuse traumatic brain injury. BMC Neurol 11:24

Pang D (1985) Pathophysiologic correlates of neurobehavioural syndromes following closed head injury. In: Ylvisaker M (ed) Head injury rehabilitation: children and adolescents. PRO-ED, Austin, TX, pp 3–70

Perlbarg V, Puybasset L, Tollard E, Lehéricy S, Benali H, Galanaud D (2009) Relation between brain lesion location and clinical outcome in patients with severe traumatic brain injury: a diffusion tensor imaging study using voxel-based approaches. Hum Brain Mapp 30(12): 3924–3933

Pike BR, Zhao X, Newcomb JK, Glenn CC, Anderson DK, Hayes RL (2000) Stretch injury causes calpain and caspase-3 activation and necrotic and apoptotic cell death in septo-hippocampal cell cultures. J Neurotrauma 17:283–298

Poirier MP (2003) Concussions: assessment, management, and recommendations for return to activity. Clin Pediatr Emerg Med 4(3):179–185

Povlishock JT (1993) Pathobiology of traumatically induced axonal injury in animals and man. Ann Emerg Med 22:980–986

Radi R, Rodriguez M, Castro L, Telleri R (1994) Inhibition of mitochondrial electron transport by peroxynitrite. Arch Biochem Biophys 24:369–380

Ratcliff G, Colantonio A, Escobar M, Chase S, Vernich L (2005) Long-term survival following traumatic brain injury. Disabil Rehabil 27:305–314

Rockhill CM, Fann JR, Fan MY, Hollingworth W, Katon WJ (2010) Healthcare costs associated with mild traumatic brain injury and psychological distress in children and adolescents. Brain Inj 24(9):1051–1060

Ross DE, Ochs AL, Seabaugh JM, Demark MF, Shrader CR, Marwitz JH, Havranek MD (2012) Progressive brain atrophy in patients with chronic neuropsychiatric symptoms after mild traumatic brain injury: a preliminary study. Brain Inj 26(12):1500–1509

Rowley G, Fielding K (1991) Reliability and accuracy of the Glasgow Coma Scale with experienced and inexperienced users. Lancet 337(8740):535–539

Ryan GA, McLean AJ, Vilenius AT, Kloeden CN, Simpson DA, Blumbergs PC, Scott G (1994) Brain injury patterns in fatally injured pedestrians. J Trauma 36(4):469–476

Scheid R, Preul C, Gruber O, Wiggins C, Yves von Cramon D (2003) Diffuse axonal injury associated with chronic traumatic brain injury, evidence from T2*-weighted gradient-echo imaging at 3T. AJNR Am J Neuroradiol 24:1049–1056

Schwab E, Çetingül HE, Afsari B, Yassa MA, Vidal R (2013) Rotation invariant features for HARDI. Information processing in medical imaging, Lect Notes in Comput Sci; 7917:705–717

Shin SS, Verstynen T, Pathak S, Jarbo K, Hricik AJ, Maserati M, Beers SR, Puccio AM, Boada FE, Okonkwo DO, Schneider W (2012) High-definition fiber tracking for assessment of neurological deficit in a case of traumatic brain injury: finding, visualizing, and interpreting small sites of damage. J Neurosurg 116(5):1062–1069

Shively S, Scher AI, Perl DP, Diaz-Arrastia R (2012) Dementia resulting from traumatic brain injury: what is the pathology? Arch Neurol 69(10):1245–1251

Sidaros A, Engberg AW, Sidaros K, Liptrot MG, Herning M, Petersen P, Paulson OB, Jernigan TL, Rostrup E (2008) Diffusion tensor imaging during recovery from severe traumatic brain injury and relation to clinical outcome: a longitudinal study. Brain 131(2):559–572

Singh J, Stock A (2006) Head trauma. www.Emedicine.com. Accessed 27 Dec 2012

Thiebaut de Schotten M, Ffytche DH, Bizzi A, Dellacqua F, Allin M, Walshe M, Murray R, Williams SC, Murphy DGM, Catani M (2011) Atlasing location, asymmetry and intersubject variability of white matter tracts in the human brain with MR diffusion tractography. Neuroimage 54(1):49–59

Thurman DJ, Coronado V, Selassie A (2007) The epidemiology of TBI: Implications for public health. In: Zasler ND, Katz DI, Zafonte RD (eds) Brain injury medicine: principles and practice. Demos, New York, NY, pp 45–55

Tollard E, Galanaud D, Perlbarg V, Sanchez-Pena P, Le Fur Y, Abdennour L, Cozzone P, Lehericy S, Chiras J, Puybasset L (2009) Experience of diffusion tensor imaging and 1H spectroscopy for outcome prediction in severe traumatic brain injury: preliminary results. Crit Care Med 37(4):1448–1455

Tuch DS, Reese TG, Wiegell MR, Makris N, Belliveau JW, Wedeen VJ (2002) High angular resolution diffusion imaging reveals intravoxel white matter fiber heterogeneity. Magn Reson Med 48(4):577–582

Tuch DS (2004) Q-ball imaging. Magn Reson Med 52(6):1358–1372
Vik A, Kvistad KA, Skandsen T, Ingebrigtsen T (2006) Diffuse axonal injury in traumatic brain injury. Tidsskr Nor Laegeforen 126(22):2940–2944, In Norwegian
Wang JY, Bakhadirov K, Devous MD, Abdi H, McColl R, Moore C, Marquez de la Plata CD, Ding K, Whittemore A, Babcock E, Rickbeil T, Dobervich J, Kroll D, Dao B, Mohindra N, Madden CJ, Diaz-Arrastia R (2008) Diffusion tensor tractography of traumatic diffuse axonal injury. Arch Neurol 65(5):619–626
Warriner EM, Velikonja D (2006) Psychiatric disturbances after traumatic brain injury: neurobehavioral and personality changes. Curr Psychiatry Rep 8:73–80
Wedeen VJ, Wang RP, Schmahmann JD, Benner T, Tseng WY, Dai G, Pandya DN, Hagmann P, D'Arceuil H, de Crespigny AJ (2008) Diffusion spectrum magnetic resonance imaging (DSI) tractography of crossing fibers. Neuroimage 41(4):1267–1277
Wilde EA, McCauley SR, Hunter JV, Bigler ED, Chu Z, Wang ZJ, Hanten GR, Troyanskaya M, Yallampalli R, Li X, Chia J, Levin HS (2008) Diffusion tensor imaging of acute mild traumatic brain injury in adolescents. Neurology 70(12):948–955
Wolf JA, Stys PK, Lusardi T, Meaney D, Smith DH (2001) Traumatic axonal injury induces calcium influx modulated by tetrodotoxin-sensitive sodium channels. J Neurosci 21(6):1923–1930
Young WY (1996) Death by calcium: a way of life. In: Narayan RK, Wilburger JE, Povlishock JT (eds) Neurotrauma. McGraw-Hill, New York, pp 1421–1431
Yuan W, Holland SK, Schmithorst VJ, Waltz NC, Cecil KM, Jones BV, Karunanayaka P, Michaud L, Wade SL (2007) Diffusion tensor MR imaging reveals persistent white matter alteration after traumatic brain injury experienced during early childhood. AJNR Am J Neuroradiol 28(10):1919–1925
Zappala G, Thiebaut de Schotten M, Eslinger PJ (2012) Traumatic brain injury and the frontal lobes: what can we gain with diffusion tensor imaging? Cortex 48:156–165
Zuercher M, Ummenhofer W, Baltussen A, Walder B (2009) The use of Glasgow coma scale in injury assessment: a critical review. Brain Inj 23(5):371–384

Part II
White Matter Injury in Stroke and Other CNS Disorders

Chapter 6
Mechanisms Underlying the Selective Vulnerability of Developing Human White Matter

Paul A. Rosenberg

Significant advances have been made in the care of premature infants aimed at increasing the probability of survival (Bode et al. 2009). However, proportionate advances have not been made in the neurological outcome of premature infants, and so the incidence of brain injury in this population remains high (Lorenz 2001; Xiong et al. 2012). The most important lesion of the premature brain is periventricular leukomalacia, which, with modern imaging techniques, has been shown to affect fully 50 % of infants born at 32 weeks gestational age or earlier. This group of very premature infants numbers over 60,000 infants in the USA. 5–10 % of this population will develop cerebral palsy, and 50 % will be impaired in their cognitive, social, and attentional abilities (Volpe 2009b). The problem of neurological damage in premature infants is enormous in terms of personal and family suffering as well as economic impact (Honeycutt et al. 2004).

This review focuses on what we have learned about why periventricular leukomalacia is a lesion characteristic of the developing human brain, the conspicuous gaps in this knowledge, and what this knowledge tells us about how to approach treating it. Here, the focus is on the acute lesion and what is hypothesized to be the initiating event in producing this lesion, which, as is made clear, is injury and death of developing oligodendrocytes. Many topics are not covered, including the generation of the chronic lesion, potential repair mechanisms and their failure in PVL, the involvement of neurons and axons in white matter, injury to grey matter structures. These are all worthy topics that have received much attention recently, but are beyond the scope of this review.

P.A. Rosenberg, M.D., Ph.D. (✉)
Neurology, Children's Hospital Boston, 300 Longwood Ave., Boston, MA 02115, USA
e-mail: paul.rosenberg@childrens.harvard.edu

S. Baltan et al. (eds.), *White Matter Injury in Stroke and CNS Disease*, Springer Series in Translational Stroke Research 4, DOI 10.1007/978-1-4614-9123-1_6,

6.1 Periventricular Leukomalacia

PVL is the predominant form of brain injury in the preterm infant, and is a lesion recognized neuropathologically by focal necrosis in the deep white matter accompanied by a diffuse lesion of the white matter characterized by astrocytosis and microgliosis (Kinney and Armstrong 2002) (Fig. 6.1). Axonal injury has also been recognized as an important component of this lesion (Banker and Larroche 1962; Kinney and Back 1998; Dammann et al. 2001; Haynes et al. 2008). An essential aspect of PVL is that it is a lesion of the early developing human brain. Whereas neuronal populations in the brain *from birth into maturity* are highly sensitive to a variety of insults, such as anoxia, hypoglycemia, and ischemia, in contrast, white matter post-term is relatively resistant. In the developing preterm brain, especially in the period of peak vulnerability to PVL, this pattern is inverted, and white matter appears to be more vulnerable than grey matter. PVL may occur in term infants, for example in the setting of cardiac surgery (Kinney et al. 2005) when cerebral myelin is still immature (Brody et al. 1987), but its peak incidence is in the very premature population born at less than 32 weeks of age (Volpe 2003). It is a lesion less seen in the mature brain, and so the inevitable question is why the developing brain is vulnerable to PVL. Developing white matter is populated primarily by developing

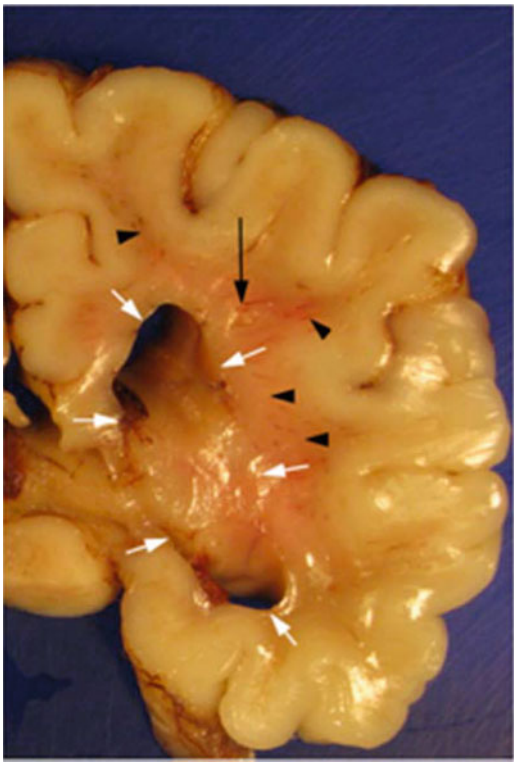


Fig. 6.1 Periventricular leukomalacia. Coronal section of the right parietal lobe of an infant born at 32 weeks and surviving 2 postnatal weeks. Focal white matter necrosis with beginning cavitation (*long black arrow*) is surrounded by diffusely injured white matter (*black arrowheads*) with pink–grey discoloration. Damage is localized to the periventricular regions (ventricular surface, is outlined with *white arrows*). Note the relative sparing of the cerebral cortical ribbon. Courtesy Dr. R. Folkerth, Children's Hospital Boston

oligodendrocytes (Back et al. 2001a; Craig et al. 2003), and many lines of evidence have converged to suggest that developing oligodendrocytes are highly vulnerable to a variety of insults, and their presence in developing white matter predisposes the developing human brain to PVL (Riddle et al. 2006).

6.2 Selective Vulnerability of Cells Within the Nervous System

One of the fundamental observations of neuropathology is that neurological diseases show selective vulnerability of neuronal populations in the central nervous system (CNS), a phenomenon known as *pathoclisis* (*patho* + *clisis*: bending or proneness) (Klatzo 2003) championed by Cecile and Oskar Vogt. There are many examples, such as the loss of dopaminergic neurons in the substantia nigra in Parkinson's disease, the loss of motor neurons in amyotrophic lateral sclerosis, the loss of medium spiny neurons in Huntington's disease, and the loss of pyramidal neurons of the hippocampus in global ischemia (Table 6.1). The fascination with selective vulnerability derives from the idea that there are intrinsic properties of specific groups of cells that make them especially vulnerable to different disease processes (Gosztonyi and Koprowski 2001; Duke et al. 2007). The observation of selective vulnerability suggested that different groups of neurons had different functions, interesting in itself, but also that, in a given disorder, some neurons were affected and some not. Therefore, the disease process did not inevitably cause death, but somehow exploited some special feature of the vulnerable neurons to cause injury. If we understood what the special feature is, then we would perhaps be able to intervene and prevent injury from occurring.

It has really only been in the past 30 years that we have attained enough mechanistic understanding of cell death to be able to intervene in experimental models and prevent death. The best understanding of the basis for selective neuronal vulnerability relates to acute injuries involving compromise of energy metabolism, such as hippocampal injury in global ischemia, carbon monoxide poisoning, and hypoglycemia, in which high levels of expression of glutamate receptors on vulnerable neurons, and successful blockade of injury by glutamate receptor antagonists in animal models provide compelling evidence for an important role for excitoxicity (Table 6.1). A cautionary example is provided by Wernicke's encephalopathy, which is thought to be due to thiamine deficiency. In animal models of thiamine deficiency, there was early evidence suggesting that excitotoxicity is the initiating event in the selective injury that occurs to structures classically injured in Wernicke's encephalopathy, such as the mammillary bodies and medial dorsal nucleus of the thalamus (Langlais and Mair 1990; Langlais and Zhang 1993; Zhang et al. 1995). However, subsequent studies indicated that the *early events* leading to the pathology of thiamine deficiency were not excitotoxic in origin because they were not blocked by glutamate receptor antagonists. These drugs may have produced benefits in the earlier studies by preventing seizures after the initial pathology had developed

Table 6.1 Selective neuronal vulnerability in disorders and disease of the central nervous system

Disease/disorder	Pathology	Underlying mechanism	References
Acute			
Global ischemia	CA1 pyramidal neurons	Excitotoxicity	Schmidt-Kastner and Freund (1991); Sheardown et al. (1990)
Carbon monoxide poisoning	CA1 pyramidal neurons	Excitotoxicity	Ishimaru et al. (1992)
Hypoglycemia	Dentate gyrus	Excitotoxicity	Auer (1986, 1991, 2004); Auer et al. (1985, 1989); Papagapiou and Auer (1990); Sandberg et al. (1986); Wieloch et al. (1985)
Wernicke's encephalopathy	Medial dorsal nucleus of thalamus; mammillary bodies	Excitotoxicity involved, but not an initiating process	Hazell and Butterworth (2009); Hazell et al. (1998); Jhala and Hazell (2011); Langlais and Mair (1990); Langlais and Zhang (1993); Todd and Butterworth (1998); Zhang et al. (1995)
Chronic			
Amyotrophic lateral sclerosis	Motor neurons	Excitotoxicity may play a role	Rothstein (1995, 2009)
Parkinson's disease	Substantia nigra	Unknown; little evidence in favor of excitotoxicity	Blandini (2010); Meredith et al. (2009); Surmeier et al. (2010)
Huntington's disease	Medium spiny neurons	Excitoxicity may play a role	DiFiglia (1990); Milnerwood and Raymond (2010); Raymond et al. (2011)
Paraneoplastic degenerations	Multiple	Antitumor immune response cross reacting with neurons	Darnell and Posner (2006); Graus and Dalmau (2012)
Paraneoplastic encephalitis	Hippocampus	Anti-NMDA receptor antibodies	Alexopoulos et al. (2011); Dalmau et al. (2007); Kataoka et al. (2008)

(Todd and Butterworth 1998). Now, the pathogenesis is thought to be a mix of oxidative stress, inflammatory processes involving microglial activation, and excitotoxicity (Hazell and Butterworth 2009; Jhala and Hazell 2011)—interestingly, not so different from the prevailing view concerning the overall pathogenesis of PVL. Regarding chronic neurodegenerative diseases, we still do not know the basis for the remarkable selective neuronal vulnerability demonstrated. Perhaps the best case for a role for abnormal excitatory synaptic signaling, including excitotoxicity, has been made for Huntington's disease (Table 6.1).

6.3 The Project of Neuroprotection

The term "neuroprotection" entered the scientific literature in 1987 with an article by Gill et al showing that the NMDA receptor antagonist MK801 is protective against hypoxic–ischemic injury to the hippocampus in the gerbil (Gill et al. 1987). Since then, over 11,000 papers have appeared that are searchable in PubMed by using the term "neuroprotection," and there are probably many times that number that are related. This accumulation of work represents a vast effort to develop effective ways to intervene to prevent brain injury, a term that encompasses injury to all cellular elements, not just neurons. The motivating idea behind much of this work is that there are mechanisms of injury to cells in the CNS that can be discovered and that this knowledge can be exploited to prevent CNS injury by interrupting these mechanisms. It is interesting to note that although modern neurology began over 150 years ago, the project of neuroprotection is relatively young. The idea that there are mechanisms of injury that can be discovered and interrupted really became very compelling about 30 years ago, with the publication of a study by Steven Rothman that used an in vitro model system of hippocampal neurons in culture (Rothman 1983) (Fig. 6.2). Not surprisingly, it was found that anoxia or cyanide causes these neurons to die. The question was asked whether synaptic activity had anything to do with the cell death, and to inhibit synaptic activity, the extracellular magnesium concentration was elevated to block calcium entry into the presynaptic terminals (Muller and Finkelstein 1974). The remarkable result was that simply raising extracellular magnesium concentration could prevent the death of hippocampal neurons grown in tissue culture following deprivation of oxygen. The interpretation of this result has changed over the years, and now at least some of the benefit of the effect

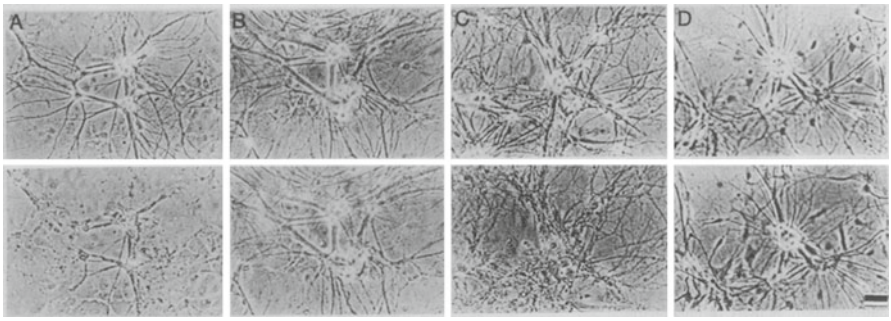


Fig. 6.2 Hippocampal neuronal death in vitro caused by anoxia or cyanide is blocked by elevated magnesium. (**a**) Phase-contrast photomicrographs of hippocampal neurons after 23 days in vitro. The same field is shown before (*top*) and after (*bottom*) 21 h of exposure to 1 mM NaCN. The neurons died and were replaced by debris. (**b**) Hippocampal neurons (23 days in vitro) before and after 21 h of exposure to 1 mM NaCN, in which the culture was first treated with 10 mM $MgCl_2$. There is virtually no change in the neurons. (**c**) Neurons (18 days in vitro) before and after exposure to the anoxic atmosphere. As in (**a**), the neurons have died, leaving only debris. (**d**) Neurons (18 days in vitro) before and after exposure to the anoxic atmosphere for 14 h, in which the culture was first treated with 10 mM $MgCl_2$. The neurons are still intact. Scale bar, 50 **μ**m. (from: Rothman 1983, Fig. 2)

of elevating extracellular magnesium would be attributed to the blocking effect of magnesium on NMDA receptor mediated currents (Nowak et al. 1984). However, the primary observation itself was a startling demonstration that neuronal death was not inevitable following near total deprivation of oxygen or poisoning of oxidative phosphorylation, but rather there are mechanisms that are set in motion that can be simply interfered with to prevent cell death. This experiment and a large number of others in tissue culture and in animal studies promoted the promise of neuroprotection based on an understanding of the mechanisms of neurological diseases (Lipton and Rosenberg 1994). Similarly, if we understand the pathogenesis of PVL on a cellular and molecular level, then we will have a rational basis for developing treatments with which to intervene to prevent this disorder.

6.4 Developing Oligodendrocytes are the Target Cell in PVL

The observation that developing oligodendrocytes represent a highly vulnerable stage in the oligodendrocyte lineage (Fig. 6.3) was made 20 years ago (Oka et al. 1993). They showed, using a paradigm of oxidative stress injury [oxidative glutamate toxicity or oxytosis (Murphy et al. 1989b; Coyle and Puttfarcken 1993; Tan et al. 2001)] that developing oligodendrocytes were much more sensitive to this form of injury than mature oligodendrocytes. This work was extended to other experimental paradigms, which, like the original paradigm—glutamate inhibition of the cystine–glutamate antiporter—all result in depletion of intracellular glutathione and oxidative stress (Yonezawa et al. 1996; Back et al. 1998). Subsequent work demonstrated that developing white matter in the human and rodent brain was

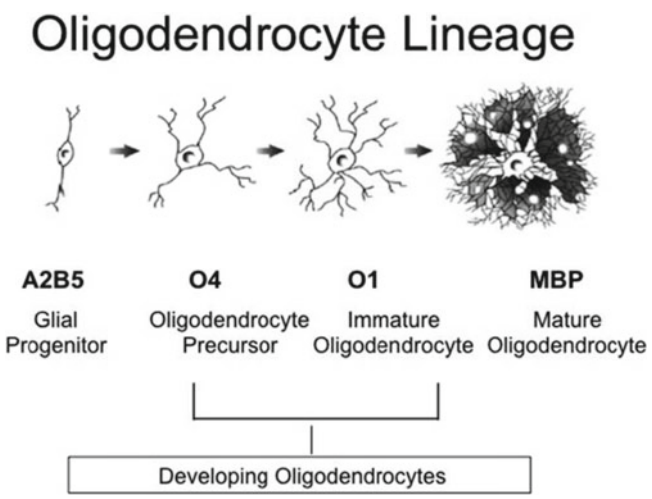


Fig. 6.3 Oligodendrocyte lineage. (from: Volpe 2008. Modified from Fig. 2.55)

populated with developing oligodendrocytes (Back et al. 2001b; Craig et al. 2003), and that developing oligodendrocytes die following hypoxic–ischemic insult (Back et al. 2002).

When the concept of selective vulnerability first emerged, it was embedded in a controversy between the Vogts and Spielmeyer over whether selective neuronal vulnerability was a manifestation of intrinsic properties of cells, or a consequence of patterns of blood flow that render certain populations more vulnerable than others (Schmidt-Kastner 1989; Schmidt-Kastner and Freund 1991). The importance of vascular factors, such as immaturity of the arterial supply to the forebrain, end-artery zones in the deep white matter, and failure of autoregulation has been emphasized by Volpe and colleagues (Tsuji et al. 1998, 2000; Volpe 2008). The issue of intrinsic versus vascular factors has been addressed with regard to PVL in an important study that showed that regions of injury in sheep white matter caused by ischemia correlated with where developing oligodendrocytes were most numerous, and not with degree of ischemia (Riddle et al. 2006; McClure et al. 2008; Buser et al. 2010). This result is further evidence emphasizing that it is developing oligodendrocytes that are the primary target of injury in the developing brain, and that cell intrinsic factors are of prime importance in the pathogenesis of PVL.

6.5 The Etiology of PVL

Remarkably, 50 years after the original description of PVL by Banker and Laroche (Banker and Larroche 1962), the etiology of PVL remains uncertain (Fig. 6.4). Pioneering work by Gilles in the 1970s demonstrated that it was possible to create a white matter lesion in kittens using endotoxin (Gilles et al. 1976, 1977). Since then, many studies have been published that are variations on the theme that inflammatory injury can produce white matter injury in animal models, and that a systemic inflammatory state in the maternal–fetal unit or postpartum in the newborn is the cause of PVL (Dammann and Leviton 2004; Malaeb and Dammann 2009; Leviton et al. 2011). A case has also been made that ischemia is the cause of PVL, based on three lines of evidence: (1) the failure of cerebral autoregulation in sick premature infants; (2) the existence of end artery watershed zones in the deep cerebral white matter corresponding to the areas of maximal injury on pathological examination of human case material; and (3) the evidence from animal studies that developing oligodendrocytes are intrinsically highly vulnerable to injury (Volpe 2009a). In addition, animal models of ischemic injury to the neonatal brain have been developed that, with adjustment of parameters, produce a somewhat selective white matter lesion (Follett et al. 2000; Elitt et al. 2003; Back et al. 2012). At this time it seems likely that there is more than one pathway to PVL, and animal studies suggest that a two-hit mechanism might be at work, in that pre-exposure to an inflammatory insult sensitizes rodent white matter to a subsequent ischemic insult (Dommergues et al. 2000; Rangon et al. 2007; Aden et al. 2010). In addition to this type of unifying mechanism, it is also possible that ischemic and inflammatory insults produce

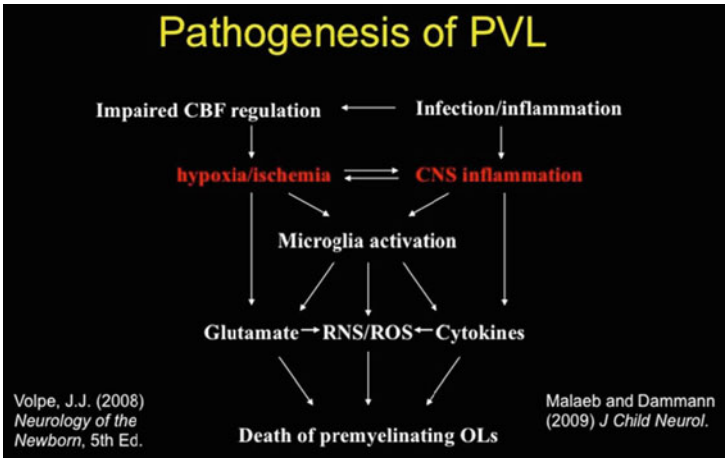


Fig. 6.4 Etiology of periventricular leukomalacia. (from: Volpe 2008. Modified from Fig. 6.35)

injury by a final common pathway (Volpe et al. 2011). An example of such a pathway is excitotoxicity. Excitotoxicity is classically produced by energy failure, as in ischemia. In addition there is the phenomenon of inflammatory excitotoxicity in which the production of nitric oxide leads to energy failure because nitric oxide is a metabolic poison; the consequences then are the same as following ischemia, which produces injury secondary to energy failure due to substrate deprivation (Bal-Price and Brown 2001; Brown and Bal-Price 2003). In considering mechanisms of injury deduced from animal models and cells in culture, it must be borne in mind that what is seen in the laboratory might not reflect what happens in humans.

6.6 The Case for Excitotoxicity

Excitotoxicity is cell death in the CNS caused by activation of glutamate receptors (Rothman and Olney 1995). The toxicity of excitatory amino acids and their analogs was discovered approximately 40 years ago (Olney et al. 1974). There are many ways to kill cells, but what is special about the phenomenon of excitoxicity is that cells are killed by the release of the endogenous excitatory transmitter, glutamate. The startling aspect of Rothman's experimental results, cited above, was that neurons were killed by their own activity. All the components necessary are already present; what's needed is a trigger of some sort to make these components behave in a way that is injurious to cells. Pathologically, lesions in any brain region are classified by degree of tissue damage; if neurons only are affected, the degree of tissue damage is called selective neuronal necrosis. As stated in Greenfield's classic neuropathology text: "The very existence of selective neuronal necrosis as a phenomenon is likely to be due in some way to the neuronal possession of receptors

for the excitatory neurotransmitter glutamate" (Auer and Sutherland 2002). Once glutamate receptor expression was mapped in the CNS, it became tempting to speculate that some well-known examples of selective vulnerability of neuronal populations in the CNS could be explained by the high density of glutamate receptors expressed in these populations (Table 6.1). One example is the sensitivity of the CA1 pyramidal neurons in the adult brain to global ischemia that is thought to be due to the high level of expression of NMDA receptors in these cells (Schmidt-Kastner and Freund 1991). Interestingly, high expression of NMDA receptors is not sufficient to produce selective vulnerability. For example, granule cells of the dentate gyrus are rich in NMDA receptors, yet do not show the vulnerability of CA1 pyramidal neurons to global ischemia. However, in the setting of hypoglycemia, large quantities of excitatory amino acids are released into the CSF, and in this setting the dentate gyrus is very vulnerable (Auer and Sutherland 2002) (Table 6.1). Why hypoglycemia and ischemia should produce different levels of glutamate release in the dentate gyrus and CA1 region is not clear. In any case, these observations suggest that exposure of vulnerable cells to extracellular excitatory amino acids is also critical, in addition to the intrinsic properties of neurons. This point is of great importance in assessing the vulnerability of oligodendrocytes and neurons in the developing brain, where sources of glutamate may be quite different than in the mature brain with its profusion of excitatory synaptic connections. Specifically, the coexpression of glutamate receptors and glutamate transporters in developing oligodendrocytes provides a source and a target for excitatory amino acids that may be released as a consequence of energy compromise, creating what has been described as a fatal feedback loop, or a suicide loop (Fern and Möller 2000).

It is generally accepted, then, that the high sensitivity of cerebral cortex and the insensitivity of white matter in the mature brain to ischemic injury is due to the differences in expression level of glutamate receptors in these two regions: high levels of glutamate receptor expression in neurons in the cortex; low levels of expression of glutamate receptors in mature oligodendrocytes. In the developing brain, the situation is reversed, and evidence has accumulated to suggest that excitotoxic injury best accounts for the sensitivity of white matter and predisposition to PVL.

The sensitivity of developing oligodendrocytes to excitotoxic injury is based on three factors (Fig. 6.5): a high expression of glutamate receptors, specifically calcium permeable AMPA receptors, a high expression of the glutamate transporter GLT-1/EAAT2, and relatively low expression of antioxidant enzymes. Oligodendrocytes grown in dissociated cell culture were found to be sensitive to excitotoxicity (Yoshioka et al. 1995; Matute et al. 1997, 2001; Matute 1998; McDonald et al. 1998; Sanchez-Gomez and Matute 1999), and this sensitivity was found to be restricted to developing oligodendrocytes; mature oligodendrocytes were found to be resistant (Rosenberg et al. 2003). The sensitivity of developing oligodendrocytes and insensitivity of mature oligodendrocytes could be explained by the higher levels of expression of glutamate receptors (Itoh et al. 2002; Rosenberg et al. 2003), and specifically calcium permeable GluR2 lacking AMPA receptors on developing oligodendrocytes (Deng et al. 2003). In an in vivo model of selective white matter injury induced by hypoxic–ischemic insult, injury could be blocked by

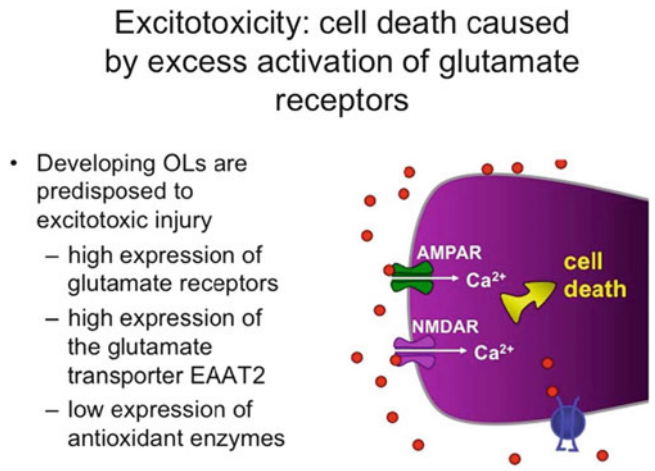


Fig. 6.5 Summary of evidence suggesting an important role for excitotoxicity as the initiating event in periventricular leukomalacia

the AMPA receptor antagonists NBQX and topiramate (Follett et al. 2000, 2004). Subsequent studies revealed that GluR2 lacking (calcium permeable) AMPA receptors on oligodendrocytes in developing white matter in the rat peak in expression at P7, an age when animals are most susceptible to selective white matter injury (Talos et al. 2006a; Dean et al. 2011). Most importantly for establishing a link between the in vitro and in vivo animal studies to the human disorder, GluR2 lacking AMPA receptors peak in expression in the developing human white matter during the period of peak vulnerability to PVL (Talos et al. 2006b). A recent study tested the efficacy of topiramate combined with hypothermia in treating term newborns who have signs of hypoxic encephalopathy (Filippi et al. 2012).

6.7 The Role of Glutamate Transporters

An essential component of the excitotoxic scenario is abnormal accumulation of glutamate in the extracellular space leading to excess activation of glutamate receptors. There are several potential sources of abnormal glutamate release, including synaptic release by exocytosis (Monyer et al. 1992), glutamate transporters "operating in reverse" due to the collapse of ion gradients in the setting of energy failure (Szatkowski et al. 1990; Attwell et al. 1993), swelling activated glutamate permeable channels in astrocytes (Kimelberg et al. 1990, 1995; Strange et al. 1996; Okada et al. 2009; Bowens et al. 2012), vesicular release from astrocytes (Parpura et al. 1994; Bezzi et al. 1998; Bezzi et al. 1999; Vesce et al. 1999; Bezzi et al. 2001; Fellin et al. 2004), leakage of glutamate across injured membranes (Tecoma et al. 1989). Synaptic release is likely to be time limited in the setting of energy failure, because

exocytosis is dependent upon nucleotide triphosphates; when ATP levels fall, synaptic release would be expected to stop (Banerjee et al. 1996; Morimoto and Ogihara 1996; Klenchin and Martin 2000; Mozhayeva et al. 2004; Meunier et al. 2005; Yao and Bajjalieh 2008). On the other hand, glutamate transporters are capable of transporting glutamate in both directions (Pines and Kanner 1990). They are secondary active transporters, which means that they rely on ionic gradients across the plasma membrane to provide the energy to power the work of concentrating glutamate inside cells (Zerangue and Kavanaugh 1996). When energy failure ensues, as in hypoxic–ischemic injury, ionic gradients collapse, and the transporters operate in reverse, becoming sources for glutamate rather than sinks. This phenomenon was first described in liposomes (Pines and Kanner 1990) and subsequently in brain slice preparations in a study that clearly recognized its importance for understanding ischemic injury (Szatkowski et al. 1990). As alluded already, an alternative way that energy failure can occur in cells is by release of nitric oxide from inflammatory cells, because nitric oxide can poison the Kreb's cycle and the electron transport chain (Beltrán et al. 2000; Riobó et al. 2001; Mander et al. 2005).

Glutamate transporters constitute a family of five genes, known in the human as EAAT1-5 (Danbolt 2001). The important transporters in the forebrain are EAAT1-3, known in the rat as GLAST, GLT-1, and EAAC1. GLAST and GLT-1 are primarily expressed in astrocytes. GLT-1 is the major transporter of the brain and is expressed in axon terminals as well (Chen et al. 2004). EAAC1 is often called the neuronal transporter. All three transporters are expressed in oligodendrocytes (DeSilva et al. 2009). As mentioned earlier, the concept of a suicide loop in developing oligodendrocytes has been suggested, whereby, in the setting of oxygen–glucose deprivation, cells release glutamate by reversal of glutamate transporters, which then acts upon glutamate receptors on the same cells to cause cell death. Evidence suggesting the existence of such a loop is that cell death could be blocked by glutamate receptor antagonists, and, importantly, also by an inhibitor of one particular glutamate transporter, GLT-1 (Fern and Möller 2000). Since all three glutamate transporters expressed in the forebrain are expressed in oligodendrocytes, it is not clear why GLT-1 in particular is important for the release of glutamate in oxygen–glucose deprivation. Now that a specific inhibitor of GLAST is available (Jensen et al. 2009), it would be interesting to test whether blocking GLAST also blocks OGD induced oligodendrocyte death, in which case there would be nothing special about GLT-1, or, whether only GLT-1 can fulfill this pathophysiological role. In addition to these in vitro studies, in vivo studies in the rat using semiquantitative immunocytochemistry showed that in the setting of hypoxia–ischemia, glutamate, which accumulates to high levels in both oligodendrocytes and axons, is depleted from both of them (Back et al. 2007), consistent with the idea that reversal of transport in oligodendrocytes is an important, though not exclusive, source for pathological accumulation of glutamate in developing white matter.

Given these experimental data implicating glutamate transporters and specifically GLT-1 in hypoxic–ischemic injury to developing oligodendrocytes, the expression of the human homolog EAAT2 and other glutamate transporters in developing white matter were investigated using in situ RNA hybridization. Remarkably, EAAT2 expression was developmentally regulated, and was increased in immature

white matter: although strongly expressed in developing oligodendrocytes in 32 week gestational age human brain, there was no detectable EAAT2 expression in oligodendrocytes in 7 month old human brain. In contrast, EAAT1 and EAAT3 expression either remained constant or, in the case of EAAT3, increased in the more mature brain (DeSilva et al. 2007). A similar pattern was found in the rat, in which GLT-1 was expressed in developing oligodendrocytes at P7, when myelin is starting to form, but no labeling was found in cells that expressed myelin basic protein, a marker of mature oligodendrocytes (DeSilva et al. 2009). These data support the concept that a fatal feedback loop exists in developing oligodendrocytes consisting of high expression of the glutamate transporter GLT-1/EAAT2 that provides a source of extracellular glutamate in the setting of energy failure, and GluR2 lacking AMPA receptors expressed on the same cells that, when activated, mediate excessive influx of calcium triggering excitotoxic injury and cell death. Our conception of excitotoxic injury in the immature brain therefore contrasts with that in the mature brain, in that in the immature brain, excitotoxicity may be a cell-autonomous process, in which certain populations of cells provide both the source and the target for pathological accumulations of glutamate. In the mature brain, the glutamate that is thought to kill neurons is derived from other cells—from excitatory terminals, or from neighboring astrocytes (Lipton and Rosenberg 1994). The existence of these cell-autonomous feedback loops may distinguish highly vulnerable targets in the developing brain and account for the particular patterns of cell death that characterize the developing brain, such as PVL. Another example of a cell-autonomous feedback loop may be layer V pyramidal cells in the developing cortex. In the last 5 years attention has been focused on the collateral neuronal damage that accompanies PVL (Pierson et al. 2007), and a careful study of neuronal loss in overlying cortex has revealed that only pyramidal neurons in layer V are depleted (Andiman et al. 2010). Glutamate receptor expression is not exclusive to layer V neurons in human cortex (Talos et al. 2006b), but it has been observed that layer V neurons, but not other neurons, heavily express EAAT2 (Desilva et al. 2012). Therefore, the loss of layer V neurons in the setting of PVL might be seen as another example of the importance of feedback loops to explain selective vulnerability in the developing brain. Alternative explanations are conceivable, for example retrograde degeneration caused by axonal disruption in the white matter.

6.8 Reconciling In Vitro and In Vivo Studies: The Role of Desensitization

An important property of membrane receptors is that upon continued exposure to their ligand their capacity to transmit a signal is diminished as a function of time (Gainetdinov et al. 2004). This property assures a time-limited signal even in the persistent presence of ligand, and probably functions to prevent excess activation of signaling networks within cells. There are at least two different phenomena that underlie this time dependent modulation of receptor activity: desensitization and inactivation.

The former refers to a structural change or posttranslational modification within the receptor itself that decreases the responsiveness of the receptor to ligand; the second refers to a process that removes receptor from the cell surface. Receptor desensitization is a prominent feature of the physiology of AMPA receptors, and has been shown to depend upon a structural rearrangement of the receptor following the binding of certain ligands, such as glutamate or AMPA (Weston et al. 2006) (Fig. 6.6, lower panel). Interestingly, for AMPA receptors, certain other ligands may activate the channel but not induce desensitization, and so produce non-desensitizing responses; the classic ligand of this type is kainate, which also is the cognate ligand for activating kainate receptors (Hollmann and Heinemann 1994).

In tissue culture, glutamate has been found to be toxic to developing oligodendrocytes, but only at high concentrations, and by a pathway (Oka et al. 1993) that is not blocked by glutamate receptor antagonists (Fig. 6.6, top panel), as is typical of oxidative glutamate toxicity. Kainate, in contrast, is toxic, and the explanation appears to be that kainate is non-densensitizing, because both glutamate and AMPA can be shown to be toxic at low concentrations by applying either of them together with a desensitization blocker, cyclothiazide (Yoshioka et al. 1995). In vivo, in animal models, hypoxia–ischemia induced injury to the white matter is blocked by AMPA receptor antagonists and so clearly is excitotoxic in nature (Follett et al. 2000, 2004) (Fig. 6.7, upper panels), implicating the endogenous transmitter glutamate, but certainly not kainate, which is naturally occurring in certain seaweeds, but not in the mammalian brain. In addition, in vitro experiments, using oxygen–glucose deprivation, have also demonstrated excitotoxic injury to oligodendrocytes (McDonald et al. 1998); here, too, the endogenous glutamate appears to be able to cause toxicity.

So, the question is why is desensitization protective in the tissue culture paradigm under normal conditions, but not in tissue culture in the setting of OGD or in vivo in the setting of hypoxic–ischemic insult? One possibility is that the desensitization process is defective in stressed cells; the other is that mechanisms downstream from activation of glutamate receptors determine whether cells die following activation of glutamate receptors—whether a given load of calcium will prove to be toxic or nontoxic. There is no evidence for or against the first possibility, which, in any case, seems unlikely given that desensitization in AMPA receptors is the result of a rapid structural rearrangement, which would seem to be immune to changes in the metabolic state of the cell (Fig. 6.6c). Evidence supporting the second possibility suggests that cells in culture are protected by the very high levels of insulin used in tissue culture that fully activate not only insulin receptors but also IGF-1 receptors. When insulin levels are reduced to physiological levels (5 ng/ml instead of the 5 μg/ml usually used), then developing oligodendrocytes in culture are killed by glutamate itself, despite normal desensitization (Ness and Wood 2002; Ness et al. 2002, 2004; Wood et al. 2007) (Fig. 6.6, middle panel). This toxicity of glutamate to developing oligodendrocytes is blocked by addition of IGF-1, consistent with the hypothesis that cross-reaction of insulin with the IGF-1 receptor is what protects oligodendrocytes in culture from the toxicity of glutamate. These studies suggest that insulin and IGF-1 levels in vivo are critical in determining whether cells live or

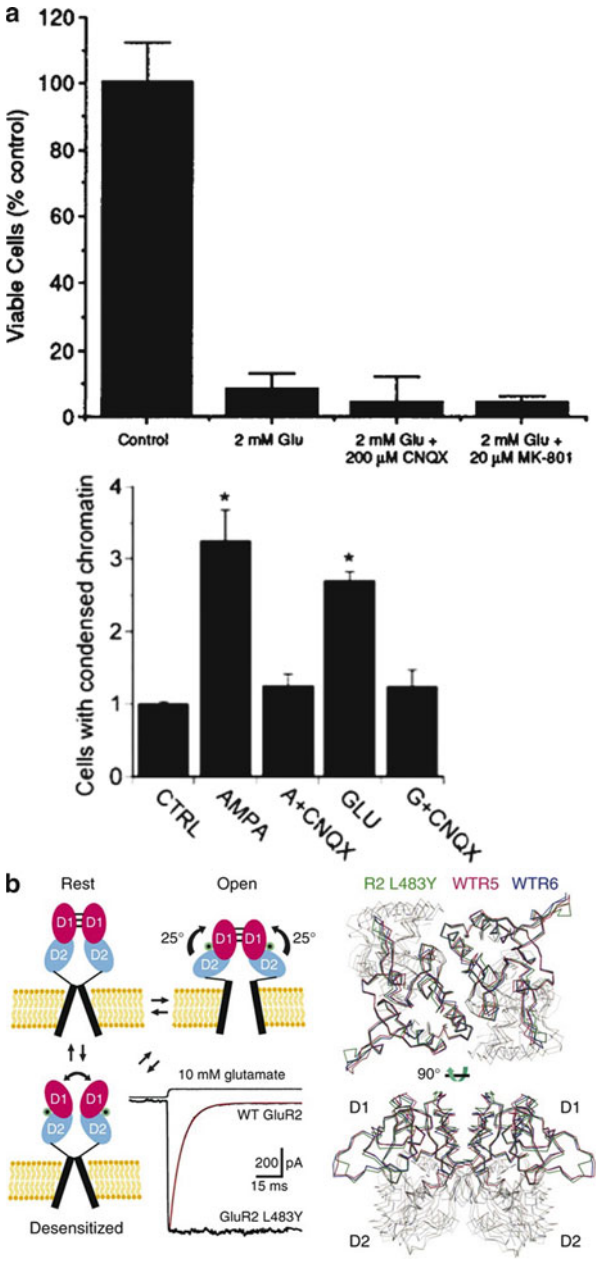


Fig. 6.6 Differing culture conditions determine whether oligodendrocytes in vitro are susceptible to glutamate induced excitotoxicity. *Upper panel* Failure of CNQX and MK-801 to protect oligodendroglia from L-glutamate toxicity. Oligodendroglia were incubated with 2 mM L-glutamate in EBSS in the presence of 200 µM CNQX or 20 µM MK-801 for 24 h, and viable cells were counted. Data are presented as the percentage of viable cells compared to control cultures. CNQX and MK-801 were not able to block L-glutamate toxicity in oligodendroglia (no significant difference compared to cultures with 2 mM glutamate by ANOVA). Values represent the mean ± SD in an experiment performed in triplicate. Similar results were observed in two other experiments. (from: Oka et al. 1993), Fig. 5). *Middle panel* Pre-oligodendrocyte death is induced by 24-h exposure to glutamate and the glutamate agonist AMPA. Pre-oligodendrocytes were exposed to 500 µM glutamate for 24 h and analyzed for

die following strong activation of glutamate receptors. Interestingly, there is evidence for decrease in IGF-1 levels in the perinatal brain in the setting of ischemia (Lee et al. 1996), and also that IGF-1 confers protection against ischemic injury (Wood et al. 2007).

6.9 NMDA Receptors on Oligodendrocytes

The expression of NMDA receptors in oligodendrocytes was not appreciated initially because of the absence of NMDA receptor mediated currents in cultured oligodendrocytes. Three papers published in 2005 and 2006, however, demonstrated that in vivo, NMDA receptors are expressed on oligodendrocytes, and that NMDA receptors play a role in ischemic injury to mature white matter (Karadottir et al. 2005; Salter and Fern 2005; Micu et al. 2006). There have been no studies demonstrating developmental stage dependent expression of NMDA receptors on oligodendrocytes. Manning et al. showed that the NMDA receptor antagonist memantine is protective in the same model of selective white matter injury in which it had been demonstrated that the non-NMDA receptor antagonists NBQX and topiramate are protective (Manning et al. 2008) (Fig. 6.7, lower panels). These observations raise the question of how AMPA/kainate receptor and NMDA receptor activation could both lead to white matter injury in this model, since when either one or the other type of specific antagonist is used, the receptors targeted by the other type of antagonist remain unblocked. The possibilities are that the receptors are on different cell types, and both have to be activated for injury to occur; that receptors are on the same cell type, and are epistatic, for example if NMDA receptor activation were

Fig. 6.6 (continued) DNA fragmentation and chromatin condensation. Cells with condensed chromatin were quantified from three fields per coverslip ($n=3$ coverslips), and results are shown as fold increase in cells with chromatin condensation. AMPA and glutamate results are from two separate representative experiments. *$P<0.001$ vs. CTRL. Error bars, SEM. (from: Ness and Wood 2002, Fig. 1f). *Lower panel* Structural basis for desensitization of glutamate receptor ion channels. (**a**) Cartoon of an AMPA receptor dimer during transitions from the resting, ligand-free state to the glutamate-bound open state and the desensitized state. Channel opening results from domain closure; desensitization is triggered by separation of domain 1 dimer contacts, allowing the pair of subunits to rotate and close the ion channel. Chart shows superimposed responses to 10 mM glutamate recorded from outside out patches from HEK cells transfected with wild-type GluR2 and the nondesensitizing L483Y mutant; the onset of desensitization for the wild-type response is fit with a single exponential of time constant 6 ms, shown in *red*. (**b**) Ca traces for dimer crystal structures of the GluR2 L483Y mutant complex with AMPA (*green*, PDB 1LB8), the wild-type GluR5 complex with glutamate (*red*, PDB 2F36) and the wild-type GluR6 complex with domoate (*blue*, PDB 1YAE). The structures were superimposed by the least-squares method using domain 1 coordinates; the view at top is from the N terminus, looking down onto the plane of the membrane; the view at bottom is rotated by 90°. Owing to differences in domain closure, the coordinates for domain 2 do not superimpose and are drawn with transparent shading. (from: Weston et al. 2006, Fig. 1)

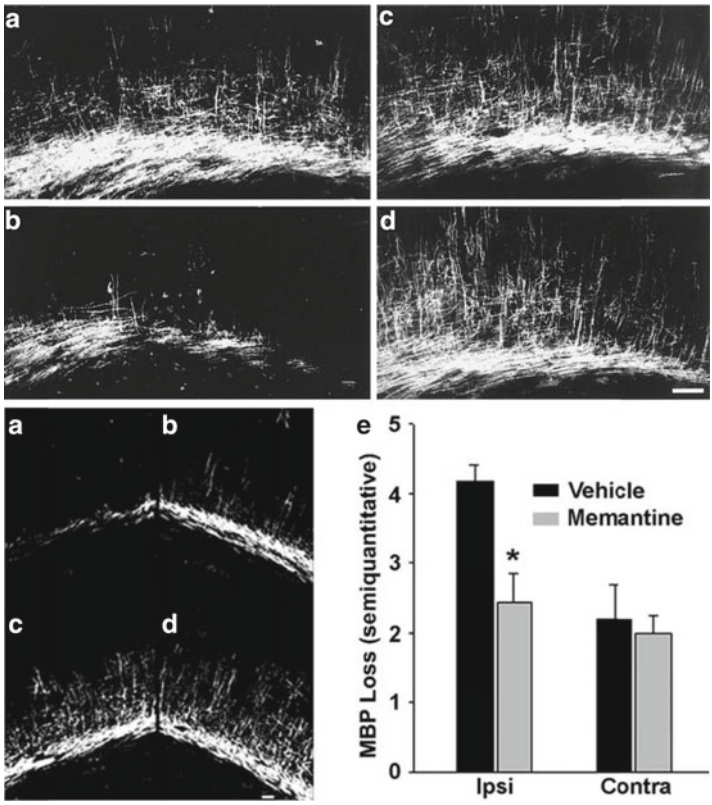


Fig. 6.7 Both a non-NMDA receptor antagonists and an NMDA receptor antagonist block selective white matter injury in a rodent model of neonatal hypoxia–ischemia**.** *Upper panel* Effect of NBQX on MBP expression in cerebral white matter after hypoxia–ischemia. (**a**)–(**d**), MBP expression in the subcortical white matter of a P11 rat after unilateral carotid ligation and hypoxia at P7, with and without NBQX treatment. MBP staining of white matter tracts contralateral (**a**) and ipsilateral (**b**) to the ligation in a vehicle-treated control and contralateral (**c**) and ipsilateral (**d**) to the ligation in a littermate post-treated with NBQX demonstrates significant attenuation of myelin loss with treatment. Scale bar, 100 mm. (from: Follett et al 2000), Fig. 4). *Lower panel.* Memantine significantly attenuates loss of MBP at 72 h after injury. (**a**)–(**d**), Representative MBP immunohistochemistry: vehicle-treated (**a**, **b**) and memantine-treated (**c, d**), ipsilateral (**a, c**) and contralateral (**b, d**) to carotid artery ligation. Scale bar, 100 m. (**e**), MBP loss based on a five-point semiquantitative scale (0 = no loss; 5 = maximum loss). Memantine significantly attenuates MBP loss ipsilateral to carotid artery ligation ($n = 11$ vehicle; $n = 14$ memantine; t test, $p < 0.008$). Error bars denote SEM. (from: Manning et al. 2008, Fig. 4)

dependent upon non-NMDA receptor activation. Since NMDA receptor activation depends on membrane depolarization to relieve the magnesium block, this latter explanation is plausible. Evidence suggesting a role for NMDA receptors in activation of microglia supports the possibility that the injury to oligodendrocytes is not cell-autonomous (Streit et al. 1992; Kaindl et al. 2012).

6.10 Immaturity of Antioxidant Defenses in the Developing Brain

The expression of glutamate receptors and glutamate transporters seems to offer a partial explanation of the selective vulnerability of developing white matter, but it is not likely to be the whole explanation. Another aspect of the vulnerability is the immaturity of systems designed to react and respond to various kinds of cellular stress. One of the most important of these is oxidative stress. In the early work documenting the sensitivity of oligodendrocytes to the effects of glutathione depletion, it was found that developing oligodendrocytes are killed more rapidly than their MBP expressing counterparts. This form of cell death appeared to be mediated by activation of 12-lipoxygenase (Wang et al. 2004), an important enzyme of arachidonic acid metabolism (Piomelli and Greengard 1990), because inhibitors of 12-lipoxygenase were protective. One explanation for the higher sensitivity of developing oligodendrocytes might be higher levels of expression of 12-lipoxygenase, but this was found to be not true (Wang et al. 2004). 12-lipoxygenase is an enzyme whose activity is regulated by the levels of peroxide in cells (Tan et al. 2001). An investigation into the expression and activity of cellular defenses against peroxide in oligodendrocytes found a striking inactivity of developing oligodendrocytes in removing extracellular peroxide from the cell medium compared with mature oligodendrocytes (Fig. 6.8), and this difference appeared to be based in low levels of

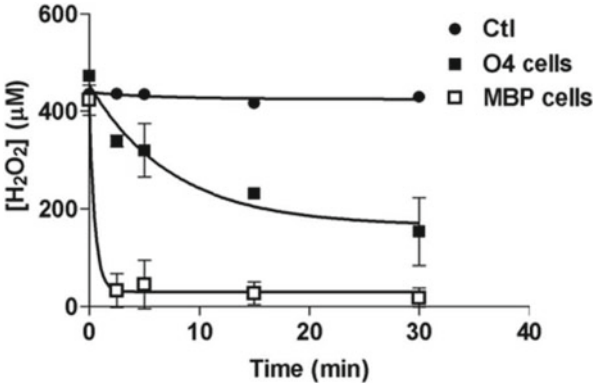


Fig. 6.8 Mature oligodendrocytes clear hydrogen peroxide more rapidly and more completely than developing oligodendrocytes. (**a**), Comparison of disposal of extracellular H_2O_2 between developing (O4+) and mature (MBP+) oligodendrocytes. Cells were incubated in EBSS containing 400 μM H_2O_2 and the amount of H_2O_2 remaining at selected time points was determined. The experiment was performed on three independently prepared cultures with comparable results. Data represent a typical experiment with means ±SD of triplicate values. (**b**) Comparison of reactive oxygen species (ROS) generation in developing and mature oligodendrocytes subjected to increasing concentrations of H_2O_2. Cells were loaded with 100 μM DCFA solution and incubated for 20 min at 37 °C. Results represent the percentage increase in the fluorescence measured at excitation and emission wavelengths of 485 and 530 nm between baseline and after 1 h of H_2O_2 exposure. Data represent means ±SD of a representative experiment replicated three times. $^{**}p<0.01$; $^{***}p<0.001$, using two-way ANOVA with Bonferroni posttest comparison. (from: Baud et al. 2004), Fig. 3a)

expression of glutathione peroxidase (Baud et al. 2004a). In this study only GPx1 was examined, and it would be of great interest to compare levels of expression of GPx4, which has been specifically implicated in the regulation of 12-LOX activity (Seiler et al. 2008). In addition to glutathione peroxidase activity, MnSOD was also found to be expressed at low levels in developing oligodendrocytes (Baud et al. 2004b). Supplementation of the activity of developing oligodendrocytes with a GPx mimic, ebselen, or MnSOD itself using viral mediated expression, conferred protection in these cells against the toxicity of glutathione depletion. The logical question then was to ask whether supplementing antioxidant activity in developing oligodendrocytes might protect them against excitotoxic injury. Using compounds with mixed catalase-superoxide dismutase activity, Deng et al. showed that developing oligodendrocytes were protected against the toxicity of oxygen-glucose deprivation (Deng et al. 2004). Therefore, another component of the special vulnerability of developing oligodendrocytes appears to be immaturity of antioxidant defenses, consistent with observations that suggest that oxidative injury is an important component of the process that leads to cell death after an excitotoxic insult (Nicholls 2004; Brennan et al. 2009; Nicholls 2009; Reyes et al. 2012). Oligodendrocytes accumulate iron, which may promote free radical generation, and iron supplementation has been shown in animal models to aggravate white matter injury in a neonatal mouse model (Dommergues et al. 1998; Khwaja and Volpe 2008). Interestingly, endotoxin induced white matter injury in the neonatal rat was associated with depletion of glutathione, and was ameliorated by administration of *N*-acetyl cysteine, a membrane permeable precursor of glutathione (Paintlia et al. 2004, 2008).

6.11 Other Insults Contribute to PVL

PVL is of uncertain etiology at present, and, in a given case, has an indefinite time of onset, with some evidence suggesting initiation antenatally in a subset of affected infants (Watanabe et al. 1999; Leviton et al. 2011). It is a complex lesion that evolves over time, and as such involves multiple pathological processes. What we have been focusing on thus far is what seems to be the most likely scenario for the first step in the pathogenesis, and the best explanation we have for the selectivity of the injury. It is likely that other processes contribute to PVL, including activation of astrocytes and the consequences of this activation, including failure of appropriate oligodendrocyte differentiation and myelination (Back et al. 2005b; Segovia et al. 2008; Buser et al. 2012), microglial invasion and activation (Haynes et al. 2003; Billiards et al. 2006; Volpe et al. 2011), axonal injury (Haynes et al. 2005, 2008; Volpe 2009b), secondary waves of oligodendrocyte death and proliferation (Segovia et al. 2008) (Fig. 6.9). It seems likely that excitotoxicity will turn out to be only one part of the pathogenesis of PVL, and important questions concern the role that other types of insults might contribute to injury of white matter, and how therapies targeting them might promote recovery and repair. It also seems likely that the most efficacious way to prevent PVL is to find ways to prevent the initial insult, the one that sets the other processes in motion.

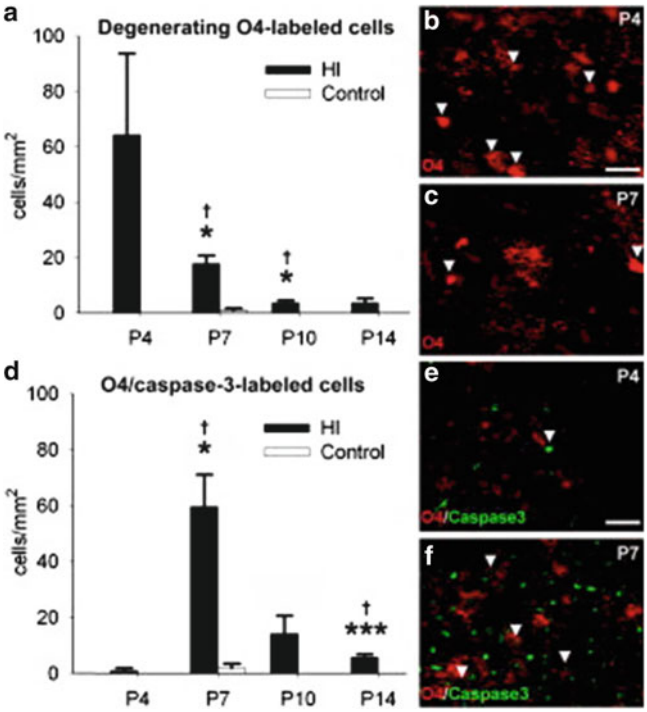


Fig. 6.9 Non-apoptotic and apoptotic waves of death in oligodendrocytes in a rodent model of neonatal hypoxia–ischemia. Perinatal hypoxia–ischemia (HI) triggers acute preoligodendrocyte (preOL) degeneration that is mostly independent of caspase-3 activation and delayed preOL degeneration that is caspase-3 dependent. (**a**) The density of O4-labeled cells with morphological features of degeneration peaked 24 h after HI (postnatal day 4 [P4]) and remained significantly increased at P7 (†$p<0.02$, univariate analysis of covariance [ANCOVA]; *$p<0.04$, paired samples t test) and P10 (†$p<0.04$, univariate ANCOVA; *$p<0.04$, paired samples t test; $n=3$ per each survival time). *Black bars* represent hypoxia–ischemia; *white bars* represent controls. (**b**) Degenerating O4-labeled cells at P4 (*arrowheads*) typically had fragmented processes and cytoplasmic O4 staining, consistent with disrupted membrane integrity. (**c**) At P7, degenerating O4-labeled cells (*arrowheads*) were reduced relative to P4. (**d**) The density of O4+/cleaved-caspase-3+ cells was low at P4, peaked at P7 (†$p<0.03$, univariate ANCOVA; *$p<0.04$, paired samples t test), and remained increased at P14 (†$p=0.000$, univariate ANCOVA; ***$p<0.01$, paired samples t test; $n=3$ per each survival time). (**e**) At P4, few O4-labeled cells (*red*) stained for cleaved caspase-3 (*green*). (**f**) At P7, numerous O4-labeled cells were caspase-3–positive. Scale bars = 100 μm. (from: Segovia et al. 2008, Fig. 1)

6.12 What Death Pathways are Activated in PVL?

Prior to the emergence of a scientific basis for the goal of neuroprotection, it was thought that cell death was a passive process of cells falling apart or ceasing to function in a nonspecific way. The emergence of our understanding of oxidative stress and its control by antioxidant programs of enzyme expression in cells was accompanied by the idea that oxidative stress might be responsible for cell injury and death in many circumstances. Oxygen-free radicals were thought to nonspecifically

react with and inactivate proteins, lipids, and DNA because of their high reactivity, something akin to a cellular fire. Given this view it would not make much sense to try to intervene to prevent cell death, except to put out the fire, leading to great interest in the use of antioxidants to prevent cell injury and death. The meager results of antioxidant therapeutics suggest that the concept that oxidative stress is a primary cause of cell injury in itself is inadequate, overly simplistic, or possibly wrong. Conceivably, the explanation for this disappointing record is that it is very difficult to get the antioxidant to the right place, at the right time, and in adequate amounts (but note that, in contrast, glutamate receptor antagonists are very effective in animal models). Most work on oxidative stress in brain injury never asks whether the observed evidence for oxidative stress reflects a primary role in the injury process, or is a secondary or even more remote consequence of injury.

In the past 30 years, cell death mechanisms have become a very active area of research. A key event was the recognition of the phenomenon of programmed cell death, which was observed to be distinctly different from necrosis (Kerr et al. 1972; Wyllie et al. 1980). Subsequently, a key protein in the pathway underlying programmed cell death in C. elegans, and the comparable pathway in mammals (Miura et al. 1993; Yuan et al. 1993) were discovered. A consensus emerged that there are three forms of cell death: apoptosis or programmed cell death, autophagy associated cell death, and necrosis, known as Type I, II, and III cell death (Hotchkiss et al. 2009). Necrosis has for a long time been thought of as a non-regulated form of cell death. Similarly, as mentioned, oxidative injury caused by reactive oxygen species (ROS) and reactive nitrogen species, has generally been conceived of as a nonspecific attack on the different constituents of the cell, resulting in overwhelming damage.

However, it has become clear that what is called necrosis can be regulated as well. This has come to be widely recognized only in the past few years, but evidence for regulated necrotic death started to emerge about 20 years ago. It proved possible to induce oxidative injury to immature cerebral neurons or cell lines in culture by glutamate that was dependent on blocking cystine uptake rather than activating glutamate receptors (Murphy et al. 1989b, 1990). The result of blocking cystine uptake was depletion of intracellular glutathione, resulting in oxidative injury and necrotic cell death. These investigators had some evidence that this process was dependent upon the metabolism of arachidonic acid, but didn't publish these data in a full paper (Murphy et al. 1989a). This form of toxicity required the activation of 12-lipoxygenase (Li et al. 1997). Arachidonic acid metabolism is highly important in cells, and there are three major pathways for the transformation of arachidonic acid in cells: the cyclooxygenase (COX) pathway of prostaglandin synthesis, the LOX pathway of leukotriene synthesis (Katsuki and Okuda 1995), and a third pathway of arachidonic acid metabolism by cytochrome P 450 arachidonic acid monooxygenase (Capdevila and Falck 2002). Lipoxygenases are important not only for the lipid mediators that they produce, but also as a catalytic source of lipid peroxides (Brash 1999). LOX appears to be negatively regulated by glutathione, and positively regulated or activated by lipid peroxides; in fact, the lipid peroxide tone of the cell sets the activity of lipoxygenases. When glutathione levels fall, lipid peroxides

rise because their accumulation is controlled by glutathione peroxidases, especially GPx4 (Seiler et al. 2008). LOX is activated by this elevation in peroxide tone and becomes a catalytic source for ROS, specifically lipid peroxides. Why there should be such a positive feedback loop, whereby elevation in lipid peroxide tone activates LOX generating more lipid peroxides is unclear, especially given the deleterious effects of 12-LOX activation. Products of lipid peroxidation accumulate in PVL (Inder et al. 2002; Back et al. 2005a; Welin et al. 2007) and are toxic to developing oligodendrocytes (Brault et al. 2004).

Developing oligodendrocytes are highly sensitive to cell death induced by glutathione depletion, and this cell death is dependent upon 12-LOX activity. Arachidonic acid itself is toxic to oligodendrocytes, and this toxicity can be blocked by 12-LOX inhibitors (Wang et al. 2004). Seiler and colleagues have shown that GPx4 specifically regulates the activity of 12-LOX, and that in neurons, the death pathway activated by GPx4 downregulation is dependent upon the translocation of AIF (Seiler et al. 2008). Studies by DeFranco looking at the effects of GSH depletion in neurons and cells lines (Ho et al. 2008) and our own studies of the mechanism of peroxynitrite toxicity to oligodendrocytes as well as neurons have shown that MAP kinases are involved in the toxicity of RNS (Zhang et al. 2004, 2006, 2007). All of these data together indicate that oxidative injury may occur by activation of a regulated pathway that involves 12-LOX, MAP kinases, and AIF (Zhang et al. 2007; Seiler et al. 2008). Inhibiting 12-LOX may be beneficial in the prevention of injury following ischemia to the mature brain (van Leyen et al. 2006; Jin et al. 2008; van Leyen et al. 2008; Pallast et al. 2009, 2010; Pekcec et al. 2012; Yigitkanli et al. 2012).

The idea that necrosis can be a regulated form of cell death has been vigorously pursued by Yuan and colleagues (Degterev et al. 2005). They started with a screen of inhibitors that blocked TNFα induced non-apoptotic cell death, and discovered the compound they named necrostatin. Subsequently, the same group showed that the target of necrostatin is RIP1 kinase (Degterev and Yuan 2008; Hitomi et al. 2008). RIP1 kinase activity is considered to be required for the upstream signaling events inducing regulated necrosis, and there are multiple pathways that may take part in the destruction phase, including lipoxygenase (Fig. 6.10).

Given the evidence that oxidative stress induced cell death in oligodendrocytes is a program of cell death involving 12-LOX activation, the question arose whether programmed cell death in oligodendrocytes is a RIP1 kinase dependent form of cell death. Necrostatin (NEC-1) was, in fact, found to be an effective inhibitor of oligodendrocyte death caused by cystine deprivation, inhibition of glutathione synthesis by buthionine sulfoximine, or exposure to arachidonic acid (Kim et al. 2010). In addition, necrostatin was very effective at blocking the production of ROS, although it is not a free radical scavenger (Degterev et al. 2005). These results suggest that the 12-LOX pathway of injury in oligodendrocytes is a necroptotic pathway, in the broadest sense that necroptosis is a regulated form of necrotic cell death. Whether RIP 1 kinase is in fact involved would require more rigorous studies to be sure that the effects of NEC-1 are due to on-target rather than off-target effects (Takahashi et al. 2012; Degterev et al. 2013; Vandenabeele et al. 2013). In any case, the relevance of necroptosis to PVL has been shown in studies in which NEC-1 was

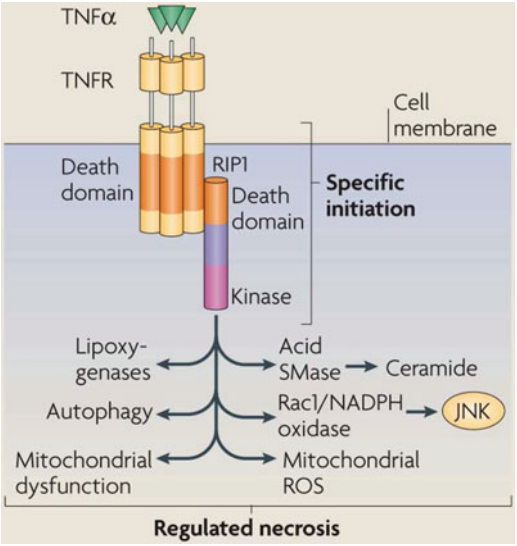


Fig. 6.10 Regulated necrotic cell death. Necroptosis. The activation of RIP1 kinase increases ROS production (from the mitochondrial respiratory chain and the RIP1–Rac1–NADPH oxidase complex) and activates c-Jun N-terminal kinase (JNK) kinase (which might be crucial for the execution of necroptotic cell death in some cell types). Other execution steps, including the activation of phospholipase A2, lipoxygenases, and acid sphingomyelinase, have been described. The exact roles of these steps remain to be elucidated. Given the similarity of many of the downstream execution steps in necroptosis to those attributed to "classic" unregulated necrosis, the main difference between these two mechanisms might be in the method of activation (regulated by internal signaling mechanisms rather than caused by overwhelming stress) of similar relatively nonspecific execution events. *SMase* sphingomyelinase, *TNFα* tumor-necrosis factor-α, *TNFR* TNFα receptor. (from: Degterev and Yuan 2008, Box 2)

protective in a model of neonatal brain injury (Northington et al. 2011; Chavez-Valdez et al. 2012). It would be of great interest to characterize the role of these various forms of cell death in oligodendrocyte death following excitotoxic injury. However, existing models of excitotoxicity to oligodendrocytes in culture are unlikely to be physiologically relevant in that they require kainate or other means to block desensitization, and therefore represent a prolonged and extremely severe insult likely to activate processes that are not necessarily relevant for understanding the pathogenesis of PVL.

6.13 Future of the Neuroprotection Project

It is frustrating, but not surprising, that we have yet to witness the emergence of effective treatments for the prevention or amelioration of any degenerative CNS disorder. It has been approximately 20 years since the discovery of the gene mutation in

Table 6.2 Why there are no therapies based on our knowledge of excitotoxicity

Clinical trial designs do not replicate narrow therapeutic time-windows observed in animal models
Clinical examination is not sensitive enough
Clinical scenarios typically involve multiple diseases (diabetes, hypertension, age)
Differences between animals and humans
Pharmacological approaches that target a single intracellular mechanism in a single cell type may be too limited

Huntington's disease (1993), which, unlike other chronic neurodegenerative diseases, is 100 % genetic. It was once thought that if we knew the gene involved in a disease then we could figure out the pathogenesis and how to cure it. Although this dream will eventually come true, it is still not an easy task. In the case of stroke, a much more complex problem, but with clear etiology, early hopes based on an emerging knowledge of excitotoxicity have come to very little. It is instructive to briefly review the explanation for this lack of progress in the treatment of stroke. A useful review has pointed to several explanations for this lack of progress (Lo et al. 2003) (Table 6.2). In the case of PVL, we have a disorder that is more difficult to understand than stroke, in that the etiology remains unclear. Without knowing the etiology, it is difficult or perhaps impossible to develop an animal model that we can be certain represents an accurate model for the disorder. However, it is important to focus on what we **can** say about it, i.e., that it represents selective injury to white matter, that selective injury to other regions and in response to a range of insults often can be best explained at this time by excitotoxic damage, and that in the case of PVL, the evidence that has emerged suggests that excitotoxic injury can best account for the selective injury that PVL represents. Therefore, it seems likely that using excitotoxic injury as the focal point in studies of pathogenesis and mechanisms of cell death will continue to be a productive route of inquiry that will lead soon to the development of effective means to prevent and treat PVL.

References

(1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's Disease Collaborative Research Group. Cell 72: 971–983

Aden U, Favrais G, Plaisant F, Winerdal M, Felderhoff-Mueser U, Lampa J, Lelievre V, Gressens P (2010) Systemic inflammation sensitizes the neonatal brain to excitotoxicity through a pro-/anti-inflammatory imbalance: key role of TNFalpha pathway and protection by etanercept. Brain Behav Immun 24:747–758

Alexopoulos H, Kosmidis ML, Dalmau J, Dalakas MC (2011) Paraneoplastic anti-NMDAR encephalitis: long term follow-up reveals persistent serum antibodies. J Neurol 258: 1568–1570

Andiman SE, Haynes RL, Trachtenberg FL, Billiards SS, Folkerth RD, Volpe JJ, Kinney HC (2010) The cerebral cortex overlying periventricular leukomalacia: analysis of pyramidal neurons. Brain Pathol 20:803–814

Attwell D, Barbour B, Szatkowski M (1993) Nonvesicular release of neurotransmitter. Neuron 11:401–407
Auer R, Kalimo H, Olsson Y, Wieloch T (1985) The dentate gyrus in hypoglycemia: pathology implicating excitotoxin-mediated neuronal necrosis. Acta Neuropathol 67:279–288
Auer RN (1986) Progress review: hypoglycemic brain damage. Stroke 17:699–708
Auer RN (1991) Excitotoxic mechanisms, and age-related susceptibility to brain damage in ischemia, hypoglycemia and toxic mussel poisoning. Neurotoxicology 12:541–546
Auer RN (2004) Hypoglycemic brain damage. Metab Brain Dis 19:169–175
Auer RN, Sutherland GR (2002) Hypoxia and related conditions. In: Graham DI, Lantos PL (eds) Greenfield's neuropathology. Arnold, New York, pp 233–280
Auer RN, Hugh J, Cosgrove E, Curry B (1989) Neuropathologic findings in three cases of profound hypoglycemia. Clin Neuropathol 8:63–68
Back SA, Riddle A, Dean J, Hohimer AR (2012) The instrumented fetal sheep as a model of cerebral white matter injury in the premature infant. Neurotherapeutics 9:359–370
Back SA, Gan X, Li Y, Rosenberg PA, Volpe JJ (1998) Maturation-dependent vulnerability of oligodendrocytes to oxidative stress-induced death caused by glutathione depletion. J Neurosci 18:6241–6253
Back SA, Luo NL, Borenstein NS, Levine JM, Volpe JJ, Kinney HC (2001) Late oligodendrocyte progenitors coincide with the developmental window of vulnerability for human perinatal white matter injury. J Neurosci 21:1302–1312
Back SA, Craig A, Kayton RJ, Luo NL, Meshul CK, Allcock N, Fern R (2007) Hypoxia-ischemia preferentially triggers glutamate depletion from oligodendroglia and axons in perinatal cerebral white matter. J Cereb Blood Flow Metab 27:334–347
Back SA, Han BH, Luo NL, Chricton CA, Xanthoudakis S, Tam J, Arvin KL, Holtzman DM (2002) Selective vulnerability of late oligodendrocyte progenitors to hypoxia-ischemia. J Neurosci 22:455–463
Back SA, Luo NL, Mallinson RA, O'Malley JP, Wallen LD, Frei B, Morrow JD, Petito CK, Roberts CT Jr, Murdoch GH, Montine TJ (2005a) Selective vulnerability of preterm white matter to oxidative damage defined by F2-isoprostanes. Ann Neurol 58:108–120
Back SA, Tuohy TM, Chen H, Wallingford N, Craig A, Struve J, Luo NL, Banine F, Liu Y, Chang A, Trapp BD, Bebo BF Jr, Rao MS, Sherman LS (2005b) Hyaluronan accumulates in demyelinated lesions and inhibits oligodendrocyte progenitor maturation. Nat Med 11:966–972
Bal-Price A, Brown GC (2001) Inflammatory neurodegeneration mediated by nitric oxide from activated glia-inhibiting neuronal respiration, causing glutamate release and excitotoxicity. J Neurosci 21:6480–6491
Banerjee A, Barry VA, DasGupta BR, Martin TF (1996) N-Ethylmaleimide-sensitive factor acts at a prefusion ATP-dependent step in Ca2+-activated exocytosis. J Biol Chem 271:20223–20226
Banker BQ, Larroche JC (1962) Periventricular leukomalacia of infancy. A form of neonatal anoxic encephalopathy. Arch Neurol 7:386–410
Baud O, Greene AE, Li J, Wang H, Volpe JJ, Rosenberg PA (2004a) Glutathione peroxidase-catalase cooperativity is required for resistance to hydrogen peroxide by mature rat oligodendrocytes. J Neurosci 24:1531–1540
Baud O, Haynes RF, Wang H, Folkerth RD, Li J, Volpe JJ, Rosenberg PA (2004b) Developmental up-regulation of MnSOD in rat oligodendrocytes confers protection against oxidative injury. Eur J Neurosci 20:29–40
Beltrán B, Mathur A, Duchen MR, Erusalimsky JD, Moncada S (2000) The effect of nitric oxide on cell respiration: a key to understanding its role in cell survival or death. Proc Natl Acad Sci U S A 97:14602–14607
Bezzi P, Vesce S, Panzarasa P, Volterra A (1999) Astrocytes as active participants of glutamatergic function and regulators of its homeostasis. Adv Exp Med Biol 468:69–80
Bezzi P, Carmignoto G, Pasti L, Vesce S, Rossi D, Rizzini BL, Pozzan T, Volterra A (1998) Prostaglandins stimulate calcium-dependent glutamate release in astrocytes. Nature 391: 281–285

Bezzi P, Domercq M, Brambilla L, Galli R, Schols D, De Clercq E, Vescovi A, Bagetta G, Kollias G, Meldolesi J, Volterra A (2001) CXCR4-activated astrocyte glutamate release via TNFalpha: amplification by microglia triggers neurotoxicity. Nat Neurosci 4:702–710

Billiards SS, Haynes RL, Folkerth RD, Trachtenberg FL, Liu LG, Volpe JJ, Kinney HC (2006) Development of microglia in the cerebral white matter of the human fetus and infant. J Comp Neurol 497:199–208

Blandini F (2010) An update on the potential role of excitotoxicity in the pathogenesis of Parkinson's disease. Funct Neurol 25:65–71

Bode MM, D'Eugenio DB, Forsyth N, Coleman J, Gross CR, Gross SJ (2009) Outcome of extreme prematurity: a prospective comparison of 2 regional cohorts born 20 years apart. Pediatrics 124:866–874

Bowens NH, Dohare P, Kuo YH, Mongin AA (2012) DCPIB, the proposed selective blocker of volume-regulated anion channels, inhibits several glutamate transport pathways in glial cells. Mol Pharmacol 83(1):22–32

Brash AR (1999) Lipoxygenases: occurrence, functions, catalysis, and acquisition of substrate. J Biol Chem 274:23679–23682

Brault S, Martinez-Bermudez AK, Roberts J 2nd, Cui QL, Fragoso G, Hemdan S, Liu HN, Gobeil F Jr, Quiniou C, Kermorvant-Duchemin E, Lachance C, Almazan G, Varma DR, Chemtob S (2004) Cytotoxicity of the E(2)-isoprostane 15-E(2t)-IsoP on oligodendrocyte progenitors. Free Radic Biol Med 37:358–366

Brennan AM, Suh SW, Won SJ, Narasimhan P, Kauppinen TM, Lee H, Edling Y, Chan PH, Swanson RA (2009) NADPH oxidase is the primary source of superoxide induced by NMDA receptor activation. Nat Neurosci 12:857–863

Brody BA, Kinney HC, Kloman AS, Gilles FH (1987) Sequence of central nervous system myelination in human infancy. I. An autopsy study of myelination. J Neuropathol Exp Neurol 46: 283–301

Brown GC, Bal-Price A (2003) Inflammatory neurodegeneration mediated by nitric oxide, glutamate, and mitochondria. Mol Neurobiol 27:325–355

Buser JR, Segovia KN, Dean JM, Nelson K, Beardsley D, Gong X, Luo NL, Ren J, Wan Y, Riddle A, McClure MM, Ji X, Derrick M, Hohimer AR, Back SA, Tan S (2010) Timing of appearance of late oligodendrocyte progenitors coincides with enhanced susceptibility of preterm rabbit cerebral white matter to hypoxia-ischemia. J Cereb Blood Flow Metab 30:1053–1065

Buser JR, Maire J, Riddle A, Gong X, Nguyen T, Nelson K, Luo NL, Ren J, Struve J, Sherman LS, Miller SP, Chau V, Hendson G, Ballabh P, Grafe MR, Back SA (2012) Arrested preoligodendrocyte maturation contributes to myelination failure in premature infants. Ann Neurol 71: 93–109

Capdevila JH, Falck JR (2002) Biochemical and molecular properties of the cytochrome P450 arachidonic acid monooxygenases. Prostaglandins Other Lipid Mediat 68–69:325–344

Chavez-Valdez R, Martin LJ, Flock DL, Northington FJ (2012) Necrostatin-1 attenuates mitochondrial dysfunction in neurons and astrocytes following neonatal hypoxia-ischemia. Neuroscience 219:192–203

Chen W, Mahadomrongkul V, Berger UV, Bassan M, DeSilva T, Tanaka K, Irwin N, Aoki C, Rosenberg PA (2004) The glutamate transporter GLT1a is expressed in excitatory axon terminals of mature hippocampal neurons. J Neurosci 24:1136–1148

Coyle JT, Puttfarcken P (1993) Oxidative stress, glutamate, and neurodegenerative disorders. Science 262:689–695

Craig A, Ling Luo N, Beardsley DJ, Wingate-Pearse N, Walker DW, Hohimer AR, Back SA (2003) Quantitative analysis of perinatal rodent oligodendrocyte lineage progression and its correlation with human. Exp Neurol 181:231–240

Dalmau J, Tuzun E, Wu HY, Masjuan J, Rossi JE, Voloschin A, Baehring JM, Shimazaki H, Koide R, King D, Mason W, Sansing LH, Dichter MA, Rosenfeld MR, Lynch DR (2007) Paraneoplastic anti-N-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. Ann Neurol 61:25–36

Dammann O, Leviton A (2004) Inflammatory brain damage in preterm newborns–dry numbers, wet lab, and causal inferences. Early Hum Dev 79:1–15
Dammann O, Hagberg H, Leviton A (2001) Is periventricular leukomalacia an axonopathy as well as an oligopathy? Pediatr Res 49:453–457
Danbolt NC (2001) Glutamate uptake. Prog Neurobiol 65:1–105
Darnell RB, Posner JB (2006) Paraneoplastic syndromes affecting the nervous system. Semin Oncol 33:270–298
Dean JM, Moravec MD, Grafe M, Abend N, Ren J, Gong X, Volpe JJ, Jensen FE, Hohimer AR, Back SA (2011) Strain-specific differences in perinatal rodent oligodendrocyte lineage progression and its correlation with human. Dev Neurosci 33:251–260
Degterev A, Maki JL, Yuan J (2013) Activity and specificity of necrostatin-1, small-molecule inhibitor of RIP1 kinase. Cell Death Differ 20:366
Degterev A, Huang Z, Boyce M, Li Y, Jagtap P, Mizushima N, Cuny GD, Mitchison TJ, Moskowitz MA, Yuan J (2005) Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury. Nat Chem Biol 1:112–119
Degterev A, Yuan J (2008) Expansion and evolution of cell death programmes. Nat Rev Mol Cell Biol 9:378–390
Deng W, Rosenberg PA, Volpe JJ, Jensen FE (2003) Calcium-permeable AMPA/kainate receptors mediate toxicity and preconditioning by oxygen-glucose deprivation in oligodendrocyte precursors. Proc Natl Acad Sci U S A 100:6801–6806
Deng W, Wang H, Rosenberg PA, Volpe JJ, Jensen FE (2004) Role of metabotropic glutamate receptors in oligodendrocyte excitotoxicity and oxidative stress. Proc Natl Acad Sci U S A 101:7751–7756
DeSilva TM, Kabakov AY, Goldhoff PE, Volpe JJ, Rosenberg PA (2009) Regulation of glutamate transport in developing rat oligodendrocytes. J Neurosci 29:7898–7908
Desilva TM, Borenstein NS, Volpe JJ, Kinney HC, Rosenberg PA (2012) Expression of EAAT2 in neurons and protoplasmic astrocytes during human cortical development. J Comp Neurol 520(17):3912–3932
DeSilva TM, Kinney HC, Borenstein NS, Trachtenberg FL, Irwin N, Volpe JJ, Rosenberg PA (2007) The glutamate transporter EAAT2 is transiently expressed in developing human cerebral white matter. J Comp Neurol 501:879–890
DiFiglia M (1990) Excitotoxic injury of the neostriatum: a model for Huntington's disease. Trends Neurosci 13:286–289
Dommergues MA, Gallego J, Evrard P, Gressens P (1998) Iron supplementation aggravates periventricular cystic white matter lesions in newborn mice. Eur J Paediatr Neurol 2:313–318
Dommergues MA, Patkai J, Renauld JC, Evrard P, Gressens P (2000) Proinflammatory cytokines and interleukin-9 exacerbate excitotoxic lesions of the newborn murine neopallium. Ann Neurol 47:54–63
Duke DC, Moran LB, Pearce RK, Graeber MB (2007) The medial and lateral substantia nigra in Parkinson's disease: mRNA profiles associated with higher brain tissue vulnerability. Neurogenetics 8:83–94
Elitt CM, Sadowska GB, Stopa EG, Pinar H, Petersson KH, Stonestreet BS (2003) Effects of antenatal steroids on ischemic brain injury in near-term ovine fetuses. Early Hum Dev 73:1–15
Fellin T, Pascual O, Gobbo S, Pozzan T, Haydon PG, Carmignoto G (2004) Neuronal synchrony mediated by astrocytic glutamate through activation of extrasynaptic NMDA receptors. Neuron 43:729–743
Fern R, Möller T (2000) Rapid ischemic cell death in immature oligodendrocytes: a fatal glutamate release feedback loop. J Neurosci 20:34–42
Filippi L et al (2012) Safety and efficacy of topiramate in neonates with hypoxic ischemic encephalopathy treated with hypothermia (NeoNATI). BMC Pediatr 12:144
Follett PL, Rosenberg PA, Volpe JJ, Jensen FE (2000) NBQX attenuates excitotoxic injury in developing white matter. J Neurosci 20:9235–9241

Follett PL, Deng W, Dai W, Talos DM, Massillon LJ, Rosenberg PA, Volpe JJ, Jensen FE (2004) Glutamate receptor-mediated oligodendrocyte toxicity in periventricular leukomalacia: a protective role for topiramate. J Neurosci 24:4412–4420

Gainetdinov RR, Premont RT, Bohn LM, Lefkowitz RJ, Caron MG (2004) Desensitization of G protein-coupled receptors and neuronal functions. Annu Rev Neurosci 27:107–144

Gill R, Foster AC, Woodruff GN (1987) Systemic administration of MK-801 protects against ischemia-induced hippocampal neurodegeneration in the gerbil. J Neurosci 7:3343–3349

Gilles FH, Leviton A, Kerr CS (1976) Endotoxin leucoencephalopathy in the telencephalon of the newborn kitten. J Neurol Sci 27:183–191

Gilles FH Jr, DRA DVM, Kerr CS (1977) Neonatal endotoxin encephalopathy. Ann Neurol 2:49–56

Gosztonyi G, Koprowski H (2001) The concept of neurotropism and selective vulnerability ("pathoclisis") in virus infections of the nervous system–a historical overview. Curr Top Microbiol Immunol 253:1–13

Graus F, Dalmau J (2012) Paraneoplastic neurological syndromes. Curr Opin Neurol 25:795–801

Haynes RL, Billiards SS, Borenstein NS, Volpe JJ, Kinney HC (2008) Diffuse axonal injury in periventricular leukomalacia as determined by apoptotic marker fractin. Pediatr Res 63:656–661

Haynes RL, Borenstein NS, Desilva TM, Folkerth RD, Liu LG, Volpe JJ, Kinney HC (2005) Axonal development in the cerebral white matter of the human fetus and infant. J Comp Neurol 484:156–167

Haynes RL, Folkerth RD, Keefe RJ, Sung I, Swzeda LI, Rosenberg PA, Volpe JJ, Kinney HC (2003) Nitrosative and oxidative injury to premyelinating oligodendrocytes in periventricular leukomalacia. J Neuropathol Exp Neurol 62:441–450

Hazell AS, Butterworth RF (2009) Update of cell damage mechanisms in thiamine deficiency: focus on oxidative stress, excitotoxicity and inflammation. Alcohol Alcohol 44:141–147

Hazell AS, Todd KG, Butterworth RF (1998) Mechanisms of neuronal cell death in Wernicke's encephalopathy. Metab Brain Dis 13:97–122

Hitomi J, Christofferson DE, Ng A, Yao J, Degterev A, Xavier RJ, Yuan J (2008) Identification of a molecular signaling network that regulates a cellular necrotic cell death pathway. Cell 135:1311–1323

Ho Y, Samarasinghe R, Knoch ME, Lewis M, Aizenman E, DeFranco DB (2008) Selective inhibition of mitogen-activated protein kinase phosphatases by zinc accounts for extracellular signal-regulated kinase 1/2-dependent oxidative neuronal cell death. Mol Pharmacol 74:1141–1151

Hollmann M, Heinemann S (1994) Cloned glutamate receptors. Annu Rev Neurosci 17:31–108

Honeycutt A, Dunlap L, CHen H, Homsi G, Grosse S, Schendel D (2004) Economic costs associated with mental retardation, cerebral plasy, hearing loss, and vision impairment–United States, 2003. Morbidity Mortality Weekly Reports 53:57–59

Hotchkiss RS, Strasser A, McDunn JE, Swanson PE (2009) Cell death. New Engl J Med 361:1570–1583

Inder T, Mocatta T, Darlow B, Spencer C, Volpe JJ, Winterbourn C (2002) Elevated free radical products in the cerebrospinal fluid of VLBW infants with cerebral white matter injury. Pediatr Res 52:213–218

Ishimaru H, Katoh A, Suzuki H, Fukuta T, Kameyama T, Nabeshima T (1992) Effects of N-methyl-D-aspartate receptor antagonists on carbon monoxide-induced brain damage in mice. J Pharmacol Exp Ther 261:349–352

Itoh T, Beesley J, Itoh A, Cohen AS, Kavanaugh B, Coulter DA, Grinspan JB, Pleasure D (2002) AMPA glutamate receptor-mediated calcium signaling is transiently enhanced during development of oligodendrocytes. J Neurochem 81:390–402

Jensen AA, Erichsen MN, Nielsen CW, Stensbol TB, Kehler J, Bunch L (2009) Discovery of the first selective inhibitor of excitatory amino acid transporter subtype 1. J Med Chem 52:912–915

Jhala SS, Hazell AS (2011) Modeling neurodegenerative disease pathophysiology in thiamine deficiency: consequences of impaired oxidative metabolism. Neurochem Int 58:248–260

Jin G, Arai K, Murata Y, Wang S, Stins MF, Lo EH, van Leyen K (2008) Protecting against cerebrovascular injury: contributions of 12/15-lipoxygenase to edema formation after transient focal ischemia. Stroke 39:2538–2543

Kaindl AM, Degos V, Peineau S, Gouadon E, Chhor V, Loron G, Le Charpentier T, Josserand J, Ali C, Vivien D, Collingridge GL, Lombet A, Issa L, Rene F, Loeffler JP, Kavelaars A, Verney C, Mantz J, Gressens P (2012) Activation of microglial N-methyl-D-aspartate receptors triggers inflammation and neuronal cell death in the developing and mature brain. Ann Neurol 72:536–549

Karadottir R, Cavelier P, Bergersen LH, Attwell D (2005) NMDA receptors are expressed in oligodendrocytes and activated in ischaemia. Nature 438:1162–1166

Kataoka H, Dalmau J, Ueno S (2008) Paraneoplastic encephalitis associated with ovarian teratoma and N-methyl-D-aspartate receptor antibodies. Eur J Neurol 15:e5–e6

Katsuki H, Okuda S (1995) Arachidonic acid as a neurotoxic and neurotrophic substance. Prog Neurobiol 46:607–636

Kerr JFR, Wyllie AH, Currie AR (1972) Apoptosis: a basic biological phenomenon with wide ranging implications in tissue kinetics. Br J Cancer 26:239–257

Khwaja O, Volpe JJ (2008) Pathogenesis of cerebral white matter injury of prematurity. Arch Dis Child Fetal Neonatal Ed 93:F153–F161

Kim S, Dayani L, Rosenberg PA, Li J (2010) RIP1 kinase mediates arachidonic acid-induced oxidative death of oligodendrocyte precursors. Int J Physiol Pathophysiol Pharmacol 2:137–147

Kimelberg HK, Rutledge E, Goderie S, Charniga C (1995) Astrocytic swelling due to hypotonic or high K^+ medium causes inhibition of glutamate and aspartate uptake and increases their release. J Cereb Blood Flow Metab 15:409–416

Kimelberg HK, Goderie SK, Higman S, Pang S, Waniewski RA (1990) Swelling-induced release of glutamate, aspartate, and taurine from astrocyte cultures. J Neurosci 10:1583–1591

Kinney HC, Back SA (1998) Human oligodendroglial development: relationship to periventricular leukomalacia. Semin Pediatr Neurol 5:180–189

Kinney HC, Armstrong DD (2002) Perinatal neuropathology. In: Graham DI, Lantos PL (eds) Greenfield's neuropathology. Arnold, London, pp 519–606

Kinney HC, Panigrahy A, Newburger JW, Jonas RA, Sleeper LA (2005) Hypoxic-ischemic brain injury in infants with congenital heart disease dying after cardiac surgery. Acta Neuropathol 110:563–578

Klatzo I (2003) Cecile & Oskar Vogt: the significance of their contributions in modern neuroscience. Acta Neurochir Suppl 86:29–32

Klenchin VA, Martin TF (2000) Priming in exocytosis: attaining fusion-competence after vesicle docking. Biochimie 82:399–407

Langlais PJ, Mair RG (1990) Protective effects of the glutamate antagonist MK-801 on pyrithiamine-induced lesions and amino acid changes in rat brain. J Neurosci 10:1664–1674

Langlais PJ, Zhang SX (1993) Extracellular glutamate is increased in thalamus during thiamine deficiency-induced lesions and is blocked by MK-801. J Neurochem 61:2175–2182

Lee WH, Wang GM, Seaman LB, Vannucci SJ (1996) Coordinate IGF-I and IGFBP5 gene expression in perinatal rat brain after hypoxia-ischemia. J Cereb Blood Flow Metab 16:227–236

Leviton A, Kuban K, O'Shea TM, Paneth N, Fichorova R, Allred EN, Dammann O (2011) The relationship between early concentrations of 25 blood proteins and cerebral white matter injury in preterm newborns: the ELGAN study. J Pediatr 158(897–903):e891–e895

Li YH, Maher P, Schubert D (1997) A role for 12-lipoxygenase in nerve cell death caused by glutathione depletion. Neuron 19:453–463

Lipton SA, Rosenberg PA (1994) Excitatory amino acids as a final common pathway for neurologic disorders. New Engl J Med 330:613–622

Lo EH, Dalkara T, Moskowitz MA (2003) Mechanisms, challenges and opportunities in stroke. Nat Rev Neurosci 4:399–415

Lorenz JM (2001) The outcome of extreme prematurity. Semin Perinatol 25:348–359

Malaeb S, Dammann O (2009) Fetal inflammatory response and brain injury in the preterm newborn. J Child Neurol 24:1119–1126

Mander P, Borutaite V, Moncada S, Brown GC (2005) Nitric oxide from inflammatory-activated glia synergizes with hypoxia to induce neuronal death. J Neurosci Res 79:208–215

Manning SM, Talos DM, Zhou C, Selip DB, Park HK, Park CJ, Volpe JJ, Jensen FE (2008) NMDA receptor blockade with memantine attenuates white matter injury in a rat model of periventricular leukomalacia. J Neurosci 28:6670–6678

Matute C (1998) Characteristics of acute and chronic kainate excitotoxic damage to the optic nerve. Proc Natl Acad Sci U S A 95:10229–10234

Matute C, Sánchez-Gómez MV, Martínez-Millán L, Miledi R (1997) Glutamate receptor-mediated toxicity in optic nerve oligodendrocytes. Proc Natl Acad Sci U S A 94:8830–8835

Matute C, Alberdi E, Domercq M, Perez-Cerda F, Perez-Samartin A, Sanchez-Gomez MV (2001) The link between excitotoxic oligodendroglial death and demyelinating diseases. Trends Neurosci 24:224–230

McClure MM, Riddle A, Manese M, Luo NL, Rorvik DA, Kelly KA, Barlow CH, Kelly JJ, Vinecore K, Roberts CT, Hohimer AR, Back SA (2008) Cerebral blood flow heterogeneity in preterm sheep: lack of physiologic support for vascular boundary zones in fetal cerebral white matter. J Cereb Blood Flow Metab 28:995–1008

McDonald JW, Althomsons SP, Hyrc KL, Choi DW, Goldberg MP (1998) Oligodendrocytes from forebrain are highly vulnerable to AMPA/kainate receptor-mediated excitotoxicity. Nat Med 4:291–297

Meredith GE, Totterdell S, Beales M, Meshul CK (2009) Impaired glutamate homeostasis and programmed cell death in a chronic MPTP mouse model of Parkinson's disease. Exp Neurol 219:334–340

Meunier FA, Osborne SL, Hammond GR, Cooke FT, Parker PJ, Domin J, Schiavo G (2005) Phosphatidylinositol 3-kinase C2alpha is essential for ATP-dependent priming of neurosecretory granule exocytosis. Mol Biol Cell 16:4841–4851

Micu I, Jiang Q, Coderre E, Ridsdale A, Zhang L, Woulfe J, Yin X, Trapp BD, McRory JE, Rehak R, Zamponi GW, Wang W, Stys PK (2006) NMDA receptors mediate calcium accumulation in myelin during chemical ischaemia. Nature 439:988–992

Milnerwood AJ, Raymond LA (2010) Early synaptic pathophysiology in neurodegeneration: insights from Huntington's disease. Trends Neurosci 33:513–523

Miura M, Zhu H, Rotello R, Hartwieg EA, Yuan J (1993) Induction of apoptosis in fibroblasts by IL-1 beta-converting enzyme, a mammalian homolog of the C. elegans cell death gene ced-3. Cell 75:653–660

Monyer H, Giffard RG, Hartley DM, Dugan LL, Goldberg MP, Choi DW (1992) Oxygen or glucose deprivation-induced neuronal injury in cortical cell cultures is reduced by tetanus toxin. Neuron 8:967–973

Morimoto T, Ogihara S (1996) ATP is required in platelet serotonin exocytosis for protein phosphorylation and priming of secretory vesicles docked on the plasma membrane. J Cell Sci 109(Pt 1):113–118

Mozhayeva MG, Matos MF, Liu X, Kavalali ET (2004) Minimum essential factors required for vesicle mobilization at hippocampal synapses. J Neurosci 24:1680–1688

Muller RU, Finkelstein A (1974) The electrostatic basis of Mg++ inhibition of transmitter release. Proc Natl Acad Sci U S A 71:923–926

Murphy T, Parikh A, Schnaar R, Coyle JT (1989a) Arachidonic acid metabolism in glutamate neurotoxicity. Ann N Y Acad Sci 559:474–477

Murphy TH, Schnaar RL, Coyle JT (1990) Immature cortical neurons are uniquely sensitive to glutamate toxicity by inhibition of cystine uptake. FASEB J 4:1624–1633

Murphy TH, Miyamoto M, Sastre A, Schnaar RL, Coyle JT (1989b) Glutamate toxicity in a neuronal cell line involves inhibition of cystine transport leading to oxidative stress. Neuron 2:1547–1558

Ness JK, Wood TL (2002) Insulin-like growth factor I, but not neurotrophin-3, sustains Akt activation and provides long-term protection of immature oligodendrocytes from glutamate-mediated apoptosis. Mol Cell Neurosci 20:476–488

Ness JK, Mitchell NE, Wood TL (2002) IGF-I and NT-3 signaling pathways in developing oligodendrocytes: differential regulation and activation of receptors and the downstream effector Akt. Dev Neurosci 24:437–445

Ness JK, Scaduto RC Jr, Wood TL (2004) IGF-I prevents glutamate-mediated bax translocation and cytochrome C release in O4+ oligodendrocyte progenitors. Glia 46:183–194

Nicholls DG (2004) Mitochondrial dysfunction and glutamate excitotoxicity studied in primary neuronal cultures. Curr Mol Med 4:149–177

Nicholls DG (2009) Spare respiratory capacity, oxidative stress and excitotoxicity. Biochem Soc Trans 37:1385–1388

Northington FJ, Chavez-Valdez R, Graham EM, Razdan S, Gauda EB, Martin LJ (2011) Necrostatin decreases oxidative damage, inflammation, and injury after neonatal HI. J Cereb Blood Flow Metab 31:178–189

Nowak L, Bregestovski P, Ascher P, Herbet A, Prochiantz A (1984) Magnesium gates glutamate-activated channels in mouse central neurones. Nature 307:462–465

Oka A, Belliveau MJ, Rosenberg PA, Volpe JJ (1993) Vulnerability of oligodendroglia to glutamate: pharmacology, mechanisms, and prevention. J Neurosci 13:1441–1453

Okada Y, Sato K, Numata T (2009) Pathophysiology and puzzles of the volume-sensitive outwardly rectifying anion channel. J Physiol 587:2141–2149

Olney JW, Rhee V, Ho OL (1974) Kainic acid: a powerful neurotoxic analogue of glutamate. Brain Res 77:507–512

Paintlia MK, Paintlia AS, Barbosa E, Singh I, Singh AK (2004) N-acetylcysteine prevents endotoxin-induced degeneration of oligodendrocyte progenitors and hypomyelination in developing rat brain. J Neurosci Res 78:347–361

Paintlia MK, Paintlia AS, Contreras MA, Singh I, Singh AK (2008) Lipopolysaccharide-induced peroxisomal dysfunction exacerbates cerebral white matter injury: attenuation by N-acetyl cysteine. Exp Neurol 210:560–576

Pallast S, Arai K, Wang X, Lo EH, van Leyen K (2009) 12/15-Lipoxygenase targets neuronal mitochondria under oxidative stress. J Neurochem 111:882–889

Pallast S, Arai K, Pekcec A, Yigitkanli K, Yu Z, Wang X, Lo EH, van Leyen K (2010) Increased nuclear apoptosis-inducing factor after transient focal ischemia: a 12/15-lipoxygenase-dependent organelle damage pathway. J Cereb Blood Flow Metab 30:1157–1167

Papagapiou MP, Auer RN (1990) Regional neuroprotective effects of the NMDA receptor antagonist MK-801 (dizocilpine) in hypoglycemic brain damage. J Cereb Blood Flow Metab 10:270–276

Parpura V, Basarsky TA, Liu F, Jeftinija K, Jeftinija S, Haydon PG (1994) Glutamate-mediated astrocyte-neuron signalling. Nature 369:744–747

Pekcec A, Yigitkanli K, Jung JE, Pallast S, Xing C, Antipenko A, Minchenko M, Nikolov DB, Holman TR, Lo EH, van Leyen K (2012) Following experimental stroke, the recovering brain is vulnerable to lipoxygenase-dependent semaphorin signaling. FASEB J 27(2):437–445

Pierson CR, Folkerth RD, Billiards SS, Trachtenberg FL, Drinkwater ME, Volpe JJ, Kinney HC (2007) Gray matter injury associated with periventricular leukomalacia in the premature infant. Acta Neuropathol 114:619–631

Pines G, Kanner BI (1990) Counterflow of L-glutamate in plasma membrane vesicles and reconstituted preparations from brain. Biochemistry 29:11209–11214

Piomelli D, Greengard P (1990) Lipoxygenase metabolites of arachidonic acid in neuronal transmembrane signalling. Trends Pharmacol Sci 11:367–373

Rangon CM, Fortes S, Lelievre V, Leroux P, Plaisant F, Joubert C, Lanfumey L, Cohen-Salmon C, Gressens P (2007) Chronic mild stress during gestation worsens neonatal brain lesions in mice. J Neurosci 27:7532–7540

Raymond LA, Andre VM, Cepeda C, Gladding CM, Milnerwood AJ, Levine MS (2011) Pathophysiology of Huntington's disease: time-dependent alterations in synaptic and receptor function. Neuroscience 198:252–273

Reyes RC, Brennan AM, Shen Y, Baldwin Y, Swanson RA (2012) Activation of neuronal NMDA receptors induces superoxide-mediated oxidative stress in neighboring neurons and astrocytes. J Neurosci 32:12973–12978

Riddle A, Luo NL, Manese M, Beardsley DJ, Green L, Rorvik DA, Kelly KA, Barlow CH, Kelly JJ, Hohimer AR, Back SA (2006) Spatial heterogeneity in oligodendrocyte lineage maturation and not cerebral blood flow predicts fetal ovine periventricular white matter injury. J Neurosci 26:3045–3055

Riobó NA, Clementi E, Melani M, Boveris A, Cadenas E, Moncada S, Poderoso JJ (2001) Nitric oxide inhibits mitochondrial NADH:ubiquinone reductase activity through peroxynitrite formation. Biochem J 359:139–145

Rosenberg PA, Dai W, Gan XD, Ali S, Fu J, Back SA, Sanchez RM, Segal MM, Follett PL, Jensen FE, Volpe JJ (2003) Mature myelin basic protein-expressing oligodendrocytes are insensitive to kainate toxicity. J Neurosci Res 71:237–245

Rothman SM (1983) Synaptic activity mediated death of hypoxic neurons. Science 220:526–527

Rothman SM, Olney JW (1995) Excitotoxicity and the NMDA receptor–still lethal after eight years. Trends Neurosci 18:57–58

Rothstein JD (1995) Excitotoxicity and neurodegeneration in amyotrophic lateral sclerosis. Clin Neurosci 3:348–359

Rothstein JD (2009) Current hypotheses for the underlying biology of amyotrophic lateral sclerosis. Ann Neurol 65(Suppl 1):S3–S9

Salter MG, Fern R (2005) NMDA receptors are expressed in developing oligodendrocyte processes and mediate injury. Nature 438:1167–1171

Sanchez-Gomez MV, Matute C (1999) AMPA and kainate receptors each mediate excitotoxicity in oligodendroglial cultures. Neurobiol Dis 6:475–485

Sandberg M, Butcher SP, Hagberg H (1986) Extracellular overflow of neuroactive amino acids during severe insulin-induced hypoglycemia: in vivo dialysis of the rat hippocampus. J Neurochem 47:178–184

Schmidt-Kastner R (1989) The problem of selective vulnerability in neuropathology: the historical discussion between C. and O. Vogt and W. Spielmeyer as seen today (review). No shinkei geka 17:109–116

Schmidt-Kastner R, Freund TF (1991) Selective vulnerability of the hippocampus in brain ischemia. Neuroscience 40:599–636

Segovia KN, McClure M, Moravec M, Luo NL, Wan Y, Gong X, Riddle A, Craig A, Struve J, Sherman LS, Back SA (2008) Arrested oligodendrocyte lineage maturation in chronic perinatal white matter injury. Ann Neurol 63:520–530

Seiler A, Schneider M, Forster H, Roth S, Wirth EK, Culmsee C, Plesnila N, Kremmer E, Radmark O, Wurst W, Bornkamm GW, Schweizer U, Conrad M (2008) Glutathione peroxidase 4 senses and translates oxidative stress into 12/15-lipoxygenase dependent- and AIF-mediated cell death. Cell Metab 8:237–248

Sheardown M, Nielsen EO, Hansen AJ, Jacobsen P, Honore T (1990) 2,3-Dihydroxy-6-nitro-7-sufamoyl-benzo(f) quinoxaline: a neuroprotectant for cerebral ischemia. Science 247:571–573

Strange K, Emma F, Jackson PS (1996) Cellular and molecular physiology of volume-sensitive anion channels. Am J Physiol 270:C711–C730

Streit WJ, Morioka T, Kalehua AN (1992) MK-801 prevents microglial reaction in rat hippocampus after forebrain ischemia. Neuroreport 3:146–148

Surmeier DJ, Guzman JN, Sanchez-Padilla J (2010) Calcium, cellular aging, and selective neuronal vulnerability in Parkinson's disease. Cell Calcium 47:175–182

Szatkowski M, Barbour B, Attwell D (1990) Non-vesicular release of glutamate from glial cells by reversed electrogenic glutamate uptake. Nature 348:443–446

Takahashi N, Duprez L, Grootjans S, Cauwels A, Nerinckx W, DuHadaway JB, Goossens V, Roelandt R, Van Hauwermeiren F, Libert C, Declercq W, Callewaert N, Prendergast GC, Degterev A, Yuan J, Vandenabeele P (2012) Necrostatin-1 analogues: critical issues on the specificity, activity and in vivo use in experimental disease models. Cell Death Dis 3:e437

Talos DM, Fishman RE, Park H, Folkerth RD, Follett PL, Volpe JJ, Jensen FE (2006a) Developmental regulation of alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor subunit expression in forebrain and relationship to regional susceptibility to hypoxic/ischemic injury. I. Rodent cerebral white matter and cortex. J Comp Neurol 497:42–60

Talos DM, Follett PL, Folkerth RD, Fishman RE, Trachtenberg FL, Volpe JJ, Jensen FE (2006b) Developmental regulation of alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor subunit expression in forebrain and relationship to regional susceptibility to hypoxic/ischemic injury. II. Human cerebral white matter and cortex. J Comp Neurol 497:61–77

Tan S, Schubert D, Maher P (2001) Oxytosis: a novel form of programmed cell death. Curr Top Med Chem 1:497–506

Tecoma ES, Monyer H, Goldberg MP, Choi DW (1989) Traumatic neuronal injury in vitro is attenuated by NMDA antagonists. Neuron 2:1541–1545

Todd KG, Butterworth RF (1998) Evaluation of the role of NMDA-mediated excitotoxicity in the selective neuronal loss in experimental Wernicke encephalopathy. Exp Neurol 149:130–138

Tsuji M, duPlessis A, Taylor G, Crocker R, Volpe JJ (1998) Near infrared spectroscopy detects cerebral ischemia during hypotension in piglets. Pediatr Res 44:591–595

Tsuji M, Saul JP, du Plessis A, Eichenwald E, Sobh J, Crocker R, Volpe JJ (2000) Cerebral intravascular oxygenation correlates with mean arterial pressure in critically ill premature infants. Pediatrics 106:625–632

van Leyen K, Kim HY, Lee SR, Jin G, Arai K, Lo EH (2006) Baicalein and 12/15-lipoxygenase in the ischemic brain. Stroke 37:3014–3018

van Leyen K, Arai K, Jin G, Kenyon V, Gerstner B, Rosenberg PA, Holman TR, Lo EH (2008) Novel lipoxygenase inhibitors as neuroprotective reagents. J Neurosci Res 86:904–909

Vandenabeele P, Grootjans S, Callewaert N, Takahashi N (2013) Necrostatin-1 blocks both RIPK1 and IDO: consequences for the study of cell death in experimental disease models. Cell Death Differ 20:185–187

Vesce S, Bezzi P, Volterra A (1999) The active role of astrocytes in synaptic transmission. Cell Mol Life Sci 56:991–1000

Volpe JJ (2003) Cerebral white matter injury of the premature infant-more common than you think. Pediatrics 112:176–180

Volpe JJ (2008) Neurology of the newborn, 5th edn. Saunders, Philadelphia

Volpe JJ (2009a) Brain injury in premature infants: a complex amalgam of destructive and developmental disturbances. Lancet Neurol 8:110–124

Volpe JJ (2009b) The encephalopathy of prematurity–brain injury and impaired brain development inextricably intertwined. Semin Pediatr Neurol 16:167–178

Volpe JJ, Kinney HC, Jensen FE, Rosenberg PA (2011) The developing oligodendrocyte: key cellular target in brain injury in the premature infant. Int J Dev Neurosci 29(4):423–440

Wang H, Li J, Follett PL, Zhang Y, Cotanche DA, Jensen FE, Volpe JJ, Rosenberg PA (2004) 12-Lipoxygenase plays a key role in cell death caused by glutathione depletion and arachidonic acid in rat oligodendrocytes. Eur J Neurosci 20:2049–2058

Watanabe K, Hayakawa F, Okumura A (1999) Neonatal EEG: a powerful tool in the assessment of brain damage in preterm infants. Brain Dev 21:361–372

Welin AK, Svedin P, Lapatto R, Sultan B, Hagberg H, Gressens P, Kjellmer I, Mallard C (2007) Melatonin reduces inflammation and cell death in white matter in the mid-gestation fetal sheep following umbilical cord occlusion. Pediatr Res 61:153–158

Weston MC, Schuck P, Ghosal A, Rosenmund C, Mayer ML (2006) Conformational restriction blocks glutamate receptor desensitization. Nat Struct Mol Biol 13:1120–1127

Wieloch T, Engelsen B, Westerberg E, Auer R (1985) Lesions of the glutamatergic cortico-striatal projections in the rat ameliorate hypoglycemic brain damage in the striatum. Neurosci Lett 58:25–30

Wood TL, Loladze V, Altieri S, Gangoli N, Levison SW, Brywe KG, Mallard C, Hagberg H (2007) Delayed IGF-1 administration rescues oligodendrocyte progenitors from glutamate-induced cell death and hypoxic-ischemic brain damage. Dev Neurosci 29:302–310

Wyllie A, Kerr J, Currie A (1980) Cell death: the significance of apoptosis. Int Rev Cytol 68:251–307

Xiong T, Gonzalez F, Mu DZ (2012) An overview of risk factors for poor neurodevelopmental outcome associated with prematurity. World J Pediatr 8:293–300

Yao J, Bajjalieh SM (2008) Synaptic vesicle protein 2 binds adenine nucleotides. J Biol Chem 283:20628–20634

Yigitkanli K, Pekcec A, Karatas H, Pallast S, Mandeville E, Joshi N, Smirnova N, Gazaryan I, Ratan RR, Witztum JL, Montaner J, Holman TR, Lo EH, van Leyen K (2012) Inhibition of 12/15-lipoxygenase as therapeutic strategy to treat stroke. Ann Neurol 73(1):129–135

Yonezawa M, Back SA, Gan XD, Rosenberg PA, Volpe JJ (1996) Cystine deprivation induces oligodendroglial death: rescue by free radical scavengers and by a diffusible glial factor. J Neurochem 67:566–573

Yoshioka A, Hardy M, Younkin DP, Grinspan JB, Stern JL, Pleasure D (1995) α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors mediate excitotoxicity in the oligodendroglial lineage. J Neurochem 64:2442–2448

Yuan J, Shaham S, Ledoux S, Ellis HM, Horvitz HR (1993) The C. elegans cell death gene ced-3 encodes a protein similar to mammalian interleukin-1-beta-converting enzyme. Cell 75:641–652

Zerangue N, Kavanaugh MP (1996) Flux coupling in a neuronal glutamate transporter. Nature 383:634–637

Zhang SX, Weilersbacher GS, Henderson SW, Corso T, Olney JW, Langlais PJ (1995) Excitotoxic cytopathology, progression, and reversibility of thiamine deficiency-induced diencephalic lesions. J Neuropathol Exp Neurol 54:255–267

Zhang Y, Aizenman E, DeFranco DB, Rosenberg PA (2007) Intracellular zinc release, 12-lipoxygenase activation and MAPK dependent neuronal and oligodendroglial death. Mol Med 13:350–355

Zhang Y, Wang H, Li J, Jiminez DA, Levitan ES, Aizenman E, Rosenberg PA (2004) Peroxynitrite induced neuronal apoptosis is mediated by intracellular zinc release and 12-lipoxygenase activation. J Neurosci 24(47):10616–10627

Zhang Y, Wang H, Li J, Dong L, Xu P, Chen W, Neve RL, Volpe JJ, Rosenberg PA (2006) Intracellular zinc release and ERK phosphorylation are required upstream of 12-lipoxygenase activation in peroxynitrite toxicity to mature rat oligodendrocytes. J Biol Chem 281: 9460–9470

Chapter 7
Neonatal Experimental White Matter Injury

Zhengwei Cai

7.1 Introduction

Each year, about 65,000 infants with very low birth weight (VLBW, <1.5 kg) are born in the U.S. With markedly improved neonatal intensive care for premature infants, the survival of these VLBW infants is strikingly improved. In the U.S., 90 % of these VLBW infants survive the neonatal period (Volpe 2008; Volpe et al. 2011). Unfortunately, 5–10 % of these survivors will later develop the spastic motor deficits categorized as cerebral palsy (CP) and up to 50 % will exhibit cognitive/behavioral defects or learning disability (Back and Rivkees 2004; Volpe 2008; Volpe et al. 2011). In recent years, the premature birth rate in the U.S. is not decreasing. It has risen 30 % since 1981 and reached to 12 % of newborn babies (World Health Organization data, 2012, http://www.who.int/maternal_child_adolescent/documents/born_too_soon/en/index.html).

Brain injury in the premature infants, especially in VLBW infants is a major clinic problem. Data from the Centers for Disease Control and Prevention show that an average of 3.3/1,000 children in the U.S. have CP (http://www.cdc.gov/ncbddd/cp/index.html), while this number in 1960s was approximately 2.2/1,000 (Silbereis et al. 2010). Periventricular white matter injury (PWMI), detectable in a significant proportion of VLBW infants, is now considered as an increasingly common cause of CP and the leading cause of chronic neurological morbidity. To better understand the underlying mechanisms involved in the WMI, many animal models of WMI induced by cerebral hypoxia–ischemia (HI) or by administration of either microbes or bacterial products have been developed during the past three decades. Hagberg et al. (2002) and Silbereis et al. (2010) have provided very comprehensive reviews on animal models of neonatal WMI. The aim of this review is to summarize the

Z. Cai, Ph.D. (✉)
Department of Pediatrics, University of Mississippi Medical Center, 2500 North State Street, Jackson, MS 39216-4505, USA
e-mail: zcai@umc.edu

S. Baltan et al. (eds.), *White Matter Injury in Stroke and CNS Disease*, Springer Series in Translational Stroke Research 4, DOI 10.1007/978-1-4614-9123-1_7,

existing animal models of neonatal WMI, emphasizing the recent developments and adaptations of these models and avoiding unnecessary replications of information reviewed previously. This review will also, to some extent, analyze and make comments on advantages and disadvantages of these models, towards improved experimental models of neonatal WMI.

7.2 Neuropathology of WMI in Human Infants

A complete description of pathological characters of PWMI in preterm infants was first reported by Banker and Larroche (1962), who named the lesions as periventricular leukomalacia (PVL). PVL refers initially to bilateral and symmetric focal cystic or non-cystic necrosis of white matter at the periventricular areas. In many cases WMI is more widespread including periventricular, subcortical, callosal white matter and the internal capsule (Hagberg et al. 2002). Although injury of white matter predominates, neuronal loss and axonal damage are often observed in patient with PVL at the cerebral cortex and deep gray matters (Inder et al. 1999; Pierson et al. 2007; Leviton and Gressens 2007; Haynes et al. 2008), which can reflect primary injury or arise as a secondary response to WMI (Volpe 2009). WMI is now recognized as a spectrum of pathology that includes: (1) focal cystic or non-cystic necrosis with loss of all cellular components, which may result in ventriculomegaly, enlarged subarachnoid space, and immature gyral development (Inder et al. 2003; Groenendaal et al. 2010), and (2) a more diffuse and cell-specific lesion that selectively triggers oligodendrocyte lineage degeneration and disturbances in myelination (Silbereis et al. 2010; Volpe et al. 2011). In recent years, neuroimaging studies have provided evidence that the incidence of focal necrotic type of PVL is declining, whereas diffuse cerebral WMI is emerging as the predominant lesion (Back and Rivkees 2004; Back 2006; Back et al. 2007). The diffuse and cell-specific lesions include acute loss of early differentiating oligodendrocytes [premyelinating oligodendrocytes (preOLs)], the O4-positive and O1-negative preOLs, which are most vulnerable (Kinney 2009) to the injurious mechanisms discussed below and are the predominant oligodendrocyte population in the infant brain at the time of peak period of occurrence of PVL (24–32 weeks of human gestation) (Back et al. 2001). This lesion may also include loss of O4-positive and O1-positive immature oligodendrocytes (Volpe et al. 2011), but they are a minor population between 18-week gestation to full term (Back et al. 2001). The diffuse and cell-specific lesions also include the failure of preOL differentiation or a persistent preOL maturation arrest. Surviving preOLs with loss of cell processes (Billiards et al. 2008) do not appear to differentiate subsequently. Although following the initial injury there was apparently a replenishment of preOL progenitors, by proliferation and/or migration, differentiation of the replenished preOL appeared to be blocked (Billiards et al. 2008). A persistent preOL maturation arrest was confirmed in a neonatal rat model of hypoxia–ischemia where remyelination after chronic hypoxia–ischemia was found to be delayed owing to a failure of preOL

differentiation (Segovia et al. 2008). A recent human study (Buser et al. 2012) comparing WMI in retrospective autopsy cases (1983–2000) with that in contemporary cases (2003–2010) showed that in the contemporary cases diffuse WMI was accompanied by a significant reduction in the burden of microscopic necrosis and axonopathy, and diffuse astrogliosis extended into the lesion surround with elevated hyaluronic acid and its receptors expressed by astrocytes. The total population of OL lineage was significantly increased in the lesions and this increase coincided with significant expansion of the preOL pool. Data from that study support that in recent year cases of WMI, microscopic necrosis does not contribute substantially to myelination failure. The primary mechanism of myelination failure involves a disrupted cellular response whereby preOLs fail to differentiate in diffuse astrogliotic lesions (Buser et al. 2012).

In addition to these features, WMI has also been found to be associated with other pathological characters such as accumulation of the axonally transported protein amyloid precursor protein, axonal transections (see Hagberg et al. 2002), and increased activation of microglia/macrophages, which are important cellular sources of proinflammatory cytokines and are involved in causing WMI by oxidative and nitrosative stress (Haynes et al. 2003). However, it remains unclear whether these cells are reactivated residents of the white matter or recruited from the blood (Hagberg et al. 2002).

7.3 Brief Description of Etiology of WMI in Human

Cerebral ischemia–reperfusion and systemic infection–inflammation are two potential, but not mutually exclusive etiologies of infant WMI (Volpe 2008). Both are closely associated with intrinsic vulnerability of cerebral white matter and preOLs during the peak period of occurrence of PVL (Volpe 2008; Volpe et al. 2011).

Premature infants have a particular propensity for developing cerebral ischemia, especially in the white matter. The ischemia–reperfusion pathway is supported by the presence of underdeveloped distal arterial fields in the cerebral white matter and a pressure-passive cerebral circulation with a deficient cerebrovascular autoregulatory system in the premature infant brain (Volpe 2008). The basal value for blood flow to cerebral white matter in the premature infant is very low and these infants are vulnerable to declines in blood pressure (and therefore to declines in blood flow to the white matter) (Volpe 2008; Khwaja and Volpe 2008). Hypocarbia in premature infants occurs as a consequence of the ventilator management of infant respiratory disease also increases the likelihood to develop cerebral white matter ischemia (Shankaran et al. 2006). The susceptibility of preOLs (O4+/O1−), the predominating oligodendrocytes at 24–32 weeks of gestation, to common consequences of hypoxic–ischemic insults, such as free radial attack (Haynes et al. 2003; Back et al. 2005; Riddle et al. 2006), excitotoxicity (Loeliger et al. 2003; Follett et al. 2000, 2004; Talos et al. 2006; Salter and Fern 2005; Karadottir and Attwell 2007) and injurious effects of microglial activation (Haynes et al. 2003; Billiards et al. 2006; Volpe et al. 2011), also supports the ischemia–reperfusion pathway.

The systemic infection–inflammation pathway is supported by the fact that WMI is predicted by histological chorioamnionitis and vasculitis in umbilical cord and chorion plate, proinflammatory cytokines in amniotic fluid and fetal blood (see Hagberg et al. 2002). A number of recent epidemiological studies have also shown an association between WMI and maternal intrauterine infection with fetal systemic inflammation or neonatal systemic infection with systemic inflammation (Wu and Colford 2000; Shah et al. 2008; Chau et al. 2009; Leviton et al. 2010). Data from a preterm fetal sheep model, in which the O4+/O1− preOLs are the predominant oligodendrocytes (Riddle et al. 2006; Back et al. 2006b), show that systemic administration of infectious agents results in WMI (Duncan et al. 2002; Rees et al. 2010; Dean et al. 2011) and support the infection–inflammation pathway. It seems that activation of brain microglia is the principal initiating event in causation or accentuation of preOL injury in the setting of systemic infection and inflammation (Kadhim et al. 2001; Haynes et al. 2003; Volpe et al. 2011).

7.4 Animal Models of WMI

Based on the about mentioned clinical information a large number of animal models of WMI have been developed. The white matter vulnerability has been shown to be related to the presence of O4+/O1− preOLs at gestational age of 24–32 weeks in human (Back et al. 2001, 2002; Kinney 2009). In most animal models, WMI can be induced either by cerebral hypoperfusion/hypoxia–ischemia or by induction of a systemic inflammatory response through administration of microbes/bacterial products at a time when the O4+/O1− preOLs are the predominant oligodendrocyte population in the animal brain.

7.4.1 Rodent Model of Neonatal Hypoxia–Ischemia

The rat model: Although PWMI with intact cerebral cortex has been reported in neonatal dogs following hypoperfusion induced by bilateral artery ligation (Yoshioka et al. 1994), the most used model in immature animals is the rat model initially developed by Vannucci's group (Rice et al. 1981) more than 30 years ago (unilateral carotid artery ligation followed by exposure to 8 % oxygen for 1–3.5 h in P7 rats). Rat brain development at early postnatal days (P7–P12) is considered to be equivalent to that of the full-term newborn human baby (Dobbing and Sands 1979; Romijn et al. 1991). In the original Rice-Vannucci (R-V) model, although necrosis of white matter was noticed 2 days after the hypoxic–ischemic (HI) insult, most research in this model was focused on gray matter injury (ipsilateral cortex, striatum, and hippocampus) resembling that seen in full-term infants with HI encephalopathy (Rice et al. 1981; Vannucci and Vannucci 2005). It has been found that brain injury produced by this procedure varies with duration of hypoxia (Rice et al. 1981; Liu et al. 2002), oxygen concentrations, and postnatal ages (Towfighi et al. 1997).

By modifications of these experimental conditions, this model has been successfully used to study perinatal WMI induced by HI. Back and coworkers (2002) suggested that white matter in the P2 rat brain, where the O4+/O1− preOLs are present in high numbers, is highly vulnerable to HI insult (unilateral carotid ligation followed by 4-h exposure to 6 % oxygen). A modified procedure in P1 rats (unilateral carotid ligation and 3.5 h of 6 % oxygen) results in loss of white matter on the affected side, but the injury is predominantly in the gray matter (Sheldon et al. 1996). A recent study has shown that milder HI insults (ischemia followed by 1.5-h exposure to 8 % oxygen in P7 rats) elicit preferential white matter injury (Skoff et al. 2007). This is consistent with the data from a previous modification of the R-V model in which unilateral carotid ligation and a shorter duration of hypoxia (6 % oxygen for 1 h) in P7 rats resulted in a selective loss of O1+ oligodendrocytes and deficient staining of myelin basic protein (MBP) in the periventricular white matter, without affecting the gray matter (Follett et al. 2000). Thus, the time point during oligodendrocyte development at which this HI procedure is carried out and severity of the hypoxic exposure are crucial for differential effects of gray versus white matter injury, and can lead to variability in results.

Losses of O4+ oligodendrocyte in the corpus callosum can be induced by bilateral carotid ligation combined with 8 % oxygen exposure for 10 min in P7 rats (Jelinski et al. 1999). Permanent bilateral carotid ligation without hypoxia in P5 rats resulted in 75 % reduction of cerebral blood flow to the subcortical white matter and brain damages mostly seen in the white matter (Uehara et al. 1999). Due to a feeding problem, the survival beyond 2–3 days was limited in that study (Uehara et al. 1999). Permanent bilateral ligation in P1 rats induced preferential WMI in corpus callosum, subcortex, internal capsule, and a significant dilation of the ventricles with limited pathology in the gray matter (Cai et al. 2001). By careful separation of the vagal nerve from the ligated arteries, long-term survival (2 weeks) was achievable (Cai et al. 2001). We found in our later studies that permanent bilateral carotid ligation followed by 10–20 min 8 % oxygen exposure in P4 rats preferentially produced severe WMI (white matter rarefaction, necrosis, and cavity formation) in 90 % animals (Lin et al. 2004; Fan et al. 2005; Cai et al. 2006). A long-term survival (21 days) and neurobehavioral evaluation were allowed (Fan et al. 2005; Cai et al. 2006).

Use of the R-V model in investigations of neonatal HI brain injury, including WMI, has many advantages. This model has been widely used and well characterized. The procedure is easy to carry out or to be modified and the low cost allows inclusion of a sufficient number of animals for dose–response evaluation of neuroprotective agents. On the other hand, this model of HI differs from clinical asphyxia with respect to the unilateral distribution of brain injury and lack of multi-organ dysfunction. It does not allow continuous cardiovascular monitoring and repeated blood sampling. Although in most cases long-term survival allows evaluation of neuropathological and functional outcomes, long-term CP-like spastic motor impairment induced by this procedure is lack or in controversy (Bona et al. 1997; Balduini et al. 2000; Ikeda et al. 2001; Lubics et al. 2005). The unilateral ligation and compensations from the undamaged hemisphere, and other unidentified factors may complicate the interpretation of the long-term behavioral outcomes (Scafidi et al. 2009).

The mouse model: The R-V model has been adapted to the mouse. Unilateral carotid ligation followed by 90 min 8 % oxygen exposure in P7 mice resulted in severe brain injury mostly in the gray matter (Ditelberg et al. 1996; Ferriero et al. 1996). With a shorter duration of hypoxia (10 % oxygen for 40–70 min) following the unilateral carotid ligation in P9 mice, this model was successfully used to study the response of immature oligodendrocyte and stem/progenitor cell in the white matter and subventricular/ventricular zone to HI (Skoff et al. 2001). An apparent advantage of using mouse models to study perinatal WMI is the increasing number of available genetically modified mice strains. Recent studies using genetically modified mice in this model have shown that many factors such as caspase-2, interleukin-18 and bone morphogenetic protein may play important roles in HI-induced WMI in the neonatal brain (Hedtjarn et al. 2005; Carlsson et al. 2011, 2012; Dizon et al. 2011). Sheldon and coworkers demonstrated that the susceptibility to and severity of injury after neonatal HI in mice are highly strain-dependent (Sheldon et al. 1998). It was found that some strains (CD1) are particularly susceptible to brain damage in this model, while others (129SV) are resistant (Sheldon et al. 1998). The marked strain differences in susceptibility to HI increases the complexity of studies of neonatal HI using the mouse model. In studies in which no wild-type litter-mates are available, careful consideration should be taken to the selection of wild-type controls.

7.4.2 Models of Perinatal Chronic Hypoxia

Premature infants with chronic lung diseases often suffer from multiple hypoxic episodes and are at high risk of developing CP. Models of hypoxia, either chronic or intermittent, have been developed (Scafidi et al. 2009) and display some pathological features of developmental WMI (Ment et al. 1998; Back et al. 2006a; Fagel et al. 2006). The chronic hypoxia model was initially developed in rats (Ment et al. 1998). Neonatal rats reared in 9.5 % oxygen from P3 until up to P63 were found to have decreased subcortical white matter and size of corpus callosum as well as progressive cerebral ventriculomegaly (Ment et al. 1998). The chronic hypoxia model was later adapted to the mouse (Weiss et al. 2004; Fagel et al. 2006; Back et al. 2006a). Hypoxic rearing (10 % oxygen) from P3 to P11–12 or to P33 resulted in ventriculomegaly and hypomyelination, which was related to abnormal OL lineage progression and a reduction in the OL progenitor pool (Back et al. 2006a), or to impaired axonal connection (Weiss et al. 2004). As mentioned above, availability of genetically manipulated mouse strain is an apparent advantage of using the mouse model of chronic hypoxia. Using mice that are deficient in the A1 adenosine receptor gene, Turner and coworkers found that the A1 adenosine receptors play prominent roles in the development of hypoxia-induced ventriculomegaly (Turner et al. 2003). The disadvantages of the chronic hypoxia model include poorly characterized inflammatory responses and relatively mild WMI (Silbereis et al. 2010). Although gliosis in this model was rarely reported (Silbereis et al. 2010), recent

studies have investigated the role of astrocytes (Raymond et al. 2011; Bi et al. 2011) and amoeboid microglial cells (Rathnasamy et al. 2011) in chronic hypoxia-induced injury to OLs and the white matter.

7.4.3 Intrauterine HI Model

Disorders of the maternal–placental–fetal unit often results in fetal brain injury. Acute placental insufficiency is frequently encountered in human pregnancy and the most direct consequence of it is fetal global hypoxia–ischemia. To mimic this scenario, intrauterine models of global hypoxia–ischemia in the rat, rabbit, and sheep have been developed.

The rat model: Intrauterine global HI in rats is induced by transient clamping of uterine arteries (Cai et al. 1995, 1998), by housing of pregnant dams in hypoxic conditions (Baud et al. 2004), or by permanent unilateral uterine artery ligation (Olivier et al. 2005, 2009). Brain injury induced by transient clamping of uterine arteries at the embryonic day 17 (E17) in rats was very mild (Cai et al. 1997), but involving alterations in nitric oxide synthase expression and neurobehavioral deficits in neonatal rats (Cai et al. 1998, 1999). Permanent unilateral uterine artery ligation at E17 resulted in death of O4+ preOLs, white matter lesion, and defective myelination with increased microglia and astrogliosis in growth-retarded neonatal rat brain (Olivier et al. 2005, 2009). Although the data clearly show the link between growth retardation and perinatal WMI, possible compensation of blood flow from the contralateral uterine horn where the artery was not ligated and the special selection of growth-retarded rat brain from the ligated uterine horn make the resulting data difficult and complicated to be interpreted as the consequences of global hypoxia followed by reperfusion at birth.

The rabbit model: An intrauterine global HI model has also been described in rabbits via abruption of placental blood flow by inflation of a balloon catheter inserted into the uterine artery at different embryonic days (Tan et al. 2005; Derrick et al. 2004, 2007). Global HI in this model resulted in still birth and multiple deficits in postnatal survivors (Tan et al. 2005). In addition to lesions throughout the cerebral gray and white matter, some offspring rabbits developed spontaneous intraventricular hemorrhage with ventriculomegaly and periventricular white matter loss. Most impressively, global HI performed at E22 or E25 (70 % and 79 % gestation, respectively) induced hypertonic motor deficits and other persistent movement deficits, resembling those seen in humans with CP, in the surviving rabbit kits (Tan et al. 2005; Derrick et al. 2007, 2009). Magnetic resonance imaging (MRI) studies show that white matter injury in the surviving rabbit kits is well-correlated with hypertonic deficits (Drobyshevsky et al. 2007; Derrick et al. 2009). Recent studies with this model show that the susceptibility of WMI in the newborn rabbit kits is closely coincided with the timing of appearance of preOLs (Buser et al. 2010) and that alterations in neuronal nitric oxide synthase are involved in the HI injury (Rao et al. 2011). In spite of the close association between WMI and motor impairment

in humans, CP-like motor deficits are usually not observed in neonatal rodent models of HI. This rabbit model is currently the only model that permits detailed clinicopathological correlations with neurobehavioral outcomes and neuroradiological assessment (Drobyshevsky et al. 2007). This model is promising, but requires specialized infrastructure and expertise. Another disadvantage of this model is that the rabbit kits can only survive 11 days and long-term neurobehavioral and potential therapeutic treatment studies are not allowed.

The sheep model: White matter injury induced by intrauterine global HI in sheep was first reported by Ting et al. (1983). Thirty-eight midgestational sheep fetuses were maternally exposed to 10 % oxygen for 2 h, delivered and sacrificed 3 days later for neuropathologic assessment. Of the 38 fetuses, the brains in 8 that reduced their mean arterial blood pressure to less than 30 mmHg were markedly damaged and the most damaged part was the hemispheric white matter (Ting et al. 1983). The instrumented fetal sheep is the most widely studied large animal model of developmental brain injury (Back et al. 2006b) and is a promising model of cerebral WMI induced by either HI or infection–inflammation in the premature infant (Back et al. 2012 and below). In the sheep model, WMI can be induced by reversible carotid artery occlusion (Reddy et al. 1998; Petersson et al. 2002; Riddle et al. 2006), umbilical cord occlusion (Ikeda et al. 1998; Mallard et al. 2003; Welin et al. 2007), maternal hypoxia (Ting et al. 1983), or hemorrhagic hypotension (Matsuda et al. 1999) between ~90 days gestation (65 % of the full gestation time) and near term (~130 days gestation, 90 % of full gestation). Several days after the insult, fetal sheep is delivered and brain injury is assessed. While the severity of cortical neuronal injury and selective neuronal loss increased by near term in this model, the preterm fetuses had a more rapid injury in the subcortical white matter (Reddy et al. 1998). It is feasible to generate selective focal or more diffuse white matter lesions that resemble the type of injury now commonly seen in surviving preterm infants (Riddle et al. 2006). The uneven distribution of periventricular WMI was closely associated with the presence of the susceptible populations of preOLs, rather than the degree of white matter ischemia (Riddle et al. 2006). Details of the instrumented fetal sheep model have been previously well-reviewed (Back et al. 2006b, 2012). Briefly, the major advantages of this model for studying WMI include: (1) neurodevelopment of the preterm sheep fetuses (65 % gestation) is similar to that of the preterm human between approximately 24 and 28 weeks of gestation (Barlow 1969), (2) compared to the rodent brain, fetal sheep has a gyrencephalic brain with more cerebral white matter to work with, and (3) the big size of the fetal sheep allows longer-term procedures to create injury and chronic instrumentation for invasive monitoring of fetal physiological conditions such as blood flow to the brain, oxygen and lactate level in the brain, and electroencephalography. With the fetal sheep model, it is possible to generate graded cerebral WMI that can be studied with reliable measurements of blood flow and metabolism in histologically defined regions of cerebral white matter (Silbereis et al. 2010). Some disadvantages of this model are the requirement of substantial cost and infrastructure to support the surgical instrumentation and postoperative care of large laboratory animals. A highly skilled surgical team, a large animal operating facility suitable

for sterile operations, specialized veterinary care and access to reliable breeders are required. The short life of the animal after delivery does not allow long-term motor functional tests.

7.4.4 WMI Induced by Premature Delivery

Prematurely delivered baboon is another large animal model of preterm WMI. In this only primate model of prematurity, baboons were delivered at ~125 days of gestation (184 days as full term gestation) and provided with ventilator-support and neonatal intensive care, with no any experimentally induced insult (Dieni et al. 2004; Inder et al. 2004, 2005). Pathological features in this model include white matter damage, hemorrhage, diffuse white matter astrogliosis or microglia activation, and ventriculomegaly. Recent studies further show that timing of ventilator therapies, common practices in neonatal intensive care, plays important roles in resulting white matter injury (Loeliger et al. 2006) and that MRI acquired following delivery is well-correlated with white matter pathology and the loss of oligodendrocytes (Griffith et al. 2012). The attractive features of this model are the highest similarity of brain development between baboons and humans, no need of any experimental insults, and permitting to study the scenario in a neonatal intensive care unit where ventilator-support is common. On the other hand, this model requires highly specialized infrastructure and expertise and has a high cost. Animals do not survive for long term (usually 2–4 weeks) and thus, the ability to carry out long-term neurobehavioral assessment is limited.

7.4.5 Models of Infection–Inflammation-Induced WMI

Intrauterine infection and inflammation is proposed to be a causative factor of premature birth and WMI. To mimic the scenario of perinatal infection and inflammation, many animal models have lately been developed by administration of microbes or bacterial products to animals during gestation or in neonatal animals through different routes of injection (see Wang et al. 2006; Burd et al. 2012).

Intravenous (*i.v.*) *injection of Lipopolysaccharide* (*LPS*) *to the sheep fetus*: In recent years, i.v. injection of LPS to fetal sheep has been extensively used to study perinatal WMI. As in the sheep model of interauterine global HI, this model also requires skilled surgical preparation and instrumentation. LPS is directly administered into the fetal veins. WMI was induced by a single i.v. injection (Mallard et al. 2003; Svedin et al. 2005; Dean et al. 2011; Keller et al. 2011) or by repeated i.v. injections of LPS for 3–5 consecutive days (Duncan et al. 2002, 2006; Rees et al. 2010; Probyn et al. 2010) at 0.65–0.85 of full gestation. Brain injury was usually assessed 10–11 days later. In one study, chronic exposure to LPS in fetal sheep was achieved through intra-amniotic osmotic pump infusion starting from E80 (0.6 of full term) for 28 days (Nitsos et al. 2006). The LPS induced brain injury ranged from

diffuse subcortical damage to PVL, including reduced numbers of white matter oligodendrocytes and increased astrogliosis and microgliosis. LPS also induces cortical neuronal injury and axonal injury (Svedin et al. 2005; Dean et al. 2011). Unlike the fetal sheep model of intrauterine HI where predominantly subcortical white matter injury was observed in preterm sheep fetuses while more severe cortical neuronal injury was observed in the near term sheep fetuses (Reddy et al. 1998), fetal exposure to LPS resulted in similar white matter injury in both the preterm and near term sheep fetuses (Svedin et al. 2005). A recent study demonstrated that fetal LPS exposure induces a pattern of injury characterized by diffuse and focal white matter injury, which closely reproduces the injury observed clinically in preterm infants and can be detected by ex vivo MRI 10 days after the exposure (Van de Looij et al. 2012). This fetal sheep model of infection–inflammation has the same advantages and disadvantages as the fetal sheep intrauterine HI model does. An additional caveat of this LPS sheep model is that LPS-containing bacteria represent only one cause (gram-negative bacteria) of inflammation in human fetuses and neonates. In a recent study killed gram-positive bacterium, su-streptococcus pyogenes (OK-432) was intrapleurally injected into preterm fetal sheep and OK-432 induced chronic central and peripheral vasodilation (Bennet et al. 2010). Surprisingly, OK-432 caused bilateral infarction in the hippocampus of only one fetus that developed seizures, but no any gray and white matter injury or gliosis of microglia and astrocytes in other fetuses. It is an indication that endotoxins from other bacteria commonly associated with inflammation in human fetus may need to be assessed in this system.

Intrauterine LPS or Live microbe exposure in rabbits and rodents: Rabbit models of intrauterine infection–inflammation have been introduced by intrauterine injection of live *E. coli* (Yoon et al. 1997; Debillon et al. 2000, 2003) or LPS (Kannan et al. 2007, 2011; Saadani-Makki et al. 2008, 2009) at E21-E28. Inflammatory responses and brain injury were assessed at different time points after bacteria or LPS injection or after birth. Intrauterine bacterial inoculation or LPS injection resulted in white matter injury including karyorrhexis, rarefaction, disorganization of white matter, hypomyelination and apoptotic cell death in the periventricular region (Yoon et al. 1997; Debillon et al. 2000, 2003; Saadani-Makki et al. 2008). MRI and positron emission tomography (PET) studies demonstrated a significant increase in activation of microglia in the periventricular region and hippocampus of the brain of newborn rabbit pups exposed to endotoxin in utero (Kannan et al. 2007; Saadani-Makki et al. 2009; Kannan et al. 2011). An apparent advantage of this rabbit model is that intrauterine LPS exposure resulted in WMI along with hypertonic motor deficits resembling those found in PVL and CP, and the PET imaging matches the severity of motor deficits in neonatal rabbits (Saadani-Makki et al. 2008; Kannan et al. 2011). A high mortality of the rabbit fetuses is a disadvantage of this model.

Consequences of intrauterine administration of LPS or bacteria on neonatal WMI have also been investigated in rodents. Regardless of different routes of administration (intracervical, intra-amnion/chorion, or intra-amniotic injection) and different does of LPS or bacteria, similar to the sheep model, the general responses in rodents following intrauterine exposure to LPS or bacteria are the increases in expression of inflammatory cytokine mRNA and protein (such as TNFα, IL-1β or IL-6) in

maternal and fetal blood circulation, the placenta and fetal brains (for comprehensive reviews, see Hagberg et al. 2002; Wang et al. 2006). Intrauterine administration of LPS or bacterium (*E. coli*) causes hypomyelination and oligodendrocyte damage in offspring rat brain as indicated by decreased immunostaining of proteolipid protein (PLP), one of the major myelin protein, and 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNPase), a marker for immature oilgodendrocytes, and myelin basic protein (MBP), a maker for mature oligodendrocytes and myelination in the neonatal brain (Bell and Hallenbeck 2002; Pang et al. 2005; Shen et al. 2007). Intrauterine LPS exposure at 79 % of gestation in mice resulted in hypomyelination, enlarged ventricles, and some cortical gray matter lesions in neonatal mice (Wang et al. 2007b). In a recent study with a mouse model of antenatal chorioamnionitis by intrauterine injection with *Ureaplasma parvum*, a more commonly seen bacterium in preterm infants (Normann et al. 2009), microglia activation, myelin deficits, and reduced numbers of interneurons were demonstrated. In a mouse model of term chorioamnionitis, intrauterine LPS injection in pregnant CD-1 mice at E18.5 (delivery day E19) induced fetal brain inflammation and neuronal injury as indicated by decreased microtubule-associated protein-2 expression and dendritic counts in the fetal brain, but without overt changes in white matter damage markers (GFAP and PLP) (Burd et al. 2011). Whether the lack of severe WMI in the term fetal mouse brain following LPS exposure is due to the resistance of the white matter in term mouse brain or due to the possibilities, as suggested by the authors, that changes in white matter may occur later in the pathogenesis of term brain injury and neurons are the primary site of initial injury (Burd et al. 2011) needs further investigation. Regardless of LPS-induced gray and white matter injury there are no consistent CP-like motor deficits or cognitive impairment associated with such lesions (Poggi et al. 2005).

Maternal systemic LPS exposure: Animal modeling to assess effects of maternal systemic infection (maternal i.p., i.v. or subcutaneous administration of LPS, influenza virus, or polyinosinic–polycytidylic acid) during pregnancy on CNS function in offspring has been done almost exclusively using rats and mice (Boksa 2010). While influenza virus and poly IC are often used to study neurobehavioral consequences and their relevance to psychiatric disorders, most perinatal white matter injury studies use LPS. Similar to intrauterine exposure, maternal systemic LPS exposure induces strong inflammatory responses in the maternal blood circulation, the placenta and in the fetal and offspring brain as indicated by the elevation of inflammatory cytokines (for comprehensive reviews, see Wang et al. 2006; Boksa 2010). For example, peak expression of IL-1β mRNA in the fetal rat brain was found as early as 1 h after LPS injection (Cai et al. 2000) and peak expression of IL-1β protein was found at 4 h post-injection (Ghiani et al. 2011). The significant elevations in IL-1β expression sustained 48–72 h (Paintlia et al. 2004; Rousset et al. 2006) or even 9–10 days (E18-19 injection and P7 detected, Kumral et al. 2007). Although at high doses of LPS (0.6 MBq) maternal injected radioactive LPS can be detected in the fetus (Kohmura et al. 2000), several studies demonstrated that LPS does not enter the fetal compartment when administered systemically to the pregnant rat dam (Goto et al. 1994; Ashdown et al. 2006). We and other investigators have shown that high doses of maternal systemic LPS administration may cause

fetal death (Cai et al. 2000; Paintlia et al. 2004). Therefore, the inflammatory responses in the fetal or offspring brain are very likely not caused by direct LPS exposure, rather by the inflammatory signals elicited in the maternal side or from the placenta and transferred to the brain of the fetus (Dammann and Leviton 1997). At various dosages (0.3 mg/kg up to 100 mg/kg), maternal LPS injection was not able to produce apparent white matter or gray matter lesions in P5–P7 rats (Eklind et al. 2001; Hagberg et al. 2002). In some other studies, increased apoptotic cell death in the periventricular area, increased astrogliosis, reduced number of O4+/O1+ immature oligodendrocytes, or hypomyelination have been reported in the offspring brain following maternal systemic LPS exposure (Cai et al. 2000; Paintlia et al. 2004; Rousset et al. 2006; Hao et al. 2010). Despite the inflammatory responses in the fetal or offspring brain following maternal LPS injection, no PVL-like severe white matter lesion has been reported. The limited accessibility of LPS to the fetal compartment and the different signaling involved in the fetal brain inflammatory responses may contribute to the less severe WMI in the fetal and offspring brain following maternal systemic LPS injection. On the other hand, maternal LPS has been demonstrated to induce a loss of pyramidal cells in the hippocampus at 8 months of age (Golan et al. 2005) and to result in a significant neuronal loss, increased astrogliosis in CA1 region and poor performance in the Morris water maze test at 20 months of age (Hao et al. 2010). Effects of maternal LPS exposure on the offspring brain development and function seem to persist in adulthood.

Neonatal central or peripheral LPS exposure: Because white matters in the rodent brain develop mostly in postnatal days (Dobbing and Sands 1979), many models of WMI induced by neonatal LPS exposure, through either central or peripheral administration, have been developed.

Intracerebral injection of LPS into the subcortical white matter in P5 rats induced strong inflammatory responses as indicated by enormous increases in expression of TNFα, IL-1β, and IL-6 mRNA and protein and expression of iNOS in the rat brain (Pang et al. 2003). IL-1β level in the brain remained significantly elevated even 9 days later (Pang et al. 2003). Brain injury induced by intracerebral injection of LPS in P2–P7 rats included ventriculomegaly, white matter rarefaction or necrosis in the periventricular region, reduced numbers in preOLs, and hypomyelination, resembling those observed in the infant brain with PVL (Lehnardt et al. 2002; Pang et al. 2003; Cai et al. 2003, 2011; He et al. 2010). Reactive astrogliosis and activated microglia/macrophages were found in the regions surrounding the damaged areas (Lehnardt et al. 2002; Pang et al. 2003; Cai et al. 2003, 2011; He et al. 2010). Microglia are the only glia that express toll-like receptor 4 (TLR-4), a receptor specifically for LPS and necessary for LPS-induced oligodendrocyte injury, in the CNS (Lehnardt et al. 2002). Intracerebral injection of LPS also induced neuronal injury in the hippocampus and the substantia nigra region (Fan et al. 2008, 2011). A recent study show that WMI induced by intracerebral injection of LPS persists even 8 weeks after the injection (Webber et al. 2009). On the other hand, although intracerbral LPS exposure induces neurobehavioral deficits in neonatal and juvenile animals, these deficits are spontaneously recoverable by P70 (Fan et al. 2011). Whether there is a causal-effect relationship between LPS-induced WMI and

LPS-induced neurological dysfunction in this model needs further investigation. Another shortcoming of this model is the extreme approach of LPS exposure via intracerebral injection, a scenario may rarely occur in real human life.

Peripheral injection of LPS: One of the first animal models of neonatal endotoxin-mediated WMI was demonstrated by Gilles et al. (1977). Intraperitoneal injection of LPS from *E. coli* into the newborn kittens resulted in necrosis of the white matter followed by astrogliosis and macrophage infiltration (Gilles et al. 1977). Peripheral injection of LPS to neonatal rodents initiates an acute inflammatory response, both systemically and centrally (Wang et al. 2006). Subcutaneous injection of live bacterial *E. coli* in P7 rats increased serum levels of IL-1β (at 24 h) and IL-6 (at 8 and 48 h), but no changes in TNFα concentrations (Bilbo et al. 2005). In examination of global gene expression, it has been shown that almost 2,000 genes are significantly regulated in the brain within the first 72 h after peripheral LPS administration and a significant number of these genes belong to inflammatory and immune categories (Eklind et al. 2006). A recent study showed that i.p. injection of LPS (0.3 mg/kg) in P7 rats induced great increases in NF-kB signaling, expression of inflammatory cytokines (TNFα and MCP1) and microglia activation in the brain at 24 h (Yang et al. 2012). Chronic subclinical inflammation induced by repeated LPS injection (0.3 mg/kg) in newborn mice from P3–P11 decreased serum levels of insulin-like growth factor 1 and resulted in axonal injury and impaired myelination in subcortical white matter as well as reduced gray matter volume (Wang et al. 2009a, b; Du et al. 2011). It is not clear how the systemic response is mediated to the brain. LPS to newborn animals has been shown to affect the permeability of the blood–brain barrier (BBB), as indicated by the detection of plasma protein in the white matter at 10–24 h after LPS injection (1 mg/kg, i.p.) in P0 and P8 rats, but not in rats old than P20 (Stolp et al. 2005). These data suggest that at least the inflammatory cytokines and other molecules generated following LPS exposure may across the BBB and mediate signals to the brain (Dammann and Leviton 1997). Data from a recent study in mice with systemic exposure to IL-1β support this theory (Favrais et al. 2011). Newborn mice received twice daily i.p. injection of IL-1β at a dose of 10 μg/kg for 5 days (from P1–P4) and has a long-lasting myelination defect that was characterized by an increased number of nonmyelinated axons and a significant reduction in the density of myelinating OLs accompanied by an increased density of OL progenitors, suggesting a partial blockade of the OL maturation process (Favrais et al. 2011). Chronic exposure to IL-1β also disrupted white matter development as detected by MRI and resulted in memory deficits (Favrais et al. 2011). On the other hand, despite the enormous inflammatory responses in the rat brain, severe white matter lesions as seen in the infant brain with PVL are rarely reported in rats following peripheral LPS exposure. Although subcutaneous administration of LPS (30–120 μg/kg) in P2–P6 rats (0.3 mg/kg) resulted in increased expression of PreOL markers (CNPase and PLP) in the rat brain at P22 and decreased expression of these markers at 12 weeks, no apparent white matter lesion and CP-like motor impairments were observed (Roberson et al. 2006). Similarly, i.p. injection of LPS (0.3 mg/kg) in P7 rats did not result in apparent brain lesions, despite the increased expression of inflammatory cytokines and microglia activation in the rat brain

(Eklind et al. 2001; Yang et al. 2012). The variation among studies indicated the complexity of the responses to LPS and the variations are likely to be related to the species, sampling regime, LPS doses and the endotoxin sources used.

7.4.6 *Models of Combined Insults of Infection–Inflammation and HI*

Preterm infants or even term infants may suffer from the combined insults of prenatal infection and perinatal HI. A number of animal studies have demonstrated that pre-exposure to LPS potentiates the consequences of subsequent HI insults and thereby exaggerates brain injury. When pregnant rats were exposed to LPS from gestation day 17 to the end of gestation at a daily dose of 200 μg/kg (i.p.), neonatal HI (unilateral carotid ligation followed by exposure to 8 % oxygen for 210 min) in P1 offspring from LPS-injected dams resulted in significantly increased neuronal damage, especially in the neocortex, as compared to those without maternal LPS exposure (Larouche et al. 2005). This combined insult of prenatal LPS exposure and neonatal HI also resulted in motor behavioral deficits in the offspring, resembling those observed in very preterm human neonates affected by subsequent CP (Girard et al. 2009). Maternal LPS exposure at gestation days 19 and 20 significantly exacerbated cortical and white matter lesions induced by intracerebral injection of ibotenate in P4 offspring as compared to those without maternal LPS exposure (Rousset et al. 2008). The synergistic effects of LPS and a subsequent HI insult on brain injury are also evidenced in neonatal rodents. LPS injection (0.3 mg/kg, i.p.) in P7 rats 4 h (Eklind et al. 2001; Yang et al. 2012) or 72 h (Eklind et al. 2005) prior to 20 min HI greatly enhanced brain injury. The sensitizing effect of LPS pre-exposure on HI brain injury was also observed in P7 rats when LPS (5 μg/pup) was given intracisternally 1 h prior to common carotid ligation and 2 h before hypoxic exposure (8 % oxygen for 60 min) (Coumans et al. 2003) or in neonatal mice when LPS (0.3 mg/kg, i.p.) was administered 1 h before HI (7.7 % oxygen for 30 min). A recent study showed that even when the insult order was reversed, HI (unilateral carotid ligation and 6 % oxygen for 35 min) first and then followed by LPS exposure (1 mg/kg, i.p.) 30 min later in P6 mice, the combined insults results in enhanced PVL-like white matter injury (Shen et al. 2010). Mechanisms underlying the sensitizing effects of LPS on neonatal HI brain injury remain unclear. The sensitizing effect has been shown to be dependent on TLR-4 and MyD88 signaling, because LPS pre-exposure failed to potentiate HI-induced gray and white matter damage in mice deficient in the TLR-4 receptor (Lehnardt et al. 2003) or in MyD88 knockout mice (Wang et al. 2009a, b).

In contrast to the sensitizing effects observed when LPS is given 4 h or 72 h before HI, reduced brain injury has been reported with a time interval of 24 h between LPS and HI (Eklind et al. 2005). This preconditioning effect appears to be dependent on glucocorticoids, a glucocorticoid receptor blocker (RU486) administered simultaneously with LPS (1 mg/kg, i.p .) at 24 h before HI abolishes the

LPS-induced tolerance effect in P7 rats (Ikeda et al. 2006). The preconditioning effect of LPS pre-exposure is also associated with the dose of LPS and the age of animals at the time of HI occurring. LPS administered at a dose of 0.05 mg/kg, but not 0.3 mg/kg, 24 h before HI reduced HI-induced microglia and macrophage activation, TNF-α expression and reactive oxygen species production in neonatal rats and resulted in better learning and memory performance and less brain damage in adulthood (Lin et al. 2009). When LPS was injected (intrauterine) to pregnant C57BL/6 mice at gestation day 15 and HI was performed in offspring at P5, 9, or 70, LPS sensitized HI-induced brain injury in neonates but conferred protection in adulthood (Wang et al. 2007a). These studies indicate that the interaction between LPS and HI is complex and many other factors might be involved in the interaction. These data also demonstrate that an LPS challenge in early life, either alone or in combination with other insults, may have life-long consequences. Our recent study shows that neonatal LPS exposure via intracerebral injection causes not only WMI in the developing rat forebrain but also chronic inflammation in the substatia nigra regions and enhances vulnerability of dopaminergic neurons in the substatia nigra to neurotoxins at an ordinarily nontoxic or sub-toxic dose in adulthood to develop Parkinson's disease-like neurodegenerative disorders (Fan et al. 2011). Our ongoing study further demonstrates that systemic injection of LPS (i.p.) in neonatal rats has similar long-lasting effects on vulnerability of the dopaminergic system in late life (unpublished data). Further investigations are required to reveal mechanisms involved in the interaction between early life LPS exposure and subsequent insults in short intervals or even in late life.

7.4.7 Excitotoxic Models of WMI

In an early study, administration of excitatory amino acid receptor agonist *N-methyl-d*-aspartate (NMDA) into P7 rat corpus striatum was found to induce lesions in striatum, hippocampus, and cortex that were 16–20 times larger than the corresponding lesions in adult rats (McDonald et al. 1988). Subcortical injection of ibotenate (agonist of the NMDA and metabotropic tropic glutamate receptors) in P0–P10 rats resulted in both cortical neuronal and periventricular white matter lesions, with a peak occurrence of white matter lesions at P5 (Marret et al. 1995). Both gray and white matter damage were prevented by DL-AP7, an NMDA receptor antagonist, but not by L-AP3, a metabotropic glutamate receptor antagonist (Marret et al. 1995), indicating the role of NMDA receptors in white matter injury. It was found later that not only the NMDA receptor, but also the AMPA/kainate type glutamate receptor are involved in the neonatal brain injury (Follett et al. 2000, 2004). The AMPA receptors are expressed in OLs in pericallosal white matter of the rat brain with a peak expression at P7, and HI in P7 rats (unilateral carotid ligation and 6 % oxygen for 1 h) resulted selective white matter injury with a marked decrease of myelin basic protein expressing OLs that was significantly attenuated by NBQX, a AMPA receptor antagonist, but not by the NMDA receptor antagonist

MK-801 (Follett et al. 2000). Intracerebral injection of AMPA to P7 rats resulted in periventricular gray and white matter injury (Follett et al. 2000; Xu et al. 2005), with a greater susceptibility of OL injury at P7 than in younger and older pups, which could be attenuated by NBQX (Follett et al. 2000). These results indicate a parallel, maturation-dependent susceptibility of immature OLs to AMPA and HI. The involvement of excitotoxicity in neonatal white matter injury was also supported by the findings that the NMDA and AMPA receptors are both expressed on OLs (Salter and Fern 2005; Karadottir and Attwell 2007) and that there is a significant increase in extracullular glutamate levels in the white matter following HI (repeated umbilical cord occlusion) in a near term fetal sheep model of HI (Loeliger et al. 2003). Intracerebral injection of either NMDA receptor agonist (such as ibotenate) or AMPA/kainate receptor agonist (such as AMPA or S-Willardiine) is currently used to produce PWMI-like lesions in neonatal rats (Chen et al. 2008; Pansiot et al. 2010) and rabbits (Sfaello et al. 2005). Nonreceptor-mediated glutamate toxicity is also involved in maturation-dependent death of preOLs and white matter injury (Volpe et al. 2011). In vitro studies (Oka et al. 1993; Back et al. 1998) demonstrated that depletion of glutathion and oxygen free radical attack resulted from glutamate-mediated inhibition of cysteine uptake are responsible for the death of preOLs. However, nonreceptor-mediated glutamate toxicity as a sole mechanism for neonatal WMI and death of the preOLs has rarely reported in in vivo animal models. As pointed in a previous review (Hagberg et al. 2002), injection of high doses of glutamate receptor agonists is highly artificial, but such paradigms may be useful to screen for potential therapeutic treatment of neonatal brain injury (Chen et al. 2010; Pansiot et al. 2010).

7.5 Summary

The above sections reviewed some of the currently available approaches to model neonatal WMI. These models have been introduced along with advances of our knowledge in WMI from human infants. Two decades ago, most experimental research on perinatal brain injury was focusing on HI injury to reflect the clinical problem of birth asphyxia, which has generated critical information about the pathophysiology of immature neuronal injury. However, only a small portion of cases suffering from CP are related to asphyxia. Later, perinatal infection was found to be an important factor in both preterm and term infants and the interest has, to some extent, moved from neuronal injury in term infants to WMI in preterms. Consequently, a number of interesting new animal models have been introduced in various species, aiming to replicate one or more features of WMI in the very immature brain, induced by either hypoperfusion or infectious agents/bacterial endotoxin. Today, advances in care have resulted in a pronounced shift in the spectrum of WMI such that the incidence of classical cystic necrotic PVL has markedly declined and focal and diffuse white matter non-cystic lesions that selectively trigger oligodendrocyte lineage degeneration and subsequent disturbances in

myelination become predominant (Silbereis et al. 2010). Animal models or refinements of the existing models that can more faithfully reproduce the major forms of WMI observed in the current population of preterm survivors are crucially needed (Back et al. 2012). Studies have shown that pre-exposure to bacteria endotoxin can either sensitize or reduce the secondary insult-induced brain injury, supporting potential roles of inflammation during fetal and neonatal life in neurologic and neuropsychiatric disorders in children and adults (Hagberg et al. 2012). Future models that more closely reproduce the spectrum of insults that appear to contribute to cerebral injury in human infants are needed. For example, they may include the influence of antecedent insults such as recurrent transient or prolonged HI, chronic in utero infection–inflammation, or placental insufficiency. In addition, genetic and modern imaging technology should be fully utilized in our future development of animal models of neonatal WMI. As important functional outcomes, neurobehavioral aspects in future animal models should be strengthened. One question about animal models may frequently be asked: which models are best? The answer is that none of them stands out as the best (Johnston et al. 2005). As discussed above, all the models have certain drawbacks, yet each has important strengths. Small rodents have less white matter than higher species and it is difficult to generate a spectrum of pathology that closely resembles the lesions observed in humans. Small rodent models have significant limitations to study the developing human brain (Ferriero 2006), but they can provide initial answers to numerous cellular and molecular questions. By contrast, large preclinical models such as fetal sheep have abundance of cerebral white matter with a developmental profile more similar to humans. These models are attractive for pathophysiological studies and clinical-translational studies, but they require the higher costs and technical challenge of surgical instrumentation. Collectively, these models provide unprecedented opportunities for rapid progress towards defining the mechanisms relevant to neonatal WMI. Appropriate uses or combined uses of these models are likely to drive progress in this area over the future years.

Acknowledgments This work was supported by a NIH grant (NS54278).

References

Ashdown H, Dumont Y, Ng M et al (2006) The role of cytokine in mediating effects of prenatal infection on the fetus: implications for schizophrenia. Mol Psychiatry 11:47–55

Back SA (2006) Perinatal white matter injury: the changing spectrum of pathology and emerging insights into pathogenetic mechanisms. Ment Retard Dev Disabil Res Rev 12:129–140

Back SA, Rivkees SA (2004) Emerging concepts in periventricular white matter injury. Semin Perinatol 28:405–414

Back SA, Gan X, Li Y et al (1998) Maturation-dependent vulnerability of oligodendrocytes to oxidative stress-induced death caused by glutathione depletion. J Neurosci 18:6241–6253

Back SA, Luo NL, Borenstein NS et al (2001) Late oligodendrocyte progenitors coincide with the developmental window of vulnerability for human perinatal white matter injury. J Neurosci 21:1302–1312

Back SA, Han BH, Luo NL et al (2002) Selective vulnerability of late oligodendrocyte progenitors to hypoxia-ischemia. J Neurosci 455–463
Back SA, Luo NL, Mallinson RA et al (2005) Selective vulnerability of preterm white matter to oxidative damage defined by F(2)-isoprostane. Ann Neurol 58:108–120
Back SA, Craig A, Luo NL et al (2006a) Protective effects of caffeine on chronic hypoxia-induced perinatal white matter injury. Ann Neurol 60:696–705
Back SA, Riddle A, Hohimer AR (2006b) Role of instrumented fetal sheep preparations in defining the pathogenesis of human periventricular white matter injury. J Child Neurol 21:582–589
Back SA, Riddle A, McClure MM (2007) Maturation-dependent vulnerability of perinatal white matter in premature birth. Stroke 38:724–730
Back SA, Riddle A, Dean J et al (2012) The instrumented fetal sheep as a model of cerebral white matter injury in the premature infant. Neurotherapeutics 9:359–370
Balduini W, De Angelis V, Mazzoni E et al (2000) Long-lasting behavioral alterations following a hypoxic/ischemic brain injury in neonatal rats. Brain Res 859:318–325
Banker BQ, Larroche JV (1962) Periventricular leukomalacia of infancy. A form of neonatal anoxic encephalopathy. Arch Neurol 7:386–410
Barlow R (1969) The foetal sheep: morphogenesis of the nervous system and histochemical aspects of myelination. J Comp Neurol 135:249–262
Baud O, Daire JL, Dalmaz Y et al (2004) Gestational hypoxia induces white matter damage in neonatal rats: a new model of periventricular leukomalacia. Brain Pathol 14:1–10
Bell MJ, Hallenbeck JM (2002) Effects of intrauterine inflammation on developing rat brain. J Neurosci Res 70:570–579
Bennet L, Cowie RV, Stone PR et al (2010) The neural and vascular effects of killed Su-Streptococcus pyogenes (OK-432) in preterm fetal sheep. Am J Physiol Regul Integr Comp Physiol 299: R662–R672
Bi B, Salmaso N, Komitova MV et al (2011) Cortical glial fibrillary acidic protein-positive cells generate neurons after perinatal hypoxic injury. J Neurosci 31:9205–9211
Bilbo SD, Biedenkapp JC, Der-Avakain A et al (2005) Neonatal infection-induced memory impairment after lipopolysaccharide in adult is prevented viacaspase-1 inhibition. J Neurosci 25:8000–8009
Billiards SS, Haynes RL, Folkerth RD et al (2006) Development of microglia in the cerebral white matter of the human fetus and infant. J Comp Neurol 497:199–208
Billiards SS, Haynes RL, Folkerth RD et al (2008) Myelin abnormalities without oligodendrocyte loss in periventricular leukomalacia. Brain Pathol 18:153–163
Boksa P (2010) Effects of prenatal infection on brain development and behavior: a review of findings from animal models. Brain Behav Immun 24:881–897
Bona E, Johansson BB, Hagberg H (1997) Sensorimotor function and neurophathology five to six weeks after hypoxia-ischemia in seven-day-old rats. Pediatr Res 42:678–683
Burd I, Brown A, Gonzalez JM et al (2011) A mouse model of term chorioamnionitis: unrevealing causes of adverse neurological outcomes. Reprod Sci 18:900–907
Burd I, Balakrishnan B, Kannan S (2012) Models of fetal brain injury, intrauterine inflammation, and preterm birth. Am J Reprod Immunol 67:287–294
Buser JR, Segovia KN, Dean JM et al (2010) Timing of appearance of late oligodendrocyte progenitors coincides with enhanced susceptibility of preterm rabbit cerebral white matter to hypoxia-ischemia. J Cereb Blood Flow Metab 30:1053–1065
Buser JR, Maire J, Riddel A et al (2012) Arrested preoligodendrocyte maturation contributes to myelination failure in premature infants. Ann Neurol 71:93–109
Cai Z, Sigrest T, Hersey K et al (1995) Intrauterine hypoxia-ischemia increases N-methyl-D-ASPARTATE-induced cGMP formation and glutamate accumulation in cultured rat cerebral granule cells. Pediatr Res 38:107–112
Cai Z, Fratkin JD, Rhodes PG (1997) Prenatal ischemia reduces neuronal injury caused by neonatal hypoxia-ischemia in rats. Neuroreport 8:1393–1398
Cai Z, Hutchins JB, Rhodes PG (1998) Intrauterine hypoxia-ischemia alters nitric oxide synthase expression and activity in fetal and neonatal rat brains. Brain Res Dev Brain Res 109:265–269

Cai Z, Feng X, Lee B et al (1999) Prenatal hypoxia-ischemia alters expression and activity of nitric oxide synthase in the young rat brain and causes learning deficits. Brain Res Bull 49:359–365
Cai Z, Pan ZL, Pang Y et al (2000) Cytokine induction in fetal rat brains and brain injury in neonatal rats after maternal lipopolysaccharide administration. Pediatr Res 47:64–72
Cai Z, Pang Y, Xiao F et al (2001) Chronic ischemia preferentially causes white matter injury in the neonatal rat brain. Brain Res 898:126–135
Cai Z, Pang Y, Lin S et al (2003) Differential roles of tumor necrosis factor-alpha and interleukin-1 beta in lipopolysaccharide-induced brain injury in the neonatal rat. Brain Res 975:37–47
Cai Z, Lin S, Fan L-W et al (2006) Minocycline alleviates hypoxic-ischemic injury to developing oligodendrocytes in the neonatal rat brain. Neuroscience 137:425–435
Cai Z, Fan L-W, Lin S et al (2011) Intranasal administration of insulin-like growth factor-1 protects against lipopolysaccharide-induced injury in the developing rat brain. Neuroscience 194:195–207
Carlsson Y, Schwendimann L, Vontell R et al (2011) Genetic inhibition of caspase-2 reduces hypoxic-ischemic and excitotoxic neonatal brain injury. Ann Neurol 70:781–789
Carlsson Y, Wang X, Schwendimann L et al (2012) Combined effect of hypothermia and caspae-2 gene deficiency on neonatal hypoxic-ischemic brain injury. Pediatr Res 71:566–572
Chau V, Positt KJ, McFadden DE et al (2009) Effect of chorioamnionitis on brain development and injury in premature newborns. Ann Neurol 66:155–164
Chen A, Dimambro N, Clowey GJ (2008) A comparison of behavioral and histological outcomes of periventricular injection of ibotenic acid in neonatal rats at postnatal days 5 and 7. Brain Res 1201:187–195
Chen A, Siow B, Blamirc AM et al (2010) Transplantation of magnetically labeled mesenchymal stem cells in a model of perinatal brain injury. Stem Cell Res 5:255–266
Coumans AB, Middelanis JS, Garnier Y et al (2003) Intracisternal application of endotoxin enhances the subsequent hypoxic-ischemic brain damage in neonatal rats. Pediatr Res 53:257–263
Dammann O, Leviton A (1997) Maternal intrauterine infection, cytokines and brain damage in the preterm newborn. Pediatr Res 42:1–8
Dean JM, Van de Looij Y, Sizonenko SV et al (2011) Delayed cortical impairment following lipopolysaccharide exposure in preterm fetal sheep. Ann Neurol 70:846–856
Debillon T, Gras-Leguen C, Verielle V et al (2000) Intrauterine infection induces programmed cell death in rabbit periventricular white matter. Pediatr Res 47:736–742
Debillon T, Gras-Leguen C, Verielle V et al (2003) Effect of maternal antibiotic treatment on fetal periventricular white matter cell death in a rabbit intrauterine infection model. Acta Paediatr 92:81–86
Derrick M, Luo NL, Bregman JC et al (2004) Preterm fetal hypoxia-ischemia causes hypertonia and motor deficits in the neonatal rabbit: a model of human cerebral palsy? J Neurosci 24:24–34
Derrick M, Drobyshevsky A, Ji X et al (2007) A model of cerebral palsy from fetal hypoxia-ischemia. Stroke 38:731–735
Derrick M, Drobyshevsky A, Ji X et al (2009) Hypoxia-ischemia causes persistent movement deficits in a perinatal rabbit model of cerebral palsy: assessed by a new swim test. Int J Dev Neurosci 27:549–557
Dieni S, Inde T, Yoder B et al (2004) The pattern of cerebral injury in a primate model of preterm birth and neonatal intensive care. J Neuropathol Exp Neurol 63:1297–1309
Ditelberg JS, Sheldon RA, Epstein CJ et al (1996) Brain injury after perinatal hypoxia-ischemia is exacerbated in cipper/zinc superoxide dismutase transgenic mice. Pediatr Res 39:204–208
Dizon ML, Maa T, Kessler JA (2011) The bone morphogenetic protein antagonist noggin protects white matter after perinatal hypoxia-ischemia. Neurobiol Dis 42:318–326
Dobbing J, Sands J (1979) Comparative aspects of the brain growth spurt. Early Hum Dev 3: 79–83
Drobyshevsky A, Derrick M, Wyrwicz AM et al (2007) White matter injury correlated with hypertonia in an animal model of cerebral palsy. J Cereb Blood Flow Metab 27:270–281
Du X, Fleiss B, Dangelo B et al (2011) Systemic stimulation of TLR2 impairs neonatal mouse brain development. PLoS One 6:e19583

Duncan JR, Cock ML, Scherlinck JP et al (2002) White matter injury after repeated endotoxin exposure in the preterm ovine fetus. Pediatr Res 52:941–949
Duncan JR, Cock ML, Suzuki K et al (2006) Chronic endotoxin exposure causes brain injury in the ovine fetus in the absence of hypoxemia. J Soc Gynecol Investig 13:87–96
Eklind S, Mallard C, Leverin A-L et al (2001) Bacterial endotoxin sensitizes the immature brain to hypoxic-ischemic injury. Eur J Neurosci 13:1101–1106
Eklind S, Mallard C, Arvidsson P et al (2005) Lipopolysaccharide induces both a primary and a secondary phase of sensitization in the developing rat brain. Pediatr Res 58:112–116
Eklind S, Hagberg H, Wang X et al (2006) Effect of lipopolysaccharide on global gene expression in the immature rat brain. Pediatr Res 60:161–168
Fagel DM, Ganat Y, Silbereis J et al (2006) Cortical neurogenesis enhanced by chronic perinatal hypoxia. Exp Neurol 199:77–91
Fan L-W, Lin S, Pang Y et al (2005) Hypoxia-ischemia induced neurological dysfunction and brain injury in the neonatal rat. Behav Brain Res 165:80–90
Fan L-W, Tien L-T, Mitchell HJ et al (2008) α-Pheyl-n-tert-butyl-nitrone ameliorates hippocampal injury and improves learning and memory in juvenile rats following neonatal exposure to lipopolysaccharide. Eur J Neurosci 27:1475–1484
Fan L-W, Tien L-T, Lin RC et al (2011) Neonatal exposure to lipopolysaccharide enhances vulnerability of nigrostriatal dopaminergic neurons to rotenone neurotoxicity in later life. Neurobiol Dis 44:304–316
Favrais G, van de Looij Y, Fleiss B et al (2011) Systemic inflammation disrupts the developmental program of white matter. Ann Neurol 70:550–565
Ferriero DM (2006) Can we define the pathogenesis of human periventricular white-matter injury using animal models? J Child Neurol 21:580–581
Ferriero DM, Holtzman DM, Black SM et al (1996) Neonatal mice lacking neuronal nitric oxide synthase are less vulnerable to hypoxic-ischemic injury. Neurobiol Dis 3:64–71
Follett P, Rosenberg PA, Volpe JJ et al (2000) NBQX attenuates excitotoxic injury in developing white matter. J Neurosci 20:9235–9241
Follett PL, Deng W, Dau W et al (2004) Glutamate receptor-mediated oligodendrocyte toxicity in periventricular leukomalacia: a protective role for topiramate. J Neurosci 24:4412–4420
Ghiani CA, Mattan NS, Nobuta H et al (2011) Early effects of lipopolysaccharide-induced inflammation on fetal brain development in rat. ASN Neuro 3(4):pii: e00068. doi:10.1042/AN20110027
Gilles FH, Averill DR Jr, Ker CS (1977) Neonatal endotoxin encephalopathy. Ann Neurol 2:49–56
Girard S, Kadhim H, Beaudet N et al (2009) Developmental motor deficits induced by combined fetal exposure to lipopolysaccharide and early neonatal hypoxia/ischemia: a novel animal model for cerebral palsy in very premature infants. Neuroscience 158:673–682
Golan HM, Lev V, Hallak M et al (2005) Specific neurodevelopment damage in mice offspring following maternal inflammation during pregnancy. Neuropharmacology 48:903–917
Goto M, Yoshioka T, Ravindranath T et al (1994) LPS injected into the pregnant rat late in gestation does not induce fetal endotoxemia. Res Commun Mol Pathol Pharmacol 85:109–112
Griffith JL, Shimony JS, Cousins SA et al (2012) MR imaging correlates of white-matter pathology in a preterm baboon model. Pediatr Res 71:185–191
Groenendaal F, Termote JU, van der Heide-Jalving M et al (2010) Complications affecting preterm neonates from 1991 to 2006: what have we gained? Acta Paediatr 99:354–358
Hagberg H, Peebles D, Mallard C (2002) Models of white matter injury: comparison of infectious, hypoxic-ischemic, and excitotoxic insults. Ment Retard Dev Disabil Res Rev 8:30–38
Hagberg H, Gressens P, Mallard C (2012) Inflammation during fetal and neonatal life: implications for neurologic and neuropsychiatric disease in children and adults. Ann Neurol 71:444–457
Hao LY, Hao XQ, Li SH et al (2010) Prenatal exposure to lipopolysaccharide results in cognitive deficits in age-increasing offspring rats. Neuroscience 166:763–770
Haynes RL, Folkerth RD, Keefe RJ et al (2003) Nitrosative and oxidative injury to premyelinating oligodendrocytes in periventricular leukomalacia. J Neuropathol Exp Neurol 62:441–450
Haynes RL, Billiards SS, Borenstein NS et al (2008) Diffuse axonal injury in periventricular leukoomalacia as determined by apoptotic marker fraction. Pediatr Res 63:656–661

He LF, Chen HI, Qian LH et al (2010) Curcumin protects pre-oligodendrocytes from activated microglia in vitro and in vivo. Brain Res 1339:60–69
Hedtjarn M, Mallard C, Arvidsson P et al (2005) White matter injury in the immature brain: role of interleukin-18. Neurosci Lett 373:16–20
Ikeda T, Murata Y, Quilligan EJ et al (1998) Physiologic and histologic changes in near-term fetal lambs exposed to asphyxia by partial umbilical cord occlusion. Am J Obstet Gynecol 178:24–32
Ikeda T, Mishima K, Yoshikawa T et al (2001) Selective and long-term learning impairment following neonatal hypoxic-ischemic brain injury in rats. Behav Brain Res 118:17–25
Ikeda T, Yang L, Ikenoue T et al (2006) Endotoxin-induced hypoxic-ischemic tolerance is mediated by up-regulation of corticosterone in neonatal rat. Pediatr Res 59:56–60
Inder TE, Huppi PS, Warfield S et al (1999) Periventriculare white matter injury in the premature infant is followed by reduced cerebral cortical gray matter volume at term. Ann Neurol 46:755–760
Inder TE, Anderson NJ, Spenser C et al (2003) White matter injury in the premature infant: a comparison between serial cranial ultrasonographic and MR findings at term. Am J Neuroradiol 24:805–809
Inder T, Neil J, Yoder B et al (2004) Non-human primate model of neonatal brain injury. Semin Perinatol 28:396–404
Inder T, Neil J, Yoder B et al (2005) Patterns of cerebral injury in a primate model of preterm birth and neonatal intensive care. J Child Neurol 20:965–967
Jelinski SE, Yager JY, Juurlink BHJ (1999) Preferential injury to oilgodendroblasts by a short hypoxic-ischemic insult. Brain Res 815:150–153
Johnston MV, Ferriero DM, Vannucci SJ et al (2005) Models of cerebral palsy: which ones are best? J Child Neurol 20:984–987
Kadhim HJ, Tabarki B, Verellen G et al (2001) Inflammatory cytokines in the pathogenesis of periventricular leukomalacia. Neurology 56:1278–1284
Kannan S, Saadani-Makki F, Muzik O et al (2007) Microglial activation in perinatal rabbit brain induced by intrauterine inflammation: detection with 11C-(R)-PK11195 and small-animal PET. J Nucl Med 48:946–954
Kannan S, Saadani-Makki F, Balakrishna B et al (2011) Magnitude of [(11)C]PK11195 binding is related to severity of motor deficits in a rabbit model of cerebral palsy induced by intrauterine endotoxin exposure. Dev Neurosci 33:231–240
Karadottir R, Attwell D (2007) Neurotransmitter receptors in the life and death of oligodendrocytes. Neuroscience 145:1426–1438
Keller M, Enot DP, Hodson MP et al (2011) Inflammatory-induced hibernation in the fetus: priming of fetal sheep metabolism correlates with developmental brain injury. PLoS One 6(12):e29503
Khwaja O, Volpe JJ (2008) Pathogenesis of cerebral white matter injury of prematurity. Arch Dis Child Fetal Neonatal Ed 93:F153–F161
Kinney HC (2009) The encephalopathy of prematurity: one pediatric neuropathalogist's perspective. Semin Pediatr Neurol 16:179–190
Kohmura Y, Kirikae T, Kirikae F et al (2000) Lipopolysaccharide (LPS)-induced intra-uterinefetal death in mice is principally due to maternal cause but not fetal sensitivity to LPS. Microbiol Immunol 44:897–904
Kumral A, Baskin H, Yesilirmak DC et al (2007) Erythropoietin attenuates lipopolysaccharide-induced white matter injury in the neonatal rat brain. Neonatology 92:269–278
Larouche A, Roy M, Kadhim H et al (2005) Neuronal injuries induced by perinatal hypoxic-ischemic insults are potentiated by prenatal exposure to lipopolysaccharide: animal model for perinatally acquired encephalopathy. Dev Neurosci 27:134–142
Lehnardt S, Lachance C, Patrizi S et al (2002) The toll-like receptor TLR4 is necessary for lipopolysaccharide-induced oligodendrocyte injury in the CNS. J Neurosci 22:2478–2486
Lehnardt S, Massillon L, Pollet P et al (2003) Activation of innate immunity in the CNS triggers neurodegeneration through a Toll-like receptor4-dependent pathway. Proc Natl Acad Sci 100:8514–8519

Leviton A, Gressens P (2007) Neuronal damage accompanies perinatal white-matter damage. Trends Neurosci 30:473–478
Leviton A, Allred EN, Kuban KC et al (2010) Microbiologic and histologic characteristics of the extremely preterm infant's placenta predict white matter damage and later cerebral palsy. The ELGAN study. Pediatr Res 67:95–101
Lin S, Rhodes PG, Lei M et al (2004) α-Phenyl-*n-tert*-butyl-nitrone attenuates hypoxic-ischemic white matter injury in the neonatal rat brain. Brain Res 1007:132–141
Lin HY, Huang CC, Chang KF (2009) Lipopolysaccharide preconditioning reduces neuroinflammation against hypoxic ischemia and provides long-term outcome of neuroprotection in neonatal rat. Pediatr Res 66:254–259
Liu Y, Silverstein FS, Skoff R et al (2002) Hypoxic-ischemic oligodendroglial injury in neonatal rat brain. Pediatr Res 51:25–33
Loeliger M, Waston CS, Reynolds JD et al (2003) Extracellular glutamate levels and neuropathology in cerebral white matter following repeated umbilical cord occlusion in the near term fetal sheep. Neuroscience 116:705–714
Loeliger M, Inder T, Cain S et al (2006) Cerebral outcomes in a preterm baboon model of early versus delayed nasal continuous positive airway pressure. Pediatrics 118:1640–1653
Lubics A, Reglodi D, Tamas A et al (2005) Neurological reflexs and early behavior in rats subjected to neonatal hypoxic-ischemic injury. Behav Brain Res 157:157–165
Mallard C, Welin AK, Peebles D et al (2003) White matter injury following systemic endotoxemia or asphyxia in the fetal sheep. Neurochem Res 28:215–223
Marret S, Mukendi R, Gadisseux JF et al (1995) Effect of ibotenate on brain development: an excitotoxic mouse model of microgyria and posthypoxic-like lesions. J Neuropathol Exp Neurol 54:358–370
Matsuda T, Okuyama K, Cho K et al (1999) Induction of antenatal periventricular leukomalacia by hemorrhagic hypotension in the chronically instrumented fetal sheep. Am J Obstet Gynecol 181:725–730
McDonald JW, Silverstein FS, Johnston MV (1988) Neurotoxicity of N-methyl-D-aspartate is markedly enhanced in developing rat central nervous system. Brain Res 459:200–203
Ment LR, Schwartz M, Makuch RW et al (1998) Association of chronic sublethal hypoxia with ventriculomegaly in the developing rat brain. Dev Brain Res 111:197–203
Nitsos I, Rees SM, Duncan J et al (2006) Chronic exposure to intra-amniotic lipopolysaccharide affects the ovine fetal brain. J Soc Gynecol Investig 13:239–247
Normann E, Lacaze-Masmonteil T, Eaton F et al (2009) A novel mouse model of Ureaplasma-induced perinatal inflammation: effects on lung and brain injury. Pediatr Res 65:430–436
Oka A, Belliveau MJ, Rosenberg PA et al (1993) Vulnerability of oligodendroglia to glutamate: pharmacology, mechanisms and prevention. J Neurosci 13:1441–1453
Olivier P, Baud O, Evrard P et al (2005) Prenatal ischemia and white matter damage in rats. J Neuropathol Exp Neurol 64:998–1006
Olivier P, Fontaine RH, Loron G et al (2009) Melatonin promotes oligodendroglial maturation of injued white matter in neonatal rats. PLoS One 4(9):e7128. doi:10.1371/jounal.pone.0007128
Paintlia MK, Paintlia AS, Barbosa E et al (2004) N-acetyl-cysteine prevents endotoxin-induced degeneration of oligodendrocyte progenitors and hypomyelination in developing rat brain. J Neurosci Res 78:347–361
Pang Y, Cai Z, Rhodes PG (2003) Disturbance of oligodendrocyte development, hypomyelination and white matter injury in the neonatal rat brain after intracerebral injection of lipopolysaccharide. Brain Res Dev Brain Res 140:205–214
Pang Y, Rodts-Palenik S, Cai Z et al (2005) Suppression of glial activation is involved in the protection of IL-10 on maternal E. coli induced neonatal white matter injury. Brain Res Dev Brain Res 157:141–149
Pansiot J, Loron G, Olivier P et al (2010) Neuroprotective effect of inhaled nitric oxide on excitotoxic-induced brain damage in neonatal rat. PLoS One 5:e10916. doi:10.1371/journal.pone.0010916

Petersson KH, Pinar H, Stopa EG et al (2002) White matter injury after cerebral ischemia in ovine fetuses. Pediatr Res 51:768–776
Pierson CR, Folkerth RD, Billiards SS, Trachtenberg FL, Drinkwater ME, Volpe JJ, Kinney HC (2007) Gray matter injury associated with periventriculare leukomalacia in the premature infant. Acta Neuropathol 114:619–631
Poggi SH, Park L, Toso L et al (2005) No phenotype associated with established lipopolysaccharide model for cerebral palsy. Am J Obstet Gynecol 192:727–733
Probyn ME, Cock ML, Duncan JR et al (2010) The anti-inflammatory agent N-acetyl cysteine exacerbates endotoxin-induced hypoxemia and hypotension and induces polycythemia in the ovine fetus. Neonatology 98:118–127
Rao S, Lin Z, Drobyshevsky A et al (2011) Involvement of neuronal nitric oxide synthase in ongoing fetal brain injury following near-term rabbit hypoxia-ischemia. Dev Neurosci 33:288–298
Rathnasamy G, Ling E-A, Kaur C (2011) Iron and iron regulatory protein in amoeboid microglial cells are linked to oligodendrocyte death in hypoxic neonatal rat periventricular white matter through production of proinflammatory cytokines and reactive oxygen/nitrogen species. J Neurosci 31:17982–17995
Raymond M, Li P, Mangin J-M et al (2011) Chronic perinatal hypoxia reduces glutamate-aspartate transporter function in astrocytes through the Janus Kinase/signal transducer and activator of transcription pathway. J Neurosci 31:17864–17871
Reddy K, Mallard C, Guan J et al (1998) Maturation change in the cortical responses to hypoperfusion injury in the fetal sheep. Pediatr Res 43:674–682
Rees S, Hale N, De Matteo R et al (2010) Erythropoietin is neuroprotective in a preterm ovine model of endotoxin-induced brain injury. J Neuropathol Exp Neurol 69:306–319
Rice JE, Vannucci RC, Brierley JB (1981) The influence of immaturity on hypoxic-ischemic brain damage in the rat. Ann Neurol 9:131–141
Riddle A, Luo NL, Manese M et al (2006) Spatial heterogeneity in oligodendrocyte lineage maturation and not cerebral blood flow predicts fetal ovine periventricular white matter injury. J Neurosci 26:3045–3055
Roberson R, Woodard JE, Toso L et al (2006) Postnatal inflammatory rat model for cerebral palsy: too different from humans. Am J Obstet Gynecol 195:1038–1044
Romijn HJ, Hofman MA, Gramsbergen A (1991) At what age is the developing cortex of the rat comparable to that of the full-term newborn human baby? Early Hum Dev 26:61–67
Rousset CI, Chalon S, Cantagrel S et al (2006) Maternal exposure to LPS induces hypomyelination in the internal capsule and programmed cell death in the deep gray matter in newborn rats. Pediatr Res 59:428–433
Rousset CI, Kassem J, Olivier P et al (2008) Antenatal bacterial endotoxin sensitizes the immature rat brain to postnatal excitotoxic injury. J Neuropathol Exp Neurol 67:994–1000
Saadani-Makki F, Kannan S, Lu X et al (2008) Intrauterine administration of endotoxin leads to motor deficits in a rabbit model: a link between prenatal infection and cerebral palsy. Am J Obstet Gynecol 199:651.e1–651.e7
Saadani-Makki F, Kannan S, Makki M et al (2009) Intrauterine endotoxin administration leads to white matter diffusivity changes in newborn rabbits. J Child Neurol 24:1179–1189
Salter MG, Fern R (2005) NMDA receptors are expressed in developing oilgodendrocyte processed and mediate injury. Nature 438:1167–1171
Scafidi J, Fagel DM, Ment LR et al (2009) Modeling premature brain injury and recovery. Int J Dev Neurosci 27:863–871
Segovia KN, McClure M, Moravec M et al (2008) Arrested oligodendrocyte lineage maturation in chronic perinatal white matter injury. Ann Neurol 63:520–530
Sfaello I, Daire J-L, Husson I et al (2005) Patterns of excitotoxins-induced brain lesions in the newborn rabbit: a neuropathological and MRI correlation. Dev Neurosci 27:160–168
Shah DK, Doyle LW, Anderson PJ et al (2008) Adverse neurodevelopment in preterm infants with postnatal sepsis or necrotizing enterocolitis is mediated by white matter abnormalities on magnetic resonance imaging at term. J Pediatr 153:170–175, 175, e171

Shankaran S, Langer JC, Kazzi SN et al (2006) Cumulative index of exposure to hypocarbia and hyperoxia as risk factors for periventricular leukomalacia in low birth weight infants. Pediatrics 118:1654–1659
Sheldon RA, Chuai J, Ferriero DM (1996) A rat model for hypoxi-ischemic brain damage in very premature infants. Biol Neonate 69:327–341
Sheldon RA, Sedik C, Ferriero DM (1998) Strain-related brain injury in neonatal mice subjected to hypoxia-ischemia. Brain Res 810:114–122
Shen Y, Yu HM, Yuan TM et al (2007) Intrauterine infection induced oligodendrocyte injury and inducible nitric oxide synthase expression in the developing rat brain. J Perinat Med 35:203–209
Shen Y, Plane JM, Deng W (2010) Mouse model of periventricular leukomalacia. J Visualized Exp 39. http://www.jove.com/index/details.stp?ID=1951, doi: 10.3791/1951
Silbereis JC, Huang EJ, Back SA et al (2010) Towards improved animal models of neonatal white matter injury associated with cerebral palsy. Dis Model Mech 3:678–688
Skoff RP, Bessert DA, Barks JD et al (2001) Hypoxic-ischemic injury in acute disruption of myelin gene expression and death of oligodendroglial precursors in neonatal mice. Int J Dev Neurosci 19:197–208
Skoff RP, Bessert D, Barks JDE et al (2007) Plasticity of neurons and glia following neonatal hypoxic-ischemic brain injury in rats. Neurochem Res 32:331–342
Stolp HB, Dziegielewska KM, Ek CT et al (2005) Long-term changes in blood–brain barrier permeability and white matter following prolonged systemic inflammation in early development in the rat. Eur J Neurosci 22:2805–2816
Svedin P, Kjellmer I, Welin AK et al (2005) Maturational effects of lipopolysaccharide on white-matter injury in fetal sheep. J Child Neurol 20:960–964
Talos DM, Follett PL, Folkerth RD et al (2006) Developmental regulation of alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor subunit expression in forebrain and relationship to regional susceptibility to hypoxic/ischemic injury. II. Human cerebral white matter and cortex. J Comp Neurol 497:61–77
Tan S, Drobyshevsky A, Jilling T et al (2005) Model of cerebral palsy in the perinatal rabbit. J Child Neurol 20:972–979
Ting P, Yamaguchi S, Bacher JD et al (1983) Hypoxic-ischemic cerebral necrosis in mid-gestational sheep fetuses: physio-pathologic correlations. Exp Neurol 80:227–245
Towfighi J, Mauger D, Vannucci RC et al (1997) Influence of age on the cerebral lesions in an immature rat model of cerebral hypoxia-ischemia: a light microscopic study. Dev Brain Res 100:149–160
Turner CP, Seli M, Ment L et al (2003) A1 adenosine receptors mediate hypoxia-induced ventriculomegaly. Proc Natl Acad Sci 100:11718–11722
Uehara H, Yoshioka H, Kawase S et al (1999) A new model of white matter injury in neonatal rats with bilateral carotid artery occlusion. Brain Res 837:213–220
Van de Looij Y, Lodygensky GA, Dean J et al (2012) High-field diffusion tensor imaging characterization of cerebral white matter injury in LPS-exposed fetal sheep. Pediatr Res. doi:10.1038/pr.2012.72
Vannucci RC, Vannucci SJ (2005) Perinatal hypoxic-ischemic brain damage: evolution of an animal model. Dev Neurosci 27:81–86
Volpe JJ (2008) Neurology of the newborn, 5th edn. Elsevier, Philadelphia
Volpe JJ (2009) Brain injury in premature infants: a complex amalgam of destructive and developmental disturbances. Lancet Neurol 8:110–124
Volpe JJ, Kenny HC, Jensen FE et al (2011) The developing oligodendrocyte: key cellular target in brain injury in the premature infant. Int J Dev Neurosci 29:423–440
Wang X, Rousset CI, Hagberg H et al (2006) Lipopolysaccharide-induced inflammation and perinatal brain injury. Semin Fetal Neonatal Med 11:343–353
Wang X, Hagberg H, Nie C et al (2007a) Dual role of intrauterine immune challenge on neonatal and adult brain vulnerability to hypoxia-ischemia. J Neuropathol Exp Neurol 66:552–561
Wang X, Hagberg H, Zhu C et al (2007b) Effects of intrauterine inflammation on the developing mouse brain. Brain Res 1144:180–185

Wang X, Hellgren G, Lofqvist C et al (2009a) White matter damage after chronic subclinical inflammation in newborn mice. J Child Neurol 24:1171–1178

Wang X, Stridh L, Li W et al (2009b) Lipopolysaccharide sensitizes neonatal hypoxic-ischemic brain injury in a MyD88-dependent manner. J Immunol 183:7471–7477

Webber DJ, Van Blitterswijk M, Chandran S (2009) Neurprotective effect of oligodendrocyte precursor cell transplantation in a long-term model of periventricular leukomalacia. Am J Pathol 175:2332–2342

Weiss J, Takizawa B, McGee A et al (2004) Neonatal hypoxia suppresses oligodendrocyte Nogo-A and increases axonal sprouting in a rodent model for human prematurity. Exp Neurol 189: 141–149

Welin AK, Svedin P, Lapatto R et al (2007) Melatonin reduces inflammation and cell death in white mater in the mid-gestation fetal sheep following umbilical cord occlusion. Pediatr Res 61:153–158

Wu YW, Colford JM (2000) Chorioamnionitis as a risk factor for cerebral palsy. A meta-analysis. JAMA 284:1417–1424

Xu G, Ong J, Liu Y-Q et al (2005) Subventricular zone proliferation after a-amino-3-hydroxy-5-methyl-4-sioxazolepropionic acid receptor-mediated neonatal brain injury. Dev Neurosci 27:228–234

Yang D, Sun Y-Y, Nemkul N et al (2012) Plasminogen activator inhibitor-1 mitigates brain injury in a rat model of infection-sensitized neonatal hypoxia-ischemia. Cereb Cortex 23:1218–1229. doi:10.1093/cercor/bhs115

Yoon BH, Kim CJ, Romero R et al (1997) Experimentally induced intrauterine infection causes fetal brain white matter lesions in rabbits. Am J Obstet Gynecol 177:797–802

Yoshioka H, Goma H, Nioka S et al (1994) Bilateral carotid artery occlusion causes periventricular leukomalcia in neonatal dogs. Brain Res 78:273–278

Chapter 8
Focal Ischemic White Matter Injury in Experimental Models

Robert Fern

The pathophysiology of central white matter is a timely subject for review. Early white matter changes are now recognized in neurological disorders ranging from Alzheimer's and Parkinson's diseases and vascular dementia (Burke in press; Lundblad in press; Strozyk in press; Jokinen in press; Back in press) to disorders such as diabetic cognitive dysfunction (McCrimmon in press), mitochondrial syndromes (Wong in press), lysosomal disorders (Renaud in press), and psychiatric illness (Walterfang in press). A plot of the use of the term "white matter" in the titles of research papers reveals an exponential growth since 2005, in large part due to this burst in interest in white matter as an early and possibly causal locus in disorders that until recently were thought to be exclusively gray matter in origin.

Despite this recent interest in novel forms of white matter pathology, the bulk of our knowledge about the mechanisms underlying white matter injury comes from models that address established white matter disorders. Pathology of white matter is rarely global, exceptions being that seen in human prion disorders (Lee in press; Gelpi in press) and the lesions produced by some forms of traumatic brain injury and toxins (Al-Hasani in press). More localized white matter injuries are common in multiple sclerosis, spinal cord injury, and cortical stroke. The purest form of ischemic lesion of white matter is more focal still: lacuna infarct. This is a form of small vessel disease involving focal injury generally localized to deep white matter structures which arise from failure of long penetrating arteries. These lesions are common, representing ~20 % of all cases of stroke (Fisher 1968; Norrving 2008; Sacco et al. 2006; Del Bene in press; Arboix and Marti-Vilalta 2009). They range in size up to 15 mm and in ~30 % of cases result in severe disability (Del Bene in press), although the majority of cases have a favorable short-term prognosis often with partial recovery coupled to a elevated mid-long-term risk of recurrence (Norrving 2008; Arboix and Marti-Vilalta 2009). The clinical features are typically a focal loss

R. Fern, Ph.D. (✉)
Cell Physiology and Pharmacology, University of Leicester, Leicester LE1 9HN, UK
e-mail: RF34@le.ac.uk

S. Baltan et al. (eds.), *White Matter Injury in Stroke and CNS Disease*, Springer Series in Translational Stroke Research 4, DOI 10.1007/978-1-4614-9123-1_8,

such as motor hemiparesis or local sensory occlusion, although the high incidence of "silent" lacunes that are subclinical (e.g., 13 % in young adults suffering their first diagnosed stroke (Putaala et al. 2009), 20 % in healthy elderly people (Vermeer et al. 2007) underscores the significance of location to the clinical relevance of these injures. Smaller "micro-infarcts" with mean diameters between 0.2 and 1 μm may be more common still, with an incidence rate of 33 % in cognitively normal elderly patients (Smith in press), although these are not exclusive to white matter. Such micro-infarcts are harder to detect but probably greatly outnumber the larger lacuna infarcts and are thought to have a similar ischemic origin (Smith in press). Cerebral subcortical micro-bleeds ranging between 0.8 and 1.5 mm in diameter are a significant clinical problem recently associated with cognitive loss in stroke patients (Gregoire in press; Patel in press).

A comprehensive review of the pathology of lacuna infarct describes a range of features including a typically irregular lesion bordered by gliotic astrocytes and macrophages in older lesions, while acute lesions feature "liquefaction" necrosis or local "softening" most often without market gliosis (Bailey in press). Considering the clinical significance of focal ischemic injury to white matter, it is surprising how little is known of the underlying mechanisms giving rise to these injuries. Recent reviews have examined the animal models available in this field, concluding that nonsurgical focal ischemia of appropriate proportion in white matter was achieved only in the spontaneously hypertensive stroke-prone rat model, where timing and location of the lesion are outside experimental control and the cellular/molecular injury pathways and the sequence of cellular events are difficult to investigate (Hainsworth and Markus 2008; Bailey et al. 2009).

8.1 Large Vessel Occlusion Models: White Matter Is Highly Sensitive to Ischemic Injury In Vivo

Some cellular information can be inferred from rodent vessel occlusion models, which are less relevant to clinical white matter injury but more experimentally malleable than the nonsurgical model. For example, rat middle cerebral artery occlusion affects both gray and white matter. Glial cell swelling in white matter is apparent within 30 min following middle cerebral artery occlusion and oligodendrocyte necrosis after 180 min; general white matter necrosis is evident after 24 h, and the progress of these pathological changes may precede that seen in overlying gray matter (Pantoni et al. 1996; Yam et al. 1998). Dewar and Dawson (1996) confirmed loss of subcortical axonal integrity using a variety of axonal immuno-markers after 120 min of ischemia in this model and pointed out that the time-course of these changes is similar to the earliest and possible reversible neuronal pathology in overlying gray matter (Dewar and Dawson 1997), while Irving et al. (1997) showed pathological changes in subcortical white matter oligodendrocytes after 40 min (Irving et al. 1997). Pathology and death are apparent in white matter astrocytes over a similar rapid period in the ischemic core (see Fern 2001), resulting in pan-necrosis.

Using a reversible rat middle cerebral artery occlusion model, Valeriani et al. (2000) and Imai et al. (2001) reported that 120 min of ischemia was sufficient to produce wide-scale glial and axonal damage in subcortical white matter assessed 22–24 h latter (Valeriani et al. 2000; Imai et al. 2001). In a 22-min transient global ischemia mouse model, striatal white matter injury was widespread after 1–3 days post ischemia (Yoshioka in press), while extensive glial and axon pathology was induced by 15 min of reversible middle cerebral artery occlusion in the rat striatum, assessed 3 days later (Schabitz et al. 2000), and 5–30 min of rat global ischemia produces white matter-reactive astrocytosis and damage in various structures after a delay of days to weeks (e.g., Walker in press; Kubo et al. 2009). As with permanent middle cerebral artery occlusion, oligodendrocytes are particularly sensitive to transient global ischemia with significant cell loss apparent 24 h after a 10-min ischemic period (Petito et al. 1998).

8.2 Genuine Focal Ischemia: Significance of the Penumbra

Transient focal ischemia achieved via endothelin-1 injection can produce focal white matter injury, with wide-scale axonal and oligodendrocyte damage after 6 h (Hughes et al. 2003; Gresle et al. 2006). Axon pathology assessed via several immuno-markers is also observed in a peri-infarct penumbra region at this time point, while axon pathology neighboring an ischemic core has been observed in other studies (Yam et al. 1997; Lin et al. 1998a; Irving et al. 2001). Frost et al. (2006) in the rat and Lecrux et al. (2008) in the spontaneously hypertensive rat used endothelin-1 injection into the internal capsule to induce a reproducible, small, focal white matter lesion that has yet to be described in detail at the histological level (Frost et al. 2006; Lecrux et al. 2008). The approach applied to subcortical white matter in mouse produces a similarly focused lesion that evolves over several days and features oligodendrocyte and axon loss in the core and a penumbra region assessed via transgenic cell markers, immuno-labeling, and electron microscopy (Sozmen et al. 2009). In this model, a microglial reaction is an early feature, and demyelination was evident in the penumbra.

To date, these endothelin-1 injection models provide the most useful experimental models of focal white matter ischemic injury. They can produce a small focal ischemic field lasting ~48 h and yield a reproducible local white matter lesion (Hainsworth and Markus 2008; Windle et al. 2006). However, actions of endothelin-1 others than those related to ischemia should be considered when evaluating the data produced via this model. For example, endothelin-1 can evoke nitric oxide production in astrocytes (Wang in press), which may be directly toxic to oligodendrocytes and axons independent of ischemia (Rosenberg et al. 1999; Garthwaite et al. 2002).

Other approaches have been pursued in the goal of finding a clinically relevant, experimentally useful model of lacuna infarct. A recent report using emboli of the canine middle cerebral artery offers hope for a predictive in vivo model of lacuna

infarct with small white matter lesions appearing within 80 min of the onset of vessel occlusion detected via diffusion-weighted imaging (Liu in press). Injection of 50 μm micro-beads into deep penetrating arteries of the macaque also evokes features of lacuna infarct assessed via MRI (Sato et al. 2009). The major pathology is a disruption of axon staining and an almost uniform white matter astrocytosis. The absence of quantification of the effects upon axon staining and the unusual nature of the astrocytosis make this model hard to assess. Permanent anterior choroidal artery occlusion in the "gyrencephalic" brain of the miniature pig produces an internal capsule infarction characterized by loss of evoked potentials within minutes followed by gradual expansion of a core lesion into a penumbra zone (Tanaka et al. 2008). The core of the white matter lesion involved damage to oligodendrocytes and axons assessed at 24 h via electron microscopy with damaged and swollen axons in the penumbra zone which appears to expand after this point. Astrocyte injury is also apparent from the published micrographs.

While not a "focal" model of white matter ischemia, cerebral hypo-perfusion achieved via carotid micro-coil results in a selective defuse white matter pathology in mice, assessed via MRI and histology (Shibata et al. 2004; Holland in press; Horsburgh in press; Reimer in press), while hypo-perfusion following permanent, bilateral occlusion of the common carotid arteries of rats results in delayed white matter pathology including axons and glia, e.g., Farkas et al. (2004). The injury features progressive disruption to myelinated axons and the glial-axonal arrangement at the node of Ranvier, although the underlying mechanisms remain unclear. Cerebral hypo-perfusion is a feature of Alzheimer's disease, and this model may be most relevant for the white matter pathology associated with neurodegenerative diseases of this type, which may also be true of the defused micro-infarct generated by cholesterol crystal in mouse CNS which involve focal neuronal loss and defused glial reaction (Rapp et al. 2008; Wang in press).

8.3 The Problem with Isolated Rodent Optic Nerve/Brain Slice Approaches: Absent Penumbra

Intensive use of isolated white matter structures such as the rodent optic nerve has provided an extensive database of molecular and cellular information regarding the potential pathways to focal white matter injury, reviewed elsewhere in this volume (ref). These global models of focal injury, where an entire white matter structure is exposed to some form of energy deprivation, exclude important local factors that might influence injury in situ. Well-recognized shortcomings of in vitro models for the study of ischemic injury include deficiencies in the ischemia itself, for example matching the extent and the temporal profile of changes in the concentration of metabolic substrate and respiratory gases to those experienced in situ. Typically, white matter injury has been induced by a standard period of anoxia or oxygen–glucose deprivation followed by restoration of control conditions. In contrast, lacuna infarct appears to involve prolonged/permanent obstruction of a single penetrating

artery with focal loss in the associated vascular field in a discreet region of cortical white matter or brain stem (Sacco et al. 2006; Del Bene in press). Occlusions leading to lacuna infarcts may be proximal, with an ischemic field that encompasses the whole penetrating vessel territory, or distal with an ischemic field restricted to the deep white matter (Del Bene in press; Caplan 1989; Serena et al. 2001; Chung in press). The former lesions may be larger and associated with clinical deterioration that can affect a sizable subset of patients (20–30 %). This progressive form of lesion may be due to expansion of an ischemic core due to thrombus propagation into collateral branches in an ischemic penumbra (Del Bene in press; Nagakane et al. 2008) and may involve excitotoxic injury within the penumbra (Serena et al. 2001). There is no evidence for reperfusion in lacuna infarcts, and the rationale for reperfusion in isolate white matter models is technical; a stable electrophysiological decline is the standard pathological readout and cannot be achieved during progressive ischemic failure. This is, however, a serious drawback of the standard approach, since the mechanisms underlying complete focal necrotic damage of white matter with subsequent expansion of a penumbra zone may differ from those underlying injury following partial necrosis and reperfusion, while there is evidence that a significant component of the injury produced by the standard model occurs in the acute reperfusion period (Stys et al. 1990). It is possible, however, that early intervention with thrombolytic agents results in reperfusion in some patients suffering lacuna infarct and has been associated with improved outcomes (Shobha in press). The standard rodent-isolated white matter model may be most appropriate to understand injury in this scenario.

Several considerations suggest that the absence of an edge effect in the standard globally ischemic white matter models is particularly problematic and that interactions between an ischemic core, a penumbra zone, and a normoxic, normo-glycemic surround may be a defining feature of lacuna infarct which is not captured in these models. Mature lacuna infarcts typically include a densely necrotic core delineated by a rim of reactive gliosis, embedded in an otherwise normal white matter. Expansion of an ischemic core into a penumbra is suggested in some cases (Del Bene in press; Nagakane et al. 2008) and in vivo models (see above). The large surface area relative to the ischemic core volume in lacuna infarct may also indicate that interactions between an ischemic core and penumbra/relatively normal white matter are likely to be particularly important in the evolution of these lesions compared to the much larger cortical strokes.

In vivo models of cortical stroke evoke waves of spreading depression within the penumbra region of partially interrupted vascular supply neighboring the ischemic core (Nedergaard 1988), which may be partially responsible for progressive cellular injury in this region (Nedergaard and Dirnagl 2005; Takano et al. 2007). Gap-junction coupling between cells also allows propagation of stress signals between damaged and healthy cells, contributing to bystander cell death (Lin et al. 1998b; Frantseva et al. 2002a, b; Perez Velazquez et al. 2006). Astrocyte networks have been implicated in this phenomena, while coupling between astrocytes and from astrocytes to oligodendrocytes in white matter is both well documented and necessary for viability (Rash et al. 1997; Magnotti in press; Tress in press). The significance of

these cellular mechanisms for expansion of an ischemic core into a penumbra region is unclear in white matter structures. The role of free radicals in this area is also poorly understood. The abundance of lipid in the myelin sheath makes white matter particularly vulnerable to oxidative damage associated with ischemia, an affect exacerbated by low levels of intrinsic antioxidants in white matter (Ueno et al. 2009). Delayed white matter damage associated with brief global ischemia is linked to oxidative stress (Imai et al. 2001; Yoshioka in press) and can be mitigated by free radical scavengers (Imai et al. 2001; Kubo et al. 2009). There is evidence that clinical use of this strategy is selectively beneficial for lacuna stroke patients (Mishina et al. 2005; Ohta et al. 2009; Nakase in press).

Some of these deficiencies in the standard isolated rodent optic nerve model of focal white matter injury are common to all isolated preparations and some can be addressed experimentally. For example, the majority of studies that have examined ischemic-type injury of isolated white matter have used either anoxia or oxygen–glucose deprivation (OGD) applied to the continually perfused rodent optic nerve. In cell culture, it is possible to mimic changes in pH and ion concentration in the extracellular space of the ischemic brain (e.g., Bondarenko and Chesler 2001a, b). We have found that a similar approach used in rodent optic nerve produced similar injury to that produced by standard OGD (Shannon et al. 2007). Presumably, therefore, the tight extracellular space of the nerve coupled to the presence of a connective tissue sheath prevents significant washout of biologically relevant ions during OGD. This is consistent with data using ion-sensitive microelectrodes which report standing concentration changes of H^+, Ca^{2+}, and K^+ in isolated rodent optic nerve during anoxia (Brown et al. 1998; Connors et al. 1982).

An alternative, and in many ways superior, preparation is the isolated corpus callosum. In coronal slices, this structure is sufficiently large to allow compound action potential recording across the central commissure. The approach has been used to demonstrate the role of ionotropic glutamate receptors in ischemic injury (e.g., McDonald et al. 1998; McCarran and Goldberg 2007; Tekkok and Goldberg 2001; Bakiri et al. 2008). This preparation has the same limitation regarding global ischemia rather than focal, although it has the advantage of containing a mixed population of axons compared to the purely myelinated axons of the optic nerve and also retaining overlying gray matter structures which may contribute to white matter injury. However, all isolated preparations where white matter injury is subject to global ischemic conditions produce injury progressively over a period of hours, while in vivo and clinical data suggest rapid focal white matter necrosis occurring in parallel with gray matter injury (see above).

8.4 Potential Avenues to Better Models

Focal ischemic injury in vitro has been reported using brain slices and local streaming of OGD solution, generating an ischemic “streak” across the slice (Richard in press). Electrophysiological recordings show rapid anoxic depolarization in the

"core" area of ischemia, with gradual expansion on the depolarization into the neighboring "penumbra." This model therefore recapitulates many of the features of clinical stroke, allowing for testing of protective agents during the evolution of the core-expansion phenomena. The potential for application of this approach to white matter injury has not been examined. The limited amount of white matter in rodent brain and the "streak" nature of the focal OGD would appear to prevent a pure white matter focal OGD in brain slices, although the approach might work on optic nerve arranged perpendicular to the flow. The high resistance of isolated white matter to ischemic injury may represent a problem with such an approach, since it demands a rigorous OGD to produce white matter injury with even very low amounts of contaminating oxygen confounding the model (personal observation).

Recent developments in in situ focal ischemia could address some of these issues via single-vessel photo-occlusion (Shih in press a, b). Systemic introduction of a photosensitizer coupled to multiphoton confocal microscopy via a cranial window allow imaging and coagulation of penetrating vessels on the surface of rat cortex, leading to small ischemic lesions of underlying gray matter. Both electrophysiology and cell imaging can be performed on the preparation and systemic pharmacology examined, although the feasibility of occluding the larger diameter deep penetrating arterioles that vascularize white matter structures and which are obstructed in lacuna infarcts may be problematic, as might imaging in the subsequent deep white matter ischemic field. One possibility would be to apply the approach to the immature nervous system which is smaller and less opaque due to the absence of myelination. Immature white matter is subject to focal ischemic injury in prominent lesions associated with cerebral palsy, suggesting a golden opportunity for the initial application of such an approach, where focal white matter model development and clinical relevance align.

References

Al-Hasani OH, Smith C (in press) Traumatic white matter injury and toxic leukoencephalopathies. Expert Rev Neurother 11:1315–1324

Arboix A, Marti-Vilalta JL (2009) Lacunar stroke. Expert Rev Neurother 9:179–196

Back SA, Kroenke CD, Sherman LS et al (in press) White matter lesions defined by diffusion tensor imaging in older adults. Ann Neuro l70:465–476

Bailey EL, Smith C, Sudlow CL, Wardlaw JM (in press) Pathology of lacunar ischemic stroke in humans-a systematic review. Brain Pathol 22:583–591

Bailey EL, McCulloch J, Sudlow C, Wardlaw JM (2009) Potential animal models of lacunar stroke: a systematic review. Stroke 40:e451–e458

Bakiri Y, Hamilton NB, Karadottir R, Attwell D (2008) Testing NMDA receptor block as a therapeutic strategy for reducing ischaemic damage to CNS white matter. Glia 56:233–240

Bondarenko A, Chesler M (2001a) Rapid astrocyte death induced by transient hypoxia, acidosis, and extracellular ion shifts. Glia 34:134–142

Bondarenko A, Chesler M (2001b) Calcium dependence of rapid astrocyte death induced by transient hypoxia, acidosis, and extracellular ion shifts. Glia 34:143–149

Brown AM, Fern R, Jarvinen JP et al (1998) Changes in [Ca2+]0 during anoxia in CNS white matter. Neuroreport 9:1997–2000

Burke RE, O'Malley K (in press) Axon degeneration in Parkinson's disease. Exp Neurol
Caplan LR (1989) Intracranial branch atheromatous disease: a neglected, understudied, and underused concept. Neurology 39:1246–1250
Chung JW, Kim BJ, Sohn CH et al (in press) Branch atheromatous plaque: a major cause of lacunar infarction (high-resolution MRI study). Cerebrovasc Dis Extra 2:36–44
Connors BW, Ransom BR, Kunis DM, Gutnick MJ (1982) Activity-dependent K+ accumulation in the developing rat optic nerve. Science 216:1341–1343
Del Bene A, Palumbo V, Lamassa M et al (in press) Progressive lacunar stroke: review of mechanisms, prognostic features, and putative treatments. Int J Stroke 7:321–329
Dewar D, Dawson DA (1997) Changes of cytoskeletal protein immunostaining in myelinated fibre tracts after focal cerebral ischaemia in the rat. Acta Neuropathol 93:71–77
Farkas E, Donka G, de Vos RA et al (2004) Experimental cerebral hypoperfusion induces white matter injury and microglial activation in the rat brain. Acta Neuropathol 108:57–64
Fern R (2001) Ischemia: astrocytes show their sensitive side. Prog Brain Res 132:405–411
Fisher CM (1968) The arterial lesions underlying lacunes. Acta Neuropathol 12:1–15
Frantseva MV, Kokarovtseva L, Naus CG et al (2002a) Specific gap junctions enhance the neuronal vulnerability to brain traumatic injury. J Neurosci 22:644–653
Frantseva MV, Kokarovtseva L, Perez Velazquez JL (2002b) Ischemia-induced brain damage depends on specific gap-junctional coupling. J Cereb Blood Flow Metab 22:453–462
Frost SB, Barbay S, Mumert ML et al (2006) An animal model of capsular infarct: endothelin-1 injections in the rat. Behav Brain Res 169:206–211
Garthwaite G, Goodwin DA, Batchelor AM et al (2002) Nitric oxide toxicity in CNS white matter: an in vitro study using rat optic nerve. Neuroscience 109:145–155
Gelpi E, Soler Insa JM, Parchi P et al (in press) Atypical neuropathological sCJD-MM phenotype with abundant white matter Kuru-type plaques sparing the cerebellar cortex. Neuropathology
Gregoire SM, Smith K, Jager HR et al (in press) Cerebral microbleeds and long-term cognitive outcome: longitudinal cohort study of stroke clinic patients. Cerebrovasc Dis 33:430–435
Gresle MM, Jarrott B, Jones NM, Callaway JK (2006) Injury to axons and oligodendrocytes following endothelin-1-induced middle cerebral artery occlusion in conscious rats. Brain Res 1110:13–22
Hainsworth AH, Markus HS (2008) Do in vivo experimental models reflect human cerebral small vessel disease? A systematic review. J Cereb Blood Flow Metab 28:1877–1891
Holland PR, Bastin ME, Jansen MA et al (in press) MRI is a sensitive marker of subtle white matter pathology in hypoperfused mice. Neurobiol Aging 32:2325, e2321–2326
Horsburgh K, Reimer MM, Holland P et al (in press) Axon-glial disruption: the link between vascular disease and Alzheimer's disease? Biochem Soc Trans 39:881–885
Hughes PM, Anthony DC, Ruddin M et al (2003) Focal lesions in the rat central nervous system induced by endothelin-1. J Neuropathol Exp Neurol 62:1276–1286
Imai H, Masayasu H, Dewar D et al (2001) Ebselen protects both gray and white matter in a rodent model of focal cerebral ischemia. Stroke 32:2149–2154
Irving EA, Yatsushiro K, McCulloch J, Dewar D (1997) Rapid alteration of tau in oligodendrocytes after focal ischemic injury in the rat: involvement of free radicals. J Cereb Blood Flow Metab 17:612–622
Irving EA, Bentley DL, Parsons AA (2001) Assessment of white matter injury following prolonged focal cerebral ischaemia in the rat. Acta Neuropathol 102:627–635
Jokinen H, Lipsanen J, Schmidt R et al (in press) Brain atrophy accelerates cognitive decline in cerebral small vessel disease: the LADIS study. Neurology 78:1785–1792
Kubo K, Nakao S, Jomura S et al (2009) Edaravone, a free radical scavenger, mitigates both gray and white matter damages after global cerebral ischemia in rats. Brain Res 1279:139–146
Lecrux C, McCabe C, Weir CJ et al (2008) Effects of magnesium treatment in a model of internal capsule lesion in spontaneously hypertensive rats. Stroke 39:448–454
Lee H, Cohen OS, Rosenmann H et al (in press) Cerebral white matter disruption in Creutzfeldt-Jakob disease. AJNR Am J Neuroradiol

Lin B, Ginsberg MD, Busto R, Dietrich WD (1998a) Sequential analysis of subacute and chronic neuronal, astrocytic and microglial alterations after transient global ischemia in rats. Acta Neuropathol 95:511–523

Lin JH, Weigel H, Cotrina ML et al (1998b) Gap-junction-mediated propagation and amplification of cell injury. Nat Neurosci 1:494–500

Liu S, Hu WX, Zu QQ et al (in press) A novel embolic stroke model resembling lacunar infarction following proximal middle cerebral artery occlusion in beagle dogs. J Neurosci Methods 209:90–96

Lundblad M, Decressac M, Mattsson B, Bjorklund A (in press) Impaired neurotransmission caused by overexpression of alpha-synuclein in nigral dopamine neurons. Proc Natl Acad Sci USA 109:3213–3219

Magnotti LM, Goodenough DA, Paul DL (in press) Deletion of oligodendrocyte Cx32 and astrocyte Cx43 causes white matter vacuolation, astrocyte loss and early mortality. Glia 59:1064–1074

McCarran WJ, Goldberg MP (2007) White matter axon vulnerability to AMPA/kainate receptor-mediated ischemic injury is developmentally regulated. J Neurosci 27:4220–4229

McCrimmon RJ, Ryan CM, Frier BM (in press) Diabetes and cognitive dysfunction. Lancet 379:2291–2299

McDonald JW, Althomsons SP, Hyrc KL et al (1998) Oligodendrocytes from forebrain are highly vulnerable to AMPA/kainate receptor-mediated excitotoxicity. Nat Med 4:291–297

Mishina M, Komaba Y, Kobayashi S et al (2005) Efficacy of edaravone, a free radical scavenger, for the treatment of acute lacunar infarction. Neurol Med Chir (Tokyo) 45:344–348, discussion 348

Nagakane Y, Naritomi H, Oe H et al (2008) Neurological and MRI findings as predictors of progressive-type lacunar infarction. Eur Neurol 60:137–141

Nakase T, Yoshioka S, Suzuki A (in press) Free radical scavenger, edaravone, reduces the lesion size of lacunar infarction in human brain ischemic stroke. BMC Neurol 11:39

Nedergaard M (1988) Mechanisms of brain damage in focal cerebral ischemia. Acta Neurol Scand 77:81–101

Nedergaard M, Dirnagl U (2005) Role of glial cells in cerebral ischemia. Glia 50:281–286

Norrving B (2008) Lacunar infarcts: no black holes in the brain are benign. Pract Neurol 8:222–228

Ohta Y, Takamatsu K, Fukushima T et al (2009) Efficacy of the free radical scavenger, edaravone, for motor palsy of acute lacunar infarction. Intern Med 48:593–596

Pantoni L, Garcia JH, Gutierrez JA (1996) Cerebral white matter is highly vulnerable to ischemia. Stroke 27:1641–1646, discussion 1647

Patel B, Lawrence AJ, Chung AW et al (in press) Cerebral microbleeds and cognition in patients with symptomatic small vessel disease. Stroke 44:356–361

Perez Velazquez JL, Kokarovtseva L, Sarbaziha R et al (2006) Role of gap junctional coupling in astrocytic networks in the determination of global ischaemia-induced oxidative stress and hippocampal damage. Eur J Neurosci 23:1–10

Petito CK, Olarte JP, Roberts B et al (1998) Selective glial vulnerability following transient global ischemia in rat brain. J Neuropathol Exp Neurol 57:231–238

Putaala J, Kurkinen M, Tarvos V et al (2009) Silent brain infarcts and leukoaraiosis in young adults with first-ever ischemic stroke. Neurology 72:1823–1829

Rapp JH, Pan XM, Neumann M et al (2008) Microemboli composed of cholesterol crystals disrupt the blood–brain barrier and reduce cognition. Stroke 39:2354–2361

Rash JE, Duffy HS, Dudek FE et al (1997) Grid-mapped freeze-fracture analysis of gap junctions in gray and white matter of adult rat central nervous system, with evidence for a "panglial syncytium" that is not coupled to neurons. J Comp Neurol 388:265–292

Reimer MM, McQueen J, Searcy L et al (in press) Rapid disruption of axon-glial integrity in response to mild cerebral hypoperfusion. J Neurosci 31:18185–18194

Renaud DL (in press) Lysosomal disorders associated with leukoencephalopathy. Semin Neurol 32:51–54

Richard MJ, Saleh TM, El Bahh B, Zidichouski JA (in press) A novel method for inducing focal ischemia in vitro. J Neurosci Methods 190:20–27

Rosenberg PA, Li Y, Ali S et al (1999) Intracellular redox state determines whether nitric oxide is toxic or protective to rat oligodendrocytes in culture. J Neurochem 73:476–484
Sacco S, Marini C, Totaro R et al (2006) A population-based study of the incidence and prognosis of lacunar stroke. Neurology 66:1335–1338
Sato Y, Chin Y, Kato T et al (2009) White matter activated glial cells produce BDNF in a stroke model of monkeys. Neurosci Res 65:71–78
Schabitz WR, Li F, Fisher M (2000) The *N*-methyl-D-aspartate antagonist CNS 1102 protects cerebral gray and white matter from ischemic injury following temporary focal ischemia in rats. Stroke 31:1709–1714
Serena J, Leira R, Castillo J et al (2001) Neurological deterioration in acute lacunar infarctions: the role of excitatory and inhibitory neurotransmitters. Stroke 32:1154–1161
Shannon C, Salter M, Fern R (2007) GFP imaging of live astrocytes: regional differences in the effects of ischaemia upon astrocytes. J Anat 210:684–692
Shibata M, Ohtani R, Ihara M, Tomimoto H (2004) White matter lesions and glial activation in a novel mouse model of chronic cerebral hypoperfusion. Stroke 35:2598–2603
Shih AY, Blinder P, Tsai PS et al (in press a) The smallest stroke: occlusion of one penetrating vessel leads to infarction and a cognitive deficit. Nat Neurosci 16:55–63
Shih AY, Driscoll JD, Drew PJ et al (in press b) Two-photon microscopy as a tool to study blood flow and neurovascular coupling in the rodent brain. J Cereb Blood Flow Metab 32:1277–1309
Shobha N, Fang J, Hill MD (in press) Do lacunar strokes benefit from thrombolysis? Evidence from the Registry of the Canadian Stroke Network. Int J Stroke
Smith EE, Schneider JA, Wardlaw JM, Greenberg SM (in press) Cerebral microinfarcts: the invisible lesions. Lancet Neurol 11:272–282
Sozmen EG, Kolekar A, Havton LA, Carmichael ST (2009) A white matter stroke model in the mouse: axonal damage, progenitor responses and MRI correlates. J Neurosci Methods 180: 261–272
Strozyk D, Dickson DW, Lipton RB et al (in press) Contribution of vascular pathology to the clinical expression of dementia. Neurobiol Aging 31:1710–1720
Stys PK, Ransom BR, Waxman SG, Davis PK (1990) Role of extracellular calcium in anoxic injury of mammalian central white matter. Proc Natl Acad Sci U S A 87:4212–4216
Takano T, Tian GF, Peng W et al (2007) Cortical spreading depression causes and coincides with tissue hypoxia. Nat Neurosci 10:754–762
Tanaka Y, Imai H, Konno K et al (2008) Experimental model of lacunar infarction in the gyrencephalic brain of the miniature pig: neurological assessment and histological, immunohistochemical, and physiological evaluation of dynamic corticospinal tract deformation. Stroke 39: 205–212
Tekkok SB, Goldberg MP (2001) Ampa/kainate receptor activation mediates hypoxic oligodendrocyte death and axonal injury in cerebral white matter. J Neurosci 21:4237–4248
Tress O, Maglione M, May D et al (in press) Panglial gap junctional communication is essential for maintenance of myelin in the CNS. J Neurosci 32:7499–7518
Ueno Y, Zhang N, Miyamoto N et al (2009) Edaravone attenuates white matter lesions through endothelial protection in a rat chronic hypoperfusion model. Neuroscience 162:317–327
Valeriani V, Dewar D, McCulloch J (2000) Quantitative assessment of ischemic pathology in axons, oligodendrocytes, and neurons: attenuation of damage after transient ischemia. J Cereb Blood Flow Metab 20:765–771
Vermeer SE, Longstreth WT Jr, Koudstaal PJ (2007) Silent brain infarcts: a systematic review. Lancet Neurol 6:611–619
Walker EJ, Rosenberg GA (in press) Divergent role for MMP-2 in myelin breakdown and oligodendrocyte death following transient global ischemia. J Neurosci Res 88:764–773
Walterfang M, Velakoulis D, Whitford TJ, Pantelis C (in press) Understanding aberrant white matter development in schizophrenia: an avenue for therapy? Expert Rev Neurother 11:971–987
Wang HH, Hsieh HL, Yang CM (in press) Nitric oxide production by endothelin-1 enhances astrocytic migration via the tyrosine nitration of matrix metalloproteinase-9. J Cell Physiol 226: 2244–2256

Wang M, Iliff JJ, Liao Y et al (in press) Cognitive deficits and delayed neuronal loss in a mouse model of multiple microinfarcts. J Neurosci 32:17948–17960

Windle V, Szymanska A, Granter-Button S et al (2006) An analysis of four different methods of producing focal cerebral ischemia with endothelin-1 in the rat. Exp Neurol 201:324–334

Wong LJ (in press) Mitochondrial syndromes with leukoencephalopathies. Semin Neurol 32:55–61

Yam PS, Takasago T, Dewar D et al (1997) Amyloid precursor protein accumulates in white matter at the margin of a focal ischaemic lesion. Brain Res 760:150–157

Yam PS, Dewar D, McCulloch J (1998) Axonal injury caused by focal cerebral ischemia in the rat. J Neurotrauma 15:441–450

Yoshioka H, Niizuma K, Katsu M et al (in press) NADPH oxidase mediates striatal neuronal injury after transient global cerebral ischemia. J Cereb Blood Flow Metab 31:868–880

Chapter 9
White Matter Injury in Global Cerebral Ischemia

Shinichi Nakao and Yan Xu

Tissues in the mammalian central nervous system (CNS) are classified by their anatomic appearance as gray and white matter. Cerebral gray matter is mainly composed of neuronal cell bodies (perikaryon), dendrites, and astroglial cells, whereas white matter is composed of myelinated axons and glia, especially oligodendrocytes that form the myelin sheath. Many acute and chronic diseases can cause white matter injuries. In this chapter, we discuss axonal injury and oligodendrocyte injury from global or diffusive cerebral ischemia in standard white matter and in other brain areas that can be also broadly defined as white matter.

White matter lesions (WMLs) develop chronically with aging, particularly in patients with arterial hypertension, diabetes mellitus, or cardiovascular diseases. These lesions are also frequently observed in stroke patients and are responsible for the impairment of motor and sensory functions and for the behavioral and cognitive deterioration. In certain diseases such as Binswanger's disease, which is a form of vascular dementia (Desmond 2002; Pantoni and Garcia 1997), WMLs are the core pathology. Global WMLs are associated with small-vessel diseases, including radiologic lacunar infarction and asymptomatic microhemorrhages (Schmidt et al. 2004; Pantoni 2010), and are most extensive and prevalent in patients with small-vessel strokes, which result in chronic cerebral hypoperfusion. Clinical manifestations of WML include white matter hyperintensities (WMH) on T_2-weighted and fluid-attenuated inversion recovery (FLAIR) magnetic resonance imaging (MRI). New imaging techniques, such as diffusion tensor imaging (DTI), magnetization transfer imaging (MTI), and functional MRI, are expected to contribute to the understanding of the pathophysiology of small-vessel diseases in general and their clinical correlates (Pantoni 2010) in WML in particular (Schmidt et al. 2011).

S. Nakao, M.D., Ph.D.
Department of Anesthesiology, Faculty of Medicine, Kinki University, Osaka, Japan

Y. Xu, Ph.D. (✉)
Department of Anesthesiology, University of Pittsburgh School of Medicine, 2048 Biomedical Science Tower 3, 3501 Fifth Avenue, Pittsburgh, PA 15213, USA
e-mail: xuy@anes.upmc.edu

S. Baltan et al. (eds.), *White Matter Injury in Stroke and CNS Disease*, Springer Series in Translational Stroke Research 4, DOI 10.1007/978-1-4614-9123-1_9,

9.1 Chronic Cerebral Hypoperfusion Model in Rodents

White matter in humans comprises about half of the CNS volume, which is about three- to fourfold greater in proportion than what is found in other mammals, including rodents (Matute 2011). Experimentally, WML can be induced in rat or mouse brains by permanent occlusion of both common carotid arteries, known as the "chronic cerebral hypoperfusion model" (Wakita et al. 1994, 1995). In this model, the cerebral blood flow (CBF) of an animal is decreased to 40–82 % of normal over a prolonged period. The animals exhibit significant learning impairment with demyelination and axonal damage in the brain but without apparent cerebral infarction, which typically occurs when the CBF is reduced to less than 20 % of normal (Wakita et al. 1994, 1995; Vicente et al. 2009). In rodent models of acute but transient global cerebral ischemia (Xu et al. 2002, 2009; Wang et al. 2002; Liachenko et al. 1998, 2001, 2003), neuronal injuries are observed in the hippocampus, cerebral cortex, caudoputamen, and thalamus. In chronic cerebral hypoperfusion, in contrast, white matter changes, such as demyelination and axonal damage, are obvious in the optic nerve and tract, corpus callosum, internal capsule, anterior commissure, and caudoputamen, with few neuronal injuries (Wakita et al. 1994, 1995; Vicente et al. 2009). Ample evidence suggests that a primary culprit of WML is oxidative stress with inflammatory responses (Dewar et al. 2003). Hypoperfusion-induced inflammation events include peripheral leukocyte recruitment, the activation of microglia and astrocytes (Hirko et al. 2008), and up-regulation of inflammatory mediators such as tumor necrosis factor α (TNF-α) and interleukin-1β (IL-1β) (Wang et al. 2010). It has been reported that WMLs are ameliorated by various agents, such as nimesulide (a cyclooxygenase-2 inhibitor) (Wakita et al. 1999), cyclosporine A and FK 506 (immunosuppressants) (Wakita et al. 1995, 1998), cilostazol (an inhibitor of type III phosphodiesterase and an antiplatelet aggregation agent) (Watanabe et al. 2006), dicholine salt of succinic acid (a specific mitochondrial respiratory substrate) (Storozheva et al. 2008), and edaravone (an antioxidant) (Ueno et al. 2009). It has been recently reported (Wang et al. 2010) that huperzine A, a natural acetylcholinesterase (AChE) inhibitor, ameliorated WML by suppressing overexpression of TNF-α, JNK, and p38 mitogen-activated protein kinases (MAPKs). AChE inhibitors have been used for clinical treatment of Alzheimer's disease (AD) and vascular dementia (Tayeb et al. 2012; Erkinjuntti et al. 2004). These inhibitors can suppress not only inflammation but also cholinergic supply (Nizri et al. 2006; Pavlov and Tracey 2005).

In conjunction with the chronic cerebral hypoperfusion model, hypocapnia induced by 2-h hyperventilation can cause aggravation of WML in the caudoputamen and neuronal injuries in the cerebral cortex and the caudoputamen (Miyamoto et al. 2001). The hypocapnia-induced injuries were less profound in rats with normal cerebral perfusion and could be prevented by pretreatment with ketamine (Miyamoto et al. 2004), an NMDA receptor antagonist. This hypocapnia model is clinically relevant to the anesthesia complications associated with the postoperative or post-intensive care unit (ICU) cognitive impairment in elderly patients, particularly those who

have hypertension, diabetes mellitus, or a cerebrovascular disease. In these patients, postoperative brain dysfunction, such as delirium, may be partly attributed to white matter injuries secondary to prolonged hypocapnia during mechanical ventilation.

9.2 White Matter Injury in Acute Focal Cerebral Ischemia

Many neuroprotective drugs, designed for acute cerebral ischemia and traumatic brain injuries (TBI) and which have shown great promise in preclinical testing, have unfortunately failed in clinical trials. One of the primary reasons for these failures is that preclinical neuroprotective studies have concentrated almost exclusively on the protection of cerebral gray matter, whereas the neuroprotective effects on cerebral white matter tracts, especially on axonal damage, are neglected and unknown (Gladstone et al. 2002). Furthermore, most of the preclinical experiments are performed using rodents, whose white matter comprises only ~14 % of total brain volume. In comparison, white matter makes up about half of the volume in human brain (~50 % gray matter and ~50 % white matter). Thus, the data from rodent studies are likely to misrepresent the relevance of white matter in human brain pathology (Matute 2011). In addition, pathophysiology of ischemic injury in white matter may differ from that in gray matter for a number of reasons (Matute 2011; Stys 1998; Dewar et al. 1999). Dewar and colleagues stressed that strategies for the preclinical development of anti-ischemic drugs should focus on the protection of not only gray matter but also white matter, specifically axons and oligodendrocytes (Dewar et al. 1999). Traditionally, white matter is viewed as being less vulnerable to ischemia than gray matter, and white matter injury is thought to occur mainly as a result of Wallerian degeneration. More recent studies (Pantoni et al. 1996), however, suggest that white matter, particularly oligodendrocytes and myelinated axons, is also highly vulnerable to ischemia. It has become increasingly clear that in addition to Wallerian changes secondary to neuronal injuries, white matter injuries can also be induced directly by ischemic insults.

General methods for quantitative or semiquantitative assessment of white matter injuries after ischemia include Klüver–Barrera staining for myelinated axons (Wakita et al. 1998, 1999; Miyamoto et al. 2001, 2004; Irving et al. 2001), increased tau immunoreactivity for cytoskeletal pathology in oligodendrocytes (Irving et al. 1997), reduced immunoreactivity of myelin basic protein (MBP) for myelinated fiber disruption (Irving et al. 2001), and accumulation of β amyloid precursor protein (βAPP) (Yam et al. 1997; Imai et al. 2002) or synaptosomal associated protein of 25 kD (SNAP25) (Yam et al. 1998) for disruption of fast axonal transport. βAPP is produced in the cell body and is normally transported along the axon, where it accumulates at sites of axonal injury. Most bench-top investigations use focal ischemia models. Short periods (2–4 h) of focal cerebral ischemia in rats have been shown to induce cytoskeletal breakdown and disturbance of fast axonal transport (Yam et al. 1998; Dewar and Dawson 1995), exhibiting βAPP accumulation in the injured axons. It has been reported that melatonin (Lee et al. 2005, a potent free radical

scavenger and an antioxidant), ebselen (Imai et al. 2001, a potent antioxidant), as well as cilostazol discussed earlier (Honda et al. 2006) can attenuate gray and white matter damage in focal ischemia.

Many factors, including excitatory amino acid release, peri-infarct waves of depolarization, specific gene expression, inflammation, and oxidative stress, contribute to brain damage after ischemia and reperfusion. White matter is considered more sensitive to oxidative stress because myelinated fibers are rich in lipid content, and oligodendrocytes have a high metabolic activity, low intracellular concentrations of glutathione, and high concentrations of iron (Pavlov and Tracey 2005). Moreover, the ischemic injury pathways may be different between gray and white matter: *N*-methyl-D-aspartate (NMDA) receptors are preferentially located at synapses, and it was once believed that oligodendrocytes lacked NMDA receptors (Patneau et al. 1994; Berger et al. 1992). Thus, blockade of Ca^{2+} entry via reversal of the Na^{+}–Ca^{2+} exchanger (Stys et al. 1992) and inhibition of sodium channels (Stys 1995) or alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptors (Follett et al. 2000; Tekkök and Goldberg 2001; McCracken et al. 2002) were thought to be more effective for white matter protection. Indeed, Yam et al. reported that MK-801, an NMDA receptor antagonist, failed to inhibit axonal injury in focal cerebral ischemia in cats (Yam et al. 2000). Although Schäbitz et al. demonstrated that CNS-1102, also an NMDA receptor antagonist, protected both cerebral gray and white matter from transient focal ischemia in rats, the authors suggested that the protection was due to a secondary prevention of white matter damage by neuroprotection of cerebral gray matter, i.e., through inhibition of Wallerian degeneration instead of direct protection of white matter (Schäbitz et al. 2000). However, more recent studies have revealed that NMDA receptors are also expressed in oligodendrocytes and myelin sheath and are activated in ischemia. These receptors are composed of NR1, NR2A-NR2C, and NR3A subunits (Káradóttir et al. 2005; Salter and Fern 2005; Micu et al. 2006). Electrophysiological and pharmacological characterizations suggest that subunit compositions of NMDA receptors are different in neurons and oligodendrocytes; the most abundant in the latter are formed by NR1, NR2C, and NR3A (Matute 2006). An interesting feature of NMDA receptors composed of NR1/NR3 in oligodendrocytes is that they are less susceptible to Mg^{2+} block than the neuronal receptors, having a substantial current even at the resting potential (Káradóttir et al. 2005) and exhibiting a higher sensitivity to glycine (Stys and Lipton 2007; Chatterton et al. 2002). Because of this, there is a renewed hope that NMDA antagonists might be a reasonable option for not only mitigating gray matter damage but also targeting white matter damage in brain ischemia. Although most clinical trials with NMDA receptor antagonists were disappointing or stopped prematurely due to side effects—likely because the same antagonists also inhibit normal physiological NMDA receptor signaling—a carefully selected group of drug candidates may still offer potential clinical benefits. For example, memantine, which has been approved by the Food and Drug Administration (FDA) in the United States and the European Medicines Agency for the treatment of AD, preferentially blocks excessive NMDA receptor activation without disrupting the normal receptor functions and hence is a promising protective agent for gray

and white matter injuries (Stys and Lipton 2007). Manning et al. showed that memantine attenuated white matter injury in a rat model of periventricular leukomalacia (PVL) (Manning et al. 2008). Combination therapies using antioxidants and minocycline in addition to memantine have been proposed as a potential therapeutic regimen for white matter ischemia (Arai and Lo 2009). Minocycline has anti-inflammatory effects and was reported to attenuate hypoxia- and ischemia-induced white matter injury in neonatal rats (Carty et al. 2008) and reduce long-term functional deficits as well as white matter injury in an endothelin-1-induced white matter injury model (Hewlett and Corbett 2006).

9.3 White Matter Injury in Acute Global Cerebral Ischemia

For critically ill patients with neurologic complications, as a consequence of cardiac arrest, diffuse axonal damage after TBI, or cytotoxic edema after ischemic stroke, accurate diagnosis of the neurological state and early prediction of potential outcome are vitally important. Often, loss of distinction between gray and white matter develops in computed tomography (CT) of comatose patients following cardiac arrest, and such a radiographic change predicts poor outcome (Torbey et al. 2000; Inamasu et al. 2011; Choi et al. 2008). Metter et al. recently reported (Metter et al. 2011) that when the severity of cerebral edema after cardiac arrest is defined by the ratio of X-ray attenuation in gray matter to that in white matter (GWR), the outcome as measured by survival to hospital discharge is robustly predicted by a critical GWR of 1.20, in agreement with the cutoff values established earlier for predicting vegetative state or death after cardiac arrest (Torbey et al. 2000; Choi et al. 2008). Interestingly, however, two patients (out of 58) in the study by Metter et al. had a GWR of 1.17 and 1.15, treated with hypothermia, and survived to hospital discharge.

MRI can show brain lesions that are not readily visible in CT scans and thus offers additional diagnosis modalities for outcome prediction (Weiss et al. 2007). MRI studies indicate that there are four phases in human global anoxic and hypoxic encephalopathy caused by cardiac arrest and resuscitation (Weiss et al. 2007; Arbelaez et al. 1999; Takahashi et al. 1993): an acute phase within 24 h after cardiac arrest and resuscitation, an early subacute phase during the first 2 weeks after the acute phase, a late subacute phase in the subsequent week, and a chronic phase from the fourth week onward (Table 9.1). In the acute and early subacute phases, brain swelling in the cortex, thalamus, and basal ganglia appears as hyperintensity in the T_2-weighted and diffusion-weighted images (DWI). In the late subacute phase, diffuse white matter abnormalities are often visible. In the chronic phase when diffuse atrophy and dilatation of the ventricles develop, DWI and T_2-weighted images are usually normal, but in some cases hyperintensity in the cortex and hypointensity in the subcortical zones appear in both T_1-weighted and T_2-weighted images. As an early prediction tool, diffuse cortical hypersignals in DWI are associated with poor clinical outcome. In a study of ten cardiac arrest patients who became comatose, T_2-weighted FLAIR images and DWI showed diffuse abnormalities in the

Table 9.1 MRI characteristics of white matter injuries after global hypoxic-ischemic encephalopathy

Causes	T_2/FLAIR	DWI	ADC map	DTI	T_1 weighted
Age-related or small-vessel disease-related chronic hypoperfusion	Mild cases: High-contrast WM lesion delineation at periventricular locations Widespread high intense areas >2 cm in severe cases, with rapidly increasing lesion volumes	In both T_2 hyperintense and even normal-appearing T_2/FLAIR areas, WMLs appear as hyperintense signal in DWI	Even though restricted diffusion occurs, WM can become slightly more hyperintense than GW due to more severe GW edema. GW ADC is usually slightly more hyperintense than WM in normal tissue	Characteristic changes are regional decrease in fractional anisotropy and increase in radial diffusivity. Changes in axial diffusivity and mean diffusivity are region dependent; higher diffusivity is found in more anterior and superior regions	Appear normal without contrast
Acute global ischemia (e.g., cardiac arrest)					
Acute phase (<1 day)	Normal in mild cases Noticeable hyperintense signals in perirolandic cortex in more severe cases	Hyperintense in cortex, thalamus, and basal ganglia, but WM sometimes shows no obvious DWI changes	Very hypointense with thickening of GW in cortex	No data	Normal appearance

Early subacute phase (1–14 days)	Hyperintensity in cortex	Progressive hyperintensity in WM bilaterally	Restricted diffusion with ADC hypointensity in the selenium of the corpus callosum	Fractional anisotropy value <0.9 is predictive of poor long-term outcome	Normal appearance without contrast Possible enhancement of subcortical regions with contrast agent
Late subacute phase (15–21 days)		Disappearance of hyperintensity	Start to normalize	Analyses of fractional anisotropy, axial and radial diffusivities, and mean diffusivity can potentially be used to measure WM remodeling during recovery	Hyperintensity in subcortical region and basal ganglia
Chronic phase (>21 days)		Approaching normal	Normal appearance	Same as in the late subacute phase	Normal appearance

For more detailed account, see Schmidt et al. (2011); Luyt et al. (2012); White et al. (2013); and Madden et al. (1822). *ADC* apparent diffusion coefficient, *DTI* diffusion tensor imaging, *DWI* diffusion-weighted imaging, *FLAIR* fluid-attenuated inversion recovery, *GM* gray matter, *WM* white matter, *WML* white matter lesions

cerebellum, thalamus, frontal and parietal cortices, and hippocampus in eight of the ten patients. None of the patients with cortical structure abnormalities recovered beyond a severely disabled state (Wijdicks et al. 2001). In another study of 12 comatose patients having MRI evaluations within 36 h after cardiac arrest, three with short resuscitation times showed a good recovery with normal DWI, whereas the remaining nine had DWI abnormalities and deteriorated into a vegetative state (Els et al. 2004). Diffusion tensor MRI is useful for detecting neurofibers in white matter. It has been recently demonstrated (Luyt et al. 2012) that white matter damage is widespread after cardiac arrest and that the selected white matter fractional anisotropy value, a parameter describing the degree of directionality of water in the tissue, can accurately predict 1-year outcome.

To date, only a few studies have been conducted to investigate white matter injuries or specific axonal and oligodendrocytic injuries following global cerebral ischemia in animals. As in focal ischemia studies, accumulation of βAPP has been used as an indicator of axonal injuries (Yam et al. 1997; Imai et al. 2002; Els et al. 2004). The mRNA encoding the Kunitz-type protease-inhibitor domain of APP was induced in the granular layers of dentate gyrus and in the pyramidal cells of the hippocampal CA1–CA3 regions 24 h after forebrain ischemia by four-vessel occlusion in rats. This mRNA, absent in sham-operated rats, peaked on day 3 and remained elevated until day 7 (Heurteaux et al. 1993). At the protein level, APP immunoreactivity was found both extracellularly and intracellularly after a 10-min cardiac arrest in rats. The extracellular accumulation of APP occurred in the hippocampus, cerebral and cerebellar cortex, basal ganglia, and thalamus (Pluta et al. 1994). The extent of APP accumulation seems to correlate with the severity of the global ischemia insult. Using bilateral occlusion of the common carotids in gerbils, Tomimoto et al. (Tomimoto et al. 1994) found that 2-min ischemia produced no detectable APP accumulation; 3-min ischemia led to a small number of neurons with intense APP immunoreactivity scattered in the CA1 of hippocampus and in layers V and VI of the frontoparietal cortex 24 h after the insult. A 24-h reperfusion after a 5- or a 15-min ischemia led to a large number of densely APP-stained neurons in the subiculum, hippocampal CA3, and layers III and V/VI of the frontoparietal cortex (Tomimoto et al. 1994). However, in all these studies, βAPP accumulation was not used as a marker for axonal damage, because βAPP accumulation in the neuronal cell body was detected. Lin et al. reported an interesting finding: after global forebrain ischemia by bilateral carotid artery occlusion plus systemic hypotension via arterial blood withdrawal, the brain of rats under a hyperglycemic condition led to the robust, widespread intraneural expression of βAPP immunoreactivity in the neocortex, hippocampus, thalamus, and striatum, but the brain of rats under a normoglycemic condition showed only weak βAPP immunoreactivity. They believe that βAPP or its glycosylation state may be involved in the more pronounced injury process under the hyperglycemia condition (Lin et al. 2001). Recent studies have shown that hyperglycemia may produce advanced glycation end products (AGEs) and high-mobility group box 1 (HMGB1) and activate the receptor for AGEs (RAGE), which generates excessive reactive oxygen species (ROS) and induces an inflammatory response, consequently worsening cerebral ischemia (Goldin et al.

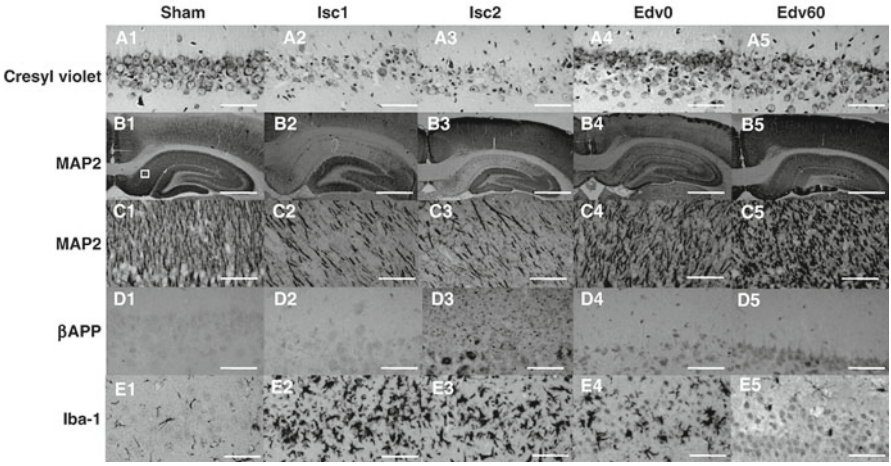


Fig. 9.1 Histologic neuronal and axonal damage in the hippocampal CA1 region following global cerebral ischemia caused by cardiac arrest and resuscitation (CAR). Representative sections of cresyl violet staining (A1–5) and immunostained sections of MAP2 (B1–5 and C1–5), βAPP (D1–5), and Iba-1 (E1–5) in the CA1 in the sham, Isc1, Isc2, Edv0, and Edv60 groups are depicted. A1: Normal pyramidal neurons in the sham group. A2 and A3: Typical appearance of neuronal damage 1 week (Isc1) and 2 weeks (Isc2) after CAR, respectively. A4 and A5: Reduction of neuronal damage by edaravone administered immediately (Edv0) and 60 min (Edv60) after CAR, respectively. B1: Normal MAP2 expression in the sham group. A white square shows the region of predetermined area for the neuronal perikaryal and axonal damage evaluation. B2 and B3: Extensive decrease in MAP2 expression after CAR in the Isc1 and Isc2 groups, respectively. B4 and B5: Mitigation of decrease in MAP2 expression by edaravone in the Edv0 and Edv60 groups. C: MAP2 expression as in B at a higher magnification. D1 and D2: Normal detection level of the βAPP accumulation in the sham and Isc1 groups, respectively, 1 week after CAR. D3: Extensive granular βAPP deposition 2 weeks after CAR in the Isc2 group. D4 and D5: Mitigation of βAPP accumulation in the edaravone-treated groups. E1: Scattered ramified microglias are found in the sham group. E2 and E3: Dense accumulation of ameboid microglia 1 week and 2 weeks after CAR. E4: Microglial activation is slightly suppressed by edaravone administered immediately after CAR. E5: Microglial activation is markedly suppressed by edaravone administered 60 min after CAR. Scale bar: 50 μm (A, C, D, and E); 1 mm (B). Figure and legend are adapted with permission from Kubo et al. (2009)

2006; Tsuruta et al. 2010; Kamide et al. 2012; Weil 2012; Liu et al. 2007; Zhang et al. 2011). We previously investigated both neuronal and axonal injury following cardiac arrest and resuscitation in rats (Kubo et al. 2009). Five minutes of cardiac arrest followed by resuscitation induced marked pyramidal neuronal injury and microglial activation but not axonal injury in the hippocampal CA1 region and the corpus callosum after 1 week. Two weeks after cardiac arrest and resuscitation, robust βAPP accumulation was observed in the stratum radiatum and the stratum orients in the CA1 and corpus callosum (Fig. 9.1). Edaravone, a free radical scavenger, which was administered 60 min after resuscitation, suppressed microglial activation and reversed the injuries in the pyramidal cell body and in the axons to the control level (Fig. 9.2). It was proposed that the axonal injury was not only due to

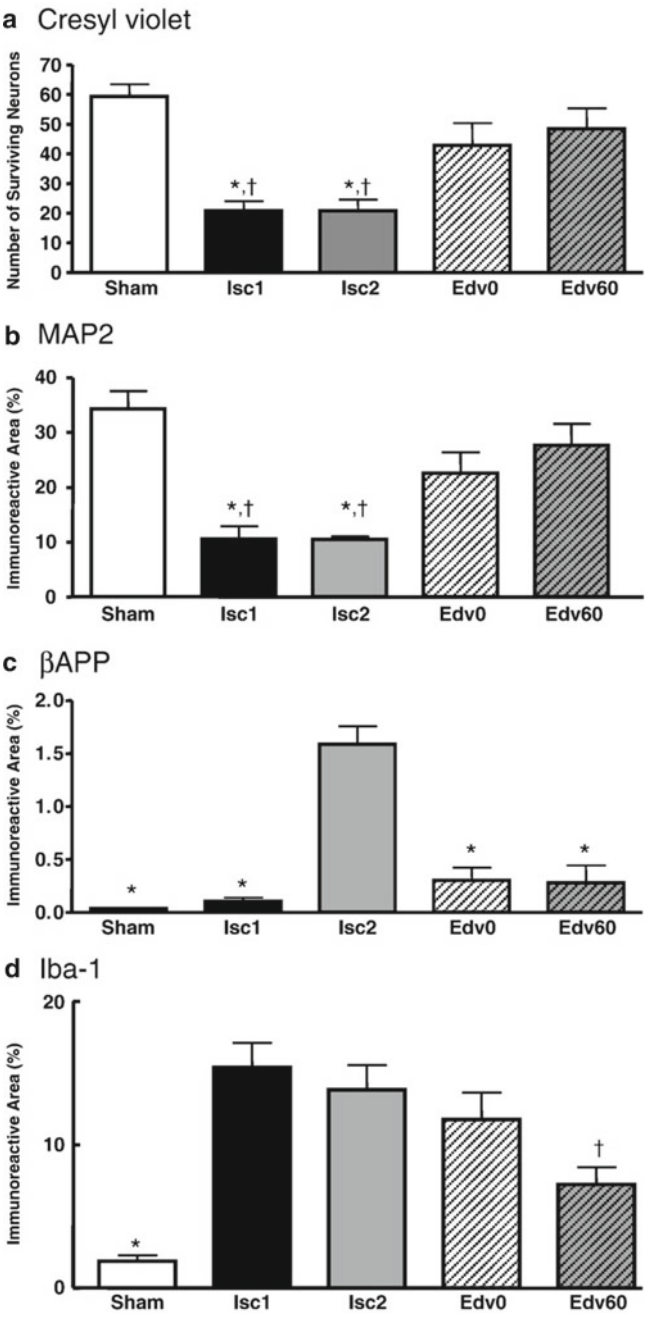


Fig. 9.2 Quantified analyses of neuronal and axonal damages in the hippocampal CA1 region following global cerebral ischemia caused by cardiac arrest and resuscitation. (**a**) The extent of neuronal perikaryal damage is quantified by counting the number of surviving neurons in a predetermined hippocampal CA1 region. *$P<0.05$ compared with the sham group; † $P<0.05$ compared

the secondary effect of neuronal cell body injury in the CA1 pyramidal layer, i.e., Wallerian degeneration, but also due to the direct effect of cardiac arrest and resuscitation. The latter becomes evident because the stratum radiatum in the CA1 region mainly contains septal and commissural fibers from the contralateral hippocampus and the Schaffer collateral fibers, which project forward from CA3 to CA1, and CA3 pyramidal neurons were not damaged. Similar to βAPP accumulation in the CA1 region, βAPP accumulation in the corpus callosum was apparent at 2 weeks after the cardiac arrest and resuscitation and was suppressed by edaravone (Kubo et al. 2009).

9.4 Diffuse Axonal Injury Following Traumatic Brain Injury

Diffuse axonal injury (DAI) occurs following TBI and is one of the most common and important pathological features of TBI (Johnson and Stewart 2012). There is a widespread axonal injury in the cerebral hemispheres, cerebellum, and brain stem. Axonal injury has been observed to increase and peak at 24 h following TBI and can persist for months or even years (Gultekin and Smith 1994; Chen et al. 2009). DAI is responsible for immediate and prolonged posttraumatic coma, independent of a mass lesion and cognitive dysfunction (Gennarelli et al. 1982). It is believed that the pathological mechanism of DAI due to TBI is the direct mechanical force at the time of injury, inducing widely distributed shear and tensile strains throughout the brain material (Johnson and Stewart 2012; Strich 1961). During rapid mechanical loading conditions like TBI, white matter axons appear vulnerable as a result of their highly anisotropic arrangement and their inherent structural design (Maxwell et al. 2003). In addition to the primary mechanical damage, axons are thought to suffer secondary chemical changes. These changes include mitochondrial damage, including an increase in mitochondrial permeability (Okonkwo and Povlishock 1999), oxidative stress and lipid peroxidation (Deng et al. 2007; Mustafa et al. 2010), and an increase in intra-axonal Ca^{2+} (Büki et al. 1999; Gitler and Spira 1998; Maxwell et al. 1991; Povlishock et al. 1999).

In conclusion, white matter, which is mostly composed of axons and oligodendrocytes, is also vulnerable to ischemia, and its injury is associated with behavioral and cognition impairment. The mechanisms of white matter injury are different in many respects from those of gray matter. In a chronic state, WMLs are thought to result from chronic cerebral hypoperfusion, most extensive and prevalent in patients

Fig. 9.2 (continued) with the Edv60 group. (**b**) Neuronal perikaryal damage, quantified by the percentage of MAP2 immunoreactive areas in a predetermined hippocampal CA1 region. *$P<0.05$ compared with the sham group; † $P<0.05$ compared with the Edv60 group. (**c**) Axonal damage, quantified by the percentage of the βAPP immunoreactive areas in a predetermined CA1. *$P<0.05$ compared with the Isc2 group. (**d**) Microglial activation, quantified by the percentage of the Iba-1 immunoreactive areas in a predetermined hippocampal CA1 region. *$P<0.05$ compared with the Isc1 and Isc2 groups; † $P<0.05$ compared with the Isc1 and Isc2 groups. Figure and legend are adapted with permission from Kubo et al. (2009)

with small-vessel strokes, and develop with aging. In acute global ischemia, white matter protection in addition to gray matter protection is necessary to achieve the so-called total brain protection.

Acknowledgment The authors would like to acknowledge the support from the National Institutes of Health (R01NS036124) and from the Department of Anesthesiology at the University of Pittsburgh.

References

Arai K, Lo EH (2009) Experimental models for analysis of oligodendrocyte pathophysiology in stroke. Exp Transl Stroke Med 1:6

Arbelaez A, Castillo M, Mukherji SK (1999) Diffusion-weighted MR imaging of global cerebral anoxia. AJNR Am J Neuroradiol 20:999–1007

Berger T, Walz W, Schnitzer J, Kettenmann H (1992) Gaba- and glutamate-activated currents in glial cells of the mouse corpus callosum slice. J Neurosci Res 31:21–27

Büki A, Siman R, Trojanowski JQ, Povlishock JT (1999) The role of calpain-mediated spectrin proteolysis in traumatically induced axonal injury. J Neuropathol Exp Neurol 58:365–375

Carty ML, Wixey JA, Colditz PB, Buller KM (2008) Post-insult minocycline treatment attenuates hypoxia-ischemia-induced neuroinflammation and white matter injury in the neonatal rat: a comparison of two different dose regimens. Int J Dev Neurosci 26:477–485

Chatterton JE, Awobuluyi M, Premkumar LS, Takahashi H, Talantova M, Shin Y, Cui J, Tu S, Sevarino KA, Nakanishi N, Tong G, Lipton SA, Zhang D (2002) Excitatory glycine receptors containing the nr3 family of nmda receptor subunits. Nature 415:793–798

Chen XH, Johnson VE, Uryu K, Trojanowski JQ, Smith DH (2009) A lack of amyloid beta plaques despite persistent accumulation of amyloid beta in axons of long-term survivors of traumatic brain injury. Brain Pathol 19:214–223

Choi SP, Park HK, Park KN, Kim YM, Ahn KJ, Choi KH, Lee WJ, Jeong SK (2008) The density ratio of grey to white matter on computed tomography as an early predictor of vegetative state or death after cardiac arrest. Emerg Med J 25:666–669

Deng Y, Thompson BM, Gao X, Hall ED (2007) Temporal relationship of peroxynitrite-induced oxidative damage, calpain-mediated cytoskeletal degradation and neurodegeneration after traumatic brain injury. Exp Neurol 205:154–165

Desmond DW (2002) Cognition and white matter lesions. Cerebrovasc Dis 13(Suppl 2):53–57

Dewar D, Dawson D (1995) Tau protein is altered by focal cerebral ischaemia in the rat: an immunohistochemical and immunoblotting study. Brain Res 684:70–78

Dewar D, Yam P, McCulloch J (1999) Drug development for stroke: importance of protecting cerebral white matter. Eur J Pharmacol 375:41–50

Dewar D, Underhill SM, Goldberg MP (2003) Oligodendrocytes and ischemic brain injury. J Cerebr Blood Flow Metabol 23:263–274

Els T, Kassubek J, Kubalek R, Klisch J (2004) Diffusion-weighted mri during early global cerebral hypoxia: a predictor for clinical outcome? Acta Neurol Scand 110:361–367

Erkinjuntti T, Roman G, Gauthier S (2004) Treatment of vascular dementia-evidence from clinical trials with cholinesterase inhibitors. J Neurol Sci 226:63–66

Follett PL, Rosenberg PA, Volpe JJ, Jensen FE (2000) Nbqx attenuates excitotoxic injury in developing white matter. J Neurosci 20:9235–9241

Gennarelli TA, Thibault LE, Adams JH, Graham DI, Thompson CJ, Marcincin RP (1982) Diffuse axonal injury and traumatic coma in the primate. Ann Neurol 12:564–574

Gitler D, Spira ME (1998) Real time imaging of calcium-induced localized proteolytic activity after axotomy and its relation to growth cone formation. Neuron 20:1123–1135

Gladstone DJ, Black SE, Hakim AM (2002) Toward wisdom from failure: lessons from neuroprotective stroke trials and new therapeutic directions. Stroke 33:2123–2136
Goldin A, Beckman JA, Schmidt AM, Creager MA (2006) Advanced glycation end products: sparking the development of diabetic vascular injury. Circulation 114:597–605
Gultekin SH, Smith TW (1994) Diffuse axonal injury in craniocerebral trauma. A comparative histologic and immunohistochemical study. Arch Pathol Lab Med 118:168–171
Heurteaux C, Bertaina V, Widmann C, Lazdunski M (1993) K+ channel openers prevent global ischemia-induced expression of c-fos, c-jun, heat shock protein, and amyloid beta-protein precursor genes and neuronal death in rat hippocampus. Proc Natl Acad Sci USA 90: 9431–9435
Hewlett KA, Corbett D (2006) Delayed minocycline treatment reduces long-term functional deficits and histological injury in a rodent model of focal ischemia. Neuroscience 141:27–33
Hirko AC, Dallasen R, Jomura S, Xu Y (2008) Modulation of inflammatory responses after global ischemia by transplanted umbilical cord matrix stem cells. Stem Cells 26:2893–2901
Honda F, Imai H, Ishikawa M, Kubota C, Shimizu T, Fukunaga M, Saito N (2006) Cilostazol attenuates gray and white matter damage in a rodent model of focal cerebral ischemia. Stroke 37:223–228
Imai H, Masayasu H, Dewar D, Graham DI, Macrae IM (2001) Ebselen protects both gray and white matter in a rodent model of focal cerebral ischemia. Stroke 32:2149–2154
Imai H, McCulloch J, Graham DI, Masayasu H, Macrae IM (2002) New method for the quantitative assessment of axonal damage in focal cerebral ischemia. J Cerebr Blood Flow Metabol 22:1080–1089
Inamasu J, Miyatake S, Nakatsukasa M, Koh H, Yagami T (2011) Loss of gray-white matter discrimination as an early ct sign of brain ischemia/hypoxia in victims of asphyxial cardiac arrest. Emerg Radiol 18:295–298
Irving EA, Yatsushiro K, McCulloch J, Dewar D (1997) Rapid alteration of tau in oligodendrocytes after focal ischemic injury in the rat: involvement of free radicals. J Cerebr Blood Flow Metabol 17:612–622
Irving EA, Bentley DL, Parsons AA (2001) Assessment of white matter injury following prolonged focal cerebral ischaemia in the rat. Acta Neuropathol 102:627–635
Johnson VE, Stewart W (2012) Smith DH. Axonal pathology in traumatic brain injury, Exp Neurol
Kamide T, Kitao Y, Takeichi T, Okada A, Mohri H, Schmidt AM, Kawano T, Munesue S, Yamamoto Y, Yamamoto H, Hamada J, Hori O (2012) Rage mediates vascular injury and inflammation after global cerebral ischemia. Neurochem Int 60:220–228
Káradóttir R, Cavelier P, Bergersen LH, Attwell D (2005) Nmda receptors are expressed in oligodendrocytes and activated in ischaemia. Nature 438:1162–1166
Kubo K, Nakao S, Jomura S, Sakamoto S, Miyamoto E, Xu Y, Tomimoto H, Inada T, Shingu K (2009) Edaravone, a free radical scavenger, mitigates both gray and white matter damages after global cerebral ischemia in rats. Brain Res 1279:139–146
Lee EJ, Lee MY, Chen HY, Hsu YS, Wu TS, Chen ST, Chang GL (2005) Melatonin attenuates gray and white matter damage in a mouse model of transient focal cerebral ischemia. J Pineal Res 38:42–52
Liachenko S, Tang P, Hamilton RL, Xu Y (1998) A reproducible model of circulatory arrest and remote resuscitation in rats for NMR investigation. Stroke 29:1229–1238, discussion 1238-1229
Liachenko S, Tang P, Hamilton RL, Xu Y (2001) Regional dependence of cerebral reperfusion after circulatory arrest in rats. J Cerebr Blood Flow Metabol 21:1320–1329
Liachenko S, Tang P, Xu Y (2003) Deferoxamine improves early postresuscitation reperfusion after prolonged cardiac arrest in rats. J Cerebr Blood Flow Metabol 23:574–581
Lin B, Ginsberg MD, Busto R (2001) Hyperglycemic but not normoglycemic global ischemia induces marked early intraneuronal expression of beta-amyloid precursor protein. Brain Res 888:107–116
Liu K, Mori S, Takahashi HK, Tomono Y, Wake H, Kanke T, Sato Y, Hiraga N, Adachi N, Yoshino T, Nishibori M (2007) Anti-high mobility group box 1 monoclonal antibody ameliorates brain infarction induced by transient ischemia in rats. FASEB J 21:3904–3916
Luyt CE, Galanaud D, Perlbarg V, Vanhaudenhuyse A, Stevens RD, Gupta R, Besancenot H, Krainik A, Audibert G, Combes A, Chastre J, Benali H, Laureys S, Puybasset L (2012) Neuro

imaging for coma E, recovery C. Diffusion tensor imaging to predict long-term outcome after cardiac arrest: a bicentric pilot study. Anesthesiology 117:1311–1321
Madden DJ, Bennett IJ, Burzynska A, Potter GG, Chen NK, Song AW (1822) Diffusion tensor imaging of cerebral white matter integrity in cognitive aging. Biochimica et Biophysica Acta 2012:386–400
Manning SM, Talos DM, Zhou C, Selip DB, Park HK, Park CJ, Volpe JJ, Jensen FE (2008) Nmda receptor blockade with memantine attenuates white matter injury in a rat model of periventricular leukomalacia. J Neurosci 28:6670–6678
Matute C (2006) Oligodendrocyte nmda receptors: a novel therapeutic target. Trends Mol Med 12: 289–292
Matute C (2011) Glutamate and atp signalling in white matter pathology. J Anat 219:53–64
Maxwell WL, Watt C, Pediani JD, Graham DI, Adams JH, Gennarelli TA (1991) Localisation of calcium ions and calcium-atpase activity within myelinated nerve fibres of the adult guinea-pig optic nerve. J Anat 176:71–79
Maxwell WL, Domleo A, McColl G, Jafari SS, Graham DI (2003) Post-acute alterations in the axonal cytoskeleton after traumatic axonal injury. J Neurotrauma 20:151–168
McCracken E, Fowler JH, Dewar D, Morrison S, McCulloch J (2002) Grey matter and white matter ischemic damage is reduced by the competitive ampa receptor antagonist, spd 502. J Cerebr Blood Flow Metabol 22:1090–1097
Metter RB, Rittenberger JC, Guyette FX, Callaway CW (2011) Association between a quantitative ct scan measure of brain edema and outcome after cardiac arrest. Resuscitation 82:1180–1185
Micu I, Jiang Q, Coderre E, Ridsdale A, Zhang L, Woulfe J, Yin X, Trapp BD, McRory JE, Rehak R, Zamponi GW, Wang W, Stys PK (2006) Nmda receptors mediate calcium accumulation in myelin during chemical ischaemia. Nature 439:988–992
Miyamoto E, Tomimoto H, Nakao Si S, Wakita H, Akiguchi I, Miyamoto K, Shingu K (2001) Caudoputamen is damaged by hypocapnia during mechanical ventilation in a rat model of chronic cerebral hypoperfusion. Stroke 32:2920–2925
Miyamoto E, Nakao S, Tomimoto H, Wakita H, Yamada M, Masuzawa M, Takahira K, Sakamoto S, Shingu K (2004) Ketamine attenuates hypocapnia-induced neuronal damage in the caudoputamen in a rat model of chronic cerebral hypoperfusion. Neurosci Lett 354:26–29
Mustafa AG, Singh IN, Wang J, Carrico KM, Hall ED (2010) Mitochondrial protection after traumatic brain injury by scavenging lipid peroxyl radicals. J Neurochem 114:271–280
Nizri E, Hamra-Amitay Y, Sicsic C, Lavon I, Brenner T (2006) Anti-inflammatory properties of cholinergic up-regulation: a new role for acetylcholinesterase inhibitors. Neuropharmacology 50:540–547
Okonkwo DO, Povlishock JT (1999) An intrathecal bolus of cyclosporin a before injury preserves mitochondrial integrity and attenuates axonal disruption in traumatic brain injury. J Cerebr Blood Flow Metabol 19:443–451
Pantoni L (2010) Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. Lancet Neurol 9:689–701
Pantoni L, Garcia JH (1997) Pathogenesis of leukoaraiosis: a review. Stroke 28:652–659
Pantoni L, Garcia JH, Gutierrez JA (1996) Cerebral white matter is highly vulnerable to ischemia. Stroke 27:1641–1646, discussion 1647
Patneau DK, Wright PW, Winters C, Mayer ML, Gallo V (1994) Glial cells of the oligodendrocyte lineage express both kainate- and ampa-preferring subtypes of glutamate receptor. Neuron 12:357–371
Pavlov VA, Tracey KJ (2005) The cholinergic anti-inflammatory pathway. Brain Behav Immun 19:493–499
Pluta R, Kida E, Lossinsky AS, Golabek AA, Mossakowski MJ, Wisniewski HM (1994) Complete cerebral ischemia with short-term survival in rats induced by cardiac arrest. I. Extracellular accumulation of Alzheimer's beta-amyloid protein precursor in the brain. Brain Res 649:323–328
Povlishock JT, Buki A, Koiziumi H, Stone J, Okonkwo DO (1999) Initiating mechanisms involved in the pathobiology of traumatically induced axonal injury and interventions targeted at blunting their progression. Acta Neurochir Suppl 73:15–20

Salter MG, Fern R (2005) Nmda receptors are expressed in developing oligodendrocyte processes and mediate injury. Nature 438:1167–1171

Schäbitz WR, Li F, Fisher M (2000) The *N*-methyl-D-aspartate antagonist cns 1102 protects cerebral gray and white matter from ischemic injury following temporary focal ischemia in rats. Stroke 31:1709–1714

Schmidt R, Scheltens P, Erkinjuntti T, Pantoni L, Markus HS, Wallin A, Barkhof F, Fazekas F (2004) White matter lesion progression: a surrogate endpoint for trials in cerebral small-vessel disease. Neurology 63:139–144

Schmidt R, Grazer A, Enzinger C, Ropele S, Homayoon N, Pluta-Fuerst A, Schwingenschuh P, Katschnig P, Cavalieri M, Schmidt H, Langkammer C, Ebner F, Fazekas F (2011) Mri-detected white matter lesions: do they really matter? J Neural Transm 118:673–681

Storozheva ZI, Proshin AT, Sherstnev VV, Storozhevykh TP, Senilova YE, Persiyantseva NA, Pinelis VG, Semenova NA, Zakharova EI, Pomytkin IA (2008) Dicholine salt of succinic acid, a neuronal insulin sensitizer, ameliorates cognitive deficits in rodent models of normal aging, chronic cerebral hypoperfusion, and beta-amyloid peptide-(25–35)-induced amnesia. BMC Pharmacol 8:1

Strich S (1961) Shearing of nerve fibres as a cause of brain damage due to head injury: a pathological study of twenty cases. Lancet 278:443–448

Stys PK (1995) Protective effects of antiarrhythmic agents against anoxic injury in cns white matter. J Cerebr Blood Flow Metabol 15:425–432

Stys PK (1998) Anoxic and ischemic injury of myelinated axons in cns white matter: from mechanistic concepts to therapeutics. J Cerebr Blood Flow Metabol 18:2–25

Stys PK, Lipton SA (2007) White matter nmda receptors: an unexpected new therapeutic target? Trends Pharmacol Sci 28:561–566

Stys PK, Waxman SG, Ransom BR (1992) Ionic mechanisms of anoxic injury in mammalian cns white matter: role of na+ channels and na(+)-ca2+ exchanger. J Neurosci 12:430–439

Takahashi S, Higano S, Ishii K, Matsumoto K, Sakamoto K, Iwasaki Y, Suzuki M (1993) Hypoxic brain damage: cortical laminar necrosis and delayed changes in white matter at sequential MR imaging. Radiology 189:449–456

Tayeb HO, Yang HD, Price BH, Tarazi FI (2012) Pharmacotherapies for alzheimer's disease: beyond cholinesterase inhibitors. Pharmacol Ther 134:8–25

Tekkök SB, Goldberg MP (2001) Ampa/kainate receptor activation mediates hypoxic oligodendrocyte death and axonal injury in cerebral white matter. J Neurosci 21:4237–4248

Tomimoto H, Wakita H, Akiguchi I, Nakamura S, Kimura J (1994) Temporal profiles of accumulation of amyloid beta/a4 protein precursor in the gerbil after graded ischemic stress. J Cerebr Blood Flow Metabol 14:565–573

Torbey MT, Selim M, Knorr J, Bigelow C, Recht L (2000) Quantitative analysis of the loss of distinction between gray and white matter in comatose patients after cardiac arrest. Stroke 31: 2163–2167

Tsuruta R, Fujita M, Ono T, Koda Y, Koga Y, Yamamoto T, Nanba M, Shitara M, Kasaoka S, Maruyama I, Yuasa M, Maekawa T (2010) Hyperglycemia enhances excessive superoxide anion radical generation, oxidative stress, early inflammation, and endothelial injury in forebrain ischemia/reperfusion rats. Brain Res 1309:155–163

Ueno Y, Zhang N, Miyamoto N, Tanaka R, Hattori N, Urabe T (2009) Edaravone attenuates white matter lesions through endothelial protection in a rat chronic hypoperfusion model. Neuroscience 162:317–327

Vicente E, Degerone D, Bohn L, Scornavaca F, Pimentel A, Leite MC, Swarowsky A, Rodrigues L, Nardin P, de Almeida LM, Gottfried C, Souza DO, Netto CA, Goncalves CA (2009) Astroglial and cognitive effects of chronic cerebral hypoperfusion in the rat. Brain Res 1251:204–212

Wakita H, Tomimoto H, Akiguchi I, Kimura J (1994) Glial activation and white matter changes in the rat brain induced by chronic cerebral hypoperfusion: an immunohistochemical study. Acta Neuropathol 87:484–492

Wakita H, Tomimoto H, Akiguchi I, Kimura J (1995) Protective effect of cyclosporin a on white matter changes in the rat brain after chronic cerebral hypoperfusion. Stroke 26:1415–1422

Wakita H, Tomimoto H, Akiguchi I, Kimura J (1998) Dose-dependent, protective effect of fk506 against white matter changes in the rat brain after chronic cerebral ischemia. Brain Res 792: 105–113

Wakita H, Tomimoto H, Akiguchi I, Lin JX, Miyamoto K, Oka N (1999) A cyclooxygenase-2 inhibitor attenuates white matter damage in chronic cerebral ischemia. Neuroreport 10:1461–1465

Wang L, Yushmanov VE, Liachenko SM, Tang P, Hamilton RL, Xu Y (2002) Late reversal of cerebral perfusion and water diffusion after transient focal ischemia in rats. J Cerebr Blood Flow Metabol 22:253–261

Wang J, Zhang HY, Tang XC (2010) Huperzine a improves chronic inflammation and cognitive decline in rats with cerebral hypoperfusion. J Neurosci Res 88:807–815

Watanabe T, Zhang N, Liu M, Tanaka R, Mizuno Y, Urabe T (2006) Cilostazol protects against brain white matter damage and cognitive impairment in a rat model of chronic cerebral hypoperfusion. Stroke 37:1539–1545

Weil ZM (2012) Ischemia-induced hyperglycemia: consequences, neuroendocrine regulation, and a role for rage. Horm Behav 62:280–285

Weiss N, Galanaud D, Carpentier A, Naccache L, Puybasset L (2007) Clinical review: prognostic value of magnetic resonance imaging in acute brain injury and coma. Crit Care 11:230

White ML, Zhang Y, Helvey JT, Omojola MF (2013) Anatomical patterns and correlated mri findings of non-perinatal hypoxic-ischaemic encephalopathy. Br J Radiol 86:20120464

Wijdicks EF, Campeau NG, Miller GM (2001) MR imaging in comatose survivors of cardiac resuscitation. AJNR Am J Neuroradiol 22:1561–1565

Xu Y, Liachenko S, Tang P (2002) Dependence of early cerebral reperfusion and long-term outcome on resuscitation efficiency after cardiac arrest in rats. Stroke 33:837–843

Xu Y, Liachenko SM, Tang P, Chan PH (2009) Faster recovery of cerebral perfusion in sod1-overexpressed rats after cardiac arrest and resuscitation. Stroke 40:2512–2518

Yam PS, Takasago T, Dewar D, Graham DI, McCulloch J (1997) Amyloid precursor protein accumulates in white matter at the margin of a focal ischaemic lesion. Brain Res 760:150–157

Yam PS, Dewar D, McCulloch J (1998) Axonal injury caused by focal cerebral ischemia in the rat. J Neurotrauma 15:441–450

Yam PS, Dunn LT, Graham DI, Dewar D, McCulloch J (2000) Nmda receptor blockade fails to alter axonal injury in focal cerebral ischemia. J Cerebr Blood Flow Metabol 20:772–779

Zhang J, Takahashi HK, Liu K, Wake H, Liu R, Maruo T, Date I, Yoshino T, Ohtsuka A, Mori S, Nishibori M (2011) Anti-high mobility group box-1 monoclonal antibody protects the blood-brain barrier from ischemia-induced disruption in rats. Stroke 42:1420–1428

Chapter 10
Experimental Global Ischemia and White Matter Injury

Ji Hae Seo, Kazuhide Hayakawa, Nobukazu Miyamoto, Takakuni Maki, Loc-Duyen D. Pham, Eng H. Lo, and Ken Arai

10.1 Introduction

In human brains, white matter constitutes about 50 % of the brain volume (Zhang and Sejnowski 2000), and white matter dysfunction due to oligodendrocyte death is highly relevant to the pathophysiology of CNS diseases (Matute 2011; Kalaria 2012). However, the mechanisms of white matter injury still mostly remain unknown compared to gray matter damage, particularly under global cerebral ischemic conditions. Neuronal loss in the hippocampal CA1 subregion after transient global ischemia is a well-known phenomena (Hatakeyama et al. 1988; Benveniste et al. 1989; Nakayama et al. 1998). Besides neuronal damage, however, it is now recognized that white matter is also vulnerable to ischemia. In fact, transient global ischemia induces white matter injury in mice (Kubo et al. 2009; Walker and Rosenberg 2009, 2010). Also in clinical site, white matter injury has been observed in patients who suffer from transient cardiac arrest (Wu et al. 2009).

In clinical situations, global ischemic conditions occur under cardiac arrest, drowning, and anesthesia-related accidents, which are followed by successful but delayed resuscitation. In unusual cases, the anoxic-ischemic brain changes secondary to cyanide and carbon monoxide exposure induce characteristic white matter lesions in both humans and animals, sometimes in the absence of gray matter damage and with a biphasic clinical course (Schwedenberg 1959; Hirano and Zimmerman 1971). In order to investigate the pathophysiology underlying these lesions after global ischemia and to translate experimental therapeutics from the laboratory to

J.H. Seo • K. Hayakawa • N. Miyamoto • T. Maki • L.-D.D. Pham
E.H. Lo • K. Arai, Ph.D. (✉)
Neuroprotection Research Laboratory, Departments of Radiology and Neurology, Massachusetts General Hospital/Harvard Medical School, 149 Thirteenth Street, MGH-CNY149, Room 2401, Charlestown, MA 02129, USA
e-mail: karai@partners.org

S. Baltan et al. (eds.), *White Matter Injury in Stroke and CNS Disease*, Springer Series in Translational Stroke Research 4, DOI 10.1007/978-1-4614-9123-1_10,

Focal Ischemia	Rat/mouse intraluminal filament MCAO
	Rat/mouse Intraluminal blood-clot MCAO
	Rat/mouse photochemical MCAO
	Rat/mouse microsphere MCAO
	Rat/mouse endothelin-1 MCAO
	Rat/mouse permanent MCAO (tamura model)
Forebrain Ischemia	Gerbil bilateral carotid artery stenosis
	Rat bilateral carotid artery occlusion
	Mouse bilateral carotid artery stenosis
Global Ischemia	Gerbil bilateral carotid artery occlusion
	Rat 4-vessel occlusion
	Mouse bilateral carotid artery occlusion
	Rat/mouse cardiac arrest
	Rat neonatal hypoxia-ischemic injury

Fig. 10.1 Animal models of focal, forebrain, and cerebral global ischemia

the human subjects, a reproducible animal model of global cerebral ischemia is essential. Similar to other brain ischemic insults, several rodent models of global cerebral ischemia have been developed (Fig. 10.1). Transient global cerebral ischemia leads to delayed cell death in selectively vulnerable brain areas, such as the hippocampal CA1 region; medium-sized neurons in the striatum; the neocortical neurons in layers 3, 5, and 6; and white matters in both experimental animals and humans (Kirino 1982; Pulsinelli et al. 1982; Horn and Schlote 1992; Petito et al. 1998; Yoshioka et al. 2011; Onken et al. 2012) (Fig. 10.2). In this chapter, we summarize current animal models of global cerebral ischemia and discuss the mechanisms of white matter injury in these models.

10.2 Animal Models of Global Ischemia

Nonhuman primates seem to be the best model for the study of white matter lesions. They have well-developed white matter and vascular architectures that closely resemble those in human brains. Indeed, accumulating evidence has shown that ischemic cell loss occurs after transient global ischemia in nonhuman primates (Zola-Morgan et al. 1992; Yamashima et al. 2003; Hara et al. 2007). However, most experiments studying global ischemia have been performed on rodents because of the ease of handling and high availability and acceptability from an economical and ethical viewpoint. Hence, compared to the nonhuman primate models, rodent animal models are now widely used to dissect brain pathology after global cerebral ischemia.

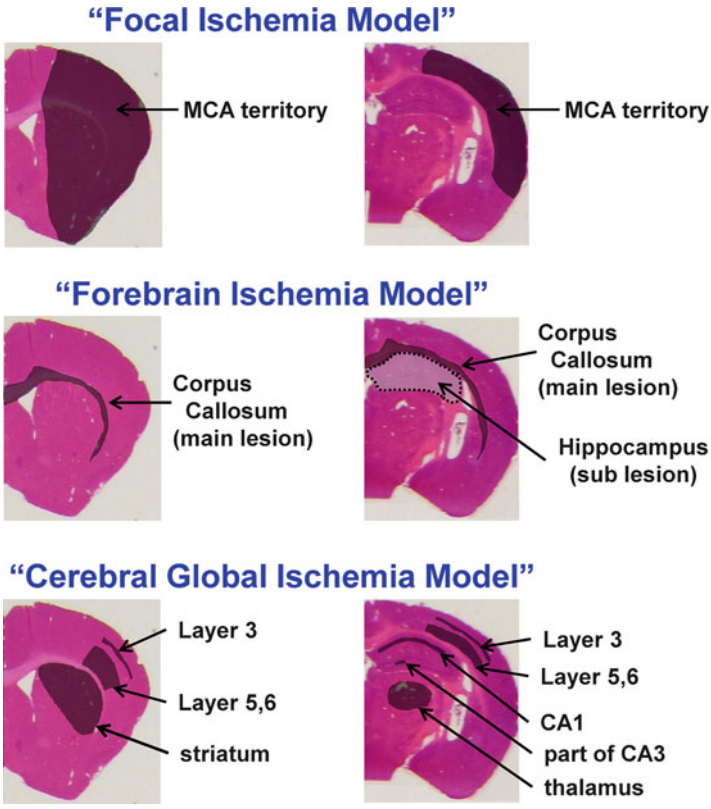


Fig. 10.2 Vulnerable brain regions in brain ischemia models. Coronal sections of mouse brain (*left*: bregma +0.98 mm, *right*: bregma −2.066 mm). Although the lesion areas in the three brain ischemia models are different, white matters are affected in all the models

Notably, the lesion area in those cerebral global ischemia models is different from other types of rodent models for brain ischemia (Fig. 10.2). But interestingly, white matters are vulnerable regions in all the brain ischemic models. Here we overview the existing rodent models of global cerebral ischemia and introduce key findings in those models.

10.2.1 Gerbil Model

The gerbil was first introduced as an experimental model for cerebral ischemia in 1966 (Levine and Payan 1966). The gerbil is a small rodent with an incomplete Circle of Willis, which lacks connections between the carotid and vertebral arteries. There are only limited studies of behavioral changes following ischemia in this model.

But the most consistent behavioral measure has been increased hyperactivity (Chandler et al. 1985; O'Neill et al. 1996). Five minutes of bilateral common carotid artery occlusion (BCAO) causes a selective pattern of cell death in the striatum, cortex, and particularly the CA1 pyramidal cells in the hippocampus, which develops slowly over 2–4 days (Kirino 1982; Kirino and Sano 1984; Kirino et al. 1986). This cell death was termed "delayed neuronal death." Also, Kitagawa et al. reported the dramatic findings that the brief nonlethal BCAO (i.e., two 2-min ischemia at 1-day interval) 2 days before 5-min global ischemia prevented the delayed neuronal death in the hippocampal CA1 region (Kitagawa et al. 1990). This landmark report, which described "classical ischemic preconditioning" phenomenon, launched the field of "ischemic tolerance" research in the brain (Gidday 2006; Dirnagl et al. 2009; Zhao 2009). This so-called preconditioning can protect the brain either almost immediately (classical preconditioning) or with a delay of 1–3 days (delayed preconditioning) after stimulation. Multiple mechanisms would mediate preconditioning by regulating energy metabolism, improving the delivery of blood flow, suppressing the pathogenic pathways, and maximizing the endogenous protection and repair potential (Dirnagl et al. 2009).

10.2.2 Rat Model

Rats have adequate collateral flow through the posterior circulation. Hence, the "4-vessel occlusion (4-VO)" or the "2-VO with hypotension" has been widely used as a rat global cerebral ischemia model (Pulsinelli and Brierley 1979; Pulsinelli et al. 1982). In the 4-VO model, bilateral vertebral arteries are electrocauterized with a bipolar coagulator, followed by bilateral common carotid artery occlusion with the hydraulic pressure occluders or surgical clips to interrupt cerebral circulation (Fig. 10.3). The rat 4-VO model closely resembles the pattern of brain damage seen in humans after cardiac arrest, while the occlusion of the vertebral arteries often leads to a subsequent variation in compensatory blood flow at the time of carotid occlusion. Past studies extensively compared the pattern of cell death in this model to focal stroke models. In the 4-VO model, the astrocytes undergo hypertrophy and the microglia become activated. There seems no significant infiltration of peripheral immune cells into the brain parenchyma, and neuronal cell death occurs slowly and matures over a period of 24 h to 7 days. In situ end labeling, which detects DNA fragmentation, showed that oligodendrocytes die at post-ischemic day 1 and are more sensitive than neurons in the cerebral cortex and thalamus (Petito et al. 1998). On the contrary, in rodent models of focal stroke, neurons appear to die within 4–5 h, and astrocytes also die in the core of the infarct. Furthermore, there is invasion of peripheral immune cells into brain parenchyma. Bendel and Bueters et al. have reported comprehensive long-term studies using the rat 2-VO with halothane-induced hypotension model, which leads to transient complete reduction of cerebral blood flow (Bendel et al. 2005a, b; von Euler et al. 2006; Bueters et al. 2008). In this model, following 11 min of BCAO with

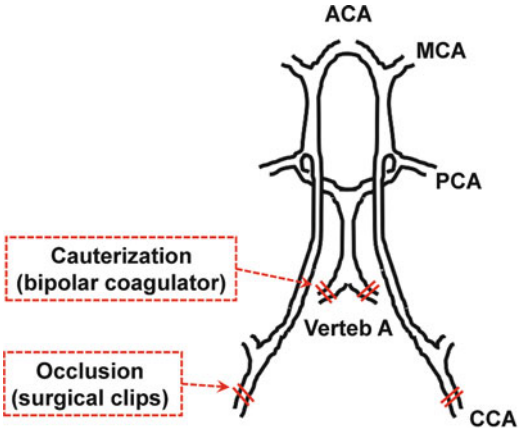


Fig. 10.3 Diagram of cerebrovascular anatomy in rats. To induce global cerebral ischemic conditions in rats, bilateral vertebral arteries are electrocauterized with a bipolar coagulator followed by bilateral common carotid artery occlusion with the hydraulic pressure occluders or surgical clips to interrupt cerebral circulation (4-vessel occlusion model). *ACA* anterior cerebral artery, *MCA* middle cerebral artery, *PCA* posterior cerebral artery, *CCA* common carotid artery, *Verteb A* vertebral artery

hypotension, a profound reduction of CA1 neurons (3–8 % of control values) occurs at 7–14 days after ischemia (DAI) accompanied with a robust impairment in spatial learning and memory. At 90 DAI, however, a restoration of CA1 neurons and functional recovery were observed (Bendel et al. 2005a, b). On the contrary, a large number of the newborn CA1 neurons at 90 DAI again vanished at 250 DAI without functional deterioration (Bueters et al. 2008). The neurodegenerative responses at 250 DAI may be associated with an accumulation of large mineralized calcium deposits and extensive long-lasting microgilal and astroglial reactions (Bueters et al. 2008). Langdon et al. also showed significant atrophy of the CA1 field of hippocampus with a persistent elevation of ED-1 staining (macrophage marker) at 270 days post ischemia following 10 min of BCAO with hypotension (Langdon et al. 2008). At this time point, the rats showed significant deficits in working and reference memory but not in general sensorimotor abilities (Langdon et al. 2008). Thus, both plastic regenerative and neurodegenerative process may persist for a long period after transient global ischemia. As seen in the gerbil model above, rat global ischemia models have been also used for preconditioning studies. In addition, the rat models are also utilized for "post"-conditioning phenomena, which is also a neuroprotective approach for lessening injury in global or focal ischemia and reperfusion. For example, Zhang et al. showed that rapid post-conditioning (six cycles of 10 s/10-s reperfusion/reocclusion) applied immediately after 10-min transient global ischemia attenuated neuronal death in the hippocampus accompanied with downregulation of caspase-3 and upregulation of Bcl-2 in a rat model of 4-VO (Zhang et al. 2012).

10.2.3 Mouse Model

In general, the mouse model is critical for analyzing cellular mechanisms in the basic research field because of the availability of genetically engineered mouse strains. For example, a loss-of-function study using protease-activated receptor-1 (PAR-1) knockout mice revealed that PAR-1 activation has a major role in brain injury after transient global cerebral ischemia (Wang et al. 2012). Another example is matrix metalloproteinase-9 (MMP-9) knockout mice, which showed significant reduction of hippocampal neuronal damage after transient global ischemia compared with wild-type mice (Lee et al. 2004). However, mouse models of global cerebral ischemia may be rather complex compared with gerbil or rat models. Mice express highly variable arterial cerebrovascular structures even within the same strain. Indeed, the mouse BCAO model resulted in less reproducible histopathological damage due to varying residual flow through collaterals from the posterior cerebral circulation (Kitagawa et al. 1998). On the other hand, the 3-VO model (Panahian et al. 1996; Yonekura et al. 2004) with temporary occlusion of the basilar artery would be well reproducible, but technically challenging. The BCAO plus hypotension model also gives typical constant brain damages, but surgical techniques and physiological monitoring are again challenging (Sheng et al. 1999; Wellons et al. 2000). The BCAO plus isoflurane-induced hypotension model, which has been most recently reported to show reproducible changes (Onken et al. 2012), also needs further verification because isoflurane inhibits the cerebral autoregulation. Ultimately, selecting mouse models of global cerebral ischemia will have to be based upon the model features and experimental purposes.

10.2.4 Cardiac Arrest Model

A nonsurgical model of global cerebral ischemia induced by cardiac arrest has been reported in rats or other species (Bottiger et al. 1999; Kofler et al. 2004). This approach eliminates the problem of intra-ischemic collateral blood flow by completely stopping blood flow to the cranium, suggesting that cardiac arrest induces widespread brain damage. Recent study using porcine model has confirmed that cardiac arrest causes white matter injury (i.e., damaged myelinated fibers and loss of myelin) as well as gray matter injury (i.e., neuronal damage, perivascular edema, glial and endothelial cells) (Sharma et al. 2011). However, it was demonstrated that the duration for inducing cardiac arrest could cause neuronal cell death in rats. Moreover, high mortality rate and complicating systemic effects such as renal, hepatic, and myocardial injury also occur, leading to the low number of subsequent publications using this approach (Kawahara et al. 1995; Small and Buchan 2000). Despite the limitation of this model, however, recent studies have reported a long-term evaluation after transient cardiac arrest. At 6 months after 10-min cardiac arrest, the middle-aged rats showed hyperactivity and decreased level of anxiety

with deficits in spatial learning and memory. At 18 months after ischemia, the old rats showed a decline of motor and cognitive functions without significant difference compared with sham-operated rats (Kiryk et al. 2011). In addition, another study demonstrated that long-lived rats exhibited inflammatory reactions, such as lymphocyte infiltration into brain parenchyma, microglial activation, and blood–brain barrier (BBB) leakage, along with neurogenic and angiogenic reactions at 9 and 13 months after 10-min cardiac arrest (Sekeljic et al. 2012).

10.2.5 Neonatal Hypoxic-Ischemic Injury Model

Human neonatal hypoxic-ischemic injury (HII) causes devastating complications such as cerebral palsy, epilepsy, and cognitive delay with an occurrence of 3–5 cases per 1,000 births (Levene et al. 1986; Rennie et al. 2007). Clinically, periventricular leukomalacia is observed in the preterm neonatal HII, whereas injury to the basal ganglia, thalamus, and cortex is most often exhibited in the full-term neonatal HII (Triulzi et al. 2006). Rat pups have been used for mimicking this phenomena. Permanent BCAO with hypoxia (8 % oxygen) in the postnatal day-4 rat pups induces periventricular leukomalacia, mimicking the clinical condition in the preterm neonates (Fan et al. 2005; Cai et al. 2006). On the other hand, permanent BCAO with hypoxia (8 % oxygen) in the postnatal day-10 pups produces predominantly gray matter injury (bilateral basal ganglia and cortex) with the addition of hippocampal injury in severely affected animals, resembling the global distribution of injury seen in the full-term newborn (Recker et al. 2009). Magnetic resonance imaging study was also performed to clarify the time course of the brain damage changes after 5, 9, and 57 weeks of recovery following the HII, indicating that brain damage and memory impairment occurred slowly and progressively after HII (Mishima et al. 2005).

10.3 Mechanisms of White Matter Damage in Global Ischemia Models

In the CNS, white matter is primarily comprised of axonal bundles ensheathed with myelin. The cells forming these sheaths are the oligodendrocytes, which tend to be arranged in rows parallel to axonal tracts. Just before and after birth, oligodendrocyte precursor cells (OPCs) multiply rapidly, differentiate into mature oligodendrocytes, and develop processes, which are then involved in the formation of myelin. Dysfunction or death of oligodendrocytes and their precursor cells (i.e., OPCs) may contribute to white matter damage under pathologic conditions including brain ischemia. Like neurons in gray matter, axons and oligodendrocytes are vulnerable to damage by excitatory amino acids, oxidative stress, and inflammatory cytokines (Dewar et al. 2003; Arai and Lo 2009; Bakiri et al. 2009). Hence, even if we protect neurons in gray matter, loss of myelin and axonal integrity would interfere with

neuronal connectivity and function. Although research has mostly focused on the mechanisms of neuronal cell death after transient global ischemia, key mechanisms of white matter damage in the animal models introduced above are now revealed. Here, we briefly summarize major mediators that induce white matter dysfunction under cerebral global ischemic conditions.

10.3.1 Glutamate

Glutamate is the most well-examined excitatory amino acid for inducing neuronal cell death. Brains have the system to clear extracellular glutamate by transporter uptake. But under pathological conditions such as cerebral ischemia, the loss of energy stores induces ionic imbalances and promotes the reversal of Na^+-dependent glutamate transporter, resulting in extracellular glutamate accumulation (Grewer et al. 2008; Baltan 2009). While smaller than gray matter, glutamate and other excitatory amino acids were reported to be accumulated in white matter in a global model of brain ischemia (Shimada et al. 1993). Glutamate has been well known to induce neuronal cell death in global ischemia models. On the other hand, there is little evidence that glutamate accumulation induces white matter dysfunction in in vivo models of global cerebral ischemia. But in vitro cell culture studies have demonstrated that glutamate affects oligodendrocyte survival in many ways. Firstly, oligodendrocytes express AMPA/kainate receptors, and overactivation of these receptors can mediate Na^+ and Ca^{2+} influx leading to oligodendrocyte death (Sanchez-Gomez and Matute 1999; Sanchez-Gomez et al. 2003). Notably, long term of Ca^{2+} influx through AMPA (GluR2/3) and kainate (GluR5/6/7) receptors causes oligodendrocyte death with increased production of oxygen free radicals and release of proapoptotic factors (Matute et al. 2007). Secondly, the cystine–glutamate exchange antiporter in oligodendrocytes may also contribute to glutamate-induced oligodendrocyte death. Excessive glutamate blocks the antiporter, which then induces glutathione depletion to augment oxidative stress (Oka et al. 1993). Thirdly, as neurons do, oligodendrocytes express *N*-methyl-D-aspartic acid (NMDA) receptors (Karadottir et al. 2005; Salter and Fern 2005; Micu et al. 2006). NMDA receptors of oligodendrocytes are activated by glutamate in white matter ischemia (Karadottir et al. 2005), and activation of these receptors can lead to rise of intracellular Ca^{2+} concentration (Micu et al. 2006). However, the involvement of NMDA receptor in glutamate-induced oligodendrocyte death is still somewhat controversial (Baltan et al. 2008), and therefore, further studies are warranted to examine whether the NMDA receptor in oligodendrocytes can be a therapeutic target for white matter damage in patients. Finally, in addition to those primary excitotoxic mechanisms, glutamate can also kill oligodendroytes via immune system-related pathways. Alberdi et al. showed that brief incubation with glutamate followed by exposure to complement is lethal to oligodendrocytes in vitro (Alberdi et al. 2006). Thus, even glutamate at nontoxic concentrations may induce oligodendrocyte damage by sensitizing these cells to complement attack.

10.3.2 Adenosine Triphosphate

Although elevations in glutamate certainly occur in white matter after global cerebral ischemia, alterations in other extracellular mediators may also be important for white matter injury. In the central nervous system, extracellular adenosine triphosphate (ATP) can act as an excitatory neurotransmitter. While it is still unknown whether ATP signaling affects white matter function under global cerebral ischemia, ATP is released by astrocytes in white matter under pathological conditions, such as stroke, trauma, and multiple sclerosis (Matute 2011). ATP activates ionotropic P2X and metabotropic P2Y receptors (Ralevic and Burnstock 1998; North 2002), and excessive ATP is cytotoxic through ionotropic P2X receptor with high permeability to Ca^{2+} (Alberdi et al. 2006). Both P2X and P2Y receptors are expressed in oligodendrocytes. Furthermore, James and Butt (2001) used isolated optic nerves to show that ATP increased intracellular Ca^{2+} concentrations in oligodendrocytes through P2Y receptors (James and Butt 2001). In addition, they also demonstrated that a P2X receptor agonist evoked a smaller but significant oligodendrocyte Ca^{2+} signal. In brain ischemia and spinal cord injury models, P2X receptors were reported to mediate signaling cascades leading to neurodegeneration (Le Feuvre et al. 2003; Wang et al. 2004). Also in oligodendrocytes, P2X was shown to be involved in cell death. Matute et al. have demonstrated that ATP or P2X agonists, but not P2Y agonists, were toxic to matured oligodendrocytes in vitro (Matute et al. 2007). Therefore, excessive ATP signaling may contribute to aggravate the extent of progressive white matter damage after global cerebral ischemia.

10.3.3 Matrix Metalloproteinases

In recent years, dysregulation of neurovascular proteases has been implicated as central in neurovascular injury after several CNS diseases, such as stroke. Within the neurovascular proteases, the matrix metalloproteinase (MMP) family has been well studied in this field. MMPs comprise a family of zinc endopeptidases with major roles in the physiology and pathology of the mammalian CNS. To date, MMP-2 (gelatinase A), MMP-3 (stromelysin 1), MMP-7 (matrilysin), MMP-9 (gelatinase B), and MMP-13 (collagenase-3) are known to contribute to infarct extent and/or BBB disruption after stroke or other neurological diseases (Anthony et al. 1997; Montaner et al. 2001; Horstmann et al. 2003; Alvarez-Sabin et al. 2004; Rosell et al. 2005). Knockouts of MMP genes or inhibition with selected drugs have proven to be significantly protective outcomes in animal models of brain ischemia. A large body of experimental data has now led to the initiation of clinical stroke trials to test minocycline, which possesses MMP inhibition properties (Fagan et al. 2010). As noted, white matter consists of lipid-rich myelin sheathes. Myelin sheaths are composed with several proteins, such as myelin basic protein (MBP) and myelin-associated glycoprotein (MAG), and the MMP family is known to break down these proteins (Gijbels et al. 1993; D'Souza and Moscarello 2006). Particularly,

MMP-2 was determined the most active enzyme for degradation of MBP in comparison of MMP-3 and MMP-9 (Chandler et al. 1995). Indeed, after transient global ischemia in a mouse BCAO model, MMP-2 enzyme activity was strongly observed in reactive astrocytes in corpus callosum, and significant suppression of delayed myelin degradation was observed after treatment with MMP inhibitor, BB-94 (Walker and Rosenberg 2010). Notably, MMPs have shown to have a beneficial role during neurovascular repair in transient global ischemic animals (Lee et al. 2006). Interestingly, the high neurogenic activity in the dentate gyrus, which is resistant to ischemic injury, is closely related with a substantial increase in MMP activity. By contrast, the disturbed maturation of progenitor cells in the CA, which is vulnerable to ischemic injury, coincides with a low level of MMP activity. Thus, the activity of MMPs might in part explain the difference in SGZ/CA1 neurogenic potential (Wojcik-Stanaszek et al. 2011). Therefore, future studies are warranted to dissect the mechanisms of MMPs on white matter repair after global cerebral ischemia.

10.3.4 Reactive Oxygen Species

White matter consists of lipid-rich myelin sheath, and therefore, it is an enormous source for reactive oxygen species (ROS). Oligodendrocyte lineage cells are sensitive to oxidative stress due to partly their low supplies of the cellular antioxidant, glutathione (Thorburne and Juurlink 1996). As mentioned, excessive glutamate leads to a depletion of intracellular glutathione, resulting in ROS accumulation and oligodendrocyte death (Back et al. 1998). Moreover, early oligodendrocyte lineage cells seem to be more susceptible to oxidative stress than mature oligodendrocytes in vitro (Kim and Kim 1991; Oka et al. 1993; Husain and Juurlink 1995; Back et al. 1998). As in gray matter, striatum white matter shows high level of oxidative stress after global cerebral ischemia (Yoshioka et al. 2011). Thus far, there has been no clear evidence that antioxidant reagents protect white matters after global ischemia. However, a clinical-usage antioxidant drug edaravone has been shown to protect axonal damage in the hippocampal region in mouse global ischemia model (Kubo et al. 2009). Therefore, although the radical spin-trap NXY-059 failed in clinical stroke trial (Proctor and Tamborello 2007), antioxidant drugs are still potent therapeutic candidates for white matters under global ischemia.

10.3.5 Inflammatory Cytokines

Inflammatory reactions after global ischemia may also contribute to white matter injury. Ischemic stress leads to inflammatory responses from nonspecific immunologic reaction, including migration of peripheral leukocytes into the brain and activation of glial cells (Iadecola and Anrather 2011). Tumor necrosis factor-alpha (TNF-α), interleukin-6, and interleukin-1β are the main cytokines, which initiate inflammatory reactions and contribute to the production of other cytokines and inflammatory

mediators (Feuerstein et al. 1998). Excessive amount of cytokines are detrimental. Indeed, a large body of experimental studies has shown that cytokines are involved in upregulation of adhesion molecules and MMPs and in activation of leukocytes and glial cells following cerebral ischemia (Wang et al. 2007). These changes may contribute to secondary brain damage, such as brain swelling, dysregulated microcirculation, and hemorrhage (Jean et al. 1998). In addition, TNF-α and interferon-gamma induce apoptosis within 72 h in human oligodendrocyte cell line (Buntinx et al. 2004). In gene studies using oligodendrocyte samples, TNF-α has shown to upregulate multiple genes including cell survival- and apoptosis-related ones (Buntinx et al. 2004). This may suggest that there exists a time-dependent mechanism that could lead to oligodendrocyte survival or death during cytokine stimulation. Moreover, those inflammatory cytokines, which are commonly released by activated glial cells, can impair glutamate uptake to trigger excitotoxic oligodendrocyte death (Takahashi et al. 2003; Matute et al. 2007). In fact in a rat transient global ischemic model, interleukin-1β mRNA signal is intensified in the white matter areas as well as gray matter (Sairanen et al. 1997). Additionally, inflammatory cytokines are also known to make glial and endothelial cells to produce MMPs through MAP kinase pathways (Genersch et al. 2000; Arai et al. 2003; Wu et al. 2004). As noted above, excessive MMP release results in breakdown of the BBB or edema formation.

10.4 Potential Therapeutic Targets for White Matter Ischemia

The combined use of cell cultures and whole-animal experiments has been dissecting the pathophysiologic mechanisms of white matter injury after global ischemia (Fig. 10.4). Of course, no validated drugs for white matter damage as yet exist. But several promising candidates have emerged, which we briefly discuss in this section.

10.4.1 Glutamate-Receptor Antagonist

As mentioned, glutamate may play a pivotal role in white matter injury after global ischemia. An uncompetitive NMDA receptor antagonist, memantine, has been well examined for the efficacy for preventing white matters from several insults, including ischemic stress. Memantine is licensed for moderate-to-severe Alzheimer's disease in the USA and the EU (Lipton 2006). Thus far, at least two studies have suggested that memantine might be protective to white matter. First, Bakiri et al. reported that memantine reduced ischemic damage to mature and precursor oligodendrocytes in brain slices assessed by patch-clamp system (Bakiri et al. 2008). Second, Manning et al. showed that memantine attenuated white matter injury in a rat model of periventricular leukomalacia (Manning et al. 2008). Furthermore, the blockade of the AMPA–kainate-type glutamate receptor would be another promising therapy for white matter damage. Inhibition of

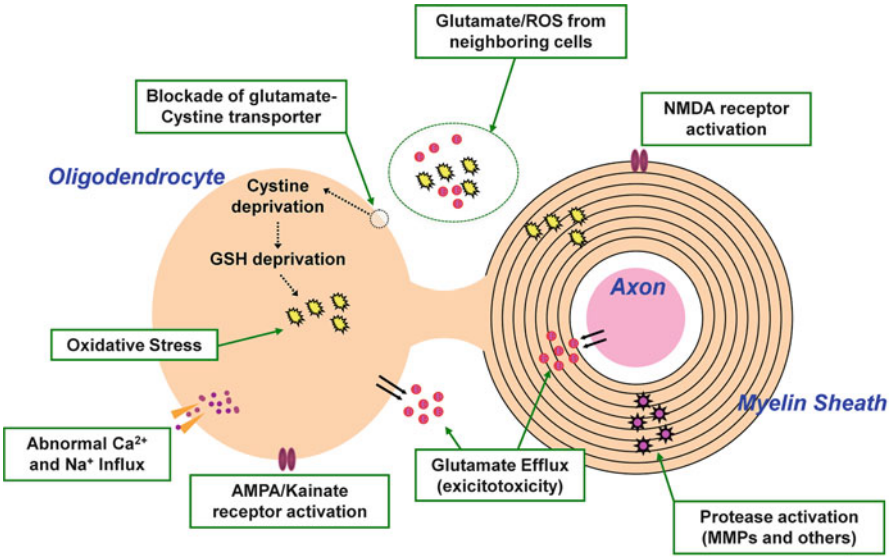


Fig. 10.4 Summary of oligodendrocyte death pathways under white matter ischemia. Under ischemic conditions, several deleterious factors/cascades are activated. Glutamate efflux, oxidative stress, and proteinase activation induce myelin loss and oligodendrocyte death, eventually resulting in white matter dysfunction. Notably, deleterious factors secreted by one cell type may affect another cell type

non-NMDA receptors may prevent not only neuronal injury but also immature oligodendroglial injury. Developing oligodendrocytes express ionotropic kainate and AMPA glutamatergic receptors (Gallo et al. 1994). In both rodents and human brain tissue, there is a direct correlation between selective vulnerability to hypoxia–ischemia and expression of AMPA receptors lacking GluR2 (Talos et al. 2006a, b). This suggests that Ca^{2+}-permeable AMPA receptor blockage may represent an age-specific therapeutic strategy for use in humans. In rodent studies, topiramate administered in vivo after hypoxia–ischemia insult has been shown to be protective against white matter injury and decreased sensorimotor deficits through AMPA–kainate receptor blockade (Follett et al. 2004). Interestingly, protective doses of topiramate did not affect normal development and maturation of oligodendrocytes (Follett et al. 2004).

10.4.2 Antioxidant Drug

Excessive reactive oxygens induce white matter dysfunction. The radical spin-trap NXY-059 failed in clinical stroke trial (Proctor and Tamborello 2007), but antioxidants can be still potent therapeutic candidates for white matter injury after global ischemia. Imai et al. used rat transient ischemia models to evaluate the efficacy of

ebselen, an antioxidant drug (Imai et al. 2001). In the study, they showed that ebselen reduced axonal damage and that oligodendrocyte pathology was also reduced. Using rat focal stroke model, Irving et al. demonstrated that a free radical scavenger phenyl-N-tert-butyl-nitron (PBN) reduced the number of tau-positive oligodendrocytes in the subcortical white matter of the ischemic hemisphere (Irving et al. 1997). Lin et al. also examined the efficacy of PBN on white matter injury by hypoxia–ischemia in the neonatal rat brain (Lin et al. 2004). In the study, the PBN treatment protected both oligodendrocytes and axons from ischemic insults. Subsequently, the same group has demonstrated that PBN also inhibited upregulation of inflammatory cytokines, such as TNF-α, interleukin-1β, and iNOS mRNA expression in the same model (Lin et al. 2006). Finally, as shown previously, clinical usage of antioxidant edaravone is reported to be protective for axonal injury in a mouse global ischemia model (Kubo et al. 2009). Notably, however, recent studies have shown that basal levels of ROS may be important for brain homeostasis and remodeling (Lo 2008). Hence, effects of antioxidant drugs on white matter remodeling after injury should be carefully examined in preclinical studies before testing them in clinical trials.

10.4.3 Immune-Suppressive Therapy

Inflammatory cascades are activated after ischemic injury to cause white matter dysfunction, and therefore, anti-inflammatory would be a promising approach for white matter protection. Minocycline is a second-generation tetracycline, which can cross the BBB (Macdonald et al. 1973; Saivin and Houin 1988). Minocycline has been shown to be beneficial in a wide range of acute neurological injuries. In rodent brain ischemic models, this drug showed anti-inflammatory effects, based on its ability to inhibit immune mediators, such as microglia (Yrjanheikki et al. 1998, 1999). Although there is no report that minocycline directly protects oligodendrocytes against ischemic stress in adult rodent stroke model, this drug was shown to attenuate hypoxia/ischemia-induced white matter injury in neonatal rats (Stolp et al. 2007; Carty et al. 2008). Further, Hewlett and Corbett have shown that delayed minocycline treatment reduced long-term functional deficits as well as white matter injury in endothelin-1-induced rat ischemia model (Hewlett and Corbett 2006). In spite of these experimental findings, it must be noted that a recent clinical trial using minocycline in amyotrophic lateral sclerosis patients failed to show efficacy (Gordon et al. 2007). A potential caveat with this study is the long-term use of minocycline. Among its many actions, minocycline is a powerful metalloproteinase inhibitor, and long-term suppression of metalloproteinases may be detrimental for neurovascular homeostasis (Zhao et al. 2006). Nevertheless, in brain ischemia, short-term applications of minocycline would be still possible. Ultimately, whether minocycline will be useful for white matter injury in brain ischemic patients will have to be answered in a carefully analyzed randomized trial.

10.4.4 Remyelination Therapy

To date, the majority of studies using cell and whole-animal models of white matter injury have mostly focused on mechanisms and targets for acute injury. However, it may not be easy to block all multifactorial pathways of brain cell death in brain ischemic patients. Therefore, an emerging emphasis on promoting recovery after white matter injury is beginning to take shape in the field. In this regard, adenosine would be a very interesting therapeutic target. An important feature of oligodendrocytes is that they express each of the different adenosine receptor (AR) subtypes (Othman et al. 2003). Treatment of OPCs in culture with adenosine accelerated their oligodendrocyte maturation through A1AR-mediated pathway (Stevens et al. 2002). Activation of A1ARs also stimulated OPC migration, without adverse affects on cell viability (Othman et al. 2003). These data have shown that adenosine plays a prominent role in this process. Erythropoietin (EPO) could be another treatment target to promote white matter remodeling. Iwai et al. investigated the state of brain oligodendrogenesis and neurological functional outcomes after administration of EPO to hypoxia–ischemia-treated neonatal rats (Iwai et al. 2010). They have demonstrated that EPO administration delayed by 24 h induced a prolonged increase in oligodendrogenesis and maturation in the ischemic hemisphere and the corpus callosum and improved neurological functional outcomes after neonatal hypoxia–ischemic injury. Other reports have demonstrated that neonatal hypoxia–ischemia showed differential effects of EPO on the various stages of maturing oligodendrocytes (Back et al. 2002). In particular, immature OPCs are more resistant to neonatal hypoxia–ischemia as compared to mature oligodendrocytic populations, and the number of late OPCs is increased by EPO treatment. EPO has also been demonstrated to possess oligodendro-protective capacity (Genc et al. 2006). Whereas the mechanism for EPO-stimulated cell replacement is still mostly unknown, both neurons and OPCs express EPO receptors (Sugawa et al. 2002). In conclusion, the signaling mechanisms underlying cell replacement in the brain may further identify potential therapeutic targets and points for intervention (van der Kooij et al. 2008).

10.5 Conclusions

Although white matter damage is a key part of most neurological disorders, including cerebral global ischemia, white matter mechanisms are relatively understudied compared to gray matters. The development of therapies for white matter protection is very challenging. One of the issues for developing new therapies for cerebral ischemia is to find pharmacological treatments that can protect multiple cell types in the white matter, especially oligodendrocyte linage cells. Oligodendrocyte and their precursors, which play important roles in myelin formation in the central nervous system, are vulnerable to ischemic stress, resulting in early loss of myelin and white matter dysfunction after ischemic stress. Inhibiting axonal damage and/or

oligodendrocyte death and accelerating the remyelination via OPC proliferation and differentiation could be critical in preventing acute neuronal disconnections as well as promoting repair and remodeling after global cerebral ischemia. In this chapter, we tried to provide a broad but brief survey of existing global cerebral ischemia models. Many studies have productively used those model systems to dissect brain pathophysiology, particularly neuronal cell death mechanisms in the gray matters. However, as discussed here, white matters are indeed damaged after global ischemic stress. The use of the animal models has been elucidating key mechanisms of white matter pathophysiology, which should help us to cross the difficult translational hurdles between basic science and clinical challenges.

Acknowledgments Supported in part by the National Institutes of Health, the American Heart Association, and the Deane Institute. Materials in this chapter have been extensively drawn from previously published reviews from the authors including Arai and Lo, Biol Pharm Bull 2009, and Arai and Lo, Exp Transl Stroke Med 2009.

References

Alberdi E, Sanchez-Gomez MV, Torre I, Domercq M, Perez-Samartin A, Perez-Cerda F, Matute C (2006) Activation of kainate receptors sensitizes oligodendrocytes to complement attack. J Neurosci 26:3220–3228

Alvarez-Sabin J, Delgado P, Abilleira S, Molina CA, Arenillas J, Ribo M, Santamarina E, Quintana M, Monasterio J, Montaner J (2004) Temporal profile of matrix metalloproteinases and their inhibitors after spontaneous intracerebral hemorrhage: relationship to clinical and radiological outcome. Stroke 35:1316–1322

Anthony DC, Ferguson B, Matyzak MK, Miller KM, Esiri MM, Perry VH (1997) Differential matrix metalloproteinase expression in cases of multiple sclerosis and stroke. Neuropathol Appl Neurobiol 23:406–415

Arai K, Lo EH (2009) Experimental models for analysis of oligodendrocyte pathophysiology in stroke. Exp Transl Stroke Med 1:6

Arai K, Lee SR, Lo EH (2003) Essential role for ERK mitogen-activated protein kinase in matrix metalloproteinase-9 regulation in rat cortical astrocytes. Glia 43:254–264

Back SA, Gan X, Li Y, Rosenberg PA, Volpe JJ (1998) Maturation-dependent vulnerability of oligodendrocytes to oxidative stress-induced death caused by glutathione depletion. J Neurosci 18:6241–6253

Back SA, Han BH, Luo NL, Chricton CA, Xanthoudakis S, Tam J, Arvin KL, Holtzman DM (2002) Selective vulnerability of late oligodendrocyte progenitors to hypoxia-ischemia. J Neurosci 22:455–463

Bakiri Y, Hamilton NB, Karadottir R, Attwell D (2008) Testing NMDA receptor block as a therapeutic strategy for reducing ischaemic damage to CNS white matter. Glia 56:233–240

Bakiri Y, Burzomato V, Frugier G, Hamilton NB, Karadottir R, Attwell D (2009) Glutamatergic signaling in the brain's white matter. Neuroscience 158:266–274

Baltan S (2009) Ischemic injury to white matter: an age-dependent process. Neuroscientist 15: 126–133

Baltan S, Besancon EF, Mbow B, Ye Z, Hamner MA, Ransom BR (2008) White matter vulnerability to ischemic injury increases with age because of enhanced excitotoxicity. J Neurosci 28: 1479–1489

Bendel O, Alkass K, Bueters T, von Euler M, von Euler G (2005a) Reproducible loss of CA1 neurons following carotid artery occlusion combined with halothane-induced hypotension. Brain Res 1033:135–142

Bendel O, Bueters T, von Euler M, Ove Ogren S, Sandin J, von Euler G (2005b) Reappearance of hippocampal CA1 neurons after ischemia is associated with recovery of learning and memory. J Cereb Blood Flow Metab 25:1586–1595

Benveniste H, Jorgensen MB, Sandberg M, Christensen T, Hagberg H, Diemer NH (1989) Ischemic damage in hippocampal CA1 is dependent on glutamate release and intact innervation from CA3. J Cereb Blood Flow Metab 9:629–639

Bottiger BW, Teschendorf P, Krumnikl JJ, Vogel P, Galmbacher R, Schmitz B, Motsch J, Martin E, Gass P (1999) Global cerebral ischemia due to cardiocirculatory arrest in mice causes neuronal degeneration and early induction of transcription factor genes in the hippocampus. Brain Res Mol Brain Res 65:135–142

Bueters T, von Euler M, Bendel O, von Euler G (2008) Degeneration of newly formed CA1 neurons following global ischemia in the rat. Exp Neurol 209:114–124

Buntinx M, Gielen E, Van Hummelen P, Raus J, Ameloot M, Steels P, Stinissen P (2004) Cytokine-induced cell death in human oligodendroglial cell lines. II: alterations in gene expression induced by interferon-gamma and tumor necrosis factor-alpha. J Neurosci Res 76:846–861

Cai Z, Lin S, Fan LW, Pang Y, Rhodes PG (2006) Minocycline alleviates hypoxic-ischemic injury to developing oligodendrocytes in the neonatal rat brain. Neuroscience 137:425–435

Carty ML, Wixey JA, Colditz PB, Buller KM (2008) Post-insult minocycline treatment attenuates hypoxia-ischemia-induced neuroinflammation and white matter injury in the neonatal rat: a comparison of two different dose regimens. Int J Dev Neurosci 26:477–485

Chandler MJ, DeLeo J, Carney JM (1985) An unanesthetized-gerbil model of cerebral ischemia-induced behavioral changes. J Pharmacol Methods 14:137–146

Chandler S, Coates R, Gearing A, Lury J, Wells G, Bone E (1995) Matrix metalloproteinases degrade myelin basic protein. Neurosci Lett 201:223–226

Dewar D, Underhill SM, Goldberg MP (2003) Oligodendrocytes and ischemic brain injury. J Cereb Blood Flow Metab 23:263–274

Dirnagl U, Becker K, Meisel A (2009) Preconditioning and tolerance against cerebral ischaemia: from experimental strategies to clinical use. Lancet Neurol 8:398–412

D'Souza CA, Moscarello MA (2006) Differences in susceptibility of MBP charge isomers to digestion by stromelysin-1 (MMP-3) and release of an immunodominant epitope. Neurochem Res 31:1045–1054

Fagan SC, Waller JL, Nichols FT, Edwards DJ, Pettigrew LC, Clark WM, Hall CE, Switzer JA, Ergul A, Hess DC (2010) Minocycline to improve neurologic outcome in stroke (MINOS): a dose-finding study. Stroke 41:2283–2287

Fan LW, Lin S, Pang Y, Lei M, Zhang F, Rhodes PG, Cai Z (2005) Hypoxia-ischemia induced neurological dysfunction and brain injury in the neonatal rat. Behav Brain Res 165:80–90

Feuerstein GZ, Wang X, Barone FC (1998) The role of cytokines in the neuropathology of stroke and neurotrauma. Neuroimmunomodulation 5:143–159

Follett PL, Deng W, Dai W, Talos DM, Massillon LJ, Rosenberg PA, Volpe JJ, Jensen FE (2004) Glutamate receptor-mediated oligodendrocyte toxicity in periventricular leukomalacia: a protective role for topiramate. J Neurosci 24:4412–4420

Gallo V, Patneau DK, Mayer ML, Vaccarino FM (1994) Excitatory amino acid receptors in glial progenitor cells: molecular and functional properties. Glia 11:94–101

Genc K, Genc S, Baskin H, Semin I (2006) Erythropoietin decreases cytotoxicity and nitric oxide formation induced by inflammatory stimuli in rat oligodendrocytes. Physiol Res 55:33–38

Genersch E, Hayess K, Neuenfeld Y, Haller H (2000) Sustained ERK phosphorylation is necessary but not sufficient for MMP-9 regulation in endothelial cells: involvement of ras-dependent and -independent pathways. J Cell Sci 113(Pt 23):4319–4330

Gidday JM (2006) Cerebral preconditioning and ischaemic tolerance. Nat Rev Neurosci 7:437–448

Gijbels K, Proost P, Masure S, Carton H, Billiau A, Opdenakker G (1993) Gelatinase B is present in the cerebrospinal fluid during experimental autoimmune encephalomyelitis and cleaves myelin basic protein. J Neurosci Res 36:432–440

Gordon PH, Moore DH, Miller RG, Florence JM, Verheijde JL, Doorish C, Hilton JF, Spitalny GM, MacArthur RB, Mitsumoto H, Neville HE, Boylan K, Mozaffar T, Belsh JM, Ravits J, Bedlack RS, Graves MC, McCluskey LF, Barohn RJ, Tandan R (2007) Efficacy of minocycline in patients with amyotrophic lateral sclerosis: a phase III randomised trial. Lancet Neurol 6:1045–1053

Grewer C, Gameiro A, Zhang Z, Tao Z, Braams S, Rauen T (2008) Glutamate forward and reverse transport: from molecular mechanism to transporter-mediated release after ischemia. IUBMB Life 60:609–619

Hara K, Yasuhara T, Matsukawa N, Maki M, Masuda T, Yu G, Xu L, Tambrallo L, Rodriguez NA, Stern DM, Kawase T, Yamashima T, Buccafusco JJ, Hess DC, Borlongan CV (2007) Hippocampal CA1 cell loss in a non-human primate model of transient global ischemia: a pilot study. Brain Res Bull 74:164–171

Hatakeyama T, Matsumoto M, Brengman JM, Yanagihara T (1988) Immunohistochemical investigation of ischemic and postischemic damage after bilateral carotid occlusion in gerbils. Stroke 19:1526–1534

Hewlett KA, Corbett D (2006) Delayed minocycline treatment reduces long-term functional deficits and histological injury in a rodent model of focal ischemia. Neuroscience 141:27–33

Hirano A, Zimmerman HM (1971) Some new pathological findings in the central myelinated axon. J Neuropathol Exp Neurol 30:325–336

Horn M, Schlote W (1992) Delayed neuronal death and delayed neuronal recovery in the human brain following global ischemia. Acta Neuropathol 85:79–87

Horstmann S, Kalb P, Koziol J, Gardner H, Wagner S (2003) Profiles of matrix metalloproteinases, their inhibitors, and laminin in stroke patients: influence of different therapies. Stroke 34: 2165–2170

Husain J, Juurlink BH (1995) Oligodendroglial precursor cell susceptibility to hypoxia is related to poor ability to cope with reactive oxygen species. Brain Res 698:86–94

Iadecola C, Anrather J (2011) The immunology of stroke: from mechanisms to translation. Nat Med 17:796–808

Imai H, Masayasu H, Dewar D, Graham DI, Macrae IM (2001) Ebselen protects both gray and white matter in a rodent model of focal cerebral ischemia. Stroke 32:2149–2154

Irving EA, Yatsushiro K, McCulloch J, Dewar D (1997) Rapid alteration of tau in oligodendrocytes after focal ischemic injury in the rat: involvement of free radicals. J Cereb Blood Flow Metab 17:612–622

Iwai M, Stetler RA, Xing J, Hu X, Gao Y, Zhang W, Chen J, Cao G (2010) Enhanced oligodendrogenesis and recovery of neurological function by erythropoietin after neonatal hypoxic/ischemic brain injury. Stroke 41:1032–1037

James G, Butt AM (2001) P2X and P2Y purinoreceptors mediate ATP-evoked calcium signalling in optic nerve glia in situ. Cell Calcium 30:251–259

Jean WC, Spellman SR, Nussbaum ES, Low WC (1998) Reperfusion injury after focal cerebral ischemia: the role of inflammation and the therapeutic horizon. Neurosurgery 43:1382–1396, discussion 1396–1387

Kalaria RN (2012) Cerebrovascular disease and mechanisms of cognitive impairment: evidence from clinicopathological studies in humans. Stroke 43:2526–2534

Karadottir R, Cavelier P, Bergersen LH, Attwell D (2005) NMDA receptors are expressed in oligodendrocytes and activated in ischaemia. Nature 438:1162–1166

Kawahara N, Ruetzler CA, Klatzo I (1995) Protective effect of spreading depression against neuronal damage following cardiac arrest cerebral ischaemia. Neurol Res 17:9–16

Kim YS, Kim SU (1991) Oligodendroglial cell death induced by oxygen radicals and its protection by catalase. J Neurosci Res 29:100–106

Kirino T (1982) Delayed neuronal death in the gerbil hippocampus following ischemia. Brain Res 239:57–69
Kirino T, Sano K (1984) Selective vulnerability in the gerbil hippocampus following transient ischemia. Acta Neuropathol 62:201–208
Kirino T, Tamura A, Sano K (1986) A reversible type of neuronal injury following ischemia in the gerbil hippocampus. Stroke 17:455–459
Kiryk A, Pluta R, Figiel I, Mikosz M, Ulamek M, Niewiadomska G, Jablonski M, Kaczmarek L (2011) Transient brain ischemia due to cardiac arrest causes irreversible long-lasting cognitive injury. Behav Brain Res 219:1–7
Kitagawa K, Matsumoto M, Tagaya M, Hata R, Ueda H, Niinobe M, Handa N, Fukunaga R, Kimura K, Mikoshiba K et al (1990) "Ischemic tolerance" phenomenon found in the brain. Brain Res 528:21–24
Kitagawa K, Matsumoto M, Yang G, Mabuchi T, Yagita Y, Hori M, Yanagihara T (1998) Cerebral ischemia after bilateral carotid artery occlusion and intraluminal suture occlusion in mice: evaluation of the patency of the posterior communicating artery. J Cereb Blood Flow Metab 18:570–579
Kofler J, Hattori K, Sawada M, DeVries AC, Martin LJ, Hurn PD, Traystman RJ (2004) Histopathological and behavioral characterization of a novel model of cardiac arrest and cardiopulmonary resuscitation in mice. J Neurosci Methods 136:33–44
Kubo K, Nakao S, Jomura S, Sakamoto S, Miyamoto E, Xu Y, Tomimoto H, Inada T, Shingu K (2009) Edaravone, a free radical scavenger, mitigates both gray and white matter damages after global cerebral ischemia in rats. Brain Res 1279:139–146
Langdon KD, Granter-Button S, Corbett D (2008) Persistent behavioral impairments and neuroinflammation following global ischemia in the rat. Eur J Neurosci 28:2310–2318
Le Feuvre RA, Brough D, Touzani O, Rothwell NJ (2003) Role of P2X7 receptors in ischemic and excitotoxic brain injury in vivo. J Cereb Blood Flow Metab 23:381–384
Lee SR, Tsuji K, Lo EH (2004) Role of matrix metalloproteinases in delayed neuronal damage after transient global cerebral ischemia. J Neurosci 24:671–678
Lee SR, Kim HY, Rogowska J, Zhao BQ, Bhide P, Parent JM, Lo EH (2006) Involvement of matrix metalloproteinase in neuroblast cell migration from the subventricular zone after stroke. J Neurosci 26:3491–3495
Levene MI, Sands C, Grindulis H, Moore JR (1986) Comparison of two methods of predicting outcome in perinatal asphyxia. Lancet 1:67–69
Levine S, Payan H (1966) Effects of ischemia and other procedures on the brain and retina of the gerbil (Meriones unguiculatus). Exp Neurol 16:255–262
Lin S, Rhodes PG, Lei M, Zhang F, Cai Z (2004) alpha-Phenyl-n-tert-butyl-nitrone attenuates hypoxic-ischemic white matter injury in the neonatal rat brain. Brain Res 1007:132–141
Lin S, Cox HJ, Rhodes PG, Cai Z (2006) Neuroprotection of alpha-phenyl-n-tert-butyl-nitrone on the neonatal white matter is associated with anti-inflammation. Neurosci Lett 405:52–56
Lipton SA (2006) NMDA receptors, glial cells, and clinical medicine. Neuron 50:9–11
Lo EH (2008) A new penumbra: transitioning from injury into repair after stroke. Nat Med 14: 497–500
Macdonald H, Kelly RG, Allen ES, Noble JF, Kanegis LA (1973) Pharmacokinetic studies on minocycline in man. Clin Pharmacol Ther 14:852–861
Manning SM, Talos DM, Zhou C, Selip DB, Park HK, Park CJ, Volpe JJ, Jensen FE (2008) NMDA receptor blockade with memantine attenuates white matter injury in a rat model of periventricular leukomalacia. J Neurosci 28:6670–6678
Matute C (2011) Glutamate and ATP signalling in white matter pathology. J Anat 219:53–64
Matute C, Alberdi E, Domercq M, Sanchez-Gomez MV, Perez-Samartin A, Rodriguez-Antiguedad A, Perez-Cerda F (2007) Excitotoxic damage to white matter. J Anat 210:693–702
Micu I, Jiang Q, Coderre E, Ridsdale A, Zhang L, Woulfe J, Yin X, Trapp BD, McRory JE, Rehak R, Zamponi GW, Wang W, Stys PK (2006) NMDA receptors mediate calcium accumulation in myelin during chemical ischaemia. Nature 439:988–992

Mishima K, Ikeda T, Aoo N, Takai N, Takahashi S, Egashira N, Ikenoue T, Iwasaki K, Fujiwara M (2005) Hypoxia-ischemic insult in neonatal rats induced slowly progressive brain damage related to memory impairment. Neurosci Lett 376:194–199

Montaner J, Alvarez-Sabin J, Molina C, Angles A, Abilleira S, Arenillas J, Gonzalez MA, Monasterio J (2001) Matrix metalloproteinase expression after human cardioembolic stroke: temporal profile and relation to neurological impairment. Stroke 32:1759–1766

Nakayama M, Uchimura K, Zhu RL, Nagayama T, Rose ME, Stetler RA, Isakson PC, Chen J, Graham SH (1998) Cyclooxygenase-2 inhibition prevents delayed death of CA1 hippocampal neurons following global ischemia. Proc Natl Acad Sci U S A 95:10954–10959

North RA (2002) Molecular physiology of P2X receptors. Physiol Rev 82:1013–1067

O'Neill M, Canney M, Earley B, Junien JL, Leonard BE (1996) The novel sigma ligand JO 1994 protects against ischaemia-induced behavioural changes, cell death and receptor dysfunction in the gerbil. Neurochem Int 28:193–207

Oka A, Belliveau MJ, Rosenberg PA, Volpe JJ (1993) Vulnerability of oligodendroglia to glutamate: pharmacology, mechanisms, and prevention. J Neurosci 13:1441–1453

Onken M, Berger S, Kristian T (2012) Simple model of forebrain ischemia in mouse. J Neurosci Methods 204:254–261

Othman T, Yan H, Rivkees SA (2003) Oligodendrocytes express functional A1 adenosine receptors that stimulate cellular migration. Glia 44:166–172

Panahian N, Yoshida T, Huang PL, Hedley-Whyte ET, Dalkara T, Fishman MC, Moskowitz MA (1996) Attenuated hippocampal damage after global cerebral ischemia in mice mutant in neuronal nitric oxide synthase. Neuroscience 72:343–354

Petito CK, Olarte JP, Roberts B, Nowak TS Jr, Pulsinelli WA (1998) Selective glial vulnerability following transient global ischemia in rat brain. J Neuropathol Exp Neurol 57:231–238

Proctor PH, Tamborello LP (2007) SAINT-I worked, but the neuroprotectant is not NXY-059. Stroke 38:e109, author reply e110

Pulsinelli WA, Brierley JB (1979) A new model of bilateral hemispheric ischemia in the unanesthetized rat. Stroke 10:267–272

Pulsinelli WA, Brierley JB, Plum F (1982) Temporal profile of neuronal damage in a model of transient forebrain ischemia. Ann Neurol 11:491–498

Ralevic V, Burnstock G (1998) Receptors for purines and pyrimidines. Pharmacol Rev 50:413–492

Recker R, Adami A, Tone B, Tian HR, Lalas S, Hartman RE, Obenaus A, Ashwal S (2009) Rodent neonatal bilateral carotid artery occlusion with hypoxia mimics human hypoxic-ischemic injury. J Cereb Blood Flow Metab 29:1305–1316

Rennie JM, Hagmann CF, Robertson NJ (2007) Outcome after intrapartum hypoxic ischaemia at term. Semin Fetal Neonatal Med 12:398–407

Rosell A, Alvarez-Sabin J, Arenillas JF, Rovira A, Delgado P, Fernandez-Cadenas I, Penalba A, Molina CA, Montaner J (2005) A matrix metalloproteinase protein array reveals a strong relation between MMP-9 and MMP-13 with diffusion-weighted image lesion increase in human stroke. Stroke 36:1415–1420

Sairanen TR, Lindsberg PJ, Brenner M, Siren AL (1997) Global forebrain ischemia results in differential cellular expression of interleukin-1beta (IL-1beta) and its receptor at mRNA and protein level. J Cereb Blood Flow Metab 17:1107–1120

Saivin S, Houin G (1988) Clinical pharmacokinetics of doxycycline and minocycline. Clin Pharmacokinet 15:355–366

Salter MG, Fern R (2005) NMDA receptors are expressed in developing oligodendrocyte processes and mediate injury. Nature 438:1167–1171

Sanchez-Gomez MV, Matute C (1999) AMPA and kainate receptors each mediate excitotoxicity in oligodendroglial cultures. Neurobiol Dis 6:475–485

Sanchez-Gomez MV, Alberdi E, Ibarretxe G, Torre I, Matute C (2003) Caspase-dependent and caspase-independent oligodendrocyte death mediated by AMPA and kainate receptors. J Neurosci 23:9519–9528

Schwedenberg TH (1959) Leukoencephalopathy following carbon monoxide asphyxia. J Neuropathol Exp Neurol 18:597–608
Sekeljic V, Bataveljic D, Stamenkovic S, Ulamek M, Jablonski M, Radenovic L, Pluta R, Andjus PR (2012) Cellular markers of neuroinflammation and neurogenesis after ischemic brain injury in the long-term survival rat model. Brain Struct Funct 217:411–420
Sharma HS, Miclescu A, Wiklund L (2011) Cardiac arrest-induced regional blood–brain barrier breakdown, edema formation and brain pathology: a light and electron microscopic study on a new model for neurodegeneration and neuroprotection in porcine brain. J Neural Transm 118:87–114
Sheng H, Laskowitz DT, Pearlstein RD, Warner DS (1999) Characterization of a recovery global cerebral ischemia model in the mouse. J Neurosci Methods 88:103–109
Shimada N, Graf R, Rosner G, Heiss WD (1993) Ischemia-induced accumulation of extracellular amino acids in cerebral cortex, white matter, and cerebrospinal fluid. J Neurochem 60:66–71
Small DL, Buchan AM (2000) Animal models. Br Med Bull 56:307–317
Stevens B, Porta S, Haak LL, Gallo V, Fields RD (2002) Adenosine: a neuron-glial transmitter promoting myelination in the CNS in response to action potentials. Neuron 36:855–868
Stolp HB, Ek CJ, Johansson PA, Dziegielewska KM, Potter AM, Habgood MD, Saunders NR (2007) Effect of minocycline on inflammation-induced damage to the blood–brain barrier and white matter during development. Eur J Neurosci 26:3465–3474
Sugawa M, Sakurai Y, Ishikawa-Ieda Y, Suzuki H, Asou H (2002) Effects of erythropoietin on glial cell development; oligodendrocyte maturation and astrocyte proliferation. Neurosci Res 44:391–403
Takahashi JL, Giuliani F, Power C, Imai Y, Yong VW (2003) Interleukin-1beta promotes oligodendrocyte death through glutamate excitotoxicity. Ann Neurol 53:588–595
Talos DM, Follett PL, Folkerth RD, Fishman RE, Trachtenberg FL, Volpe JJ, Jensen FE (2006a) Developmental regulation of alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor subunit expression in forebrain and relationship to regional susceptibility to hypoxic/ischemic injury. II. Human cerebral white matter and cortex. J Comp Neurol 497:61–77
Talos DM, Fishman RE, Park H, Folkerth RD, Follett PL, Volpe JJ, Jensen FE (2006b) Developmental regulation of alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor subunit expression in forebrain and relationship to regional susceptibility to hypoxic/ischemic injury. I. Rodent cerebral white matter and cortex. J Comp Neurol 497:42–60
Thorburne SK, Juurlink BH (1996) Low glutathione and high iron govern the susceptibility of oligodendroglial precursors to oxidative stress. J Neurochem 67:1014–1022
Triulzi F, Parazzini C, Righini A (2006) Patterns of damage in the mature neonatal brain. Pediatr Radiol 36:608–620
van der Kooij MA, Groenendaal F, Kavelaars A, Heijnen CJ, van Bel F (2008) Neuroprotective properties and mechanisms of erythropoietin in in vitro and in vivo experimental models for hypoxia/ischemia. Brain Res Rev 59:22–33
von Euler M, Bendel O, Bueters T, Sandin J, von Euler G (2006) Profound but transient deficits in learning and memory after global ischemia using a novel water maze test. Behav Brain Res 166:204–210
Walker EJ, Rosenberg GA (2009) TIMP-3 and MMP-3 contribute to delayed inflammation and hippocampal neuronal death following global ischemia. Exp Neurol 216:122–131
Walker EJ, Rosenberg GA (2010) Divergent role for MMP-2 in myelin breakdown and oligodendrocyte death following transient global ischemia. J Neurosci Res 88:764–773
Wang X, Arcuino G, Takano T, Lin J, Peng WG, Wan P, Li P, Xu Q, Liu QS, Goldman SA, Nedergaard M (2004) P2X7 receptor inhibition improves recovery after spinal cord injury. Nat Med 10:821–827
Wang Q, Tang XN, Yenari MA (2007) The inflammatory response in stroke. J Neuroimmunol 184:53–68
Wang J, Jin H, Hua Y, Keep RF, Xi G (2012) Role of protease-activated receptor-1 in brain injury after experimental global cerebral ischemia. Stroke 43:2476–2482
Wellons JC 3rd, Sheng H, Laskowitz DT, Burkhard Mackensen G, Pearlstein RD, Warner DS (2000) A comparison of strain-related susceptibility in two murine recovery models of global cerebral ischemia. Brain Res 868:14–21

Wojcik-Stanaszek L, Sypecka J, Szymczak P, Ziemka-Nalecz M, Khrestchatisky M, Rivera S, Zalewska T (2011) The potential role of metalloproteinases in neurogenesis in the gerbil hippocampus following global forebrain ischemia. PloS one 6:e22465

Wu CY, Hsieh HL, Jou MJ, Yang CM (2004) Involvement of p42/p44 MAPK, p38 MAPK, JNK and nuclear factor-kappa B in interleukin-1beta-induced matrix metalloproteinase-9 expression in rat brain astrocytes. J Neurochem 90:1477–1488

Wu O, Sorensen AG, Benner T, Singhal AB, Furie KL, Greer DM (2009) Comatose patients with cardiac arrest: predicting clinical outcome with diffusion-weighted MR imaging. Radiology 252:173–181

Yamashima T, Tonchev AB, Tsukada T, Saido TC, Imajoh-Ohmi S, Momoi T, Kominami E (2003) Sustained calpain activation associated with lysosomal rupture executes necrosis of the post-ischemic CA1 neurons in primates. Hippocampus 13:791–800

Yonekura I, Kawahara N, Nakatomi H, Furuya K, Kirino T (2004) A model of global cerebral ischemia in C57 BL/6 mice. J Cereb Blood Flow Metab 24:151–158

Yoshioka H, Niizuma K, Katsu M, Sakata H, Okami N, Chan PH (2011) Consistent injury to medium spiny neurons and white matter in the mouse striatum after prolonged transient global cerebral ischemia. J Neurotrauma 28:649–660

Yrjanheikki J, Keinanen R, Pellikka M, Hokfelt T, Koistinaho J (1998) Tetracyclines inhibit microglial activation and are neuroprotective in global brain ischemia. Proc Natl Acad Sci U S A 95:15769–15774

Yrjanheikki J, Tikka T, Keinanen R, Goldsteins G, Chan PH, Koistinaho J (1999) A tetracycline derivative, minocycline, reduces inflammation and protects against focal cerebral ischemia with a wide therapeutic window. Proc Natl Acad Sci U S A 96:13496–13500

Zhang K, Sejnowski TJ (2000) A universal scaling law between gray matter and white matter of cerebral cortex. Proc Natl Acad Sci U S A 97:5621–5626

Zhang W, Wang B, Zhou S, Qiu Y (2012) The effect of ischemic post-conditioning on hippocampal cell apoptosis following global brain ischemia in rats. J Clin Neurosci 19:570–573

Zhao H (2009) Ischemic postconditioning as a novel avenue to protect against brain injury after stroke. J Cereb Blood Flow Metab 29:873–885

Zhao BQ, Wang S, Kim HY, Storrie H, Rosen BR, Mooney DJ, Wang X, Lo EH (2006) Role of matrix metalloproteinases in delayed cortical responses after stroke. Nat Med 12:441–445

Zola-Morgan S, Squire LR, Rempel NL, Clower RP, Amaral DG (1992) Enduring memory impairment in monkeys after ischemic damage to the hippocampus. J Neurosci 12:2582–2596

Chapter 11
White Matter Injury After Experimental Intracerebral Hemorrhage

Kenneth R. Wagner

Abbreviations

APP	Amyloid precursor protein
BBB	Blood–brain barrier
CNS	Central nervous system
GFAP	Glial fibrillary acidic protein
H&E	Hematoxylin and eosin
HO-1	Heme oxygenase-1
ICAM-1	Intracellular adhesion molecule-1
IL-1	Interleukin-1
ICH	Intracerebral hemorrhage
LFB	Luxol fast blue
MAPK	Mitogen-activated protein kinases
MCP-1	Monocyte chemoattractant protein
MRI	Magnetic resonance imaging
MMPs	Matrix metalloproteinases
NF-kappaB	Nuclear factor-kappaB
NOS	Nitric oxide synthase
Nrf2	Nuclear factor (erythroid-derived 2)-like factor 2
PPARgamma	Peroxisome proliferator-activated receptor-gamma
ROS	Reactive oxygen species
PARs	Proteinase-activated receptors
STICH	Surgical trial in intracerebral hemorrhage

K.R. Wagner, Ph.D. (✉)
Research (151), Department of Veterans Affairs Medical Center, 3200 Vine Street, Cincinnati, OH 45220, USA

Department Neurology, University of Cincinnati College of Medicine, Cincinnati, OH, USA
e-mail: wagnerkr@uc.edu

S. Baltan et al. (eds.), *White Matter Injury in Stroke and CNS Disease*, Springer Series in Translational Stroke Research 4, DOI 10.1007/978-1-4614-9123-1_11,

TBI	Traumatic brain injury
TNF-alpha	Tumor necrosis factor-alpha
TUNEL	Terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine (dUTP)-biotin nick end labeling

11.1 Introduction

Of the three stroke subtypes, spontaneous intracerebral hemorrhage (ICH) has the highest death rate and the poorest prognosis in survivors (Foulkes et al. 1988). Indeed, half of ICH patients die and only 10–20 % return to normal activities of daily living (Broderick et al. 1993; Lisk et al. 1994). Although the incidence of spontaneous ICH is estimated at ~10–15 % of all strokes, approximately 2 million patients are affected yearly worldwide (Qureshi et al. 2009). Besides spontaneous ICH, intracerebral bleeds also occur following treatment with thrombolytic agents for ischemic stroke and myocardial infarction (Shoamanesh et al. 2013; Berger 2003). At present, there are no approved pharmacologic or generally accepted surgical treatments.

Hemorrhages into the cerebral white matter (lobar) occur in approximately one-third of all ICH patients (Lunardi 2012). This number is comparable to the frequency of basal ganglia bleeds (Kase and Caplan 1994) and is the most frequent site of hemorrhage in the young (Toffol et al. 1987). Clinically, neurological deterioration due to edema occurs twice as often in patients with lobar bleeds (Mayer et al. 1994). It is also noteworthy that recent clinical studies are demonstrating that chronic white matter abnormalities (e.g., leukoaraiosis) significantly increase the risk of both familial and sporadic ICH. In this regard, Longstreth and colleagues (Folsom et al. 2012) have reported that greater MRI-defined burden of leukoaraiosis is a risk factor for spontaneous ICH suggesting a pathogenetic continuum between the development of these disorders.

White matter damage, which has been well described following traumatic brain and spinal cord injuries and ischemic stroke, is a key predictor of morbidity (Medana and Esiri 2003). Similarly, both primary damage to white matter by the bleed/hematoma and the secondary injury events that follow can lead to Wallerian degeneration that contributes to long-term morbidity in ICH patients (Fukui et al. 1994; Kazui et al. 1994). Pyramidal tract degeneration observed by computed tomography develops very early (Kazui et al. 1994), and the extent of degeneration on magnetic resonance (MR) imaging at 3 months or later after ICH correlates with the 1-year Barthel Index score (Fukui et al. 1994). Recently, MR-diffusion tensor imaging (DTI) with its ability to visualize white matter tracts in three dimensions has been used to predict motor outcome in ICH patients (Koyama et al. 2011; Kuzu et al. 2012). It is expected that DTI will likely become an important imaging tool in the future for white matter prognosis following ICH.

This chapter reviews experimental studies of white matter injury from ICH in animal models. Presently, there is a dearth of reports that specifically focus on

experimental white matter injury. As in gray matter injury following experimental ICH (recently reviewed in Aronowski and Zhao 2011; Leonardo et al. 2012; Keep et al. 2012), it is likely that some similar mechanisms including physical damage due to hematoma formation and expansion, the impact of rapidly occurring excitotoxicity, and secondary, delayed injury due to edema development and inflammatory events also participate in white matter injury. These latter secondary processes may extend and prolong the injury and impair recovery and thereby contribute to poor outcomes. As described in this chapter, these white matter responses to ICH are only beginning to be examined in animal models.

11.2 ICH Animal Models

A comprehensive review of experimental ICH models was published by Kaufman and Schochet in 1992 (Kaufman and Schochet 1992). Our group has also published several reviews that have updated the subject (Andaluz et al. 2002; Wagner and Brott 2007; Wagner and Zuccarello 2009). In the past few years critical reviews have also been published by ourselves (Adeoye et al. 2011) and others (Leonardo et al. 2012; James et al. 2008; Kirkman et al. 2011; MacLellan et al. 2010, 2012) that discuss the benefits and pitfalls of experimental animal ICH models.

To study ICH experimentally, the standard model has been to infuse autologous blood directly into gray matter brain regions, most frequently the striatum. A variety of species have been employed including rat, rabbit, cat, dog, primate, and more recently mouse (reviewed in Kaufman and Schochet 1992; Andaluz et al. 2002). Recently, new rat models of cerebellar hemorrhage (Lekic et al. 2011) and pontine hemorrhage (Lekic et al. 2013) have also been introduced. Other models have targeted the motor cortex (Belayev et al. 2005; Xue and Del Bigio 2000) and the hippocampus (Song et al. 2007, 2008).

A second model, the collagenase ICH model, is an experimental model that has had considerable use since its development by Rosenberg and colleagues (Rosenberg et al. 1990, 1993). In this model an injection of bacterial collagenase into the striatum causes local dissolution of the extracellular matrix. Over several hours, blood vessel rupture occurs resulting in an intracerebral bleed. This model has almost exclusively been used in rodents.

In contrast to the many studies in gray matter ICH models, detailed examinations of white matter injury in ICH models are relatively limited. In 2005, the NINDS workshop committee on ICH urged that white matter injury receive increased attention in experimental studies (Participants 2005). This need was again emphasized by MacLellan and colleagues in their recent review (MacLellan et al. 2012). They indicated that only about 6 % of experimental ICH studies have quantified white matter injury (e.g., corpus callosum area/volume). Furthermore, they also questioned whether the current ICH models in rodents "adequately reflect human pathology" because of the paucity of white matter in this species (MacLellan et al. 2012). In this regard, it is noteworthy that while white matter comprises approximately

one-half of the forebrain composition in humans in rodents this volume is only about 2 % (Zhang and Sejnowski 2000). Thus, since rodents are the most commonly used species for ICH research, the majority of ICH studies have focused on neuronal injury and neuronal protection.

A few descriptions of white matter involvement in the collagenase ICH model have been reported. Masuda et al. (2007) described a model of stereotaxic injection of collagenase into the cortex of the mouse that produced damage to the cortex and also to the subcortical white matter and hippocampus. Rosenberg and colleagues (Brown et al. 1995) infused collagenase into the caudate/putamen in rats and described the presence of edema at 2 h in posterior brain regions along white matter tracts. Widespread white matter edema has been described for 1 week on MRI after collagenase-induced ICH in rats (Del Bigio et al. 1996). The contribution of collagenase to this edema is noteworthy since blood–brain barrier (BBB) opening is prolonged (7 days) in this model (Rosenberg et al. 1993). In this regard, collagenase-induced ICH in the striatum caused a greater reduction in the corpus callosum volume at 6 weeks versus blood infusion (MacLellan et al. 2008).

Recently, white matter injury in the rat ICH model (both blood infusion and collagenase) has received increased attention. A host of reports from the Schlichter lab at the University of Toronto have elegantly provided considerable new data on the pathogenesis of post-ICH white matter damage (Wasserman and Schlichter 2007a, b, 2008; Wasserman et al. 2007; Wasserman and Schlichter 2008; Lively and Schlichter 2012a, b; Moxon-Emre and Schlichter 2011). In addition, the early development of white matter injury in the internal capsule and thalamus has been described in a rat ICH model (Masuda et al. 2007). White matter injury, and iron chelator treatment, was reported in both the collagenase and blood infusion models (Wu et al. 2012).

White matter involvement in the porcine brain was described by Mun-Bryce and colleagues who investigated somatic evoked potentials and MMP-9 levels after inducing ICH with collagenase plus heparin injection into the primary somatosensory (SI) cortex (Mun-Bryce et al. 2004a, b; Mun-Bryce et al. 2001). In these reports, the injection produces hemorrhage primarily in the white matter of the SI cortex (Mun-Bryce et al. 2001). By 24 h an increase in MMP-9 expression was observed in white matter of the ipsilesional SI and secondary somatosensory cortex (SII) and in the contralesional SI gray matter as well.

11.3 Porcine Lobar Blood Infusion ICH Model

To study white matter injury, our laboratory developed a porcine lobar ICH model in which we infuse up to 3.0 cc of arterial blood into the frontal hemispheric white matter yielding reproducible hematoma volumes (Wagner et al. 1996). This model has clinical relevance and comparable edema development and neuropathologic outcomes as human ICH (Wagner and Brott 2007; Wagner and Zuccarello 2009; Wagner and Broderick 2001). Pigs have well-developed white matter that enables

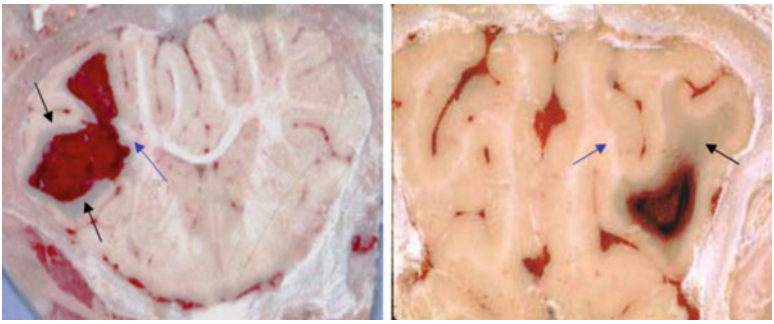


Fig. 11.1 Representative coronal sections through pig brains frozen in situ at 1 h (*left*: caudate level) and 3 h (*right*: frontal pole) following blood infusion into the frontal white matter. These sections show prominent early edema seen as a visible ("translucent") physical change in white matter adjacent to the hematoma (*black arrows*). This zone has a marked increase (>12 %) in water contents (Fig. 11.3, below). White matter regions with smaller increases (~7 %) in water content are indicated by the *blue arrows* (Wagner et al. 1996)

significant clot volumes to be induced for biochemical analyses as well as for preclinical testing of surgical therapies with and without new pharmacologic treatments (Wagner et al. 1999a). We describe in further detail below our findings on ICH-induced white matter pathophysiology, pathochemistry, and surgical clot evacuation following ICH.

11.3.1 Porcine Model: Edema Development

In general, edema development and brain pathologic responses to ICH in the pig model are comparable to other experimental models and to human ICH (Masuda et al. 2007; Enzmann et al. 1981; Jenkins et al. 1989; Weller 1992). In our initial report on this ICH model in 1996, we described the rapid development (already at 1 h) of edema in the perihematomal white matter (Wagner et al. 1996). In our studies, we employ in situ freezing of the brain to fix the tissue. After cutting 5 mm thick coronal sections through the frozen head using a band saw, topographical sampling of the tissue can be then carried out for biochemical/molecular studies. In these thick frozen sections, we noted that perihematomal edema development was visible as a physical alteration in the white matter that we called "translucency" (Fig. 11.1) (Wagner et al. 1996). T2-weighted MR images at 2 h post ICH demonstrated the rapid development of perihematomal hyperintensity (Fig. 11.2) (Wagner et al. 1997). Water content determinations showed that these perihematomal regions were markedly edematous with a greater than 10 % increase in water content (>85 %) compared with the contralateral white matter (73 %) (Fig. 11.3). Measurements of the perihematomal edema demonstrated increases in volume (by 50 %) over the first 24 h (Fig. 11.3). This increase in edema volume is likely due to delayed BBB

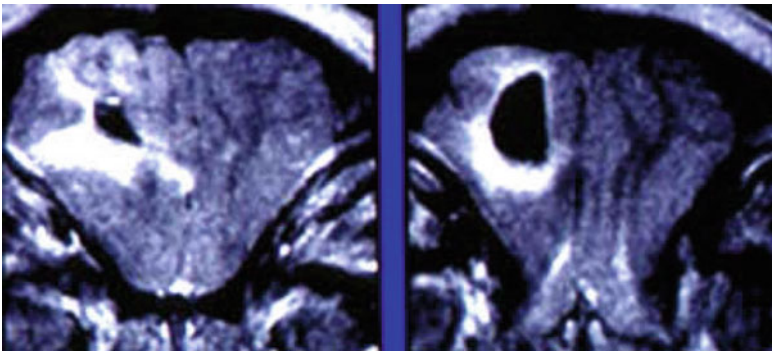


Fig. 11.2 This figure presents early (2 h) high-resolution T2-weighted images from 3.0 Tesla Bruker MR scans. The clot appears hypointense with hyperintensity indicative of early perihematomal edema development surrounding the hematoma

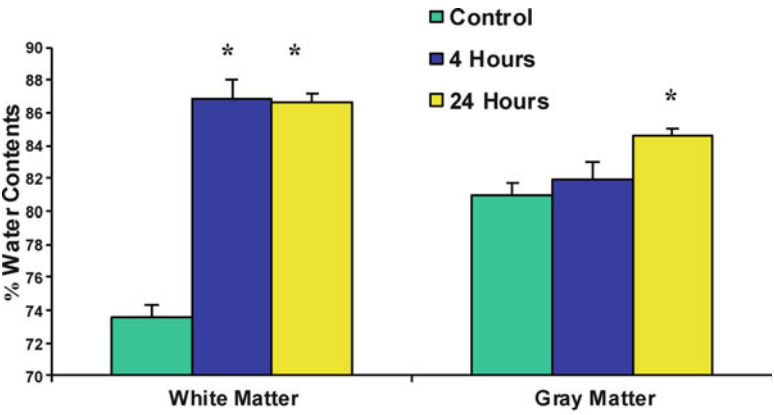


Fig. 11.3 Water contents of edematous white matter adjacent to and gyral gray matter overlying the clot at 4 and 24 h. Perihematomal "translucent" white matter rapidly developed >10 % increases in water contents that remained constant during 24 h post ICH. From 4 to 24 h, gyral gray matter showed substantial (4 %) increases in edema. Values are means ± SD ($N = 5–6$); *$p < 0.05$ vs. control

opening as evidenced by Evans blue staining (Fig. 11.4). Others have reported hyperintensities on T2-weighted imaging surrounding the hematoma and extending along white matter fiber tracts to posterior brain regions (Brown et al. 1995).

An important finding from these early studies was that within the early hours following ICH, perihematomal edematous white matter was strongly immunoreactive for serum proteins (e.g., fibrinogen, Fig. 11.5). Importantly, our finding that intravascularly delivered Evans blue dye failed to penetrate into the brain tissue during the first 8 h post ICH suggested that this serum protein accumulation and edema development were not due to increased BBB permeability (Wagner et al. 1996). Rather this edema was due to extracellular serum protein accumulation following

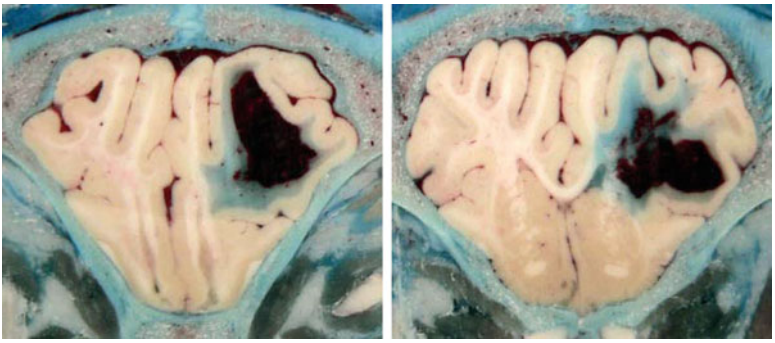


Fig. 11.4 Coronal sections at two levels from an in situ frozen brain at 24 h following ICH. These sections demonstrate the hematoma and perihematomal edema in the frontal white matter in the pig ICH model. The hematoma and edema volumes in this animal were 1.48 and 2.48 cc, respectively. Notable features include midline shift, BBB opening as evidenced by Evans blue containing edema in the ipsilateral white matter, and movement of Evans blue containing edema fluid into the lateral ventricle

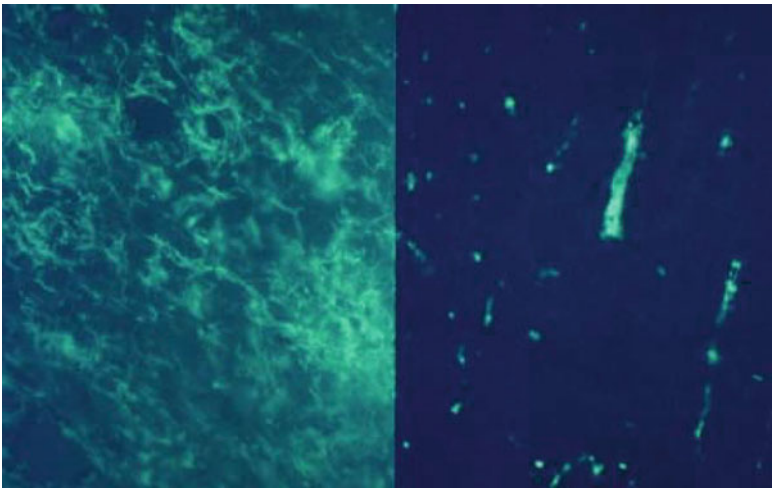


Fig. 11.5 Immunofluorescence for fibrin(ogen) in perihematomal white matter. *Left*: Plasma and clot-derived serum proteins accumulate in perihematomal white matter as evidenced by immunofluorescence for fibrin(ogen). *Right*: In contralateral white matter fluorescence is present only in blood vessels in this in situ frozen brain. The polyclonal antibody recognizes both fibrinogen and fibrin (Wagner et al. 1996)

clot retraction and the "squeezing out" of serum proteins. A similar conclusion that perihematomal edema development was plasma derived was suggested by imaging studies of human ICH (Butcher et al. 2004). In this regard, it is noteworthy that the inciting mechanisms underlying white matter edema development following ICH may be significantly different than those underlying gray matter damage. Because of

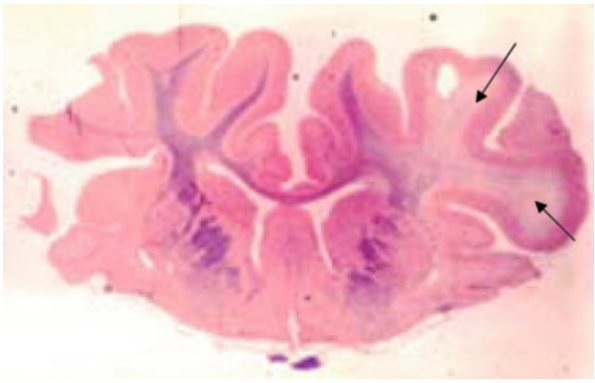


Fig. 11.6 Coronal section showing marked edema (*black arrows*) in white matter ipsilateral to the clot (lost during processing) leading to diffuse myelin pallor (H&E/Luxol Fast Blue stain)

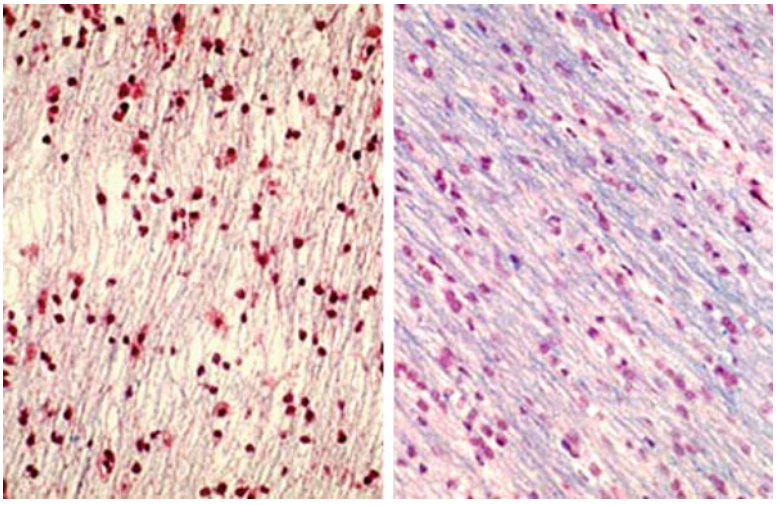


Fig. 11.7 Photomicrograph of Fig. 11.6 showing edema-induced increases in interstitial spaces, reactive glia with "pink" cytoplasm, and loss of myelin stain. *Right*: Control white matter

the "loose" structure of white as compared to gray matter, plasma proteins from the clot can diffuse in the extracellular "space" between axons and contribute to perihematomal edema development.

In the pig ICH model, hematoma volumes at 3 days were essentially unchanged from the volumes measured in the first 8 h. Thus, several days are required to activate the blood removal processes (Wagner et al. 2003a; Wagner and Dwyer 2004). Histologically, marked edema continues to be present in white matter surrounding and distant from the hematomas at 3 days (Figs. 11.6 and 11.7). The largest volume of the hematoma is located anteriorly, with edema development spreading posteriorly in the extracellular space along the white matter fiber tracts. Since

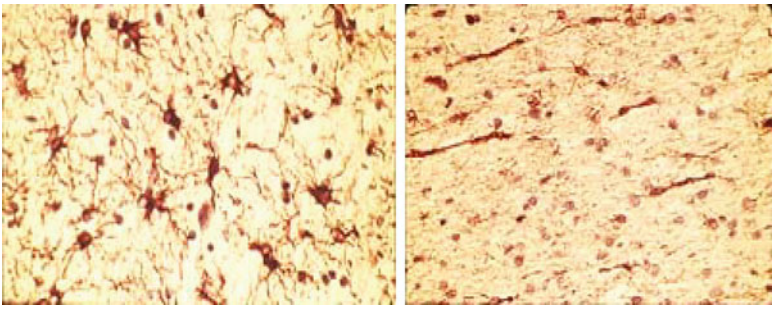


Fig. 11.8 *Left*: Markedly increased GFAP staining and enlarged astrocytic processes indicative of reactive astrocytosis in plasma protein containing edematous white matter at 3 days following an ICH. *Right*: Background GFAP staining in contralateral control white matter

white matter is more vulnerable to vasogenic edema than is gray matter (Kimelberg 1995), this model, with its significant amount of white matter, is especially useful for studying the development of white matter edema and injury. By 3 days, our model's edematous white matter shows decreased Luxol fast blue staining suggestive of myelin injury (Figs. 11.6 and 11.7). Reactive astrocytosis is also present as demonstrated by markedly increased GFAP immunoreactivity in thickened processes (Fig. 11.8). At 7 days, hematoma volumes remain little changed and neovascularization is present. At 2 weeks, hematoma resolution with glial scar and cyst formation are comparable to literature descriptions in animal models and in human ICH (Garcia et al. 1994; Jenkins et al. 1990).

11.3.2 White Matter Axonal Damage (Amyloid Precursor Protein Accumulation) Following ICH in the Porcine Model

In our porcine ICH model, we noted amyloid precursor protein (APP)-positive immunostaining already at 2 h following blood infusion into the frontal white matter (Fig. 11.9). APP, a transmembrane glycoprotein with unknown physiologic function (O'Brien and Wong 2011), is constitutively synthesized by neurons and transported by fast anterograde axonal flow to the synapse (Koo et al. 1990; Selkoe 1994). It is normally undetectable since this axonal transport maintains low local concentrations. However, axonal injury following head trauma or cerebral ischemia that results in impaired transport leads to immunostainable APP accumulation in axonal retraction bulbs within the first few hours (Gentleman et al. 1993; Otsuka et al. 1991; Sherriff et al. 1994; Stephenson et al. 1992). Thus, in our model, blood infusion into the white matter over 15 min to generate a hematoma causes displacement and stretching of axons resulting in impaired axonal transport and APP accumulation.

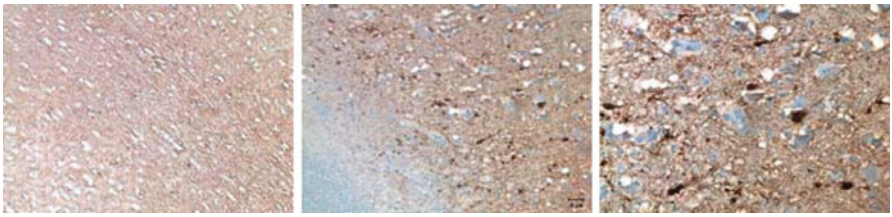


Fig. 11.9 APP immunostaining: Bain sections are from control (*left*) and from perihematomal white matter at 2 h after ICH (*middle*, *right*). *Left*: Only background staining is present in control white matter. *Middle*: In white matter adjacent to the clot (lower *left* of photo), immunostaining for APP accumulation is present that is indicative of axonal injury. *Right*: Higher magnification showing APP immunostaining in expanded axonal processes

Interestingly, our results suggest that this APP accumulation, which is indicative of axonal injury, is present earlier in the blood infusion as compared to the collagenase model (Wasserman and Schlichter 2007a). Presumably, this result is due to the considerably slower hematoma formation that occurs in this model since bleeding occurs over several hours, e.g., up to 6 h (Wasserman and Schlichter 2007a).

11.3.3 White Matter Axonal Damage (APP Accumulation) and Demyelination Following ICH in the Rat Collagenase Model

Wasserman and Schlichter (Wasserman and Schlichter 2007a) in their study of white matter injury in young and aged rats reported a detailed histologic and immunocytochemical study of the acute and chronic phases of perihematomal axonal damage and demyelination after ICH. These workers employed staining for APP and degraded myelin basic protein (dMBP) between 6 h and 1 and 3 days post ICH to monitor damaged axons and damaged myelin, respectively.

In 1 day APP-positive white matter fiber tracts were present a few hundred microns from the hematoma. By 3 days this positive APP immunostaining was present at further distances. Interestingly, they noted that axonal damage occurred without demyelination at the hematoma edge suggesting that axonal injury can be a primary event. Other interesting observations from their studies were that axonal injury began before the strong microglial/macrophage response that has been commonly described following ICH. Furthermore, aged rats had worse axonal damage versus younger animals despite similar degrees of gray matter injury. The authors concluded that this difference in axonal injury may explain the poorer functional recovery of older animals after ICH.

11.4 Intracerebral Hemorrhage Pathophysiology in White Matter

11.4.1 Perihematomal Hypoxia/Ischemia: Human Studies

Whether hypoxia or ischemia develops around intracerebral hematomas has been controversial. In human ICH, Powers and colleagues (Powers et al. 2001) concluded that reduced perihematomal blood flow (5–22 h post ictus) was coupled to reduced oxygen consumption. Similarly, attempts to describe an early perihemorrhagic penumbra that would link reduced perfusion and edema development also do not support the theory of focal ischemia (Herweh et al. 2007, 2010). However, the complexity of this issue in the human population was recently addressed (Prabhakaran and Naidech 2012). The authors noted that there is a vulnerable population in ICH patients who are considered to be in a "stroke-prone" state. In these patients with atherosclerotic risk factors and/or cerebral amyloid angiopathy (CAA), perfusion around the hematoma is not uniform. Ongoing studies of blood pressure control to reduce hematoma expansion in such patients with preexisting cerebral microangiopathy suggest that their thresholds for brain ischemia may be lower (Prabhakaran and Naidech 2012).

11.4.2 Perihematomal Hypoxia/Ischemia: Animal Studies

In rat blood infusion models, moderate ischemia reportedly develops in the early minutes and remains through the first few hours post ICH (Nath et al. 1986, 1987; Yang et al. 1994). In contrast, ischemia was not present in a dog model ICH (Qureshi et al. 1999). In our pig ICH model, perihematomal white matter high-energy phosphate concentrations were reduced only by 25 % at 1 h suggesting a possible early reduction in perihematomal perfusion but not to levels that would be considered damaging (Wagner et al. 1998). A return of blood flow to control levels is supported by our bolus-contrast perfusion MRI findings that demonstrated no reduction at 2 h post infusion (Wagner and Broderick 2001; Wagner et al. 1997). In general, ICH does not appear to produce severe ischemia, and in fact hyperemia has been observed in the rodent ICH model (Nath et al. 1987) and in white matter in our porcine model (Wagner and Broderick 2001; Wagner et al. 1997).

11.4.3 White Matter Lactate Accumulation After ICH: Glutamate and Hypermetabolism

We have measured lactate levels in edematous perihematomal white matter regions in our porcine ICH model (Wagner et al. 1998). Within 1 h after induction of ICH, lactate levels were markedly increased (by tenfold). We concluded that lactate accumulation in edematous white matter was not caused by enhanced anaerobic

glycolysis because ATP and phosphocreatine levels demonstrated that an energy deficit was not present. Interestingly, we speculated that since glutamate in the blood is severalfold higher than normal levels in the brain's extracellular space, ionotropic glutamate receptors in white matter (described below) are stimulated and aerobic glycolysis is enhanced leading to lactate accumulation. Support for this hypothesis is seen in the microdialysis findings by Qureshi et al. (2003) who reported that glutamate levels were elevated by fourfold ipsilateral to the hematoma already at 30 min after ICH and remained elevated through 5 h.

These early metabolic events in white matter are supported by findings from Sharp and colleagues (Ardizzone et al. 2004) who demonstrated an acute phase of increased perihematomal [14C]-2-deoxyglucose uptake that peaked at 3 h following hematoma induction in a rat blood infusion model. This increased glucose utilization was blocked by pretreatment with glutamate receptor antagonists. These data suggest a linkage between blood glutamate, its receptor activation, and rapid stimulation of glucose metabolism in perihematomal tissue after ICH that underlies increased lactate production in the ipsilateral white matter (Wagner et al. 1998). Interestingly, in a clinical study, neurochemical monitoring of the CSF in 75 ICH patients demonstrated a relationship between the levels of excitatory amino acids, lactate, and neurologic outcomes (Chiang et al. 2006).

11.4.4 White Matter Glutamate Receptors and ICH-Induced Injury

Increasing evidence suggests that excess glutamate released during various brain insults may contribute to white matter injury (Ransom and Baltan 2009; Matute and Ransom 2012). Glutamate receptors were initially detected on myelinating oligodendrocytes. Blocking these receptors was protective in traumatic brain injury (TBI) and ischemic stroke (Ransom and Baltan 2009). Stys and colleagues furthered this concept when they discovered that axons themselves expressed glutamate receptors (Ransom and Baltan 2009). Thus, acutely elevated levels of glutamate following ICH may participate in white matter injury through excitotoxic mechanisms that are similar to those that are well described in gray matter (Stys and Lipton 2007; Lipton 2006).

11.5 The Role of Plasma Proteins in White Matter Injury Following ICH

11.5.1 Clot Formation and Coagulation Cascade Activation

Bleeding into the brain parenchyma in ICH, or similarly after brain or spinal cord trauma and in the absence of anticoagulant medication, leads to rapid activation of the coagulation cascade. This blood clotting process importantly stops the

bleeding, thereby reducing the potential size of the hematoma and preventing additional tissue injury or even death. However, as a consequence of blood vessel rupture, plasma proteins enter the brain parenchyma along with the red blood cells. This accumulation of plasma proteins in white matter is not innocuous. Plasma proteins are edemogenic due to osmotic or specific toxic effects (Xi et al. 2002; Lee et al. 1996). In addition, as described below, parenchymal fibrinogen/fibrin accumulation following ICH may also be an important contributor to this edema development.

Experimental findings, by ourselves and others, with infusions of plasma, heparinized blood, and specific plasma proteins, e.g., thrombin and plasmin, demonstrate that clot formation and coagulation cascade activation are important pathophysiologic events in the development of perihematomal edema (Xi et al. 1998, 2002; Wagner et al. 2003b, 2005; Xue and Del Bigio 2001). Considerable evidence both in vitro and in vivo suggests an important role for thrombin in brain injury following ICH (recently reviewed in Katsuki 2010). Thrombin's action to induce brain tissue injury involves proteinase-activated receptors (PARs) on various cells including microglia and macrophages and activation of various mitogen-activated protein kinases (MAPKs) (Katsuki 2010). Activated MAPKs can regulate pro-inflammatory cytokine and nitric oxide (NO) production which can underlie perihematomal brain injury following ICH (outlined in Katsuki 2010). In addition, work from Sharp and colleagues and their findings with an Src family kinase inhibitor have implicated Src kinase signaling in multiple mechanisms of thrombin-induced injury after ICH including acute BBB injury as well as chronic BBB repair (Liu et al. 2010; Liu and Sharp 2011).

Besides these coagulation cascade proteins, various non-coagulation-related proteins also enter the brain parenchyma with the bleed including albumin and immunoglobulins. Accumulations of these proteins in the extracellular space are also believed to be significant contributors to ICH pathophysiology. In this regard, imaging studies in human ICH suggest that perihematomal edema development is plasma derived (Butcher et al. 2004).

Specifically, we have reported that infusions of plasma into the frontal white matter induce marked edema, while similar masses of packed red blood cells do not (Fig. 11.10). This edema increases by 1.5-fold between 4 and 24 h with plasma being as edemogenic as whole blood. These results suggest that perihematomal edema development is not driven by the mass effect of the infusate but rather by its composition, i.e., plasma versus red cells (Sinar et al. 1987). Furthermore, we have also observed Evans blue penetration into the edematous area of plasma protein infusions suggesting that a reactive substance(s) in plasma is (are) able to induce BBB opening (Fig. 11.10). Interestingly, based on their recent finding that neutrophil depletion decreased IgG extravasation and white matter injury, Schlichter and colleagues (Moxon-Emre and Schlichter 2011) have suggested that "proteins involved in blood clotting and lysis appear to be harmful to the brain evoking leukocyte infiltration, microglial activation, and cell apoptosis."

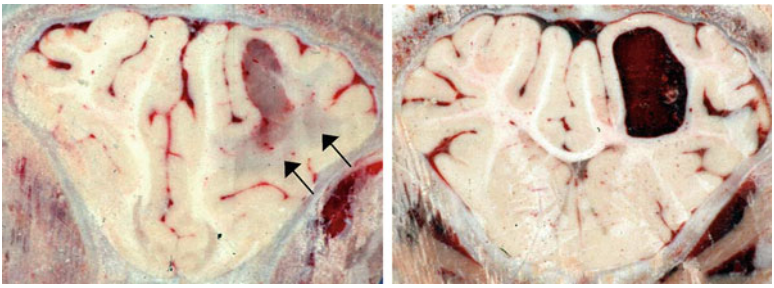


Fig. 11.10 *Left*: Representative coronal section from a brain frozen in situ at 24 h following plasma infusion into frontal cerebral white matter. This frozen brain slice demonstrates a plasma mass and areas of markedly edematous white matter adjacent to the mass (*arrows*). *Arrows* also indicate the edematous white matter sampled for the biochemical studies below. *Right*: Coronal section from a brain frozen in situ at 24 h following a comparable infusion of red blood cells that failed to induce edema. These results demonstrate that plasma has a "toxicity" that is more important than mass effect in acute edema development (modified figure from Wagner et al. 2002)

11.5.2 Fibrinogen/Fibrin

We have observed rapid fibrinogen/fibrin accumulation in perihematomal white matter after ICH (Fig. 11.5) (Wagner et al. 1996). Considerable work during the past decade from the Akassoglou laboratory has demonstrated that plasma proteins, and in particular fibrinogen, are not just markers of increased BBB permeability or vascular damage (Davalos and Akassoglou 2012). Accumulating evidence suggests that fibrinogen may play a causative role in inducing inflammation and inhibiting neurite outgrowth following TBI, spinal cord injury, and hemorrhagic stroke as well as various neurologic diseases including Alzheimer's disease and multiple sclerosis (Ryu et al. 2009). Indeed, various reports by this group have shown that vascular damage or BBB disruption resulting in thrombin's conversion of soluble fibrinogen to insoluble (Zhang and Sejnowski 2000) fibrin and fibrin deposition plays a "causative" role in CNS injury as one of the earliest pathology-inducing events (Adams et al. 2004, 2007). In this regard, they have suggested that fibrinogen is a primary regulator of inflammation events (Adams et al. 2004, 2007), remyelination (Akassoglou et al. 2002; Akassoglou and Strickland 2002), neurodegeneration (Schachtrup et al. 2010), and astrocyte activation (Schachtrup et al. 2010). These results are supported by the findings that mice genetically or pharmacologically depleted of fibrinogen show a dramatic reduction in astrocytosis and neurocan deposition after injury (Schachtrup et al. 2010). These results identify fibrinogen-bound latent TGF-beta as the molecular inducer of the inhibitory properties of the gliotic scar after vascular damage (Schachtrup et al. 2010).

11.6 The Inflammatory Response in White Matter Following ICH

The inflammatory response following ICH is believed to contribute significantly to secondary brain injury following ICH. Several excellent reviews of this response have been published (Aronowski and Zhao 2011; Aronowski and Hall 2005; Wang 2010; Wang and Dore 2007). Inflammatory processes following ICH have been mostly studied in gray matter in rodent models. We have published several reports that describe various early inflammatory events in white matter in our porcine ICH model (Wagner and Broderick 2001; Wagner et al. 1999b, c, 2001, 2003b, 2004, 2005; Wagner 2007). The Schlichter group has recently examined the inflammatory response in white matter in detail in the rat collagenase ICH model (Lively and Schlichter 2012a, b; Moxon-Emre and Schlichter 2011).

It is possible that some of the early inflammatory responses to ICH are similar in gray and white matter. Whether the primary site of the bleed is in gray or white matter, white matter edema will most certainly develop (Del Bigio et al. 1996). Indeed, perihematomal edema involving white matter is well described in human ICH patients (Butcher et al. 2004; Gebel et al. 2000). An important component of the early inflammatory response is the removal of damaged molecules from the brain parenchyma that contribute to edema development. CSF flow through white matter tracts to the ventricles is believed to be an important route in this process. In general, in both human ICH and in animal models, activation of resident brain microglia and infiltration into the brain first by neutrophils and then by macrophages appears to be the order of events after ICH (Del Bigio et al. 1996; Wasserman and Schlichter 2007a; Gong et al. 2000). This inflammatory response can be prolonged (Del Bigio et al. 1996; Wasserman et al. 2007; Wang and Dore 2007; Gong et al. 2000) and, in particular, as seen in the activation of resident microglia (Del Bigio et al. 1996; Wasserman et al. 2007; Wang and Dore 2007; Gong et al. 2000).

The inflammatory response following ICH, especially initially, is suggested to be damaging rather than beneficial. As an early component of the inflammatory response after ICH, circulating neutrophils rapidly enter the brain (Moxon-Emre and Schlichter 2011; Wang 2010; Wang and Dore 2007). In a recent study, the Schlichter lab demonstrated the important contribution of neutrophils to injury after ICH. They showed that decreasing the neutrophil number by more than 60 % with an anti-polymorphonuclear leukocyte antibody reduced BBB breakdown, axon injury, and inflammation after ICH (Moxon-Emre and Schlichter 2011). These authors described that the white matter showed dramatic sparing in the neutrophil-depleted animals. Specifically, the white matter tracts were more structurally intact with less bundle infiltration by microglia/macrophages and reduced myelin fragmentation. They concluded that neutrophil depletion reduced MMP-9 activity which contributed to BBB protection, reduced axon damage, astrocytic activation, and microglial/macrophage infiltration.

11.6.1 White Matter Inflammation: Biochemical and Molecular Studies

11.6.1.1 Intracerebral Hemorrhage Pathochemistry: Oxidative Stress

Considerable evidence implicates oxidative stress in secondary pathochemical events following ischemic stroke and TBI (Chan 2001). Although relatively unstudied following ICH, reactive oxygen species (ROS) also likely play an important but complex role in white and gray matter injury. Various molecules including proteins, lipids, and DNA are important targets for ROS that can be generated by metal-catalyzed reactions (potentially pro-oxidant iron in ICH). During the early hours after ICH, this pro-oxidant iron may not be that contained in the red cells themselves but, as recently reported, may be from the interaction of the plasma proteins holo-transferrin and thrombin (Nakamura et al. 2005). For proteins, metal-catalyzed reactions with hydrogen peroxide that generate hydroxyl radicals can preferentially attack amino acid residues at the metal-binding site. Oxidative damage to proteins can alter their enzymatic activity and increase their susceptibility to proteolysis by targeting them for proteolytic degradation (Stadtman 2006). In this regard, our findings have shown significantly increased protein carbonyl formation already at 30 min and 1 h after blood infusion in perihematomal white matter (Fig. 11.11) (Wagner et al. 2002).

An important source for ROS and nitrosative stress following ICH may be the microglia. Activated microglia are a hallmark of various brain disorders including

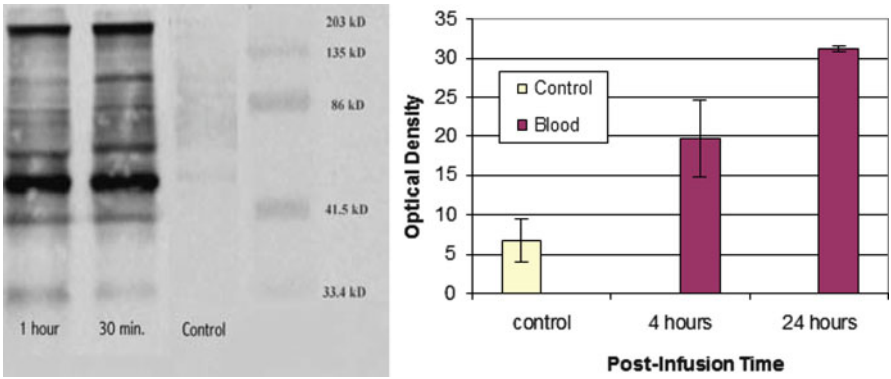


Fig. 11.11 *Left*: Example of an Oxyblot from control white matter and from perihematomal white matter sampled at 30 min and 1 h following ICH. Standard oxidized proteins are in *right* lane. A greater number and increased density of bands are present in ipsilateral white matter. *Right*: Semiquantitation of Oxyblot optical densities from ICH. Edematous white matter shows an early marked increase in protein oxidation that appears to further increase over the first 24 h. These results demonstrate that rapid development of oxidative stress in white matter surrounding the hematoma may be an important signaling event for NF-kappaB activation (Fig. 11.13) and upregulation of NF-kappaB-dependent gene expression after ICH

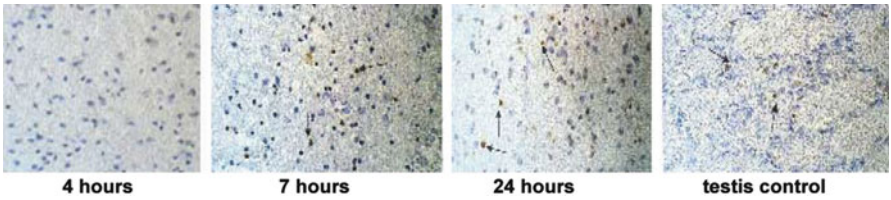


Fig. 11.12 TUNEL-positive cells (*arrows*) in edematous white matter at several times following ICH. Photomicrographs (40× objective) demonstrate DNA fragmentation with positive TUNEL staining at 7 and 24 h (*black arrows*). TUNEL-positive cells were more numerous at 24 h than at 4 h. Morphologically, the immunopositive cells appear to be oligodendrocytes. A positive control (testis) was run simultaneously with the brain sections

ischemic injury, neurodegenerative diseases, and multiple sclerosis (Gonzalez-Scarano and Baltuch 1999). Microglia undergo rapid activation that may be both a response to and also a cause of CNS injury. They secrete potentially toxic molecules including oxygen and nitrogen free radicals, TNF-α, and other cytokines and chemokines. Relevant to our discussion of the role of plasma proteins in edema development and tissue injury that was discussed above is that they appear to activate microglia (Murakami et al. 1998; Richmon et al. 1998; Si et al. 1997). Si et al. (1997) reported rapid and marked superoxide production following serum additions to microglial cultures. Our findings showing early upregulation of heme oxygenase-1 (HO-1) and cytokine expression suggest that early microglial cell activation may be present in perihematomal white matter (Wagner 2007; Wagner et al. 2002, 2004).

In our blood infusion model, our biochemical studies demonstrate DNA fragmentation indicative of both necrosis and apoptosis and positive TUNEL staining in morphologically appearing oligodendrocytes following hematoma induction (Fig. 11.12) (Wagner 2007; Wagner et al. 1999b, 2004). Evidence for a role of plasma proteins alone in these early biochemical events is supported by our findings that infused plasma but not red blood cells not only induces marked perihematomal edema (described above) but also induces early DNA fragmentation (Wagner et al. 1999b). Overall, these findings suggest that the earliest events in white matter within hours after an intracerebral bleed are not related to red cell lysis and the various processes related to hemoglobin release and heme metabolism that occur on a significantly more delayed time frame (Wagner et al. 2003a; Wagner and Dwyer 2004).

11.6.2 Transcription Factor Activation by ICH

11.6.2.1 Nuclear Factor-KappaB

As described above, inflammatory processes are important secondary events following cerebral insults that contribute significantly to neuropathologic outcome. Many inflammatory responses including cytokine upregulation appear to be mediated

through alterations in gene expression with NF-kappaB being a principal regulator. The transcription factor, NF-kappaB, is a central mediator of the rapid and coordinated induction of CNS genes that primarily respond to pathogenic stimuli (Barone and Feuerstein 1999; Baeuerle 1998; Denk et al. 2000; Mattson et al. 2000; Mattson and Meffert 2006; Pizzi and Spano 2006; Yenari and Han 2006). The cascades of events that involve NF-kappaB following stroke were recently reviewed (Harari and Liao 2010).

The signals that trigger NF-kappaB activation can be classified into two major groups: (1) signals that are also active in the periphery including cytokines and oxidative stress (Karin et al. 2001) and (2) CNS-specific signals including depolarization and neurotransmitters (glutamate and others) (Yenari and Han 2006). As such, NF-kappaB activation may be an important determiner of the cell death pathways in brain injury from ICH. Several NF-kappaB target genes, including p53 and c-Myc, are well-established modulators of apoptosis (Qin et al. 1999). Both ICAM-1 and MCP-1 genes have binding sites for NF-kappaB and are well described to be upregulated following ICH and other insults to brain. The neuroprotective antioxidant, LY341122, that can block activation of NF-kappaB in vitro completely prevented activation of NF-kappaB following cerebral ischemia (Stephenson et al. 2000). Findings with NF-kappaB translocation inhibitors support the role for NF-kappaB activation and neuronal injury following excitotoxic and dopamine-induced oxidative stress (Qin et al. 1999). Overall, these results demonstrate that NF-kappaB activation occurs in several brain pathologies and support previous observations that approaches that prevent NF-kappaB activation may inhibit CNS cell death following various insults.

Transcription factor activation and expression in gray and white matter following ICH have been determined on nuclear extracts from perihematomal brain tissue using NF-kappaB DNA bindings assays, RT-PCR, and Western blotting. Aronowski and colleagues have reported a comprehensive study on distinct patterns of alterations in NF-kappaB activation and subunit expression following ICH (Zhao et al. 2007a). These workers demonstrated that the mRNA and protein for p50, p52, p65, c-Rel, and RelB NF-kappaB subunits, as well as I-kappaB-alpha, were all upregulated after ICH, with a time course ranging from minutes to days following ICH, depending on the subunit. They also showed that NF-kappaB activation leads to nuclear translocation and TUNEL positive (fragmented DNA) containing cells (Hickenbottom et al. 1999).

In this regard, we too have observed rapid activation of NF-kappaB (Fig. 11.13) and DNA binding along with the appearance of TUNEL-positive cell staining in edematous white matter following ICH in our porcine model (Wagner 2007; Wagner et al. 2004). Thus, similar ultra-early NF-kappaB activation along with DNA damage is present in both gray and white matter in the early hours following ICH. A summary diagram is presented in Fig. 11.14 that depicts the several events described above that occur in white matter following ICH and that are linked through NF-kappaB activation to downstream secondary processes including pro-inflammatory cytokine.

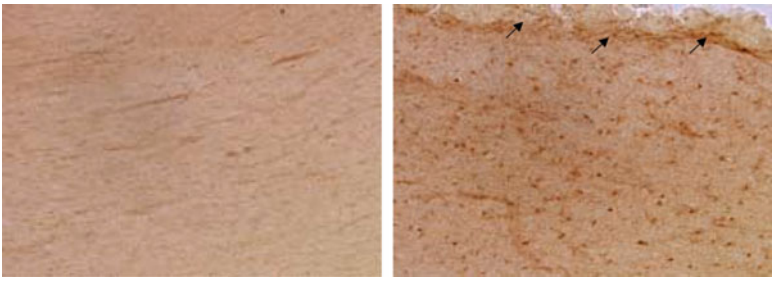


Fig. 11.13 NF-kappaB-p65 immunostaining in contralateral (*left*) and perihematomal (*right*) white matter at 1 h after ICH. *Arrows* (*right*) designate the edge of the hematoma. Increased cellular immunostaining for activated NF-kappaB can be seen in white matter adjacent to the hematoma. Biochemical studies demonstrate that NF-kappaB is activated at 30 min in perihematomal brain tissue and remains elevated during the first 24 h following ICH (Wagner et al. 2004)

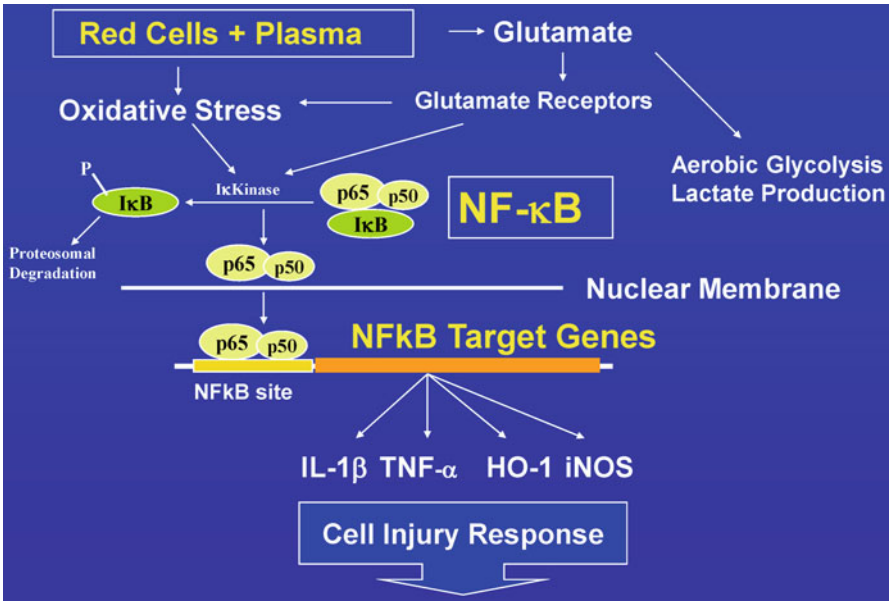


Fig. 11.14 This schematic diagram depicts the proposed role of the transcription factor, NF-kappaB, in the cell injury intracellular signaling events after ICH. Evidence referenced in our review suggests that the blood's red cell and plasma components can stimulate glutamate receptor activation and oxidative stress and activate NF-kappaB leading to downstream upregulation of gene expression. This figure is modified for postulated ICH mechanisms from that presented previously (Wagner 2007) and from that presented by Sharp and colleagues for molecular mechanisms in cerebral ischemia (Sharp et al. 2000)

11.6.2.2 PPARgamma and Nuclear Factor (Erythroid-Derived 2)-Like Factor 2

Although not studied as yet in white matter, another transcription factor, peroxisome proliferator-activated receptor-gamma (PPARgamma), is linked to inflammation but in this case anti-inflammatory activity. When activated, PPARgamma promotes expression of the peroxisome-enriched antioxidant enzyme, catalase, and reduces inflammation, behavioral dysfunction, and neuronal loss after intracerebral hemorrhage in rats (Zhao et al. 2006). These workers showed that 15-deoxy-delta(12,14)-prostaglandin J2 (15d-PGJ2), which acts as a physiologic agonist for PPARgamma, can reduce activation of the transcription factor, NF-kappaB. It also prevented neutrophil infiltration and reduced cell apoptosis.

In ICH, work mainly from the Aronowski laboratory has demonstrated that activation of the pleiotropic transcription factor, nuclear factor (erythroid-derived 2)-like factor 2 (Nrf2), plays a key role in protecting cells from cytotoxic/oxidative damage (Aronowski and Zhao 2011). In these studies, Nrf2-deficient mice developed greater ICH-induced injury (Zhao et al. 2007b). Interestingly, these workers reported that sulforaphane, which activates Nrf2, reduced myeloperoxidase-positive neutrophil count, oxidative damage, and behavioral deficits caused by blood injection in a rat ICH model. Furthermore, Nrf2-deficient mice which develop severe neurologic deficits after ICH did not benefit from the protective effect of sulforaphane.

At present, the anti-inflammatory activities of PPARgamma and Nrf2 have been defined in gray matter ICH injury models. These transcription factor activities that protect neuronal cells may also benefit astrocytes and oligodendrocytes and thereby provide protection against white matter injury and improve recovery after ICH.

11.6.3 Pro-inflammatory Cytokines, TNF-alpha and IL-1-beta, in White Matter After ICH

The two major pro-inflammatory cytokines, TNF-alpha and interluekin-1 beta (IL-1 beta), are generally considered to play a role in edema development and brain tissue injury from various insults and diseases including ICH (Wang 2010; Wang and Dore 2007; Holmin and Mathiesen 2000; Castillo et al. 2002; Rothwell 1999). IL-1 is also an important mediator of diverse forms of acute neurodegeneration and chronic neurological conditions (Rothwell 1999; Rothwell et al. 1997). In addition, IL-1 can cause vasogenic edema when injected into the brain (Holmin and Mathiesen 2000), and TNF can induce apoptosis in oligodendrocytes and demyelination (Selmaj and Raine 1988). Cytokine expression by glial cells has been well documented with the astrocyte being a major source of inducible IL-6. Factors shown to induce IL-6 expression by astrocytes include in particular TNF and IL-1 (Van Wagoner and Benveniste 1999). Early upregulation of these cytokines following ICH has been described by ourselves and others (Wagner 2007; Wagner et al. 1999c, 2004; Hua et al. 2006).

An important source of these cytokines is microglia which are rapidly activated, i.e., within 15 min post ICH (Aronowski and Zhao 2011; Wang 2010; Wang and Dore 2007). In this regard, Mayne and co-workers (Mayne et al. 2001a) reported that ICH in a rat striatal collagenase plus heparin model elevated TNF-alpha mRNA and protein in microglia, neutrophils, and macrophages. Interestingly, they found that administration of a TNF-alpha-specific antisense oligodeoxynucleotide (ORF4-PE) decreased perihematomal TNF-alpha levels, reduced neuronal cell death, and improved neurobehavioral outcome. Additionally, Mayne and co-workers (Mayne et al. 2001b) provided further evidence for the contribution of TNF-alpha and its linkage through adenosine A2A receptor activation in which pharmacologic treatment reduced pro-inflammatory events and decreased cell death following ICH.

A role of thrombin in early pro-inflammatory cytokine expression is supported by findings from Hua and co-workers (Wu et al. 2008) who demonstrated that cultured rat microglial cells treated with thrombin increased TNF-alpha and IL-1 beta levels in the culture medium. In addition, these workers reported that perihematomal TNF-alpha levels measured by ELISA were correlated with edema development after ICH or thrombin infusions into the rat striatum (Hua et al. 2006). Furthermore, a contributing role of TNF-alpha in edema development was supported by a reduction in edema development in TNF-alpha knockout compared with wild-type mice (Hua et al. 2006). These workers also showed that the tuftsin 1–3 fragment decreased thrombin-induced upregulation of TNF-alpha and IL-1 beta levels in vivo. The microglial inhibitory factor tuftsin 1–3 fragment has been shown previously by Tsirka and co-workers to be protective in ICH animal models (Wang et al. 2003; Wang and Tsirka 2005). In contrast, expression of the inflammatory mediator, macrophage inflammatory protein-2 (MIP-2), correlates with NF-kappaB activation and edema development after ICH (Wu et al. 2009). The roles of chemokines and their receptors in ICH have been recently reviewed by Yao and Tsirka (2012).

We have examined the early time course of TNF-alpha protein expression in perihematomal edematous white matter following ICH in our porcine model (Wagner 2007; Wagner et al. 1999c, 2004). We observed that TNF-alpha protein expression by ELISA using a porcine monoclonal TNF-alpha antibody showed a rapid fourfold upregulation of TNF-alpha protein expression in edematous white matter already at 2 h following hematoma induction. Immunocytochemically, this early TNF-alpha protein expression in perihematomal white matter was present in morphologically appearing micro- or astroglia cells.

A role of IL-1 beta in edema from either ICH or thrombin infusions is supported by the findings that overexpression of the interleukin-1 receptor antagonist (IL-1ra) significantly attenuated edema development (Masada et al. 2001, 2003). We (and others) have demonstrated early upregulation of IL-1 beta in perihematomal gray and white matter following ICH (Wagner 2007; Wagner et al. 2006).

11.6.4 Matrix Metalloproteinases

MMPs are a family of zinc endopeptidases which can modify the extracellular matrix and have been implicated in brain injury from ischemic and hemorrhagic stroke, brain trauma, and other disorders (Tejima et al. 2007; Wang et al. 2000; Rosenberg 2002; Rosenberg and Mun-Bryce 2004; del Zoppo et al. 2007). MMPs are generally expressed at low levels, but upregulation of MMP gene transcription occurs after ICH and contributes to secondary brain injury including edema formation, and are an integral component of the inflammatory response (Florczak-Rzepka et al. 2012). Power et al. (2003) reported that ICH elevated MMP-2, -3, -7, and -9 mRNA levels (Power et al. 2003). Specifically, astrocytic MMP-9 (gelatinase B) has been suggested to contribute to edema development, since edema is reduced in MMP-9 knockout mice (Tejima et al. 2007).

Interestingly, Mun-Bryce et al. (2004a) used somatic evoked potentials in hemorrhagic stroke to show the depressed cortical response, also contralateral to the hematoma site, which was followed by overexpression of MMP-9 and MMP-2, implying that neuroinflammation triggered by blood extravasation in the course of ICH is not limited to the directly damaged brain tissue. This may suggest that the inflammation in the course of ICH is not only localized to the lesion but also involves distant areas.

11.6.5 Toll-Like Receptors in ICH

Toll-like receptors (TLRs) are evolutionarily conserved transmembrane proteins involved in signal transduction in innate immunity and inflammatory responses (Hanke and Kielian 2011). Binding of ligands to these receptor molecules results in downstream activation of transcription factors including NF-kappaB, leading to transcription of a host of inflammation-associated genes including cytokines (Hanke and Kielian 2011; Teng et al. 2009; Lin et al. 2012; review Fang et al. 2013). TLR4 knockout mice had reduced ICH-induced cerebral edema and neurological deficit scores following ICH (Lin et al. 2012). Microglia, as the primary cerebral parenchymal responders to pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs), are known to express all TLR family members (Hanke and Kielian 2011). In human ICH, expression levels of TLR2 and TLR4 in both monocytes and neutrophils were associated with poor clinical outcome and greater remaining hematoma volume (Rodriguez-Yanez et al. 2012). Astrocytes express these proteins but to a somewhat more limited degree (Hanke and Kielian 2011). In white matter, a few reports have described the presence of and response to injury of TLRs by microglia and astrocytes (Zhang et al. 2012; Wirths et al. 2010).

11.7 ICH Treatments

Various comprehensive reviews of ICH treatments including targets and therapies have been published recently (Katsuki 2010; Sangha and Gonzales 2011; Brouwers and Goldstein 2012; Hwang et al. 2011; Babu et al. 2012). These excellent reviews provide current overviews of ICH therapeutics. Overall, except for a few studies, white matter treatment has not been carefully studied. Our goal in this review is to briefly describe those experimental treatments that specifically have examined the outcome of white matter after ICH. Figure 11.15 presents a schematic summary of proposed pathophysiologic, cellular, and molecular processes along with therapies that have demonstrated effectiveness following ICH against white matter injury in experimental animal models.

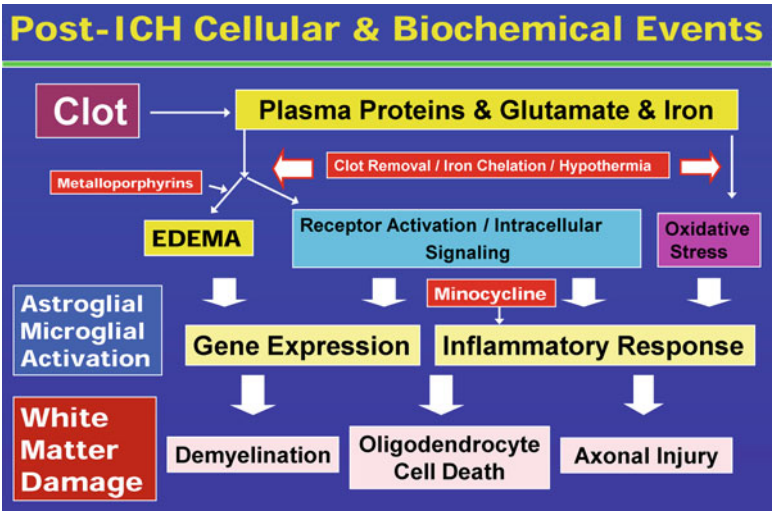


Fig. 11.15 This diagram presents a general schematic of hypothetical cellular and biochemical responses that may lead to white matter injury after ICH. Experimental treatments (*red* boxes) including surgical clot removal, iron chelation, hypothermia, minocycline, and metalloporphyrins have been demonstrated to provide white matter protection in ICH models. In this figure, surgical clot removal, iron chelation, and hypothermia are proposed to interrupt the events that lead to white matter edema development, oxidative stress, and intracellular signaling cascades that likely involve astroglia and microglial cells. Minocycline attenuates the inflammatory response in these cells. These therapeutic approaches may prevent enhanced pro-inflammatory gene expression and cellular inflammatory responses that lead ultimately to white matter damage including axonal injury, demyelination, and oligodendrocyte cell death

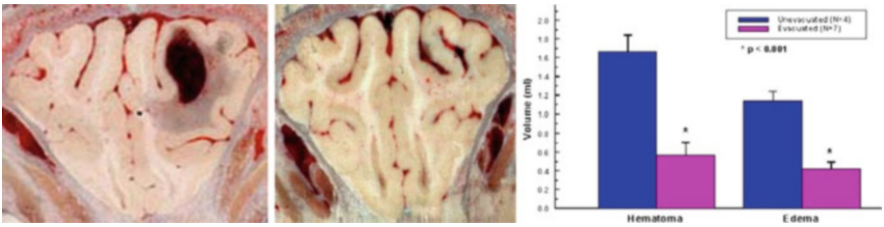


Fig. 11.16 *Left*: Coronal section from porcine brain at 24 h showing a hematoma in the frontal white matter surrounded by Evans blue containing perihematomal edema indicative of BBB opening. *Middle*: Clot aspiration following tPA-induced lysis markedly reduced hematoma and edema volumes and prevented BBB opening. *Right*: Quantitation of hematoma and edema volumes in the clot evacuation series. Values are means ± SEM $*p < 0.001$, $N = 5–6$ (Wagner et al. 1999a)

11.7.1 Surgical Treatment for ICH

Surgical clot removal for treating ICH has had a controversial history. The 2005 findings from the STICH trial indicated that surgical and medical management of ICH were equivocal (Mendelow et al. 2005; Mendelow and Unterberg 2007). Several reasons for this outcome have been discussed (Broderick 2005). On the other hand, the most recent findings from the ongoing multicenter MISTIE II trial that utilizes a minimally invasive approach and t-PA-induced clot lysis are very encouraging. Hanley and colleagues (Mould et al. 2013) recently reported that successful hematoma evacuation leads to significant edema volume reduction. In addition, at the 2013 International Stroke Meeting, Dr. Hanley presented the trial's 365-day results that demonstrated an improving long-term beneficial clinical outcome versus 180 days and a 14 % upward shift across all modified Rankin Score levels. Fewer MISTIE-treated subjects were in long-term care facilities and these had shorter hospital stays with a significant cost savings.

Based on earlier findings that tPA was used successfully (>24 h) in treating human intracerebral hematomas and was more effective for clot lysis than urokinase (Lippitz et al. 1994; Rohde et al. 1995; Schaller et al. 1995), we conducted an experimental study in our pig lobar ICH model in the late 1990s (Wagner et al. 1999a). We reported that early (3.5 h) tPA-induced clot lysis and aspiration was highly effective in reducing clot volumes by >70 % in white matter at 24 h (Fig. 11.16). Importantly, white matter edema volume was also reduced by >70 %, and BBB opening was prevented (Wagner et al. 1999a). Early tPA treatment is important, since aging thrombi lose their plasminogen, and continued clot retraction chemically changes fibrin molecules reducing their proteolytic reactivity.

Although it seems counterintuitive to use a thrombolytic agent to liquefy the clot in hemorrhagic stroke, if a fibrinolytic agent is not used, it is extremely difficult to remove intraparenchymal clots. Surgery without tPA increases brain trauma and only partially removes hematomas (~30 %) (Matsumoto and Hondo 1984). In addition, only when clots are fibrinolyzed are lasting ICP decreases achieved (Mohadjer et al. 1992).

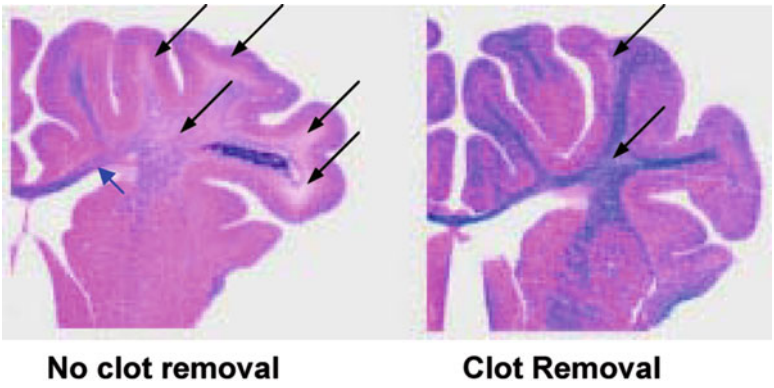


Fig. 11.17 H&E/Luxol Fast Blue-stained myelin at 7 days after ICH without (*left*) and with clot removal (*right*). *Arrows* indicate decreased myelin staining that is especially marked in the brain without clot removal. Large-headed *blue arrow* in the untreated brain indicates border between normal and reduced myelin staining. In this example, clot removal reduced the area of myelin pallor by >80 % (77 mm^2 versus 11 mm^2)

Furthermore, mechanical methods to remove hematomas can damage red blood cells which can lead to marked edema and tissue injury (Wu et al. 2002).

Interestingly, recent findings from Vespa and colleagues demonstrate that frameless stereotactic aspiration and thrombolysis of deep intracerebral hemorrhage are associated with reduced levels of extracellular cerebral glutamate and unchanged lactate/pyruvate ratios (Miller et al. 2007). As described above, we have reported that markedly elevated tissue lactate develops during the first hours following ICH (Wagner et al. 1998) and that blocking glutamate receptor activation reduces perihematomal hypermetabolism following ICH (Ardizzone et al. 2004; Wagner 2007).

Thus, our previous surgical treatment study (Wagner et al. 1999a) and our demonstration of rapid interstitial fibrin(ogen) deposition following ICH (Fig. 11.16) (Wagner et al. 1996) provide support for the use of tPA due to its ability to (1) facilitate clot removal by stereotactic aspiration; (2) enable the majority of the clot to be removed, thereby significantly reducing mass effect; and (3) catalyze extracellular fibrinolysis which may protect axons against injury as described above (Akassoglou et al. 2000). In a preliminary study, we have observed that experimental clot removal in our porcine model was effective in reducing reduced white matter injury as measured histologically (Fig. 11.17). In addition, surgical clot aspiration after tPA lysis can be used as a tool to gain insights into early inflammatory responses so that medicines for these pathophysiological events and for downstream gene expression could be developed to extend the window for ICH treatment.

It should be noted that in contrast to our findings, other experimental studies conducted in a porcine model have suggested that tPA use for clot lysis and removal enhances the development of delayed edema (Thiex et al. 2003). However, the hematoma model employed by these investigators utilized a rapid balloon inflation

to produce a cavity within the white matter that serves as a reservoir for the infused blood. This traumatic injury produced by balloon inflation is a different insult than that produced by blood infusion alone in which a dissection or a spreading of the white fiber tracts is produced as the blood is infused.

In addition, recent studies in tPA-deficient mice also indicate that proteases such as plasminogen activators and plasmin may induce CNS injury (Kaur et al. 2004). In contrast, in a thrombotic stroke model study using mice with appropriately matched genetic backgrounds, tPA deficiency exacerbated injury. Furthermore, tPA/plasmin's fibrinolytic activity can also be beneficial, since proteolysis plays a critical role in fibrin(ogen) clearance (Akassoglou et al. 2000).

11.7.2 Hypothermia

Hypothermia has a long history of use in the medical treatment of stroke and TBI. Excellent reviews of hypothermia treatment of global and focal cerebral ischemia in experimental animals and mechanisms of hypothermic neuroprotection have been published (refs). The significance of hypothermia in clinical ICH treatment is the recent findings that hypothermia of 35 °C for 10 days in 12 patients with spontaneous ICH >25 ml completely prevented perihematomal edema development (Kollmar et al. 2010).

Global hypothermia has been tested in ICH treatment in the collagenase ICH model (MacLellan et al. 2004, 2006). Hypothermia treatment reduced BBB disruption and infiltration of inflammatory cells and modestly reduced edema. However, in this model, hypothermia provided limited to no functional benefit in the behavioral tests.

We have reported that local brain cooling with an experimental device protected the BBB and markedly reduced vasogenic edema development in white matter in our porcine ICH model (Wagner and Zuccarello 2005). Furthermore, we also found that local hypothermia also markedly downregulated RNA expression of the pro-inflammatory cytokine, IL-1 beta (Wagner et al. 2006).

11.7.3 Pharmacologic Treatments

Similar to preclinical studies in ischemic stroke, many pharmacologic treatments for ICH have been studied in experimental animals. These studies have been almost exclusively conducted in striatal injury models. At present (May 2013), and similar to the situation in ischemic stroke (except for tPA), there are no FDA-approved standard pharmacologic treatments for ICH.

A recent meta-analysis of experimental therapies in ICH animal models reviewed more than 13,000 publications (Frantzias et al. 2011). This report described 88 controlled studies that examined the effectiveness of 64 medical interventions.

From this analysis the authors found that the most effective therapies in more than one study which improved both structural outcomes and neurobehavioral scores were anti-inflammatory drugs and iron chelators among others (Frantzias et al. 2011). Below, we have briefly reviewed the efficacies of anti-inflammatory drugs, iron chelators, and metalloporphyrins which have been studied in white matter injury treatment paradigms.

11.7.3.1 Minocycline

The most studied drug for reducing white matter injury after ICH is the anti-inflammatory drug, minocycline, which has been examined by Schlichter and colleagues (Wasserman and Schlichter 2007a, b; Wasserman et al. 2007). Minocycline protected the BBB and reduced edema following ICH in the rat (Wasserman and Schlichter 2007a). This finding is significant since vasogenic edema appears to be an important mediator of secondary injury and clinical deterioration in ICH patients (Leira et al. 2004). These workers reported that minocycline effectively reduced early TNF-alpha upregulation and also delayed MMP-12 upregulation in damaged microvessels. These authors suggested that minocycline, by reducing neutrophil infiltration, reduced TNF-alpha production since neutrophils are the major source of TNF-alpha after ICH.

11.7.3.2 Iron Chelators

Iron accumulates in brain tissue during breakdown and removal of the hematoma after ICH and contributes to tissue injury and brain atrophy (Hua et al. 2007, 2008). Normally, free iron levels are very low in normal rat CSF. However, they are markedly increased after ICH and remain elevated for at least 28 days (Wan et al. 2006). Deferoxamine (DFX) is an FDA-approved iron chelator that is used to treat acute iron intoxication and chronic iron overload due to transfusion-dependent anemia.

Recent studies by Xi and colleagues in rat ICH models have demonstrated a significant improvement in ICH outcome including reductions in brain edema and atrophy, neuronal death, and neurological deficits following treatment with DFX (Song et al. 2007; Hua et al. 2007, 2008; Nakamura et al. 2004). In addition, Xi and colleagues (Wan et al. 2006, 2008) reported that the c-Jun-N-terminal kinase (JNK) signaling pathway that mediates cell death after ischemic stroke was activated after ICH and after an intracerebral infusion of ferrous iron. Administration of DFX after ICH reduced free iron contents in CSF, suppressed JNK activation, and improved ICH-induced neurological deficits. This group has examined the efficacy of DFX on white matter in a large animal (pig) blood infusion ICH model (Gu et al. 2009). They reported that DFX was effective in reducing the number of Perls', ferritin, and Fluoro-Jade C-positive cells in white matter. These results demonstrate that DFX is effective in reducing injury in different animal models with different sized clots. These studies have provided the preclinical support for the presently ongoing clinical trial of the iron chelator, DFX (Selim 2009; Selim et al. 2011).

Other workers have also examined the effectiveness of iron chelators on white matter injury. Masada et al. (2007) reported that oral administration of metal chelator, clioquinol, that reduces hydroxyl radical production, ameliorates motor dysfunction after a small hemorrhage near the internal capsule in rat. Wu et al. (2012) reported that the lipid-soluble ferrous iron chelator 2,2′-dipyridyl can reduce white matter injury and improve functional outcome after ICH.

It should be noted that there is some controversy regarding the effectiveness of iron chelators for ICH treatment. Several studies have not found significant improvement in various outcome variables in the collagenase ICH model (Wu et al. 2012; Warkentin et al. 2010; Auriat et al. 2012). Presently, the basis for these differences between laboratories remains unknown.

11.7.3.3 Metalloporphyrins and Hematoma Resolution

Metalloporphyrins are potent heme oxygenase inhibitors that have been found to reduce edema and injury in cerebral ischemia models and in ICH models (Wagner and Dwyer 2004; Huang et al. 2002; Koeppen et al. 2004). The metalloporphyrin, tin-mesoporphyrin (SnMP), is a neuroprotectant that has also been used clinically to treat hyperbilirubinemia (Wagner et al. 2000). We investigated the effectiveness of SnMP on edema development in white matter following ICH in our porcine model (Wagner et al. 2000). Interestingly, SnMP treatment significantly reduced not only edema but also hematoma size, thereby reducing the intracerebral mass following ICH.

In this regard, work in the Aronowski laboratory has focused on an interesting treatment approach for ICH, i.e., hematoma resolution as a therapeutic target (Zhao et al. 2007c, 2009). Specifically, these workers have studied the involvement of microglia/macrophages in hematoma removal hypothesizing that the neurotoxicity of blood products that develops within hours to days after ICH is important in tissue injury. Their findings suggest that after red cell lysis, hemoglobin, heme, and iron are released which initiate perihematomal cell death. They hypothesize that "a treatment that stimulates phagocytosis will lead to faster removal of blood from the ICH-affected brain, thus limiting/preventing hemolysis from occurring." These workers have reported that ICH treatment with PPARgamma agonists (e.g., rosiglitazone) upregulate CD36 in microglia/macrophages, thereby enhancing their ability to phagocytose red blood cells. This treatment in a mouse ICH model resulted in improved hematoma resolution and reduced deficits. Further studies of hematoma resolution and its long-term impact on white matter are necessary.

Acknowledgments The studies from the author's laboratory that are described in this review were supported by National Institutes of Health grant R01NS30652 and funds from the Office of Research and Development, Medical Research Service, Department of Veterans Affairs.

References

Adams RA, Passino M, Sachs BD, Nuriel T, Akassoglou K (2004) Fibrin mechanisms and functions in nervous system pathology. Mol Interv 4:163–176

Adams RA, Bauer J, Flick MJ, Sikorski SL, Nuriel T, Lassmann H, Degen JL, Akassoglou K (2007) The fibrin-derived gamma377-395 peptide inhibits microglia activation and suppresses relapsing paralysis in central nervous system autoimmune disease. J Exp Med 204:571–582

Adeoye O, Clark JF, Khatri P, Wagner KR, Zuccarello M, Pyne-Geithman GJ (2011) Animal models of hemorrhagic stroke: do current models mirror the human pathologies? Transl Stroke Res 2:17–25

Akassoglou K, Strickland S (2002) Nervous system pathology: the fibrin perspective. Biol Chem 383:37–45

Akassoglou K, Kombrinck K, Degen J, Strickland S (2000) Tissue plasminogen activator-mediated fibrinolysis protects against axonal degeneration and demyelination after nerve injury. J Cell Biol 149:1157–1166

Akassoglou K, Yu WM, Akpinar P, Strickland S (2002) Fibrin inhibits peripheral nerve remyelination by regulating Schwann cell differentiation. Neuron 33:861–875

Andaluz N, Zuccarello M, Wagner KR (2002) Experimental animal models of intracerebral hemorrhage. Neurosurg Clin N Am 13:385–393

Ardizzone TD, Lu A, Wagner KR, Tang Y, Ran R, Sharp FR (2004) Glutamate receptor blockade attenuates glucose hypermetabolism in perihematomal brain after experimental intracerebral hemorrhage in rat. Stroke 35:2587–2591

Aronowski J, Hall CE (2005) New horizons for primary intracerebral hemorrhage treatment: experience from preclinical studies. Neurol Res 27:268–279

Aronowski J, Zhao X (2011) Molecular pathophysiology of cerebral hemorrhage: secondary brain injury. Stroke 42:1781–1786

Auriat AM, Silasi G, Wei Z, Paquette R, Paterson P, Nichol H, Colbourne F (2012) Ferric iron chelation lowers brain iron levels after intracerebral hemorrhage in rats but does not improve outcome. Exp Neurol 234:136–143

Babu R, Bagley JH, Di C, Friedman AH, Adamson C (2012) Thrombin and hemin as central factors in the mechanisms of intracerebral hemorrhage-induced secondary brain injury and as potential targets for intervention. Neurosurg Focus 32:E8

Baeuerle PA (1998) Pro-inflammatory signaling: last pieces in the NF-kappaB puzzle? Curr Biol 8:R19–R22

Barone FC, Feuerstein GZ (1999) Inflammatory mediators and stroke: new opportunities for novel therapeutics. J Cereb Blood Flow Metab 19:819–834

Belayev L, Saul I, Busto R, Danielyan K, Vigdorchik A, Khoutorova L, Ginsberg MD (2005) Albumin treatment reduces neurological deficit and protects blood–brain barrier integrity after acute intracortical hematoma in the rat. Stroke 36:326–331

Berger AK (2003) Thrombolysis in elderly patients with acute myocardial infarction. Am J Geriatr Cardiol 12:251–256

Broderick JP (2005) The STICH trial: what does it tell us and where do we go from here? Stroke 36:1619–1620

Broderick JP, Brott TG, Duldner JE, Tomsick T, Huster G (1993) Volume of intracerebral hemorrhage. A powerful and easy-to-use predictor of 30-day mortality. Stroke 24:987–993

Brouwers HB, Goldstein JN (2012) Therapeutic strategies in acute intracerebral hemorrhage. Neurotherapeutics 9:87–98

Brown MS, Kornfeld M, Mun-Bryce S, Sibbitt RR, Rosenberg GA (1995) Comparison of magnetic resonance imaging and histology in collagenase-induced hemorrhage in the rat. J Neuroimaging 5:23–33

Butcher KS, Baird T, MacGregor L, Desmond P, Tress B, Davis S (2004) Perihematomal edema in primary intracerebral hemorrhage is plasma derived. Stroke 35:1879–1885

Castillo J, Davalos A, Alvarez-Sabin J, Pumar JM, Leira R, Silva Y, Montaner J, Kase CS (2002) Molecular signatures of brain injury after intracerebral hemorrhage. Neurology 58:624–629
Chan PH (2001) Reactive oxygen radicals in signaling and damage in the ischemic brain. J Cereb Blood Flow Metab 21:2–14
Chiang MF, Chiu WT, Lin FJ, Thajeb P, Huang CJ, Tsai SH (2006) Multiparametric analysis of cerebral substrates and nitric oxide delivery in cerebrospinal fluid in patients with intracerebral haemorrhage: correlation with hemodynamics and outcome. Acta Neurochir (Wien) 148:615–621, dicussion 621
Davalos D, Akassoglou K (2012) Fibrinogen as a key regulator of inflammation in disease. Semin Immunopathol 34:43–62
Del Bigio MR, Yan HJ, Buist R, Peeling J (1996) Experimental intracerebral hemorrhage in rats. Magnetic resonance imaging and histopathological correlates. Stroke 27:2312–2319
del Zoppo GJ, Milner R, Mabuchi T, Hung S, Wang X, Berg GI, Koziol JA (2007) Microglial activation and matrix protease generation during focal cerebral ischemia. Stroke 38:646–651
Denk A, Wirth T, Baumann B (2000) NF-kappaB transcription factors: critical regulators of hematopoiesis and neuronal survival. Cytokine Growth Factor Rev 11:303–320
Enzmann DR, Britt RH, Lyons BE, Buxton JL, Wilson DA (1981) Natural history of experimental intracerebral hemorrhage: sonography, computed tomography and neuropathology. AJNR Am J Neuroradiol 2:517–526
Fang H, Wang PF, Zhou Y, Wang YC, Yang QW (2013) Toll-like receptor 4 signaling in intracerebral hemorrhage-induced inflammation and injury. J Neuroinflammation 10:27
Florczak-Rzepka M, Grond-Ginsbach C, Montaner J, Steiner T (2012) Matrix metalloproteinases in human spontaneous intracerebral hemorrhage: an update. Cerebrovasc Dis 34:249–262
Folsom AR, Yatsuya H, Mosley TH Jr, Psaty BM, Longstreth WT Jr (2012) Risk of intraparenchymal hemorrhage with magnetic resonance imaging-defined leukoaraiosis and brain infarcts. Ann Neurol 71:552–559
Foulkes MA, Wolf PA, Price TR, Mohr JP, Hier DB (1988) The Stroke Data Bank: design, methods, and baseline characteristics. Stroke 19:547–554
Frantzias J, Sena ES, Macleod MR, Al-Shahi Salman R (2011) Treatment of intracerebral hemorrhage in animal models: meta-analysis. Ann Neurol 69:389–399
Fukui K, Iguchi I, Kito A, Watanabe Y, Sugita K (1994) Extent of pontine pyramidal tract Wallerian degeneration and outcome after supratentorial hemorrhagic stroke. Stroke 25:1207–1210
Garcia JH, Ho KL, Caccamo DV (1994) Intracerebral hemorrhage: pathology of selected topics. In: Kase CS, Caplan LR (eds) Intracerebral hemorrhage. Butterworth-Heinemann, Boston, MA, pp 45–72
Gebel JM, Brott TG, Sila CA, Tomsick TA, Jauch E, Salisbury S, Khoury J, Miller R, Pancioli A, Duldner JE et al (2000) Decreased perihematomal edema in thrombolysis-related intracerebral hemorrhage compared with spontaneous intracerebral hemorrhage. Stroke 31:596–600
Gentleman SM, Nash MJ, Sweeting CJ, Graham DI, Roberts GW (1993) Beta-amyloid precursor protein (beta APP) as a marker for axonal injury after head injury. Neurosci Lett 160:139–144
Gong C, Hoff JT, Keep RF (2000) Acute inflammatory reaction following experimental intracerebral hemorrhage in rat. Brain Res 871:57–65
Gonzalez-Scarano F, Baltuch G (1999) Microglia as mediators of inflammatory and degenerative diseases. Annu Rev Neurosci 22:219–240
Gu Y, Hua Y, Keep RF, Morgenstern LB, Xi G (2009) Deferoxamine reduces intracerebral hematoma-induced iron accumulation and neuronal death in piglets. Stroke 40:2241–2243
Hanke ML, Kielian T (2011) Toll-like receptors in health and disease in the brain: mechanisms and therapeutic potential. Clin Sci (Lond) 121:367–387
Harari OA, Liao JK (2010) NF-kappaB and innate immunity in ischemic stroke. Ann N Y Acad Sci 1207:32–40
Herweh C, Juttler E, Schellinger PD, Klotz E, Jenetzky E, Orakcioglu B, Sartor K, Schramm P (2007) Evidence against a perihemorrhagic penumbra provided by perfusion computed tomography. Stroke 38:2941–2947

Herweh C, Juttler E, Schellinger PD, Klotz E, Schramm P (2010) Perfusion CT in hyperacute cerebral hemorrhage within 3 h after symptom onset: is there an early perihemorrhagic penumbra? J Neuroimaging 20:350–353

Hickenbottom SL, Grotta JC, Strong R, Denner LA, Aronowski J (1999) Nuclear factor-kappaB and cell death after experimental intracerebral hemorrhage in rats. Stroke 30:2472–2477

Holmin S, Mathiesen T (2000) Intracerebral administration of interleukin-1beta and induction of inflammation, apoptosis, and vasogenic edema. J Neurosurg 92:108–120

Hua Y, Wu J, Keep RF, Nakamura T, Hoff JT, Xi G (2006) Tumor necrosis factor-alpha increases in the brain after intracerebral hemorrhage and thrombin stimulation. Neurosurgery 58:542–550

Hua Y, Keep RF, Hoff JT, Xi G (2007) Brain injury after intracerebral hemorrhage: the role of thrombin and iron. Stroke 38:759–762

Hua Y, Keep RF, Hoff JT, Xi G (2008) Deferoxamine therapy for intracerebral hemorrhage. Acta Neurochir Suppl 105:3–6

Huang FP, Xi G, Keep RF, Hua Y, Nemoianu A, Hoff JT (2002) Brain edema after experimental intracerebral hemorrhage: role of hemoglobin degradation products. J Neurosurg 96:287–293

Hwang BY, Appelboom G, Ayer A, Kellner CP, Kotchetkov IS, Gigante PR, Haque R, Kellner M, Connolly ES (2011) Advances in neuroprotective strategies: potential therapies for intracerebral hemorrhage. Cerebrovasc Dis 31:211–222

James ML, Warner DS, Laskowitz DT (2008) Preclinical models of intracerebral hemorrhage: a translational perspective. Neurocrit Care 9:139–152

Jenkins A, Maxwell WL, Graham DI (1989) Experimental intracerebral haematoma in the rat: sequential light microscopical changes. Neuropathol Appl Neurobiol 15:477–486

Jenkins A, Mendelow AD, Graham DI, Nath FP, Teasdale GM (1990) Experimental intracerebral haematoma: the role of blood constituents in early ischaemia. Br J Neurosurg 4:45–51

Karin M, Takahashi T, Kapahi P, Delhase M, Chen Y, Makris C, Rothwarf D, Baud V, Natoli G, Guido F et al (2001) Oxidative stress and gene expression: the AP-1 and NF-kappaB connections. Biofactors 15:87–89

Kase CS, Caplan LR (1994) Intracerebral hemorrhage. Butterworth-Heinemann, Newton, MA

Katsuki H (2010) Exploring neuroprotective drug therapies for intracerebral hemorrhage. J Pharmacol Sci 114:366–378

Kaufman HH, Schochet SS (1992) Pathology, pathophysiology and modeling. In: Kaufman HH (ed) Intracerebral hematomas: etiology, pathophysiology, clinical presentation and treatment. Raven, New York, pp 13–20

Kaur J, Zhao Z, Klein GM, Lo EH, Buchan AM (2004) The neurotoxicity of tissue plasminogen activator? J Cereb Blood Flow Metab 24:945–963

Kazui S, Kuriyama Y, Sawada T, Imakita S (1994) Very early demonstration of secondary pyramidal tract degeneration by computed tomography. Stroke 25:2287–2289

Keep RF, Hua Y, Xi G (2012) Intracerebral haemorrhage: mechanisms of injury and therapeutic targets. Lancet Neurol 11:720–731

Kimelberg HK (1995) Current concepts of brain edema. Review of laboratory investigations. J Neurosurg 83:1051–1059

Kirkman MA, Allan SM, Parry-Jones AR (2011) Experimental intracerebral hemorrhage: avoiding pitfalls in translational research. J Cereb Blood Flow Metab 31:2135–2151

Koeppen AH, Dickson AC, Smith J (2004) Heme oxygenase in experimental intracerebral hemorrhage: the benefit of tin-mesoporphyrin. J Neuropathol Exp Neurol 63:587–597

Kollmar R, Staykov D, Dorfler A, Schellinger PD, Schwab S, Bardutzky J (2010) Hypothermia reduces perihemorrhagic edema after intracerebral hemorrhage. Stroke 41:1684–1689

Koo EH, Sisodia SS, Archer DR, Martin LJ, Weidemann A, Beyreuther K, Fischer P, Masters CL, Price DL (1990) Precursor of amyloid protein in Alzheimer disease undergoes fast anterograde axonal transport. Proc Natl Acad Sci U S A 87:1561–1565

Koyama T, Tsuji M, Nishimura H, Miyake H, Ohmura T, Domen K (2011) Diffusion tensor imaging for intracerebral hemorrhage outcome prediction: comparison using data from the corona radiata/internal capsule and the cerebral peduncle. J Stroke Cerebrovasc Dis 22:72–79

Kuzu Y, Inoue T, Kanbara Y, Nishimoto H, Fujiwara S, Ogasawara K, Ogawa A (2012) Prediction of motor function outcome after intracerebral hemorrhage using fractional anisotropy calculated from diffusion tensor imaging. Cerebrovasc Dis 33:566–573

Lee KR, Colon GP, Betz AL, Keep RF, Kim S, Hoff JT (1996) Edema from intracerebral hemorrhage: the role of thrombin. J Neurosurg 84:91–96

Leira R, Davalos A, Silva Y, Gil-Peralta A, Tejada J, Garcia M, Castillo J, Stroke Project, C.D.G.o.t.S.N.S (2004) Early neurologic deterioration in intracerebral hemorrhage: predictors and associated factors. Neurology 63:461–467

Lekic T, Rolland W, Hartman R, Kamper J, Suzuki H, Tang J, Zhang JH (2011) Characterization of the brain injury, neurobehavioral profiles, and histopathology in a rat model of cerebellar hemorrhage. Exp Neurol 227:96–103

Lekic T, Rolland W, Manaenko A, Krafft PR, Kamper JE, Suzuki H, Hartman RE, Tang J, Zhang JH (2013) Evaluation of the hematoma consequences, neurobehavioral profiles, and histopathology in a rat model of pontine hemorrhage. J Neurosurg 118:465–477

Leonardo CC, Robbins S, Dore S (2012) Translating basic science research to clinical application: models and strategies for intracerebral hemorrhage. Front Neurol 3:85

Lin S, Yin Q, Zhong Q, Lv FL, Zhou Y, Li JQ, Wang JZ, Su BY, Yang QW (2012) Heme activates TLR4-mediated inflammatory injury via MyD88/TRIF signaling pathway in intracerebral hemorrhage. J Neuroinflammation 9:46

Lippitz BE, Mayfrank L, Spetzger U, Warnke JP, Bertalanffy H, Gilsbach JM (1994) Lysis of basal ganglia haematoma with recombinant tissue plasminogen activator (rtPA) after stereotactic aspiration: initial results. Acta Neurochir (Wien) 127:157–160

Lipton SA (2006) Paradigm shift in neuroprotection by NMDA receptor blockade: memantine and beyond. Nat Rev Drug Discov 5:160–170

Lisk DR, Pasteur W, Rhoades H, Putnam RD, Grotta JC (1994) Early presentation of hemispheric intracerebral hemorrhage: prediction of outcome and guidelines for treatment allocation. Neurology 44:133–139

Liu DZ, Sharp FR (2011) The dual role of SRC kinases in intracerebral hemorrhage. Acta Neurochir Suppl 111:77–81

Liu DZ, Ander BP, Xu H, Shen Y, Kaur P, Deng W, Sharp FR (2010) Blood–brain barrier breakdown and repair by Src after thrombin-induced injury. Ann Neurol 67:526–533

Lively S, Schlichter LC (2012a) Age-related comparisons of evolution of the inflammatory response after intracerebral hemorrhage in rats. Transl Stroke Res 3:132–146

Lively S, Schlichter LC (2012b) SC1/hevin identifies early white matter injury after ischemia and intracerebral hemorrhage in young and aged rats. J Neuropathol Exp Neurol 71:480–493

Lunardi P (2012) Lobar hemorrhages. Front Neurol Neurosci 30:145–148

MacLellan CL, Girgis J, Colbourne F (2004) Delayed onset of prolonged hypothermia improves outcome after intracerebral hemorrhage in rats. J Cereb Blood Flow Metab 24:432–440

MacLellan CL, Davies LM, Fingas MS, Colbourne F (2006) The influence of hypothermia on outcome after intracerebral hemorrhage in rats. Stroke 37:1266–1270

MacLellan CL, Silasi G, Poon CC, Edmundson CL, Buist R, Peeling J, Colbourne F (2008) Intracerebral hemorrhage models in rat: comparing collagenase to blood infusion. J Cereb Blood Flow Metab 28:516–525

MacLellan CL, Silasi G, Auriat AM, Colbourne F (2010) Rodent models of intracerebral hemorrhage. Stroke 41:S95–S98

MacLellan CL, Paquette R, Colbourne F (2012) A critical appraisal of experimental intracerebral hemorrhage research. J Cereb Blood Flow Metab 32:612–627

Masada T, Hua Y, Xi G, Yang GY, Hoff JT, Keep RF (2001) Attenuation of intracerebral hemorrhage and thrombin-induced brain edema by overexpression of interleukin-1 receptor antagonist. J Neurosurg 95:680–686

Masada T, Hua Y, Xi G, Yang GY, Hoff JT, Keep RF, Nagao S (2003) Overexpression of interleukin-1 receptor antagonist reduces brain edema induced by intracerebral hemorrhage and thrombin. Acta Neurochir Suppl 86:463–467

Masuda T, Hida H, Kanda Y, Aihara N, Ohta K, Yamada K, Nishino H (2007) Oral administration of metal chelator ameliorates motor dysfunction after a small hemorrhage near the internal capsule in rat. J Neurosci Res 85:213–222

Matsumoto K, Hondo H (1984) CT-guided stereotaxic evacuation of hypertensive intracerebral hematomas. J Neurosurg 61:440–448

Mattson MP, Meffert MK (2006) Roles for NF-kappaB in nerve cell survival, plasticity, and disease. Cell Death Differ 13:852–860

Mattson MP, Culmsee C, Yu Z, Camandola S (2000) Roles of nuclear factor kappaB in neuronal survival and plasticity. J Neurochem 74:443–456

Matute C, Ransom BR (2012) Roles of white matter in central nervous system pathophysiologies. ASN Neuro 4:89–101

Mayer SA, Sacco RL, Shi T, Mohr JP (1994) Neurologic deterioration in noncomatose patients with supratentorial intracerebral hemorrhage. Neurology 44:1379–1384

Mayne M, Ni W, Yan HJ, Xue M, Johnston JB, Del Bigio MR, Peeling J, Power C (2001a) Antisense oligodeoxynucleotide inhibition of tumor necrosis factor-alpha expression is neuroprotective after intracerebral hemorrhage. Stroke 32:240–248

Mayne M, Fotheringham J, Yan HJ, Power C, Del Bigio MR, Peeling J, Geiger JD (2001b) Adenosine A2A receptor activation reduces proinflammatory events and decreases cell death following intracerebral hemorrhage. Ann Neurol 49:727–735

Medana IM, Esiri MM (2003) Axonal damage: a key predictor of outcome in human CNS diseases. Brain 126:515–530

Mendelow AD, Unterberg A (2007) Surgical treatment of intracerebral haemorrhage. Curr Opin Crit Care 13:169–174

Mendelow AD, Gregson BA, Fernandes HM, Murray GD, Teasdale GM, Hope DT, Karimi A, Shaw MD, Barer DH (2005) Early surgery versus initial conservative treatment in patients with spontaneous supratentorial intracerebral haematomas in the International Surgical Trial in Intracerebral Haemorrhage (STICH): a randomised trial. Lancet 365:387–397

Miller CM, Vespa PM, McArthur DL, Hirt D, Etchepare M (2007) Frameless stereotactic aspiration and thrombolysis of deep intracerebral hemorrhage is associated with reduced levels of extracellular cerebral glutamate and unchanged lactate pyruvate ratios. Neurocrit Care 6:22–29

Mohadjer M, Braus DF, Myers A, Scheremet R, Krauss JK (1992) CT-stereotactic fibrinolysis of spontaneous intracerebral hematomas. Neurosurg Rev 15:105–110

Mould WA, Carhuapoma JR, Muschelli J, Lane K, Morgan TC, McBee NA, Bistran-Hall AJ, Ullman NL, Vespa P, Martin NA et al (2013) Minimally invasive surgery plus recombinant tissue-type plasminogen activator for intracerebral hemorrhage evacuation decreases perihematomal edema. Stroke 44:627–634

Moxon-Emre I, Schlichter LC (2011) Neutrophil depletion reduces blood–brain barrier breakdown, axon injury, and inflammation after intracerebral hemorrhage. J Neuropathol Exp Neurol 70:218–235

Mun-Bryce S, Wilkerson AC, Papuashvili N, Okada YC (2001) Recurring episodes of spreading depression are spontaneously elicited by an intracerebral hemorrhage in the swine. Brain Res 888:248–255

Mun-Bryce S, Roberts LJ, Hunt WC, Bartolo A, Okada Y (2004a) Acute changes in cortical excitability in the cortex contralateral to focal intracerebral hemorrhage in the swine. Brain Res 1026:218–226

Mun-Bryce S, Wilkerson A, Pacheco B, Zhang T, Rai S, Wang Y, Okada Y (2004b) Depressed cortical excitability and elevated matrix metalloproteinases in remote brain regions following intracerebral hemorrhage. Brain Res 1026:227–234

Murakami K, Kawase M, Kondo T, Chan PH (1998) Cellular accumulation of extravasated serum protein and DNA fragmentation following vasogenic edema. J Neurotrauma 15:825–835

Nakamura T, Keep RF, Hua Y, Schallert T, Hoff JT, Xi G (2004) Deferoxamine-induced attenuation of brain edema and neurological deficits in a rat model of intracerebral hemorrhage. J Neurosurg 100:672–678

Nakamura T, Xi G, Park JW, Hua Y, Hoff JT, Keep RF (2005) Holo-transferrin and thrombin can interact to cause brain damage. Stroke 36:348–352

Nath FP, Jenkins A, Mendelow AD, Graham DI, Teasdale GM (1986) Early hemodynamic changes in experimental intracerebral hemorrhage. J Neurosurg 65:697–703

Nath FP, Kelly PT, Jenkins A, Mendelow AD, Graham DI, Teasdale GM (1987) Effects of experimental intracerebral hemorrhage on blood flow, capillary permeability, and histochemistry. J Neurosurg 66:555–562

O'Brien RJ, Wong PC (2011) Amyloid precursor protein processing and Alzheimer's disease. Annu Rev Neurosci 34:185–204

Otsuka N, Tomonaga M, Ikeda K (1991) Rapid appearance of beta-amyloid precursor protein immunoreactivity in damaged axons and reactive glial cells in rat brain following needle stab injury. Brain Res 568:335–338

Participants NIW (2005) Priorities for clinical research in intracerebral hemorrhage: report from a National Institute of Neurological Disorders and Stroke workshop. Stroke 36:e23–e41

Pizzi M, Spano P (2006) Distinct roles of diverse nuclear factor-kappaB complexes in neuropathological mechanisms. Eur J Pharmacol 545:22–28

Power C, Henry S, Del Bigio MR, Larsen PH, Corbett D, Imai Y, Yong VW, Peeling J (2003) Intracerebral hemorrhage induces macrophage activation and matrix metalloproteinases. Ann Neurol 53:731–742

Powers WJ, Zazulia AR, Videen TO, Adams RE, Yundt KD, Aiyagari V, Grubb RL Jr, Diringer MN (2001) Autoregulation of cerebral blood flow surrounding acute (6–22 h) intracerebral hemorrhage. Neurology 57:18–24

Prabhakaran S, Naidech AM (2012) Ischemic brain injury after intracerebral hemorrhage: a critical review. Stroke 43:2258–2263

Qin ZH, Chen RW, Wang Y, Nakai M, Chuang DM, Chase TN (1999) Nuclear factor kappaB nuclear translocation upregulates c-Myc and p53 expression during NMDA receptor-mediated apoptosis in rat striatum. J Neurosci 19:4023–4033

Qureshi AI, Wilson DA, Hanley DF, Traystman RJ (1999) No evidence for an ischemic penumbra in massive experimental intracerebral hemorrhage. Neurology 52:266–272

Qureshi AI, Ali Z, Suri MF, Shuaib A, Baker G, Todd K, Guterman LR, Hopkins LN (2003) Extracellular glutamate and other amino acids in experimental intracerebral hemorrhage: an in vivo microdialysis study. Crit Care Med 31:1482–1489

Qureshi AI, Mendelow AD, Hanley DF (2009) Intracerebral haemorrhage. Lancet 373: 1632–1644

Ransom BR, Baltan SB (2009) Axons get excited to death. Ann Neurol 65:120–121

Richmon JD, Fukuda K, Maida N, Sato M, Bergeron M, Sharp FR, Panter S, Noble LJ (1998) Induction of heme oxygenase-1 after hyperosmotic opening of blood–brain barrier. Br Res 780:108–118

Rodriguez-Yanez M, Brea D, Arias S, Blanco M, Pumar JM, Castillo J, Sobrino T (2012) Increased expression of Toll-like receptors 2 and 4 is associated with poor outcome in intracerebral hemorrhage. J Neuroimmunol 247:75–80

Rohde V, Schaller C, Hassler WE (1995) Intraventricular recombinant tissue plasminogen activator for lysis of intraventricular haemorrhage. J Neurol Neurosurg Psychiatry 58:447–451

Rosenberg GA (2002) Matrix metalloproteinases in neuroinflammation. Glia 39:279–291

Rosenberg, G.A., and Mun-Bryce, S. 2004. Matrix metalloproteinases in neuroinflammation and cerebral ischemia. *Ernst. Schering. Res Found. Workshop*:1-16.

Rosenberg GA, Mun-Bryce S, Wesley M, Kornfeld M (1990) Collagenase-induced intracerebral hemorrhage in rats. Stroke 21:801–807

Rosenberg GA, Estrada E, Kelley RO, Kornfeld M (1993) Bacterial collagenase disrupts extracellular matrix and opens blood–brain barrier in rat. Neurosci Lett 160:117–119

Rothwell NJ (1999) Annual review prize lecture cytokines: killers in the brain? J Physiol 514: 3–17

Rothwell N, Allan S, Toulmond S (1997) The role of interleukin 1 in acute neurodegeneration and stroke: pathophysiological and therapeutic implications. J Clin Invest 100:2648–2652

Ryu JK, Davalos D, Akassoglou K (2009) Fibrinogen signal transduction in the nervous system. J Thromb Haemost 7(Suppl 1):151–154
Sangha N, Gonzales NR (2011) Treatment targets in intracerebral hemorrhage. Neurotherapeutics 8:374–387
Schachtrup C, Ryu JK, Helmrick MJ, Vagena E, Galanakis DK, Degen JL, Margolis RU, Akassoglou K (2010) Fibrinogen triggers astrocyte scar formation by promoting the availability of active TGF-beta after vascular damage. J Neurosci 30:5843–5854
Schaller C, Rohde V, Meyer B, Hassler W (1995) Stereotactic puncture and lysis of spontaneous intracerebral hemorrhage using recombinant tissue-plasminogen activator. Neurosurg 36:328–333
Selim M (2009) Deferoxamine mesylate: a new hope for intracerebral hemorrhage: from bench to clinical trials. Stroke 40:S90–S91
Selim M, Yeatts S, Goldstein JN, Gomes J, Greenberg S, Morgenstern LB, Schlaug G, Torbey M, Waldman B, Xi G et al (2011) Safety and tolerability of deferoxamine mesylate in patients with acute intracerebral hemorrhage. Stroke 42:3067–3074
Selkoe DJ (1994) Cell biology of the amyloid beta-protein precursor and the mechanism of Alzheimer's disease. Annu Rev Cell Biol 10:373–403
Selmaj KW, Raine CS (1988) Tumor necrosis factor mediates myelin and oligodendrocyte damage in vitro. Ann Neurol 23:339–346
Sharp FR, Lu A, Tang Y, Millhorn DE (2000) Multiple molecular penumbras after focal cerebral ischemia. J Cereb Blood Flow Metab 20:1011–1032
Sherriff FE, Bridges LR, Sivaloganathan S (1994) Early detection of axonal injury after human head trauma using immunocytochemistry for beta-amyloid precursor protein. Acta Neuropathol 87:55–62
Shoamanesh A, Kwok CS, Lim PA, Benavente OR (2013) Postthrombolysis intracranial hemorrhage risk of cerebral microbleeds in acute stroke patients: a systematic review and meta-analysis. Int J Stroke 8:348–356
Si QS, Nakamura Y, Kataoka K (1997) Albumin enhances superoxide production in cultured microglia. Glia 21:413–418
Sinar EJ, Mendelow AD, Graham DI, Teasdale GM (1987) Experimental intracerebral hemorrhage: effects of a temporary mass lesion. J Neurosurg 66:568–576
Song S, Hua Y, Keep RF, Hoff JT, Xi G (2007) A new hippocampal model for examining intracerebral hemorrhage-related neuronal death: effects of deferoxamine on hemoglobin-induced neuronal death. Stroke 38:2861–2863
Song S, Hua Y, Keep RF, He Y, Wang J, Wu J, Xi G (2008) Deferoxamine reduces brain swelling in a rat model of hippocampal intracerebral hemorrhage. Acta Neurochir Suppl 105:13–18
Stadtman ER (2006) Protein oxidation and aging. Free Radic Res 40:1250–1258
Stephenson DT, Rash K, Clemens JA (1992) Amyloid precursor protein accumulates in regions of neurodegeneration following focal cerebral ischemia in the rat. Brain Res 593:128–135
Stephenson D, Yin T, Smalstig EB, Hsu MA, Panetta J, Little S, Clemens J (2000) Transcription factor nuclear factor-kappa B is activated in neurons after focal cerebral ischemia. J Cereb Blood Flow Metab 20:592–603
Stys PK, Lipton SA (2007) White matter NMDA receptors: an unexpected new therapeutic target? Trends Pharmacol Sci 28:561–566
Tejima E, Zhao BQ, Tsuji K, Rosell A, van Leyen K, Gonzalez RG, Montaner J, Wang X, Lo EH (2007) Astrocytic induction of matrix metalloproteinase-9 and edema in brain hemorrhage. J Cereb Blood Flow Metab 27:460–468
Teng W, Wang L, Xue W, Guan C (2009) Activation of TLR4-mediated NFkappaB signaling in hemorrhagic brain in rats. Mediators Inflamm 2009:473276
Thiex R, Kuker W, Muller HD, Rohde I, Schroder JM, Gilsbach JM, Rohde V (2003) The long-term effect of recombinant tissue-plasminogen-activator (rt-PA) on edema formation in a large-animal model of intracerebral hemorrhage. Neurol Res 25:254–262
Toffol GJ, Biller J, Adams HP Jr (1987) Nontraumatic intracerebral hemorrhage in young adults. Arch Neurol 44:483–485

Van Wagoner NJ, Benveniste EN (1999) Interleukin-6 expression and regulation in astrocytes. J Neuroimmunol 100:124–139
Wagner KR (2007) Modeling intracerebral hemorrhage: Glutamate, nuclear factor-kappa B signaling and cytokines. Stroke 38:753–758
Wagner KR, Broderick JP (2001) Hemorrhagic stroke: pathophysiological mechanisms and neuroprotective treatments. In: Lo EH, Marwah J (eds) Neuroprotection. Prominent Press, Scottsdale, pp 471–508
Wagner KR, Brott TG (2007) Animal models of intracerebral hemorrhage. In: Bhardwaj A, Alkayed N, Kirsch J, Traystman R (eds) Acute stroke: bench to bedside. Informa, New York, pp 111–121
Wagner KR, Dwyer BE (2004) Hematoma removal, heme, and heme oxygenase following hemorrhagic stroke. Ann N Y Acad Sci 1012:237–251
Wagner KR, Zuccarello M (2005) Focal brain hypothermia for neuroprotection in stroke treatment and aneurysm repair. Neurol Res 27(3):238–245
Wagner KR, Zuccarello M (2009) Intracerebral hemorrhage: animal models and experimental treatments. In: Carhuapoma JR, Mayer SA, Hanley DF (eds) Textbook of intracerebral hemorrhage. Cambridge University Press, New York, pp 193–205
Wagner KR, Xi G, Hua Y, Kleinholz M, De C, Myers RE, Broderick JP, Brott TG (1996) Lobar intracerebral hemorrhage model in pigs: rapid edema development in perihematomal white matter. Stroke 27:490–497
Wagner KR, Hua Y, Xi G, Dunn RS, Holland SK, Hall NC, Samuel SJ, Williams PM, de Courten-Myers GM, Brott TG et al (1997) Pathophysiologic mechanisms underlying edema development in experimental intracerebral hemorrhage: magnetic resonance studies. Stroke 28:264
Wagner KR, Xi G, Hua Y, Kleinholz M, de Courten-Myers GM, Myers RE (1998) Early metabolic alterations in edematous perihematomal brain regions following experimental intracerebral hemorrhage. J Neurosurg 88:1058–1065
Wagner KR, Xi G, Hua Y, Zuccarello M, de Courten-Myers GM, Broderick JP, Brott TG (1999a) Ultra-early clot aspiration after lysis with tissue plasminogen activator in a porcine model of intracerebral hemorrhage: edema reduction and blood–brain barrier protection. J Neurosurg 90:491–498
Wagner KR, Bryan DW, Hall CL, de Courten-Myers GM, Broderick JP (1999b) White matter injury after intracerebral hemorrhage: infused plasma but not red blood cells induces early DNA fragmentation. J Cereb Blood Flow Metab 19(Suppl 1):S55
Wagner KR, Hua Y, Hall NC, de Courten-Myers GM (1999c) Induction of tumor necrosis factor-alpha in perihematomal edematous white matter following experimental intracerebral hemorrhage. Soc Neurosci Abs 25:1849
Wagner KR, Hua Y, de Courten-Myers GM, Broderick JP, Nishimura RN, Lu SY, Dwyer BE (2000) Tin-mesoporphyrin, a potent heme oxygenase inhibitor, for treatment of intracerebral hemorrhage: in vivo and in vitro studies. Cell Mol Biol (Noisy-le-grand) 46:597–608
Wagner KR, Knight J, Packard BA, de Courten-Myers GM, Smulian AG, Broderick JP (2001) Rapid nuclear factor kappaB activation and cytokine and heme oxygenase-1 gene expression in edematous white matter after porcine intracerebral hemorrhage. Stroke 32:327
Wagner KR, Packard BA, Hall CL, Smulian AG, Linke MJ, De C, Packard LM, Hall NC (2002) Protein oxidation and heme oxygenase-1 induction in porcine white matter following intracerebral infusions of whole blood or plasma. Dev Neurosci 24:154–160
Wagner KR, Sharp FR, Ardizzone TD, Lu A, Clark JF (2003a) Heme and iron metabolism: role in cerebral hemorrhage. J Cereb Blood Flow Metab 23:629–652
Wagner KR, Dean C, Beiler S, Knight J, Packard BA, Hall CL, de Courten-Myers GM (2003b) Rapid activation of pro-inflammatory signaling cascades in perihematomal brain regions in a porcine white matter intracerebral hemorrhage model. J Cereb Blood Flow Metab 23(Suppl 1):277
Wagner KR, Beiler S, Dean C, Hall CL, Knight J, Packard BA, Smulian AG, Linke MJ, de Courten-Myers GM (2004) NF k B activation and pro-inflammatory cytokine gene upregulation in white matter following porcine intracerebral hemorrhage. In: Krieglstein J, Klumpp S

(eds) Pharmacology of cerebral ischemia 2004. Medpharm Scientific Publishers, Stuttgart, pp 185–194

Wagner KR, Dean C, Beiler S, Bryan DW, Packard BA, Smulian AG, Linke MJ, de Courten-Myers GM (2005) Plasma infusions into porcine cerebral white matter induce early edema, oxidative stress, pro-inflammatory cytokine gene expression and DNA fragmentation: implications for white matter injury with increased blood–brain-barrier permeability. Curr Neurovasc Res 2:149–155

Wagner KR, Beiler S, Beiler C, Kirkman J, Casey K, Robinson T, Larnard D, de Courten-Myers GM, Linke MJ, Zuccarello M (2006) Delayed profound local brain hypothermia markedly reduces interleukin-1beta gene expression and vasogenic edema development in a porcine model of intracerebral hemorrhage. Acta Neurochir Suppl 96:177–182

Wan S, Hua Y, Keep RF, Hoff JT, Xi G (2006) Deferoxamine reduces CSF free iron levels following intracerebral hemorrhage. Acta Neurochir Suppl 96:199–202

Wan S, Zhan R, Zheng S, Hua Y, Xi G (2008) Activation of c-Jun-N-terminal kinase in a rat model of intracerebral hemorrhage: the role of iron. Neurosci Res 63(2):100–105

Wang J (2010) Preclinical and clinical research on inflammation after intracerebral hemorrhage. Prog Neurobiol 92:463–477

Wang J, Dore S (2007) Inflammation after intracerebral hemorrhage. J Cereb Blood Flow Metab 27:894–908

Wang J, Tsirka SE (2005) Tuftsin fragment 1–3 is beneficial when delivered after the induction of intracerebral hemorrhage. Stroke 36:613–618

Wang X, Jung J, Asahi M, Chwang W, Russo L, Moskowitz MA, Dixon CE, Fini ME, Lo EH (2000) Effects of matrix metalloproteinase-9 gene knock-out on morphological and motor outcomes after traumatic brain injury. J Neurosci 20:7037–7042

Wang J, Rogove AD, Tsirka AE, Tsirka SE (2003) Protective role of tuftsin fragment 1–3 in an animal model of intracerebral hemorrhage. Ann Neurol 54:655–664

Warkentin LM, Auriat AM, Wowk S, Colbourne F (2010) Failure of deferoxamine, an iron chelator, to improve outcome after collagenase-induced intracerebral hemorrhage in rats. Brain Res 1309:95–103

Wasserman JK, Schlichter LC (2007a) Minocycline protects the blood–brain barrier and reduces edema following intracerebral hemorrhage in the rat. Exp Neurol 207:227–237

Wasserman JK, Schlichter LC (2007b) Neuron death and inflammation in a rat model of intracerebral hemorrhage: effects of delayed minocycline treatment. Brain Res 1136:208–218

Wasserman JK, Schlichter LC (2008) White matter injury in young and aged rats after intracerebral hemorrhage. Exp Neurol 214:266–275

Wasserman JK, Zhu X, Schlichter LC (2007) Evolution of the inflammatory response in the brain following intracerebral hemorrhage and effects of delayed minocycline treatment. Brain Res 1180:140–154

Wasserman JK, Yang H, Schlichter LC (2008) Glial responses, neuron death and lesion resolution after intracerebral hemorrhage in young vs. aged rats. Eur J Neurosci 28:1316–1328

Weller RO (1992) Spontaneous intracranial hemorrhage. In: Adams Jh DLW (ed) Greenfield's neuropathology. Oxford Univ Press, New York, pp 269–301

Wirths O, Breyhan H, Marcello A, Cotel MC, Bruck W, Bayer TA (2010) Inflammatory changes are tightly associated with neurodegeneration in the brain and spinal cord of the APP/PS1KI mouse model of Alzheimer's disease. Neurobiol Aging 31:747–757

Wu J, Hua Y, Keep RF, Schallert T, Hoff JT, Xi G (2002) Oxidative brain injury from extravasated erythrocytes after intracerebral hemorrhage. Brain Res 953:45–52

Wu J, Yang S, Xi G, Song S, Fu G, Keep RF, Hua Y (2008) Microglial activation and brain injury after intracerebral hemorrhage. Acta Neurochir Suppl 105:59–65

Wu H, Cong Y, Wang D, Zhao R, Qi J (2009) Correlation of macrophage inflammatory protein-2 expression and brain edema in rats after intracerebral hemorrhage. Int J Clin Exp Pathol 2:83–90

Wu H, Wu T, Li M, Wang J (2012) Efficacy of the lipid-soluble iron chelator 2,2′-dipyridyl against hemorrhagic brain injury. Neurobiol Dis 45:388–394

Xi G, Wagner KR, Keep RF, Hua Y, De C, Broderick JP, Brott TG, Hoff JT, Muizelaar JP (1998) Role of blood clot formation on early edema development after experimental intracerebral hemorrhage. Stroke 29:2580–2586

Xi G, Keep RF, Hoff JT (2002) Pathophysiology of brain edema formation. Neurosurg Clin N Am 13:371–383

Xue M, Del Bigio MR (2000) Intracortical hemorrhage injury in rats: relationship between blood fractions and brain cell death. Stroke 31:1721–1727

Xue M, Del Bigio MR (2001) Acute tissue damage after injections of thrombin and plasmin into rat striatum. Stroke 32:2164–2169

Yang GY, Betz AL, Chenevert TL, Brunberg JA, Hoff JT (1994) Experimental intracerebral hemorrhage: relationship between brain edema, blood flow, and blood–brain barrier permeability in rats. J Neurosurg 81:93–102

Yao Y, Tsirka SE (2012) The CCL2-CCR2 system affects the progression and clearance of intracerebral hemorrhage. Glia 60:908–918

Yenari MA, Han HS (2006) Influence of hypothermia on post-ischemic inflammation: role of nuclear factor kappa B (NFkappaB). Neurochem Int 49:164–169

Zhang K, Sejnowski TJ (2000) A universal scaling law between gray matter and white matter of cerebral cortex. Proc Natl Acad Sci U S A 97:5621–5626

Zhang Z, Zhang ZY, Wu Y, Schluesener HJ (2012) Immunolocalization of Toll-like receptors 2 and 4 as well as their endogenous ligand, heat shock protein 70, in rat traumatic brain injury. Neuroimmunomodulation 19:10–19

Zhao X, Ou Z, Grotta JC, Waxham N, Aronowski J (2006) Peroxisome-proliferator-activated receptor-gamma (PPARgamma) activation protects neurons from NMDA excitotoxicity. Brain Res 1073–1074:460–469

Zhao X, Zhang Y, Strong R, Zhang J, Grotta JC, Aronowski J (2007a) Distinct patterns of intracerebral hemorrhage-induced alterations in NF-kappaB subunit, iNOS, and COX-2 expression. J Neurochem 101:652–663

Zhao X, Sun G, Zhang J, Strong R, Dash PK, Kan YW, Grotta JC, Aronowski J (2007b) Transcription factor Nrf2 protects the brain from damage produced by intracerebral hemorrhage. Stroke 38:3280–3286

Zhao X, Sun G, Zhang J, Strong R, Song W, Gonzales N, Grotta JC, Aronowski J (2007c) Hematoma resolution as a target for intracerebral hemorrhage treatment: role for peroxisome proliferator-activated receptor gamma in microglia/macrophages. Ann Neurol 61:352–362

Zhao X, Grotta J, Gonzales N, Aronowski J (2009) Hematoma resolution as a therapeutic target: the role of microglia/macrophages. Stroke 40:S92–S94

Chapter 12
White Matter Repair in Subcortical Stroke

Elif G. Sözmen and S. Thomas Carmichael

White matter stroke is a common clinical problem that constitutes up to one-third of all stroke cases (Schneider et al. 2004). Distinct histological changes are found within ischemic white matter such as focal edema, demyelination, axonal damage, loss of oligodendrocytes, and local activation of astrocytes and microglia (Sozmen et al. 2009; Fernando et al. 2006; Jellinger 2007). Unlike grey matter, treatment avenues for white matter stroke have not been explored, such as the opportunities for axonal rescue coupled with successful OPC differentiation and remyelination. The hallmarks of white matter stroke, namely, loss of oligodendrocytes and consequent axonal demyelination, are shared features with late-stage multiple sclerosis (MS), traumatic brain injury (TBI), spinal cord injury (SCI), and Alzheimer's disease. Despite the vast differences in etiology and subsequent immune responses across these diseases, white matter stroke triggers the brain's endogenous, albeit limited, repair capacity similarly to MS and SCI through a distinct cell population termed oligodendrocyte progenitor cells (OPCs) (Sozmen et al. 2009). For all the reasons above, widespread interest has been devoted into the therapeutic potential of OPCs that are ubiquitously scattered throughout the CNS (Gensert and Goldman 1997; Nishiyama 1999; Windrem et al. 2004; Rivers et al. 2008).

The mammalian process of axon myelination is a well-studied subject. The embryonic lineage of myelinating oligodendrocytes that describes the origin of OPCs, their migration, proliferation, and postnatal development are heavily investigated. Although most OPCs successfully differentiate into myelinating oligodendrocytes, a considerable amount remain as uncommitted glia in the adult as a possible reservoir of endogenous progenitors. A fundamental question in biology is whether recapitulating development can overcome the hurdles to repair and regeneration presented in a disease state. The adult OPC is a classic example for answering such a question since this cell comprises 4–8 % of total cells found in the

E.G. Sözmen • S.T. Carmichael (✉)
Department of Neurology, University of California, Los Angeles,
Los Angeles, CA, USA
e-mail: scarmichael@mednet.ucla.edu

S. Baltan et al. (eds.), *White Matter Injury in Stroke and CNS Disease*, Springer Series in Translational Stroke Research 4, DOI 10.1007/978-1-4614-9123-1_12,

CNS as persists in a progenitor state (Dawson et al. 2003). Much of the current information regarding repair mechanisms after white matter injury is derived from toxin/immune-mediated demyelination and SCI models. Many studies indicate that experimental demyelination initiates a robust regenerative response that includes cues for resident OPCs to proliferate, migrate, and partially differentiate into myelinating oligodendrocytes (Franklin 2002). For instance, spontaneous remyelination is documented in early stages of MS as shown in its primary animal model, experimental autoimmune encephalomyelitis (EAE), as well as cuprizone- and lysolecithin-mediated demyelination. Studies conducted in Shiverer mice elegantly demonstrate the extent of OPC myelinating potential in this myelin-deficient mouse strain upon progenitor transplants (Windrem et al. 2004). However, as robust as the OPC regenerative response appears to be, postmortem MS and experimental studies consistently indicate that the failure of OPC differentiation in vivo is the major roadblock to recovery after demyelination. It is currently unclear how complex cell fate decisions are controlled, not only during development but also after a demyelinating injury, as some OPCs complete maturation while some remain undifferentiated or in a progenitor state. Far less is known about the consequences of focal white matter stroke on OPC repair potential and remyelinating events as compared to toxin- or immune-mediated damage.

12.1 OPCs as Adult Progenitors

The OPC is a mysterious cell type that resides in the parenchyma in a quiescent state unless activated. It is often identified by the cell surface proteoglycan NG2 and high levels of the receptor platelet-derived growth factor receptor alpha (PDGFRa) (Sim et al. 2006). During perinatal developmental oligodendrogenesis, the NG2 marker is replaced with Olig1-, GST-pi-, CC1-, and myelin-specific proteins like CNPase, MOG, PLP, and MBP as indicators of a developmental progression toward mature oligodendrocytes (Dimou et al. 2008). Although they share the same list of markers, adult OPCs differ from their perinatal counterparts in cell cycle duration, rates of migration, differentiation, and diverse responses to growth factors (Simon et al. 2011).

In comparison to other cell types in the brain, OPCs contain unique molecular signaling systems. These may provide an opportunity for novel drug targets in white matter repair. Particularly, receptor/ligand systems play major roles in interactions with the local white matter environment and shape OPC commitment toward proliferation or differentiation. For example, OPCs highly express protein tyrosine phosphatase zeta (PTPz), fibroblast growth factor receptor 3 (FGFR 3), PDGFRa, and a unique profile of bone morphogenic protein (BMP) 4 inhibitors that differentially prime these cells for proliferation and maintain the precursor state (McClain et al. 2012). Similarly, OPCs use constitutive Notch1 signaling to activate downstream genes like Mash1 and Hes1 to sustain a less differentiated state (Stidworthy et al. 2004; Zhang et al. 2009). At the later oligodendrocyte stage, additional receptor/

ligand systems become important that potentially can improve the repair process. For instance, endogenous leukemia inhibitory factor (LIF) and ciliary neurotrophic factor (CNTF) receptor signaling are shown to promote survival of oligodendrocytes and limit damage in demyelinating injuries (Butzkueven et al. 2002; Tripathi 2008; Deverman and Patterson 2012). All these mentioned systems have been defined in culture systems or in inflammatory white matter disease models. There has been no systematic work on OPC or mature oligodendrocyte signaling systems in white matter stroke. However, such signaling systems are in a position to synergistically regulate regenerative responses in OPC-mediated white matter repair.

12.2 Major Differences Between White Matter Stroke and Immune-Mediated Demyelination: What Does It Mean for White Matter Repair After Stroke?

MS is defined as selective degeneration of myelin sheaths with relative initial sparing of axons due to dysregulated immune system, at least during the primary demyelination stage of the disease. An important pathological feature of MS is gathered from postmortem studies regarding the early remyelination taking place in the shadow plaques. In these areas of myelin rarefaction, degenerated myelin sheaths are seldom completely replaced and repair is restricted to the rim of the bordering lesion (Patrikios et al. 2006). It is currently unclear when oligodendrocytes are lost after the primary demyelination in MS. The degree of axonal degeneration varies during the course of the disease: it is initiated at the early lesions when acute inflammatory episodes are present, yet continues during later stages even in the absence of overt inflammation (Androdias et al 2010).

Unlike immune-mediated demyelination, white matter stroke does not involve toxic oligodendrocyte cell death. The fact that ischemia affects all cell types leads to white matter lesions exhibiting nonspecific cell death in the affected area. For instance oligodendrocyte and OPC death are observed shortly after focal white matter stroke produced in mice (Sozmen et al. 2009; Souza-Rodrigues et al. 2008). This is followed by loss of myelin and concurrent axonal swelling and dystrophy within the first week of injury (Sozmen et al. 2009; Tanaka et al. 2008). The extent of long-term axonal degeneration and repair has not been assessed in the context of white matter stroke. In this mouse model of the disease, the infarct results in a lesion core devoid of axons and oligodendrocytes (Fig. 12.1a, b). Nonetheless, the lesion rim consists of lightly demyelinated axons that can be a candidate site of post-stroke remyelination akin to the shadow plaques seen in MS (Fig. 12.1b).

In animal models of multiple sclerosis or primary white matter injury (cuprizone, lysolecithin), OPCs respond with cell division, migration to lesion sites, and differentiation into myelin-producing mature oligodendrocytes (reviewed by Franklin and ffrench-Constant 2008). This response has led to the characterization of injury-induced OPCs as “reactive OPCs” (Fancy 2004). Environmental signals govern the behavior of reactive OPCs following injury (Arnett et al. 2003; Sim et al. 2006).

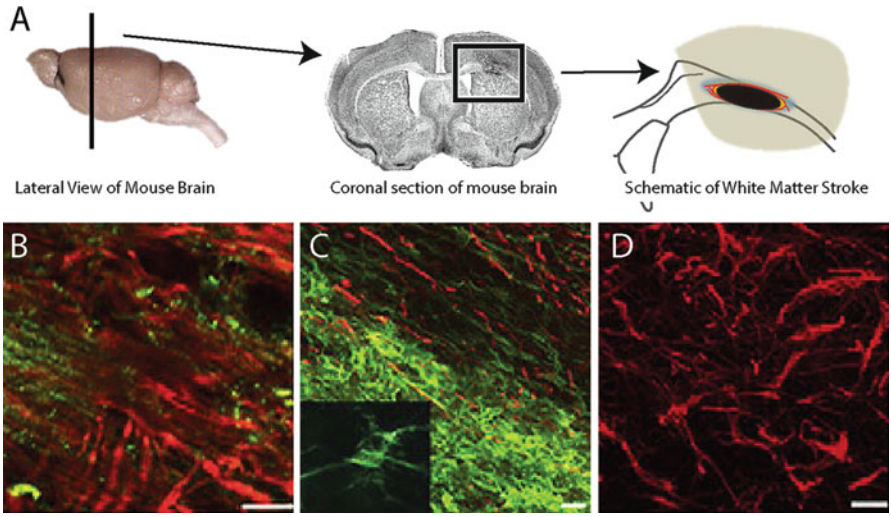


Fig. 12.1 Axonal changes and glial response following white matter stroke. (**a**) Schematic illustration of white matter stroke lesion. The lateral view of the mouse brain on the *left* serves to localize the position of the coronal section (*black line* in the lateral view). The region of the *box* is enlarged in the *rightmost* image of (**a**). The stroke core (*black*) is devoid of axons, myelin, and oligodendrocytes. The peri-infarct zone (*yellow*) contains axons (*red*) with demyelination and increased levels of injured axon marker SMI-32. The stroke lesion is dense with microglia/macrophages that are found in close proximity with peri-infarct axons. High-molecular-weight hyaluronan is deposited in the ipsilateral white matter (*blue*), while the astrocytic response detected with GFAP marker spans an area far larger than the infarct (*beige*). (**b**) Seven days after stroke, axons in the peri-infarct zone labeled with anti-neurofilament antibody (*red*) show significantly decreased levels of myelin indicated by MBP labeling (*green*). (**c**) SMI-32 labeled axons (*red*) are associated with Iba-1-positive activated microglia/macrophage (*green*). Seven-day post-lesion tissue is shown. (**d**) GFAP labeling of 7-day stroke lesion demonstrates astrocytic process hypertrophy after white matter stroke (Sozmen et al. 2009). Scale bars: 40 μm

Because each injury and lesion paradigm varies in terms of cause and overall cellular responses, the specific signals that induce NG2+ OPC division likely vary between conditions. For instance, demyelination-induced changes in the microenvironment, such as upregulated production of BMP-4, FGF-2, PDGF, IGF-1, and heparin-binding growth factor pleiotrophin, can be the prominent factors for OPC migration and proliferation (Franklin and Hinks 1999; Arnett et al. 2004; Fancy 2004; Sim et al. 2006; Albrecht 2003). Yet studies in spinal cord injury models indicate that non-demyelinating inflammatory injury can as well initiate significant OPC amplification and differentiation (Schonberg 2007). In contrast to MS, white matter stroke does not induce a prolonged T and B cell response, which likely translates into a different set of chemokines found in the ischemic white matter lesion. Despite the differences of near-complete loss of axons, ischemia-induced global cell death, and the nature of immune response, OPC differentiation remains limited after white matter stroke in the mouse (Sozmen et al. 2009).

12.3 Promoters of OPC Differentiation

In vitro studies of OPCs illustrate that this cell type is highly responsive to external stimuli, in which cell differentiation can be induced by merely changing the culture medium from PDGF- and FGF2-rich to a more complex multifactorial cocktail (De Vellis 1980; Barres 1999; Colognato 2004). There are a few single factors that are capable of inducing OPC differentiation in vitro such as several subtypes of integrin (fFrench Constant 2004) and vitronectin (Gutowski et al. 1999) (both are components of the extracellular matrix), glutamate release on OPCs (Bergles 2005; Gallo 2009), and secreted factors as in retinoic acid (Barres et al. 1994; Huang et al. 2011) and neurotrophin 3 (Wilson et al. 2003) among many others. However, a combination of factors is required for successful OPC differentiation and remyelination in vivo. Moreover, the multifactorial environment permissive to white matter repair appears to show redundancy. For instance extrinsic regulatory systems such as the extracellular matrix protein dystroglycan, secreted factors CNTF and LIF, as well as chemokines such as CXCL12 have been shown to enhance oligodendrocyte survival and myelination, yet they are often insufficient to promote remyelination on their own (McTigue and Tripathi 2008; Patterson 2005; Woodruff 2004).

Similar to immune-mediated demyelination, white matter stroke in mice triggers a robust regeneration response by resident OPCs that become "reactive" (Sozmen et al. 2009). NG2+ OPCs are recruited in the lesion and surrounding peri-infarct white matter in high numbers within the first week of injury. Furthermore, following white matter stroke OPCs receive proper signals to proliferate as evidenced by BrdU incorporation, an indication of mitotic activity. These results from animal studies indicate OPC proliferation and mobilization steps that are necessary for oligodendrogenesis function normally in the peri-infarct white matter. Therefore, the failure of remyelination after stroke is not likely due to a deficiency of OPCs but instead absent pro-myelinating signals and/or the presence of inhibitory cues acting on OPCs.

Based on the studies investigating acute and chronic injury states of demyelination, the concept of a permissive window to enhance white matter repair emerges as fundamentally important in white matter stroke. The most obvious temporal changes occurring in white matter stroke are astrocytic and microglia responses that are likely to change the milieu of the peri-infarct white matter into an inhibitory environment to the remyelination process. It is yet unclear if a window of opportunity exists after white matter stroke, in addition to neutralizing the effects of excitotoxic injury, so that OPC responses can be modulated to favor differentiation.

12.4 Inhibitors of Oligodendrocyte Regeneration

A number of studies have identified specific OPC differentiation inhibitors in postmortem MS lesions, MS models of disease, models of SCI, and neonatal hypoxic-ischemic injury. It is likely that white matter stroke lesions contain some of the

Table 12.1 Review of prominent negative regulators of OPC differentiation and the factors that promote myelination according to the published reports

Molecular class	Active or associated molecule	Effect	Reference
OPC differentiation			
High-molecular-weight hyaluronan	TLR2	↓	Back et al. (2005), Sloane et al. (2010)
PSA NCAM		↓	Charles et al. (2002), Koutsoudaki et al. (2010)
Notch canonical	Jagged 1	↓	John et al. (2002), Park and Appel (2003)
Notch noncanonical	TIP30	↓	Nakahara et al. (2009)
Semaphorin	Sema3A, Sema 4D	↓	Williams et al. (2007), Syed et al. (2011), Taniguchi et al. (2009)
Wnt	Tcf4, GSK3B	↓	He et al. (2007), Fancy et al. (2009)
SRF		↓	Stritt et al. (2009)
Myelin-associated inhibitors		↓	Kotter et al. (2006), Baer et al. (2009), Syed et al. (2008)
LINGO	p75 NTR	↓	Mi et al. (2005), Bourikas et al. (2010)
NOGO	Nogo-A	↓	Pernet et al. (2008), Yang et al. (2010)
CXCR2		↓	Liu et al. (2010)
DR6		↓	Mi et al. (2011)
CNTF		↓	McTigue and Tripathi (2008)
OPC maturation and survival			
LIF		↑	Kerr and Patterson (2005)
IL-6		↑	Valerio et al. (2002)
Retinoic acid	RXR gamma	↑	Barres et al. (1994), Huang et al. (2011)
Notch noncanonical	F3/contactin	↑	Hu et al. (2003)
BDNF		↑	Chan et al. (2001)
Neuregulins		↑	Wang et al. (2007)
MMPs	MMP 9, MMP 12	↑	Larsen et al. (2006)
CXCL12		↑	Gottle et al. (2010)
NT3		↑	Wilson et al. (2003)
Wnt	Axin2	↑	Fancy et al. (2011)

mentioned changes in the microenvironment that can negatively impact the oligodendrocyte regeneration and remyelination. For the purpose of this chapter, only the major inhibitors will be mentioned. Please refer to Table 12.1 for additional pro- and anti-myelination factors.

12.5 PSA NCAM

Polysialylated neural cell adhesion molecule (PSA NCAM) is an unusual example of an anti-myelination molecule derived by injured axons. Postmortem examination of chronic MS lesions indicates that demyelinated axons paradoxically express high

levels of PSA NCAM that may play a role in stunting OPC differentiation (Charles et al. 2002). Congruent with this finding, oligodendrocyte regeneration and formation of nodes of Ranvier could be enhanced when specific antibodies were used to mask PSA NCAM or when the functional PSA domain was cleaved with endoneuroaminidase N in vitro (Charles 2000). Additionally, Koutsoudaki and colleagues demonstrated elimination of the PSA-synthesizing enzyme in a transgenic mouse line successfully promotes remyelination above baseline in cuprizone-mediated demyelination (Koutsoudaki et al. 2010). The peri-infarct axons remaining in the white matter stroke show limited preservation of nodal and paranodal structures. It is to be found if these select axons are expressing PSA NCAM similar to inactive MS lesions.

12.6 Hyaluronan

Healthy brain tissue contains a steady amount of hyaluronan, an extracellular matrix glycosaminoglycan, for scaffolding and cell support. However, excessive amounts of high-molecular-weight hyaluronan is produced by reactive astrocytes upon injury that is implicated in the inhibition of OPC differentiation (Back et al. 2005). In vitro studies show that high-molecular-weight hyaluronan reduces the expression of mature oligodendrocyte marker MBP and the complexity of myelin sheaths when added to differentiating rat OPCs. Similarly, degradation of hyaluronan with the enzyme hyaluronidase reverses the inhibitory effects on OPC differentiation in the OPC–astrocyte co-culture system (Back et al. 2005). Accumulated high-molecular-weight hyaluronan acts on cell surface receptor CD44 to relay intracellular signaling. It is proposed that hyaluronan–CD44 autocrine signaling regulates astrocytosis after injury to maintain quiescence. For instance, administration of hyaluronidase into quiescent astrocyte cultures in vitro or into normal rat spinal cords in vivo induces proliferation of astrocytes (Struve et al. 2005). Conversely, addition of high-molecular- but not low-molecular-weight hyaluronan into proliferating astrocyte cultures inhibits mitotic activity. Besides astrocytes, OPCs are also shown to express receptor CD44, which appear to have an anti-myelinating effect rather than cell cycle control. For instance, CD44 overexpression in myelinating glia in the CNPase-CD44 transgenic mice induces noninflammatory dysmyelination and progressive demyelination (Tuohy et al. 2004).

Still little is known about the underlying mechanism through which hyaluronan blocks OPC maturation. In addition to CD44, several other cell surface receptors bind to hyaluronan such as Toll-like receptor (TLR) 2 and 4, during the process of dendritic cell, microglia, and macrophage activation (Scheibner et al. 2006; Taylor et al. 2007). Furthermore, hyaluronan binding to TLRs stunts differentiation of several cell types as in osteoblasts and keratinocytes (Passi et al. 2004; Falconi and Aubin 2007). In the same way, hyaluronan acting as a TLR2 ligand is able to inhibit OPC differentiation in vitro, which can be partially reversed by TLR2-binding antibodies and completely prevented in OPCs derived from TLR2-null mouse pups

(Sloane et al. 2010). When hyaluronan was injected into lysolecithin-induced lesions in TLR2 mutant mice, remyelination took place in a similar fashion to lesions generated in wild-type animals that did not receive hyaluronan (Sloane et al. 2010). In sum, CD44 and TLR2 are candidate receptor signaling targets of hyaluronan in white matter injury that may synergistically contribute to the remyelination failure in disease.

Relevant to the findings mentioned above, an elevated amount of hyaluronan was found in chronic MS lesions as well as in mice with EAE (Back et al. 2005). Increased CD44 expression was also detected in the glial scar astrocytes within the chronic MS lesions (Tuohy et al. 2004). Further studies in postmortem chronic MS lesions also show the presence of TLR2 in cells of oligodendrocyte lineage (Sloane et al. 2010). White matter infarcts in the mice display astrocytic processes that are hypertrophied, as a dense population of astrocytes spans the overlying cortex to adjacent striatum, an area far larger than the stroke volume itself. This area corresponding to reactive astrocytosis exhibits significant accumulation of high-molecular-weight hyaluronan that does not resolve at later stages of injury. It is to be determined if the newly born OPCs and the surviving oligodendrocytes express altered levels of CD44 or TLR2 in the peri-infarct white matter and whether neutralizing the respective signaling cascades can ameliorate the OPC differentiation block in vivo.

12.7 Notch1 Signaling

During white matter development, Notch1 receptor interaction with ligands Delta and Jagged activates the canonical Notch pathway, which favors OPC proliferation by inducing *Hes1* and *Hes5* gene expression (D'Souza et al. 2008). Downstream effects of canonical Notch signaling when bound to Jagged-1 have also been implicated in remyelination failure during early stages of MS (John et al. 2002). Astrocytes have been suggested as potential source of OPC Notch1 activation; since Jagged-1 production was increased in cultured astrocytes in response to TGF-b and hypertrophic astrocytes in MS lesions are shown to express Jagged-1 (John et al. 2002). The most convincing evidence regarding an anti-remyelination role of the Notch–Jagged1 system comes from the study conducted by Zhang and colleagues (Zhang et al. 2009) using a transgenic mouse line in which targeted inactivation of Notch1 is driven by oligodendrocyte lineage-specific Olig1 promoter. When Notch1 was inactivated, both OPC differentiation and remyelination processes were accelerated following lysolecithin injury (Zhang et al. 2009). Consistent with the developmental studies, the inhibitory effects of Notch1–Jagged1 signaling appear to be significant during the early stages of oligodendrocyte development in disease, given that conditional ablation of Notch1 under the control of oligodendrocyte-specific PLP promoter does not result in further remyelination (Stidworthy et al. 2004).

In contrast to Notch–Jagged interaction, the noncanonical Notch pathway activation via axon-derived ligand F3/contactin coordinates commitment of cells of the

oligodendrocyte lineage by inducing differentiation of OPCs into mature myelinating oligodendrocytes (Hu et al. 2003). A recent study examining chronic human MS lesions paradoxically found increased levels of contactin and of Notch1-positive OPCs (Nakahara et al. 2009). Further investigation suggests that nuclear localization of the Notch1 intracellular domain necessary for myelogenesis was interrupted in these inactive lesions by abnormal OPC expression of TIP30, an importin inhibitor (Nakahara et al. 2009).

Taken together, both canonical and noncanonical Notch signaling might be influencing OPC differentiation after white matter stroke. Whether such inhibition takes place due to increased production of Jagged1 by hypertrophic astrocytes, a lack of contactin on demyelinated axons, or aberrant expression of TIP30 in OPCs remains to be elucidated in ischemic white matter disease.

12.8 Wnt Signaling

Canonical Wnt–catenin pathway is involved in many developmental processes. It is not surprising that canonical Wnt signaling and the expression of its intranuclear mediator Tcf4 also play a role in oligodendrogenesis during development and are implicated in effective myelin repair after multiple modes of injury (Fancy et al. 2009). Several lines of evidence indicate that Wnt pathway acts cell autonomously in OPCs. Particularly, Tcf4/b-catenin-mediated Wnt signaling functions in the timing of OPC differentiation. When Tcf4 is associated with beta-catenin, it forms a complex that is typically associated with the activation of Wnt downstream genes (van de Wetering et al. 2002) and acts as a negative regulator of myelin gene expression (He et al. 2007). Fancy and colleagues demonstrated that Tcf4 is upregulated in OPCs residing in active MS plaques, in developing rodent brain, and in lesioned adult rodent white matter (Fancy et al. 2009). It is suggested that binding of Wnt ligands to OPCs promotes the stabilization of the beta-catenin/Tcf4 complex, maintaining the OPCs in an undifferentiated state. This hypothesis is confirmed by their use of a transgenic mouse line expressing constitutively active form of beta-catenin in OPC lineage of cells, which results in hypomyelination during development and impaired remyelination after injury. Follow-up remyelination experiments conducted in APC^{Min} mice, which lack the negative regulatory function of beta-catenin antagonist APC, confirm the anti-myelinating role of Wnt pathway (Fancy et al. 2009). Another autoregulatory checkpoint is accomplished by Wnt-induced *AXIN2* expression, which in turn controls the degradation of beta-catenin in various tissue types (Lustig et al. 2002). Therefore, *AXIN2* expression serves as a readout of Wnt pathway activations as well as indication of inhibitory feedback. Fancy and colleagues report abundant *AXIN2* mRNA levels in the OPCs found in neonatal white matter injury samples and in active MS lesions (Fancy et al. 2011). Furthermore, deletion of Axin2 in transgenic mice leads to delayed OPC maturation and perturbed remyelination kinetics following spinal cord demyelination (Fancy et al. 2011).

It is unclear whether dysregulation of Wnt signaling is sufficient to account for OPC differentiation arrest or Wnt signals work synergistically with other negative regulators acting via myelin debris, inflammatory cells, etc. Despite the more likely scenario of multifactorial inhibition of oligodendrogenesis, delivery of an exogenous Axin2 stabilizer was enough to promote myelination in cerebellar slice cultures after acute hypoxia and repair in spinal cords after lysolecithin administration (Fancy et al. 2011). For the purpose of post-stroke white matter repair, it remains to be determined if Wnt pathway is dysregulated in newly born OPCs that maintains the progenitors in an undifferentiated state.

References

Albrecht PJ, Murtie JC, Ness JK, Redwine JM, Enterline JR, Armstrong RC, Levison SW (2003) Astrocytes produce CNTF during the remyelination phase of viral-induced spinal cord demyelination to stimulate FGF-2 production. Neurobiol Dis 13(2):89–101

Androdias G, Reynolds R, Chanal M, Ritleng C, Confavreux C, Nataf S (2010) Meningeal T cells associate with diffuse axonal loss in multiple sclerosis spinal cords. Ann Neurol 68:465–476

Arnett HA, Wang Y, Matsushima GK, Suzuki K, Ting JP (2003) Functional genomic analysis of remyelination reveals importance of inflammation in oligodendrocyte regeneration. J Neurosci 23:9824–9832

Arnett HA, Fancy SP, Alberta JA, Zhao C, Plant SR, Kaing S, Raine CS, Rowitch DH, Franklin RJ, Stiles CD (2004) bHLH transcription factor Olig1 is required to repair demyelinated lesions in the CNS. Science 306:2111–2115

Back SA, Tuohy TM, Chen H, Wallingford N, Craig A, Struve J, Luo NL, Banine F, Liu Y, Chang A, Trapp BD, Bebo BF Jr, Rao MS, Sherman LS (2005) Hyaluronan accumulates in demyelinated lesions and inhibits oligodendrocyte progenitor maturation. Nat Med 11:966–972

Baer AS, Syed YA, Kang SU, Mitteregger D, Vig R, Ffrench-Constant C, Franklin RJ, Altmann F, Lubec G, Kotter MR (2009) Myelin-mediated inhibition of oligodendrocyte precursor differentiation can be overcome by pharmacological modulation of Fyn-RhoA and protein kinase C signalling. Brain 132:465–481

Barres BA, Lazar MA, Raff MC (1994) A novel role for thyroid hormone, glucocorticoids and retinoic acid in timing oligodendrocyte development. Development 120:1097–1108

Barres BA, Mi H (1999) Purification and characterization of astrocyte precursor cells in the developing rat optic nerve. J Neurosci 19(3):1049–1061

Bergles DE, Lin SC, Huck JH, Roberts JD, Macklin WB, Somogyi P (2005) Climbing fiber innervation of NG2-expressing glia in the mammalian cerebellum. Neuron 46(5):773–785

Bourikas D, Mir A, Walmsley AR (2010) LINGO-1-mediated inhibition of oligodendrocyte differentiation does not require the leucine-rich repeats and is reversed by p75(NTR) antagonists. Mol Cell Neurosci 45:363–369

Butzkueven H, Zhang JG, Soilu-Hanninen M, Hochrein H, Chionh F, Shipham KA, Emery B, Turnley AM, Petratos S, Ernst M, Bartlett PF, Kilpatrick TJ (2002) LIF receptor signaling limits immune-mediated demyelination by enhancing oligodendrocyte survival. Nat Med 8:613–619

Chan JR, Cosgaya JM, Wu YJ, Shooter EM (2001) Neurotrophins are key mediators of the myelination program in the peripheral nervous system. Proc Natl Acad Sci U S A 98:14661–14668

Charles P, Reynolds R, Seilhean D, Rougon G, Aigrot MS, Niezgoda A, Zalc B, Lubetzki C (2002) Re-expression of PSA-NCAM by demyelinated axons: an inhibitor of remyelination in multiple sclerosis? Brain 125:1972–1979

Charles P, Hernandez MP, Stankoff B, Aigrot MS, Colin C, Rougon G, Zalc B, Lubetzki C (2000) Negative regulation of central nervous system myelination by polysialylated-neural cell adhesion molecule. Proc Natl Acad Sci 97(13):7585–7590

Colognato H, ffrench-Constant C (2004) Mechanisms of glial development. Curr Opin Neurobiol 14(1):37–44

Colognato H, Ramachandrappa S, Olsen IM, ffrench-Constant C (2004) Integrins direct Src family kinases to regulate distinct phases of oligodendrocyte development. J Cell Biol 167(2):365–375

D'Souza B, Miyamoto A, Weinmaster G (2008) The many facets of Notch ligands. Oncogene 27:5148–5167

Dawson MR, Polito A, Levine JM, Reynolds R (2003) NG2-expressing glial progenitor cells: an abundant and widespread population of cycling cells in the adult rat CNS. Mol Cell Neurosci 24:476–488

Deverman BE, Patterson PH (2012) Exogenous leukemia inhibitory factor stimulates oligodendrocyte progenitor cell proliferation and enhances hippocampal remyelination. J Neurosci 32:2100–2109

De Vellis J, McCarthy KD (1980) Preparation of separate astroglial and oligodendroglial cell cultures from rat cerebral tissue. J Cell Biol 85(3):890–902

Dimou L, Simon C, Kirchhoff F, Takebayashi H, Gotz M (2008) Progeny of Olig2-expressing progenitors in the gray and white matter of the adult mouse cerebral cortex. J Neurosci 28:10434–10442

Falconi D, Aubin JE (2007) LIF inhibits osteoblast differentiation at least in part by regulation of HAS2 and its product hyaluronan. J Bone Miner Res 22:1289–1300

Fancy SP, Zhao C, Franklin RJ (2004) Increased expression of Nkx2.2 and Olig2 identifies reactive oligodendrocyte progenitor cells responding to demyelination in the adult CNS. Mol Cell Neurosci 27(3):247–254

Fancy SP, Harrington EP, Yuen TJ, Silbereis JC, Zhao C, Baranzini SE, Bruce CC, Otero JJ, Huang EJ, Nusse R, Franklin RJ, Rowitch DH (2011) Axin2 as regulatory and therapeutic target in newborn brain injury and remyelination. Nat Neurosci 14:1009–1016

Fernando MS, Simpson JE, Matthews F, Brayne C, Lewis CE, Barber R, Kalaria RN, Forster G, Esteves F, Wharton SB, Shaw PJ, O'Brien JT, Ince PG (2006) White matter lesions in an unselected cohort of the elderly: molecular pathology suggests origin from chronic hypoperfusion injury. Stroke 37:1391–1398

Fancy SP, Baranzini SE, Zhao C, Yuk DI, Irvine KA, Kaing S, Sanai N, Franklin RJ, Rowitch DH (2009) Dysregulation of the Wnt pathway inhibits timely myelination and remyelination in the mammalian CNS. Genes Dev 23:1571–1585

Franklin RJ (2002) Remyelination of the demyelinated CNS: the case for and against transplantation of central, peripheral and olfactory glia. Brain Res Bull 57:827–832

Franklin RJ, Hinks GL (1999) Understanding CNS remyelination: clues from developmental and regeneration biology. J Neurosci Res 58:207–213

Franklin RJ, Ffrench-Constant C (2008) Remyelination in the CNS: from biology to therapy. Nat Rev Neurosci 9:839–855

Gallo V, Gadea A, Aguirre A, Haydar TF (2009) Gallo 2009 should be: Endothelin-1 regulates oligodendrocyte development. J Neurosci 29(32):10047–10062

Gensert JM, Goldman JE (1997) Endogenous progenitors remyelinate demyelinated axons in the adult CNS. Neuron 19:197–203

Gottle P, Kremer D, Jander S, Odemis V, Engele J, Hartung HP, Kury P (2010) Activation of CXCR7 receptor promotes oligodendroglial cell maturation. Ann Neurol 68:915–924

Gutowski NJ, Newcombe J, Cuzner ML (1999) Tenascin-R and C in multiple sclerosis lesions: relevance to extracellular matrix remodelling. Neuropathol Appl Neurobiol 25:207–214

He Y, Dupree J, Wang J, Sandoval J, Li J, Liu H, Shi Y, Nave KA, Casaccia-Bonnefil P (2007) The transcription factor Yin Yang 1 is essential for oligodendrocyte progenitor differentiation. Neuron 55:217–230

Hu QD et al (2003) F3/contactin acts as a functional ligand for Notch during oligodendrocyte maturation. Cell 115:163–175

Huang JK, Jarjour AA, Nait Oumesmar B, Kerninon C, Williams A, Krezel W, Kagechika H, Bauer J, Zhao C, Evercooren AB, Chambon P, Ffrench-Constant C, Franklin RJ (2011) Retinoid X receptor gamma signaling accelerates CNS remyelination. Nat Neurosci 14:45–53

Jellinger KA (2007) The enigma of vascular cognitive disorder and vascular dementia. Acta Neuropathol 113:349–388

John GR, Shankar SL, Shafit-Zagardo B, Massimi A, Lee SC, Raine CS, Brosnan CF (2002) Multiple sclerosis: re-expression of a developmental pathway that restricts oligodendrocyte maturation. Nat Med 8:1115–1121

Kerr BJ, Patterson PH (2005) Leukemia inhibitory factor promotes oligodendrocyte survival after spinal cord injury. Glia 51:73–79

Kotter MR, Li WW, Zhao C, Franklin RJ (2006) Myelin impairs CNS remyelination by inhibiting oligodendrocyte precursor cell differentiation. J Neurosci 26:328–332

Koutsoudaki PN, Hildebrandt H, Gudi V, Skripuletz T, Skuljec J, Stangel M (2010) Remyelination after cuprizone induced demyelination is accelerated in mice deficient in the polysialic acid synthesizing enzyme St8siaIV. Neuroscience 171:235–244

Larsen PH, DaSilva AG, Conant K, Yong VW (2006) Myelin formation during development of the CNS is delayed in matrix metalloproteinase-9 and -12 null mice. J Neurosci 26(8):2207–2214

Liu L, Belkadi A, Darnall L, Hu T, Drescher C, Cotleur AC, Padovani-Claudio D, He T, Choi K, Lane TE, Miller RH, Ransohoff RM (2010) CXCR2-positive neutrophils are essential for cuprizone-induced demyelination: relevance to multiple sclerosis. Nat Neurosci 13:319–326

Lustig B, Jerchow B, Sachs M, Weiler S, Pietsch T, Karsten U, van de Wetering M, Clevers H, Schlag PM, Birchmeier W, Behrens J (2002) Negative feedback loop of Wnt signaling through upregulation of conductin/axin2 in colorectal and liver tumors. Mol Cell Biol 22:1184–1193

McClain CR, Sim FJ, Goldman SA (2012) Pleiotrophin suppression of receptor protein tyrosine phosphatase-beta/zeta maintains the self-renewal competence of fetal human oligodendrocyte progenitor cells. J Neurosci 32:15066–15075

McTigue DM, Tripathi RB (2008) The life, death, and replacement of oligodendrocytes in the adult CNS. J Neurochem 107:1–19

Mi S, Lee X, Hu Y, Ji B, Shao Z, Yang W, Huang G, Walus L, Rhodes K, Gong BJ, Miller RH, Pepinsky RB (2011) Death receptor 6 negatively regulates oligodendrocyte survival, maturation and myelination. Nat Med 17(7):816–821

Mi S, Miller RH, Lee X, Scott ML, Shulag-Morskaya S, Shao Z, Chang J, Thill G, Levesque M, Zhang M, Hession C, Sah D, Trapp B, He Z, Jung V, McCoy JM, Pepinsky RB (2005) LINGO-1 negatively regulates myelination by oligodendrocytes. Nat Neurosci 8:745–751

Nakahara J, Kanekura K, Nawa M, Aiso S, Suzuki N (2009) Abnormal expression of TIP30 and arrested nucleocytoplasmic transport within oligodendrocyte precursor cells in multiple sclerosis. J Clin Invest 119:169–181

Nishiyama A, Chang A, Trapp BD (1999) NG2+ glial cells: a novel glial cell population in the adult brain. J Neuropathol Exp Neurol 58(11):1113–1124

Park HC, Appel B (2003) Delta-Notch signaling regulates oligodendrocyte specification. Development 130:3747–3755

Passi A, Sadeghi P, Kawamura H, Anand S, Sato N, White LE, Hascall VC, Maytin EV (2004) Hyaluronan suppresses epidermal differentiation in organotypic cultures of rat keratinocytes. Exp Cell Res 296:123–134

Patrikios P, Stadelmann C, Kutzelnigg A, Rauschka H, Schmidbauer M, Laursen H, Sorensen PS, Bruck W, Lucchinetti C, Lassmann H (2006) Remyelination is extensive in a subset of multiple sclerosis patients. Brain 129:3165–3172

Patterson PH, Kerr BJ (2005) Leukemia inhibitory factor promotes oligodendrocyte survival after spinal cord injury. Glia 51(1):73–79

Pernet V, Joly S, Christ F, Dimou L, Schwab ME (2008) Nogo-A and myelin-associated glycoprotein differently regulate oligodendrocyte maturation and myelin formation. J Neurosci 28: 7435–7444

Rivers LE, Young KM, Rizzi M, Jamen F, Psachoulia K, Wade A, Kessaris N, Richardson WD (2008) PDGFRA/NG2 glia generate myelinating oligodendrocytes and piriform projection neurons in adult mice. Nat Neurosci 11:1392–1401

Scheibner KA, Lutz MA, Boodoo S, Fenton MJ, Powell JD, Horton MR (2006) Hyaluronan fragments act as an endogenous danger signal by engaging TLR2. J Immunol 177:1272–1281

Schneider AT, Kissela B, Woo D, Kleindorfer D, Alwell K, Miller R, Szaflarski J, Gebel J, Khoury J, Shukla R, Moomaw C, Pancioli A, Jauch E, Broderick J (2004) Ischemic stroke subtypes: a population-based study of incidence rates among blacks and whites. Stroke 35:1552–1556

Schonberg DL, Popovich PG, McTigue DM (2007) Oligodendrocyte generation is differentially influenced by toll-like receptor (TLR) 2 and TLR4-mediated intraspinal macrophage activation. J Neuropathol Exp Neurol 66(12):1124–1135

Sim FJ, Lang JK, Waldau B, Roy NS, Schwartz TE, Pilcher WH, Chandross KJ, Natesan S, Merrill JE, Goldman SA (2006) Complementary patterns of gene expression by human oligodendrocyte progenitors and their environment predict determinants of progenitor maintenance and differentiation. Ann Neurol 59:763–779

Simon C, Gotz M, Dimou L (2011) Progenitors in the adult cerebral cortex: cell cycle properties and regulation by physiological stimuli and injury. Glia 59:869–881

Sloane JA, Batt C, Ma Y, Harris ZM, Trapp B, Vartanian T (2010) Hyaluronan blocks oligodendrocyte progenitor maturation and remyelination through TLR2. Proc Natl Acad Sci U S A 107: 11555–11560

Souza-Rodrigues RD, Costa AM, Lima RR, Dos Santos CD, Picanco-Diniz CW, Gomes-Leal W (2008) Inflammatory response and white matter damage after microinjections of endothelin-1 into the rat striatum. Brain Res 1200:78–88

Sozmen EG, Kolekar A, Havton LA, Carmichael ST (2009) A white matter stroke model in the mouse: axonal damage, progenitor responses and MRI correlates. J Neurosci Methods 180:261–272

Stidworthy MF, Genoud S, Li WW, Leone DP, Mantei N, Suter U, Franklin RJ (2004) Notch1 and Jagged1 are expressed after CNS demyelination, but are not a major rate-determining factor during remyelination. Brain 127:1928–1941

Stritt C, Stern S, Harting K, Manke T, Sinske D, Schwarz H, Vingron M, Nordheim A, Knoll B (2009) Paracrine control of oligodendrocyte differentiation by SRF-directed neuronal gene expression. Nat Neurosci 12:418–427

Struve J, Maher PC, Li YQ, Kinney S, Fehlings MG, Ct K, Sherman LS (2005) Disruption of the hyaluronan-based extracellular matrix in spinal cord promotes astrocyte proliferation. Glia 52: 16–24

Syed YA, Baer AS, Lubec G, Hoeger H, Widhalm G, Kotter MR (2008) Inhibition of oligodendrocyte precursor cell differentiation by myelin-associated proteins. Neurosurg Focus 24:E5

Syed YA, Hand E, Mobius W, Zhao C, Hofer M, Nave KA, Kotter MR (2011) Inhibition of CNS remyelination by the presence of semaphorin 3A. J Neurosci 31:3719–3728

Tanaka Y, Imai H, Konno K, Miyagishima T, Kubota C, Puentes S, Aoki T, Hata H, Takata K, Yoshimoto Y, Saito N (2008) Experimental model of lacunar infarction in the gyrencephalic brain of the miniature pig: neurological assessment and histological, immunohistochemical, and physiological evaluation of dynamic corticospinal tract deformation. Stroke 39:205–212

Taniguchi Y, Amazaki M, Furuyama T, Yamaguchi W, Takahara M, Saino O, Wada T, Niwa H, Tashiro F, J-i M, Kogo M, Matsuyama T, Inagaki S (2009) Sema4D deficiency results in an increase in the number of oligodendrocytes in healthy and injured mouse brains. J Neurosci Res 87:2833–2841

Taylor KR, Yamasaki K, Radek KA, Di Nardo A, Goodarzi H, Golenbock D, Beutler B, Gallo RL (2007) Recognition of hyaluronan released in sterile injury involves a unique receptor complex dependent on Toll-like receptor 4, CD44, and MD-2. J Biol Chem 282:18265–18275

Tripathi RB, McTigue DM (2008) Chronically increased ciliary neurotrophic factor and fibroblast growth factor-2 expression after spinal contusion in rats. J Comp Neurol 510(2):129–144

Tuohy TM, Wallingford N, Liu Y, Chan FH, Rizvi T, Xing R, Bebo B, Rao MS, Sherman LS (2004) CD44 overexpression by oligodendrocytes: a novel mouse model of inflammation-independent demyelination and dysmyelination. Glia 47:335–345

Valerio A, Ferrario M, Dreano M, Garotta G, Spano P, Pizzi M (2002) Soluble interleukin-6 (IL-6) receptor/IL-6 fusion protein enhances in vitro differentiation of purified rat oligodendroglial lineage cells. Mol Cell Neurosci 21:602–615

van de Wetering M, Sancho E, Verweij C, de Lau W, Oving I, Hurlstone A, van der Horn K, Batlle E, Coudreuse D, Haramis AP, Tjon-Pon-Fong M, Moerer P, van den Born M, Soete G, Pals S, Eilers M, Medema R, Clevers H (2002) The beta-catenin/TCF-4 complex imposes a crypt progenitor phenotype on colorectal cancer cells. Cell 111:241–250

Wang Z, Colognato H, Ffrench-Constant C (2007) Contrasting effects of mitogenic growth factors on myelination in neuron-oligodendrocyte co-cultures. Glia 55(5):537–545

Williams A, Piaton G, Aigrot MS, Belhadi A, Theaudin M, Petermann F, Thomas JL, Zalc B, Lubetzki C (2007) Semaphorin 3A and 3F: key players in myelin repair in multiple sclerosis? Brain 130:2554–2565

Wilson HC, Onischke C, Raine CS (2003) Human oligodendrocyte precursor cells in vitro: phenotypic analysis and differential response to growth factors. Glia 44:153–165

Windrem MS, Nunes MC, Rashbaum WK, Schwartz TH, Goodman RA, McKhann G II, Roy NS, Goldman SA (2004) Fetal and adult human oligodendrocyte progenitor cell isolates myelinate the congenitally dysmyelinated brain. Nat Med 10:93–97

Woodruff RH, Fruttiger M, Richardson WD, Franklin RJ (2004) Platelet-derived growth factor regulates oligodendrocyte progenitor numbers in adult CNS and their response following CNS demyelination. Mol Cell Neurosci 25(2):252–262

Yang Y, Liu Y, Wei P, Peng H, Winger R, Hussain RZ, Ben LH, Cravens PD, Gocke AR, Puttaparthi K, Racke MK, McTigue DM, Lovett-Racke AE (2010) Silencing Nogo-A promotes functional recovery in demyelinating disease. Ann Neurol 67:498–507

Zhang Y, Argaw AT, Gurfein BT, Zameer A, Snyder BJ, Ge C, Lu QR, Rowitch DH, Raine CS, Brosnan CF, John GR (2009) Notch1 signaling plays a role in regulating precursor differentiation during CNS remyelination. Proc Natl Acad Sci U S A 106:19162–19167

Chapter 13
White Matter Injury in Subarachnoid Hemorrhage in Humans

Gian Marco De Marchis and Stephan A. Mayer

13.1 Early Brain Injury

The majority (86 %) of poor-grade subarachnoid hemorrhage (SAH) patients (Hunt–Hess 4 or 5) have evidence of ischemic brain injury on diffusion-weighted MRI when performed within 96 h of bleeding onset. These lesions are often bi-hemispheric, symmetric, and are located in multiple vascular territories, most commonly in the midline anterior cerebral artery and medial middle cerebral artery territories (Fig. 13.1) (Wartenberg et al. 2010; Schmidt et al. 2007). Close inspection of representative brain images in this figure shows that the preponderance of injury affects the gray matter, with DWI and FLAIR sequences often showing a selective gyral pattern of injury. However, clear examples of more severe injury involving infarction of both the gray and underlying white matter can be observed (see Fig. 13.1, case 18, left parieto-occipital lobe). This pattern of injury likely reflects the lower metabolic demands and relative resistance of the white matter to ischemic injury.

The clinical relevance of early brain injury has been underestimated because it is rarely visible on CT scans of SAH patients (<5 %) (Schmidt et al. 2007). The causes of early brain injury remain unclear, but there are good candidate mechanisms. Aneurysm bleeding may raise the intracranial pressure to the point that it reaches and offsets mean arterial pressure, leading to transient intracranial circulatory arrest. The intracranial pressure raise may also result into hypothalamic dysfunction and catecholamine surge, leading to neurogenic stunned myocardium with reduction of cardiac output and cerebral ischemia (Mayer et al. 1999; Lee et al. 2006). Ultra-early vasospasm, visible on digital-subtraction angiography (DSA) within 48 h from aneurysm bleeding in 10 % of SAH patients, may also contribute to early

G.M. De Marchis, M.D. • S.A. Mayer, M.D. (✉)
Division of Neurocritical Care, Department of Neurology, New York Presbyterian Hospital/Columbia University Medical Center, New York, NY, USA
e-mail: Sam14@columbia.edu

S. Baltan et al. (eds.), *White Matter Injury in Stroke and CNS Disease*, Springer Series in Translational Stroke Research 4, DOI 10.1007/978-1-4614-9123-1_13,

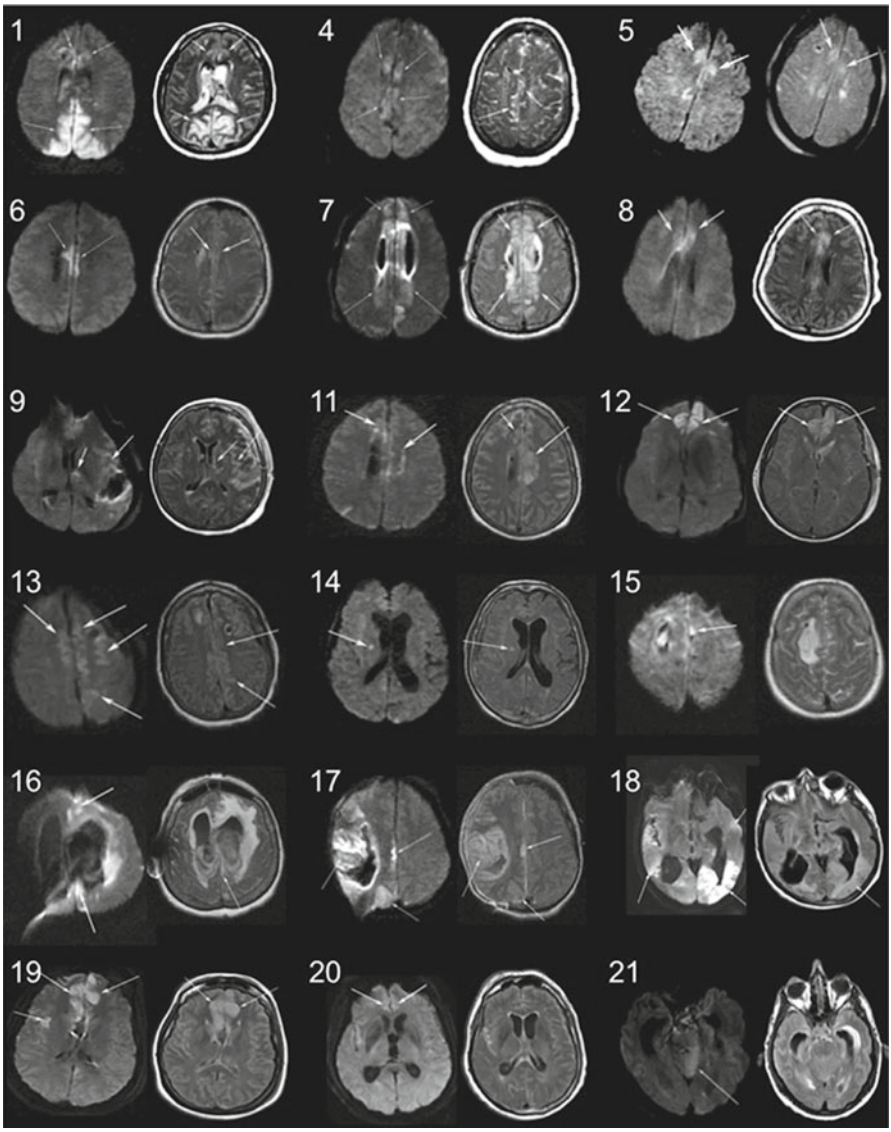


Fig. 13.1 Representative diffusion-weighted imaging (DWI) and fluid attenuated inversion recovery (FLAIR) imaging of ischemic lesions in 18 poor-grade SAH patients with hemorrhage-related ischemic injury. The predominant pattern involved patchy or confluent bilateral ischemic injury involving the anterior cerebral artery territories. In patients 5, 7, 8, 9, 12, 14 imaging was performed in 1 or more days after aneurysm repair (from Wartenberg et al., Neurocritical Care 2010)

brain injury. Ultra-early vasospasm usually resolves within the first 24 h, has an unclear relationship with delayed vasospasm, and may have distinct pathophysiologies (Baldwin et al. 2004). Cortical spreading depression might contribute to early

brain injury through neuronal energy failure, tone increase in intracranial resistance vessels, and hypoperfusion ("spreading ischemia"), but the existence of this process in early brain injury has yet to be documented (Dreier 2011).

Early brain injury after SAH was also visible on MR from canine experimental models. T2-weighted MR sequences were used to estimate vasogenic edema and ADC-weighted MR sequences to estimate cytotoxic edema. T2 and ADC values were measured within the white and gray matter at multiple time points: prior to SAH induction and at 2 and 7 days after SAH induction. In the white matter, the T2 lengthening seen at day 2 and its resolution by day 7 was interpreted as possible early, transient vasogenic edema. In the white and gray matters, the progressive ADC increase was read as possible progressive cytotoxic edema. Overall, this temporal evolution suggested a "shift from early vasogenic edema to a cytotoxic edema in the cortex" by day 7 (Jadhav et al. 2008).

Many aspects of early brain injury remain unclear at this stage given the challenges of performing emergency MRI imaging in critically ill SAH patients. In poor-grade SAH patients, the association between early brain injury and outcome is of clinical interest because it may unveil novel therapeutic pathways in the early stage of SAH.

13.2 Hydrocephalus as Cause of White Matter Injury

Of the three main forms of brain injury that occur after SAH, hydrocephalus is the process that appears to be most closely related to selective white matter injury. Acute hydrocephalus develops in about 20 % of SAH cases, typically in the first 48 h from the initial bleeding (Suarez et al. 2006). The main risk factor for hydrocephalus is large volumes of subarachnoid and intraventricular blood, which results in impairment of cerebrospinal fluid outflow (Jartti et al. 2004). Symptoms always include deterioration of the level of consciousness, possibly with forced downgaze, spastic and cogwheel rigidity, hyper-reflexia, clonus, and flexor or extensor motor posturing as manifestations of white matter pathway torsion and displacement. These motor signs are typically more prominent in the lower than upper extremities. CT shows distension of the brain ventricles, best quantified by the bicaudate index as the "width of the frontal horns at the level of the caudate nuclei, divided by the corresponding diameter of the brain" (van Gijn et al. 1985). In the clinical setting, a bicaudate index of >0.2 is often regarded as a sign of hydrocephalus.

In 49 SAH patients with acute hydrocephalus, CT perfusion showed that mean cerebral blood flow (CBF) was significantly lower in the periventricular white matter and basal ganglia, but not in the cortex, compared to 89 SAH patients without acute hydrocephalus. Hydrocephalus impairs blood flow in the proximity of the ventricles rather than in the cortical gray matter, which probably reflects a combination of flow reductions coupled to impaired tissue metabolism as well as high periventricular tissue pressures, which may limit microcirculatory flow (van Asch et al. 2010). Periventricular blood flow impairment, along with direct mechanical stress, may lead

to periventricular white matter injury through damage of axons and myelin. Increasing severity and duration of hydrocephalus are associated with increasing periventricular white matter injury (Del Bigio 2001). Urgent external ventricular drain (EVD) insertion can be life-saving in SAH patients with acute hydrocephalus leading to brain stem herniation, and EVD can lead to a rapid improvement in the level of consciousness (Komotar et al. 2009). However, it remains unclear to what extent EVD reduces the amount of periventricular white matter injury and if white matter injury due to the physical forces exerted by hydrocephalus is reversible.

13.3 Circulating Biomarkers as Markers of White Matter Injury

Specific biochemical evidence for axonal injury in patients with SAH and acute hydrocephalus is available. The levels of a biomarker for axonal damage, neurofilament heavy chain $(NfH)^{SM135}$, were measured in the CSF gained through EVD of SAH patients with hydrocephalus. NfH^{SM135} levels correlated with the initial severity of SAH as measured on the grading scale of the World Federation of Neurological Surgeons. All patients with a bad outcome showed an increase in NfH^{SM135} CSF levels, and this increase was significant 7 days after SAH onset. Of note, only 8 % of patients with a good outcome showed an increase in the NfH^{SM135} levels (Petzold et al. 2006).

Additional biochemical markers of white matter injury in SAH are matrix metalloprotease (MMP)-9 and S100B. MMP-9, a proteolytic enzyme released from leukocytes and smooth muscle cells, disrupts the blood–brain barrier by degrading the endothelial basal lamina and the extracellular matrix. As early as in the first day after SAH, MMP-9 blood levels are higher than in healthy controls and remain elevated throughout 15 days after SAH. The early rise of MMP-9 blood levels mirrors the early onset of white brain injury. Later on during the course of SAH, brain ischemia from vasospasm further contributes to the elevation of MMP-9 (Fischer et al. 2013).

S100B proteins are a group of calcium-binding proteins in the cytosol of astrocytes. Damage to astrocytes results into extracellular S100B release and leakage of S100B into the blood flow. Mean S100B blood levels over the first 15 SAH days are associated with poor outcome 12 months after SAH. These findings indicate that glial white matter injury might play an important role in terms of outcome after SAH (Sanchez-Pena et al. 2008).

13.4 Delayed Cerebral Injury

Large-vessel vasospasm plays an important role in mediating delayed cerebral ischemia (DCI) after SAH. In recent years, however, it has been increasingly appreciated that microcirculatory dysfunction and tissue spreading depression also contribute to delayed cellular injury and DCI.

Definitions and Their Clinical Relevance: Many different terms are used clinically to refer to vasospasm after SAH, depending on the specific mode of diagnosis and focus on clinical signs and vessel or tissue pathology. Common definitions include the following (Frontera et al. 2009):

- "Transcranial Doppler (TVD) spasm" is defined as any mean flow velocity >120 cm/s. TCD spasms occur in approximately 45 % of SAH patients.
- "Angiographic spasm" is defined as visible narrowing of a large- or a medium-sized vessel on DSA. Angiographic spasms occur in over 50–70 % of SAH patients.
- "Symptomatic vasospasm" is defined as neurological worsening attributed to vasospasm after other causes were eliminated. Symptomatic vasospasm is described in 15–50 % of SAH patients.
- "DCI" is defined as symptomatic vasospasm, delayed infarction from vasospasm, or both. This definition addresses the issue of comatose patients who develop cerebral infarction in the absence of clear symptoms at onset. DCI occurs in 20 % of SAH patients.

Among the definitions above, DCI is most strongly associated with disability or death at 3 months: the odds of death and disability were 2.2 times higher among patients with DCI compared to patients without DCI adjusting for age, Hunt–Hess, and size of the ruptured aneurysm (Frontera et al. 2009).

Risk Factors for DCI from Vasospasm: The most important risk factors for DCI from vasospasm are large amounts of blood in the basilar cisterns and brain ventricles. The additive risk of cisternal and intraventricular blood is reflected by the modified Fisher scale, in which the highest DCI risk is found in the highest risk category (grade 4) with thick cisternal clot and bilateral intraventricular hemorrhage. Other accepted risk factors for DCI include poor clinical grade on admission, untreated dehydration and hypovolemia, and neurogenic cardiopulmonary dysfunction. A systemic literature review found additional evidence for an association between DCI and smoking, diabetes mellitus, hyperglycemia, early systemic inflammatory response syndrome, and hydrocephalus (de Rooij et al. 2012) Preliminary evidence suggests that tonic-cloonic activity at onset of SAH increases the risk of DCI (De Marchis et al. 2013).

Detection of Vasospasm: Vasospasm usually progressively increases in severity between 3 and 10 days after the initial bleeding, maintains at peak severity between days 7 and 14, and resolves thereafter until day 21. Large-vessel vasospasm can be detected by neurological exam, DSA, TCD, and CT angiography/perfusion. DSA is the diagnostic gold standard. TCD can be performed at the bedside and is ideal for daily monitoring, but its dependence on the examiner's skill is reflected into the wide range of sensitivity and specificity for the detection of vasospasm (Saqqur et al. 2007):

Table 13.1 Overview of the principal drugs investigated for the prevention of vasospams

Drug	Efficacy	Safety	Key sentence	Level of evidence[a]
Calcium channel blockers, mainly nimodipine	Reduce the risk of poor outcome (number needed to treat = 19).	IV administration not deemed safe (FDA MedWatch 2006 Feb 15).	"The evidence for nimodipine is not beyond all doubt, but given the potential benefits and modest risks of this treatment, oral nimodipine is currently indicated in patients with aneurysmal SAH." is the *verbatim* bottom line of the COCHRANE review of 16 randomized clinical trials (Dorhout Mees et al. 2007a).	*Mid-level evidence*
Magnesium sulfate	Does not improve outcome.	–	"Intravenous magnesium sulphate does not improve clinical outcome after aneurysmal subarachnoid haemorrhage, therefore routine administration of magnesium cannot be recommended." is the *verbatim* bottom line of the MASH trial (Dorhout Mees et al. 2012).	*Likely reliable evidence*
Endothelin receptor antagonists (clazosentan and TAK-044)	Do not reduce unfavorable outcomes or mortality.	Increased incidence of hypotension and pneumonia.	"ETAs [Endothelin Receptor Antagonists] appear to reduce DIND [delayed ischemic neurological deficit] and angiographic vasospasm but there were adverse events and the impact on clinical outcome is unclear."—from the COCHRANE meta-analysis of four randomized clinical trial including the CONSCIOUS trials (Guo et al. 2012).	*Likely reliable evidence*
Cilostazol	Reduces symptomatic and angiographic vasospasm but does not improve clinical outcomes at 1, 3, and 6 months compared to standard of care.	No severe adverse outcome reported.	"Oral administration of cilostazol is effective in preventing cerebral vasospasm with a low risk of severe adverse events" (Senbokuya et al. 2013).	*Mid level* because the randomized trial was not double blinded

Antiplatelet agents	No significant improvement of clinical outcomes (nonsignificant trend).	Nonsignificantly increased risk of hemorrhagic complications. No conclusive data on aneurysm rebleeding available.	"This review shows a trend towards better outcome in patients treated with antiplatelet agents, possibly due to a reduction in secondary ischaemia. However, results were not statistically significant, thus no definite conclusions can be drawn. Also, antiplatelet agents could increase the risk of haemorrhagic complications. On the basis of the current evidence treatment with antiplatelet agents in order to prevent secondary ischaemia or poor outcome cannot be recommended." is the *verbatim* bottom line of a COCHRANE of 7 randomized clinical trials (Dorhout Mees et al. 2007b).	*Mid-level evidence*
Methylprednisolone, starting 6 h after diagnosis of aneurysm rupture, administered daily for 3 days	No significant reduction of symptomatic vasospasm. Significant reduction of poor outcome at 1 year (secondary end point).	No serious adverse events reported.	"A safe and simple treatment with methylprednisolone did not reduce the incidence of symptomatic vasospasm but improved ability and functional outcome at 1 year after SAH" (Gomis et al. 2010).	*Likely reliable evidence.* Notice that this is a negative trial because the primary end point, reduction of vasospasm, was not met. The study was not powered to show improvement in outcome: it remains to be proven that methylprednisolone improves outcome.
Statins	No statistically relevant effect on vasospasm, DCI, functional outcome, and mortality.	–	"The results of the present systematic review do not lend statistically significant support to the finding of a beneficial effect of statins in patients with aneurysmal subarachnoid hemorrhage as reported in a previous meta-analysis." (Vergouwen et al. 2010) is the *verbatim* bottom line of a systemic review and meta-analysis of four randomized clinical trials.	*Mid-level evidence*

[a]Level of evidence according to *DynaMed* [Internet]. Ipswich (MA): EBSCO Publishing. 1995—Subarachnoid Hemorrhage; [updated 2013 Jan 22; cited 2013 Jan 21]; available from http://web.ebscohost.com/dynamed/search/basic?sid=b2524aee-ae70-4645-b240-aa0e12d5a2cc%40sessionmgr111&vid=9&hid=114. Registration and login required

Blood vessel	Sensitivity (%)	Specificity (%)
Middle cerebral artery (mean arterial velocity of >120–130 cm/s)	38–91	94–100
Anterior cerebral artery (mean arterial velocity of >120–140 cm/s)	13–83	65–100
Vertebral and basilar cerebral artery (mean arterial velocity of >60 cm/s)	44	87.5

More than half of vasospasm in the middle cerebral artery can be missed by TCD, primarily because only the proximal segments of the vessel can be insonated. Moreover, not all elevations in the mean arterial velocity are due to vasospasm; for instance, mean arterial velocities can be increased due to hyperemia without vasospasm, e.g., because of induced arterial hypertension. This shortcoming is addressed by the ratio between mean arterial velocities in the middle cerebral artery and the cervical segment of the internal carotid artery (Lindegaard Index). A Lindegaard Index of >4 is associated with vasospasm of the middle cerebral artery (Lindegaard et al. 1989). Less dependent on the examiner's skills, CT angiography and CT perfusion are increasingly used for the detection of vasospasm. A meta-analysis showed that CT angiography has a sensitivity for vasospasm of the proximal vessels of 90 % and specificity of 93 %. Proximal vessels included the A1 segment of the anterior cerebral artery, the M1 segment of the middle cerebral artery, the anterior communicating artery, the vertebral artery, the basilar artery, the P1 segment of the posterior cerebral artery, and the posterior communicating artery (Greenberg et al. 2010). CT perfusion has a sensitivity of 74 % and a specificity of 93 % for vasospasm compared to the DSA.

Therapy: An overview of the principal drugs investigated for the prevention of vasospasm is available in Table 13.1. At this time, there is no drug whose efficacy in reducing vasospasm and improving outcome is proven beyond any doubt.

References

Baldwin ME, Macdonald RL, Huo D et al (2004) Early vasospasm on admission angiography in patients with aneurysmal subarachnoid hemorrhage is a predictor for in-hospital complications and poor outcome. Stroke 35(11):2506–2511

De Marchis GM, Pugin D, Lantigua H et al (2013)Tonic-clonic activity at subarachnoid hemorrhage onset: impact on complications and outcome. PLoS One 8(8):e71405

de Rooij NK, Rinkel GJ, Dankbaar JW, Frijns CJ (2013) Delayed cerebral ischemia after subarachnoid hemorrhage: a systematic review of clinical, laboratory, and radiological predictors. Stroke 44(1):43–54

Del Bigio MR (2001) Pathophysiologic consequences of hydrocephalus. Neurosurg Clin N Am 12(4):639–649, vii

Dorhout Mees SM, Rinkel GJ, Feigin VL et al (2007) Calcium antagonists for aneurysmal subarachnoid haemorrhage. Cochrane Database Syst Rev (3): CD000277

Dorhout Mees SM, van den Bergh WM, Algra A, Rinkel GJE (2007) Antiplatelet therapy for aneurysmal subarachnoid haemorrhage. Cochrane Database Syst Rev (4): CD00618

Dorhout Mees SM, Algra A, Vandertop WP et al (2012) Magnesium for aneurysmal subarachnoid haemorrhage (MASH-2): a randomised placebo-controlled trial. Lancet 380(9836):44–49

Dreier JP (2011) The role of spreading depression, spreading depolarization and spreading ischemia in neurological disease. Nat Med 17(4):439–447

Fischer M, Dietmann A, Beer R et al (2013) Differential regulation of matrix-metalloproteinases and their tissue inhibitors in patients with aneurysmal subarachnoid hemorrhage. PloS one 8(3):e59952

Frontera JA, Fernandez A, Schmidt JM et al (2009) Defining vasospasm after subarachnoid hemorrhage: what is the most clinically relevant definition? Stroke 40(6):1963–1968

Gomis P, Graftieaux JP, Sercombe R, Hettler D, Scherpereel B, Rousseaux P (2010) Randomized, double-blind, placebo-controlled, pilot trial of high-dose methylprednisolone in aneurysmal subarachnoid hemorrhage. J Neurosurg 112(3):681–688

Greenberg ED, Gold R, Reichman M et al (2010) Diagnostic accuracy of CT angiography and CT perfusion for cerebral vasospasm: a meta-analysis. AJNR Am J Neuroradiol 31(10): 1853–1860

Guo J, Shi Z, Yang K, Tian JH, Jiang L (2012) Endothelin receptor antagonists for subarachnoid hemorrhage. Cochrane Database Syst Rev (9): CD008354

Jadhav V, Sugawara T, Zhang J, Jacobson P, Obenaus A (2008) Magnetic resonance imaging detects and predicts early brain injury after subarachnoid hemorrhage in a canine experimental model. J Neurotrauma 25(9):1099–1106

Jartti P, Karttunen A, Jartti A, Ukkola V, Sajanti J, Pyhtinen J (2004) Factors related to acute hydrocephalus after subarachnoid hemorrhage. Acta Radiol 45(3):333–339

Komotar RJ, Schmidt JM, Starke RM et al (2009) Resuscitation and critical care of poor-grade subarachnoid hemorrhage. Neurosurgery 64(3):397–410

Lee VH, Connolly HM, Fulgham JR, Manno EM, Brown RD, Wijdicks EFM (2006) Tako-tsubo cardiomyopathy in aneurysmal subarachnoid hemorrhage: an underappreciated ventricular dysfunction. J Neurosurg 105(2):264–270

Lindegaard KF, Nornes H, Bakke SJ, Sorteberg W, Nakstad P (1989) Cerebral vasospasm diagnosis by means of angiography and blood velocity measurements. Acta Neurochir 100(1–2):12–24

Mayer SA, Lin J, Homma S et al (1999) Myocardial injury and left ventricular performance after subarachnoid hemorrhage. Stroke 30(4):780–786

Petzold A, Keir G, Kay A, Kerr M, Thompson EJ (2006) Axonal damage and outcome in subarachnoid haemorrhage. J Neurol Neurosurg Psychiatry 77(6):753–759

Sanchez-Pena P, Pereira AR, Sourour NA et al (2008) S100B as an additional prognostic marker in subarachnoid aneurysmal hemorrhage. Crit Care Med 36(8):2267–2273

Saqqur M, Zygun D, Demchuk A (2007) Role of transcranial Doppler in neurocritical care. Crit Care Med 35(5 Suppl):S216–S223

Schmidt JM, Rincon F, Fernandez A et al (2007) Cerebral infarction associated with acute subarachnoid hemorrhage. Neurocrit Care 7(1):10–17

Senbokuya N, Kinouchi H, Kanemaru K et al (2013) Effects of cilostazol on cerebral vasospasm after aneurysmal subarachnoid hemorrhage: a multicenter prospective, randomized, open-label blinded end point trial. J Neurosurg 118(1):121–130

Suarez JI, Tarr RW, Selman WR (2006) Aneurysmal subarachnoid hemorrhage. N Eng J Med 354(4):387–396

van Asch CJJ, van der Schaaf IC, Rinkel GJE (2010) Acute hydrocephalus and cerebral perfusion after aneurysmal subarachnoid hemorrhage. AJNR Am J Neuroradiol 31(1):67–70

van Gijn J, Hijdra A, Wijdicks EF, Vermeulen M, van Crevel H (1985) Acute hydrocephalus after aneurysmal subarachnoid hemorrhage. J Neurosurg 63(3):355–362

Vergouwen MDI, de Haan RJ, Vermeulen M, Roos YBWEM (2010) Effect of statin treatment on vasospasm, delayed cerebral ischemia, and functional outcome in patients with aneurysmal subarachnoid hemorrhage: a systematic review and meta-analysis update. Stroke 41(1):e47–e52

Wartenberg KE, Sheth SJ, Michael Schmidt J et al (2010) Acute ischemic injury on diffusion-weighted magnetic resonance imaging after poor grade subarachnoid hemorrhage. Neurocrit Care 14(3):407–415

Chapter 14
Degenerative Brain Diseases and White Matter Injury

George Bartzokis and Po H. Lu

14.1 Introduction

The human brain has historically been dichotomized into white and gray matter based on the whitish appearance of the exceptionally high lipid content of the myelin sheaths encasing neuronal axons. Unfortunately this nomenclature propagated an artificial "split" in clinical and scientific thinking by promoting the segregation of neurons, whose bodies reside in gray matter, from glia. This dichotomy was particularly unfortunate for the study of the human brain whose gray matter structures including the cortex continue adding oligodendrocytes and myelin well into adulthood and even old age.

The artificial dichotomy between neurons and glia obscures the continuous dynamic communication between all brain cell types that culminates in the achievement and continued maintenance of optimal function of neural networks. This dichotomy has unfortunately also pervaded diagnostic and research efforts by categorizing diseases into many canonical gray matter diseases such as Alzheimer's and Parkinson's disease (AD and PD) and a few canonical white matter diseases such as multiple sclerosis (MS) thus markedly skewing the research focus toward neurons.

G. Bartzokis, M.D. (✉)
Department of Psychiatry, Semel Institute for Neuroscience and Human Behavior, The David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

Greater Los Angeles VA Healthcare System, West Los Angeles, CA, USA
e-mail: gbar@ucla.edu

P.H. Lu, Psy.D.
Department of Neurology, The David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

S. Baltan et al. (eds.), *White Matter Injury in Stroke and CNS Disease*, Springer Series in Translational Stroke Research 4, DOI 10.1007/978-1-4614-9123-1_14,

This chapter will attempt to rebalance these historic biases which undermine a more comprehensive systems biology examination of the dynamic and complex interdependence of the brain's cellular elements as well as their interplay with peripheral and environmental factors. By necessity the chapter will shift the focus onto glia and especially oligodendrocytes and the myelin they produce. This shift is not meant to diminish in any way the key role of neurons that form the basis of the brain's neural networks. Rather, the refocus is necessitated by the ultimate clinical imperative to improve/optimize brain function.

Optimal brain function is based on the timing and synchronous arrival of multiple action potentials at their myriads of destinations. Given the vastly different lengths of the brain's neural networks, the speed of neural transmission determines the timing and synchronization of action potential arrivals (see Sect. 14.3). The speed of transmission is dependent on the axonal size and properties of its myelin sheath that are optimized through complex and poorly understood neuro-glial signaling mechanisms. These signaling mechanisms as well as the cellular and chemical environment of the brain undergo considerable age-related changes driven primarily by a uniquely prolonged myelination process that underlies the exceptional cognitive and behavioral abilities of the human species (Miller et al. 2012; Kemper 1994; Bartzokis et al. 2001). Unfortunately these changes also predispose humans to several unique developmental as well as age-related degenerative brain disorders.

Brain *aging* is the dominant risk factor for the major degenerative brain diseases such as AD, PD, and dementia with Lewy bodies (DLB). The pathognomonic amyloid beta (Aβ) and tau protein deposits that are used to define AD and α-synuclein (αSyn) that is used to define PD and DLB most commonly co-occur with each other together with additional abnormal protein deposits as well as considerable damage due to vascular disease. This multifaceted pathology continues to keep the actual causes of these diseases ambiguous. In addition to aging, many powerful predisposing genetic as well as environmental/lifestyle risk factors have been identified. The genetic factors are currently not easily modifiable and include both genetic mutations involved in production of proteins such as Aβ that cause rare familial phenotypes as well as other common and rare allele variants that somehow increase risk for the highly prevalent sporadic disease phenotypes (Tanzi 2012).

AD is by far the most prevalent and best studied of these diseases. The identified gene variants that increase risk for the sporadic late onset form of AD (LOAD) often have multiple physiologic roles that fall roughly into several general functions: *lipid metabolism* (apolipoprotein E (ApoE), ATP-binding cassette subfamily A member 7 (ABCA7), apolipoprotein J or clusterin (CLU)); *inflammation/immunity* (complement receptor 1 (CR1) (Lambert et al. 2009), triggering receptor expressed on myeloid cells-2 (TREM2) (Guerreiro et al. 2012; Jonsson et al. 2012), and angiotensin converting enzyme (ACE) (Tian et al. 2004; Corneveaux et al. 2010; Saavedra 2012)); and *cellular signaling* (phosphatidylinositol-binding clathrin assembly lymphoid myeloid leukemia protein (PICALM) (Harold et al. 2009)) which was recently discovered to influence *iron metabolism* (Scotland et al. 2012; Lehmann et al. 2010; Castellani et al. 2012).

It is important to note that after aging itself, ApoE is not only the dominant risk factor for LOAD (increasing risk approximately tenfold), but it seems to also be a powerful risk factor for pathologically "pure" DLB (increasing risk sixfold), as well as pathologically "pure" PD (increasing risk threefold) (Tsuang et al. 2013). ApoE also seems to increase risk for multiple other brain disorders ranging from MS to traumatic brain injury as well as brain aging itself. These all share an increased need for brain and specifically myelin "repair", a process that requires remyelination of myelin segments by a newly differentiated oligodendrocyte (reviewed in Franklin and Ffrench-Constant 2008). A better understanding of the processes leading to the exceptional predisposition of the human brain to develop these highly prevalent age-related degenerative brain disorders (AD, PD, DLB) can be achieved by considering the human brain's exceptional cellular composition and the evolutionary changes that made it possible (reviewed in Bartzokis 2004a, 2011b).

14.2 Exceptional White Matter and Myelination of the Human Brain

The brain is classically divided into gray matter (defined as the regions containing neuronal cell bodies and almost all synaptic connections) and white matter (composed primarily of the very long neuronal appendage (axon) that acts as a "wire" connecting widely dispersed neurons plus oligodendrocytes that produce the axon's "insulating" myelin sheaths). The human brain's roughly 100 billion neurons are a small minority of brain cells (10 %). Glia, which are present in both gray and white matter, account for the rest with the following approximate proportions: astrocytes (45 %), oligodendrocytes (35 %), microglia (5 %), and progenitor (NG2) cells (5 %) the vast majority of which differentiate into oligodendrocytes. During brain evolution from nematodes to humans, the number of glia have increased 50-fold more than the number of neurons suggesting that glia have an increasingly important role in the function of larger-brain species (reviewed in Bartzokis 2004a, 2011b).

Myelin may have initially evolved to promote speed of reactions such as those needed for escape or predatory reflexes (Harris and Attwell 2012). As increasingly sophisticated cognitive networks evolved, the role of myelin in optimizing timing and neural network synchrony may have become the paramount reason for its continued exuberant elaboration throughout evolution. Continual life-long oligogenesis is a distinctive oligodendrocyte feature that is central to brain development and plasticity throughout life. Unlike neurons, whose numbers are essentially established at birth, in healthy primates, large numbers of progenitor (NG2) cells (see Sect. 14.4) are produced to support the decades-long processes of postnatal myelination as well as remyelination (Levine et al. 2001; Vinet et al. 2010). In primates, the NG2 cells continue to divide, increasing the number of differentiated oligodendrocytes by as much as 50 % *during adulthood* (O'Kusky and Colonnier 1982; Peters and Sethares 2004;

Peters et al. 2008; Vostrikov et al. 2007; Vostrikov and Uranova 2011) (reviewed in Peters 2009). By dividing and differentiating into oligodendrocytes, NG2 cells can support both continued myelination of additional axons or portions thereof (e.g., intracortical) as well as remyelinate axons when myelin sheaths are damaged and lost (Peters and Sethares 2003; Peters et al. 2008).

During evolution, hyperscaling of white matter relative to gray matter seems to be a feature of all mammals (Zhang and Sejnowski 2000). Gray matter scales in direct proportion to the rest of the brain while white matter volume hyperscales (Barton and Harvey 2000; Schoenemann et al. 2005; Smaers et al. 2010). It is thus not surprising that compared to other primates species, the human brain is not only the largest, but it also has *proportionately* more white matter and especially more frontal lobe white matter. Thus, frontal white matter can be considered a principal component in explaining species differences in brain size and accounts for higher structural connectivity (Smaers et al. 2010) (reviewed in Bartzokis et al. 2011).

The development of frontal white matter seems to be protracted in both chimpanzee and humans (Sakai et al. 2011). However, while myelin is commonly thought of as a component of white matter, gray matter is also extensively myelinated and this process is especially prominent and protracted in the human brain (Miller et al. 2012). The key role of this intracortical myelin (ICM) in optimizing brain function has been generally overlooked (reviewed in Bartzokis 2011b, 2012). Nevertheless humans differ from their closest primate relatives the chimpanzee whose intracortical myelination ceases in young adulthood while in human brain the process continues well into adulthood (Miller et al. 2012). In humans full maturity (as judged by peak frontal lobe white matter *volume*) is not reached until the fifth to sixth decades (Bartzokis et al. 2001). This extensive and protracted myelination has imposed exceptionally high metabolic demands and is associated with vulnerabilities that make the human species highly susceptible to distinctive and highly prevalent brain disorders throughout its lifespan. These include disorders of inappropriate myelin development such as schizophrenia and bipolar disorder (Alba-Ferrara and de Erausquin 2013; Voineskos et al. 2013; Versace et al. 2013) (reviewed in Bartzokis 2002, 2005, 2012) as well as age-related degenerative disorders such as AD, PD, DLB, Huntington's disease (HD), Frontotemporal dementia (FTD), and amyotrophic lateral sclerosis (ALS) (Kang et al. 2013) (reviewed in Bartzokis 2002, 2004b; Bartzokis et al. 2007c; Bartzokis 2011a).

14.2.1 Epigenetic Modifications of Oligodendrocytes Throughout the Lifespan

The continued oligogenesis, myelination, and repair/remyelination processes produce important consequences that may be particularly significant to our understanding of myelin. Repair processes remove the debris from broken down myelin prior to remyelination; therefore, these repair plus remyelination processes may "mask" the key role of myelin maintenance in healthy as well as disease states (reviewed in

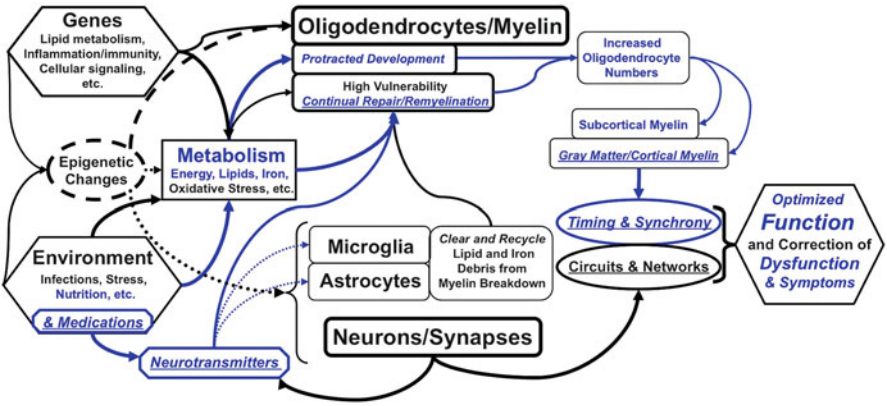


Fig. 14.1 Gene and environment interact to optimize brain function. This schematic depicts the continual and dynamic interrelated processes supporting myelination. As a schematic focused on the key yet underappreciated role of oligodendrocytes and myelin this figure does not depict a myriad of additional relationships such as the ones between genes, environment, epigenetic changes, and metabolism and all five interdependent CNS cell types (NG2 progenitor cells are subsumed under oligodendrocytes) and their specialized structures such as synapses. In the continually dividing and differentiating oligodendrocyte cell line, epigenetic modifications on gene expression can be introduced in each subsequent generation of differentiating cells and thus reflect environmental conditions at different stages of the lifespan (Metzler et al. 2013; Yang et al. 2013; Makinodan et al. 2012). These epigenetic modifications can help explain how at different periods in life, various environmental perturbations such as infections, physical and psychological trauma, metabolic disorders such as hypertension, education/mental/physical activity, and diet can significantly influence temporally distant future risk of developing degenerative brain diseases. *Note: Blue arrows*, *boxes*, and *italics* summarize some of the available modes of interventions at the pharmacotherapeutic and nutritional levels and their potential therapeutic consequences

Bartzokis 2011a). The principal evidence for these processes is indirect and consists of thinner myelin sheaths and shorter segments characteristic of the remyelination process that can be precisely quantified only using electron microscopy. Since remyelinated segments are produced by *newly differentiated* oligodendrocytes, the generalized age-related increase in oligodendrocytes numbers (by as much as 50 % *during adulthood*) are also indirect evidence of the pervasive repair/remyelination of the CNS (reviewed in Peters 2009; Peters and Kemper 2012). These *lifelong repair/remyelination processes* also enrich the prospect that new "generations" of oligodendrocytes are subject to different epigenetic modifications of gene expression. Therefore, as a cell class, oligodendrocytes may be exceptionally susceptible to environmental–genetic interactions throughout the lifespan (Fig. 14.1).

A model of brain development that includes oligodendrocytes and the myelin they produce reframes the human lifespan in terms of seamless quadratic-like (inverted U) myelination trajectories of the myriad of neural networks that underlie cognition and behavior. This perspective redefines human brain "development" as roughly the first *five decades* of life. Within this framework dysregulations occurring during the increasingly complex stages of the myelination process contribute to

several early-life neuropsychiatric disorders defined by overlapping (comorbid) cognitive and behavioral symptom clusters (reviewed in Bartzokis 2002, 2005, 2011b). This myelin-centered perspective "cuts" across current symptom-based classifications of neuropsychiatric diseases and helps explain why the dysfunction manifest in the entire cadre of symptoms that define classic psychiatric disorders (psychosis, depression, obsessions, compulsions, poor impulse control, etc.) can reappear in the dementias of old age (Bartzokis 2011a). This perspective suggests that both *developmental* deficits/dysregulation of myelination of neural networks that contribute to the earlier-life psychiatric disorders, as well as *degenerative* breakdown and loss of myelin of *the same networks* occurring in the dementias of old age, can result in similar behavioral and cognitive symptoms despite entirely different etiologies (Bartzokis 2011a, b).

The detection and study of myelination-associated disorders is made more complex by the lifelong dynamism of myelination, repair, and remyelination as well as epigenetic modifications (Fig. 14.1). Epigenetic modifications of gene expression result from environmental effects that alter nuclear chromatin through methylation and demethylation reactions and histone modifications. These modifications alter gene expression of cells as they differentiate and thus affect subsequent cell function (Grayson et al. 2009; Kim et al. 2008; Liu et al. 2007; Popko 2008; Shen et al. 2006, 2008). Environmental insults (defined as deviations from optimal conditions and include brain and peripheral diseases as well as environmental stressors such as malnutrition and psychosocial trauma) that occur in earlier stages of development can thus have both immediate as well as long-term effects on developmental/repair/remyelination processes through epigenetic changes of gene expression.

Myelination itself can also contribute to age-related changes in the brain's own intrinsic environment (reviewed in Bartzokis 2011a; Bartzokis and Lu 2009). Because oligodendrocytes require large amounts of iron in order to differentiate (Sow et al. 2006), later differentiating oligodendrocytes precursors (NG2 cells) differentiate in a brain environment with more oligodendrocytes and higher iron levels (reviewed in Bartzokis 2011a). Nevertheless, the precursors that differentiate in adulthood or even old age continue to have "developmental" requirements (e.g., nutrition and iron, see next section) similar to the ones present in infancy although their environment (internal (CNS) and external (stress, diet, etc.)) is markedly different. These requirements may persist or even increase in older ages as an increasingly iron-rich CNS environment may promote oxidative stress and thus accelerate the need for repair/remyelination. The changing environments could introduce epigenetic changes that may contribute to the commonly observed age-related decline in myelin repair/remyelination efficiency (Shen et al. 2008). Without adequate interventions this slowed repair/remyelination ability would make age-related cognitive decline and dementia inevitable for the vast proportion of the population (reviewed in Bartzokis 2011a).

In summary, myelin may arguably represent the "weakest link" of both brain development as well as age-related degeneration and thus contribute to many of the normal as well disease-related changes in brain function over the entire human lifespan.

Including glia and myelin in a conceptual "myelin model" of the human brain can help explain normal brain function, clinical and pathologic phenomenology of multiple diseases, as well as their shared responsiveness to pharmaceutical and other (e.g., nutritional) interventions (reviewed in Bartzokis 2011a; Bartzokis et al. 2011).

14.2.2 Metabolic Requirements of an Exceptionally Myelinated Brain Increase Vulnerability

Compared to other brain cells oligodendrocytes have extreme metabolic requirements of energy, iron, omega-3 fatty acids (especially docosahexaenoic acid (DHA)), and cholesterol which render them the most vulnerable cell type in the brain (reviewed in Bartzokis 2004b, 2011a, b). The production and maintenance of the myelin sheath(s) that is up to 600× the surface area of oligodendrocyte soma membrane and 100× the weight of the soma (Morell and Toews 1984; Wiggins 1982) makes the energy requirements of oligodendrocytes 2–3-fold higher than other brain cells (Connor and Menzies 1996; Sanchez-Abarca et al. 2001). An evolutionary switch occurred among primates changing lactate dehydrogenase function from supporting primarily anaerobic to oxidative metabolism (Syner and Goodman 1966). Thus in brain, astrocytes produce lactate to supply metabolic needs of neurons (Ames 2000) and especially oligodendrocytes, which use of 80 % of all lactate to synthesize lipids at a sixfold greater rate than neurons and astrocytes while their use of glucose for this purpose is twofold greater (Sanchez-Abarca et al. 2001; Rinholm et al. 2011).

Cells with high metabolism such as oligodendrocytes and neurons are intrinsically more vulnerable. This is due in part to their elevated levels of damaging oxidative reactions because approximately 2–3 % of oxygen consumed in normal mitochondrial respiration is obligatorily transformed into free radicals. Such metabolic stress makes oligodendrocytes highly vulnerable to a variety of insults ranging from hypoperfusion, toxic products of activated microglia and inflammation, other free radicals, to heavy metals and excitotoxicity (for review see Bartzokis 2004b, 2011a). These metabolic demands are even higher for oligodendrocyte precursors that are actively myelinating axon segments. Precursors produce three times their own weight in membrane lipids each day and are even more exquisitely vulnerable than mature cells (reviewed in Bartzokis 2004a, 2011a).

Oxidative stress can be exacerbated by the presence of iron that accelerates free radical damage. Oligodendrocytes have the highest iron content of all brain cell types (reviewed in Todorich et al. 2009) and as much as 70 % of brain iron may be associated with myelin (de los Monteros et al. 2000; Quintana et al. 2006). Developmental and repair/remyelination processes increase the number of oligodendrocytes with age and may underlie the age-related increase in brain iron (Hallgren and Sourander 1958; Bartzokis et al. 2007d). Iron levels are further increased in degenerative diseases of old age such as AD (Quintana et al. 2006;

Oakley et al. 2007; Bartzokis et al. 2007b) possibly because of increasing numbers of oligodendrocytes that are produced during remyelination attempts. In addition to promoting the production of toxic free radicals, elevated iron may also compromise a variety of essential functions such as phagocytosis and lysosomal activity (Chen et al. 2009) contributing to accumulation of lipofuscin (an undegradable intracellular byproduct of oxidation reactions) (Terman and Brunk 2004; Johnstone and Milward 2010). These changes can help explain the age-related slowing of myelin repair (Shields et al. 1999; Shen et al. 2008) and the exceptional vulnerability of later-myelinating oligodendrocytes such as the intracortical ones (see Sect. 14.3). Not surprisingly, oxidative stress has been consistently observed in psychiatric disorders of development such as schizophrenia and bipolar disorder as well as degenerative disorders of older age such as AD, PD, and DLB (reviewed in Bartzokis 2004b, 2011a).

The human brain is particularly vulnerable to oxidative stress as it consumes a disproportionate amount of the body's total energy expenditure (20 %) compared to other species (13 % in monkeys and 2–8 % in other vertebrates). This striking shift in resource use was needed to support our exuberant myelination and was made possible by evolutionary adaptations in lipid and energy metabolism. Brain-driven evolutionary shifts in human metabolism were partly "subsidized" by substantial reductions in the length and energy consumed by the gastrointestinal system. This made foods with high-nutritional content as well as increased absorption of nutrients through cooking key human requirements (Leonard et al. 2007). The evolutionary shifts in proportions of metabolic resources dedicated to brain are even more striking in developing infants. At 18 months human infants reach a peak requirement of 60 % of the body's energy dedicated to brain development and growth. To support these high brain requirements through periods of nutritional deprivation the human species has evolved increased body fat deposits compared to other primates: 10 % in men, 20 % in women, peaking at 25 % in 18 month infants (Leonard et al. 2007). Body fat serves as a source of lipids as well as storage for essential omega-3 fatty acids such as DHA, a crucial brain building block that is concentrated in myelin as part of plasmalogen phospholipids (reviewed in Bartzokis 2011b). In women, DHA stores in body fat are twice as large as in men. These DHA stores are released primarily during pregnancy and lactation to support newborn brain development, and this sequestration of DHA in women may make them *more* vulnerable than men to cognitive deficits when subjected to DHA deficient diets (Lassek and Gaulin 2012).

The brain also contains a disproportionate amount (25 %) of the body's cholesterol (Bjorkhem 2006). The bulk of brain cholesterol is incorporated into myelin at disproportionately high concentrations (twofold higher than other plasma membranes) because it is required for myelin formation and stability. Since the brain produces all of its cholesterol de novo, it is not surprising that additional adaptations have evolved for more efficient cholesterol metabolism, transport, and recycling. Apolipoprotein E (ApoE) alleles are responsible for most of the brain's cholesterol transport. Humans evolved ApoE3 and E2 alleles which are carried by

approximately 70 and 10 % of the population respectively with less than 20 % carrying the ancestral (primate) ApoE4 allele (reviewed in Bartzokis 2004a, 2011b). ApoE4 is the sole allele present in nonhuman primates who nevertheless do *not* develop AD as ApoE4 is sufficiently efficient to support the metabolic needs of a brain with 20-25 % less myelin than humans. On the other hand, despite its presence in less than 20 % of the human population ApoE4 accounts for as much as 50 % of the genetic risk of AD (Lahiri et al. 2004; Raber et al. 2004; Ashford 2004) and the great majority of LOAD cases with an onset before age 80 (Raber et al. 2004). The ApoE4 allele also confers greater risk for a wide variety of other brain insults that put a premium on efficient lipid recycling of membrane/lipid debris to speed repairs (Tsuang et al. 2013) (reviewed in Bartzokis 2011b).

Compared to other species, these evolutionary adaptations permitted humans to devote a greater proportion of their brain to white matter (approximately 50 %), half of which is composed of myelin. This investment made it possible to achieve the exceptional information processing capacity that underlines the intellectual and behavioral repertoires which defines the human species (reviewed in Bartzokis 2011b). In humans, the brain's myelinated white matter volume continues expanding until middle age (Bartzokis et al. 2001) despite the fact that in late childhood (age 12) the human skull becomes rigid, ending further brain expansion. Myelin volume expansion is coordinated with the "pruning" (elimination) of 30–40 % of the synapses and axons that continues well into adulthood (age 38) (Petanjek et al. 2012). This pruning process can be reconceptualized as a key permissive step (imposed by the cessation of skull enlargement) for the myelination-driven cognitive and behavioral development of humans on their way to becoming healthy adults (reviewed in Bartzokis 2005). Thus, in order for myelin expansion to continue into adulthood, synapses and axons are sacrificed. It is important to note that such massive losses of synapses and axons do *not* result in cognitive decline or dementia. On the contrary, the losses allow the brain to optimize neural network timing and synchrony by continuing to increase myelin content until it peaks in middle age at approximately 25 % of brain volume (reviewed in Bartzokis 2005; Bartzokis and Lu 2009).

In nonhuman primates the synapse and axon pruning processes occur simultaneously in all cortical regions (Rakic 2002). In humans however, these processes are heterochronous and substantial elimination of synaptic spines continues in late-myelinating prefrontal cortex into the fourth decade (Petanjek et al. 2012). The human brain differs from other primate species not only in the higher proportion dedicated to myelin but also in the temporal extent and locations of this added myelination. Unlike our closest primate relative the chimpanzee, humans continue developing their *intracortical* myelin (ICM) well past the late teens and into middle age (Miller et al. 2012; Kemper 1994; Bartzokis et al. 2001). This specialized ICM may be especially pertinent to optimizing human neural network synchrony as well as providing the last opportunity to compensate for any damage-induced delay in subcortical signal transmission by increasing *intracortical* transmission speed and thus restoring optimal network synchrony (reviewed in Bartzokis 2012) (see Sect. 14.3).

14.3 Both White and Gray Matter Myelination Determines Function of the Brain "Internet" Throughout the Lifespan

Although the brain is routinely conceptualized as a singular entity, it is composed of a myriad of interacting neural networks that have different myelin development and degeneration/repair/remyelination trajectories. Imaging and postmortem studies show that even at the gross lobar level, the different trajectories reach peak myelination at different ages with frontal and temporal lobes myelinating last (Bartzokis et al. 2001; Kemper 1994). These different trajectories are supported by oligodendrocytes that become increasingly more complex the later in life they differentiate. They range from robust oligodendrocytes that myelinate a single axon segment with over 100 wraps of myelin membrane in the early-myelinating motor and sensory regions/networks to more vulnerable oligodendrocytes that myelinate upwards of 50 different axon segments with less than 10 wraps in late-myelinating intracortical regions (Fig. 14.2) (reviewed in Butt and Berry 2000). The structurally more complex and metabolically overextended later-myelinating oligodendrocytes and their myelin are especially vulnerable during both developmental (Uranova et al. 2004; Stark et al. 2004) (Vostrikov et al. 2007; Vostrikov and Uranova 2011) as well as degenerative phases (Marner et al. 2003; Bartzokis et al. 2004, 2012) of the lifespan myelination trajectories (reviewed in Bartzokis 2011b). From the perspective of the exceptionally myelinated human species, the development and maintenance/repair of myelin's integrity may be the single-most important as well as most vulnerable element for acquiring and maintaining optimal cognitive and behavioral functions (reviewed in Bartzokis 2004a, b, 2011a; Bartzokis et al. 2012).

Human brain myelination has a quadratic-like (inverted "U") trajectory across the lifespan with increasing myelin content that peaks in middle-age (Kemper 1994; Bartzokis et al. 2001, 2012). The "connectivity" provided by myelination consists of increased action potential transmission speed (over 100-fold) as well as decreased refractory time (34-fold) which increases the number of action potentials that can be transmitted per unit time (in Internet terminology this would represent expanded "bandwidth"). Myelination thus potentially increases our brain's "Internet" information processing capacity by over 3,000-fold, making human myelination indispensable for developing our species' elaborate cognitive functions (reviewed in Bartzokis et al. 2011). Human cognitive functions are also highly dependent on *later*-myelinating oligodendrocytes. These cells myelinate the circuitry of our neural networks all the way to the neuron bodies located in gray matter structures such as the cortex. The extensive intracortical myelination process occurs *after* childhood (O'Kusky and Colonnier 1982; Miller et al. 2012) and basically "upgrades" neural networks with immediate response capacity such that they are essentially "on line" and process information much more quickly and precisely (reviewed in Bartzokis 2011b).

Most important, however, continued myelination and repair processes allow neural networks to remain "plastic" (e.g., adapt) (Scholz et al. 2009; Bengtsson et al. 2005) and thus control the timing of action potential arrival at their destination (Fig. 14.2). Timing is a key metric of brain function and directly influences a wide range of

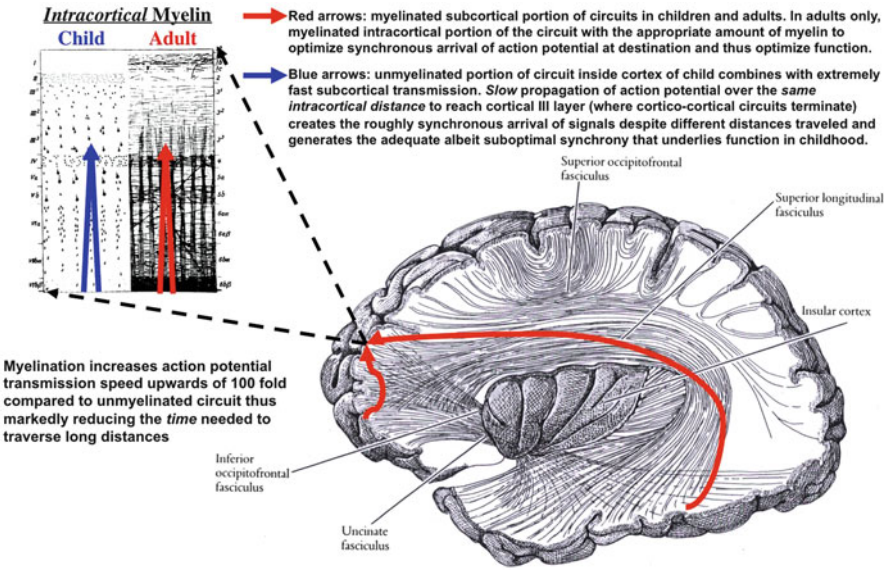


Fig. 14.2 Intracortical myelin (ICM) elaborated during adulthood has a key role in optimizing network synchrony and compensating for subcortical insults. Cortical myelination underlies a key mechanism of brain plasticity and its disturbance could have important consequences for disease pathophysiology (Bartzokis 2012). The importance of intracortical myelin optimizing brain function and compensating for subcortical transmission delays is supported by observations from multiple sclerosis (MS), a canonical myelin disease, and Alzheimer's disease (AD), a canonical "cortical" disease. Until recently myelin-destroying intracortical MS lesions, which postmortem data show represent as much as 60 % of MS lesions, were under-appreciated due in part to difficulty in detecting them on MRI (Wegner et al. 2006; Moll et al. 2008) (reviewed in Simon et al. 2010). Prospective studies show that absence of such cortical lesions is associated with a favorable clinical and cognitive outcome independent of deep white matter lesion accumulation (Calabrese et al. 2009). Conversely, the presence and progression of *intracortical* MS lesions are most strongly associated with cognitive decline (including processing speed and memory) (Roosendaal et al. 2009). These phenomena can be parsimoniously explained by the plasticity of ICM and its ability to compensate for subcortical delays in transmission and reestablishing network synchrony by augmenting ICM. Thus, only when the optimizing/compensatory role of ICM is lost to intracortical demyelination would subcortical delays fully manifest as degraded network synchrony and function and thus become observable as clinical symptoms. Adapted from Bartzokis (2012)

cortical operations (Klausberger and Somogyi 2008). The plasticity of myelin makes it possible to *synchronize* the arrival of action potentials at disparate sites across many brain regions and thus makes cognitive integration of divergent information streams (e.g., gestalts) possible. Complex neuro-glial signaling (between neurons and oligodendrocytes and their precursors) allows for continual optimization of timing and synchrony of network function and thus facilitates learning and behavioral improvements (Als et al. 2004; Szeligo and Leblond 1977; Diamond et al. 1972). In gray matter regions where the vast majority of neurotransmitters are released, neurotransmitters themselves can serve as a key avenue of neuro-glial signaling to direct the appropriate myelination that will optimize timing and synchrony of neural networks (reviewed in Bartzokis 2011b).

The first step towards network synchronization is achieved in childhood by myelinating the *subcortical* white matter portion of axons connecting widely distributed brain regions into functional networks (Fig. 14.2). This initial subcortical myelination can be initiated/directed by neuronal signals themselves (Ziskin et al. 2007; Fields and Burnstock 2006) (reviewed in Butt 2011) and results in the remarkably faster conduction (>100 times faster than unmyelinated axon) between widely separated gray matter regions. Once subcortical myelination is achieved, the total conduction time between these highly dispersed regions becomes primarily dependent on the much longer time (roughly ten times) action potentials spend traversing the short but *unmyelinated* portion of axons within cortex. This *intracortical* distance to a *specific* neuronal layer is roughly *constant*. The constant intracortical distance to layer III (which receives most of the cortico-cortical input), together with the slow intracortical signal propagation, establishes the initial roughly synchronous arrival of action potentials to all cortical regions despite different distances (Salami et al. 2003; Kimura and Itami 2009). The rough network synchrony achieved by this process underlies the vast repertoire of cognitive and behavioral abilities that can be achieved in childhood albeit few of these functions or their integration are "perfected"/optimized at that early stage of life (reviewed in Bartzokis 2011b, 2012).

The short *intracortical* portion of axonal propagation (that is largely unmyelinated in childhood) exerts a markedly disproportionate influence on network synchronicity. Beyond childhood, even faster transmission as well as exquisitely more precisely synchronized timing can be achieved by adding the appropriate amounts of myelin to the intracortical portion of fibers. As Fig. 14.2 depicts, this *later-developed* acceleration and "fine grained" synchronization of cognitive and behavioral networks may continue to be refined over the entire first six decades of life by cortical oligodendrocytes. This later-differentiating subgroup of oligodendrocytes seem to differ in subtle ways from their subcortical counterparts (Bauer et al. 2002; Power et al. 2002; Noble et al. 2003; Kessaris et al. 2006; Schulz et al. 2011) as may the composition of the myelin they produce (Hartman et al. 1982; Butt and Berry 2000).

Differences between oligodendrocytes may be most acutely pertinent to the protracted ICM development in humans. Oligodendrocyte heterogeneity based on location of origin such as dorsal versus ventral origin in spinal cord (Tripathi et al. 2011) or corpus callosum versus overlying cortex (Kessaris et al. 2006) has been reported. These differences can have important physiologic consequences. For example, the oligodendrocytes residing in gray matter seem to differ from subcortical white matter oligodendrocytes in their iron efflux abilities (Schulz et al. 2011) resulting in higher iron accumulation in gray matter oligodendrocytes. This difference may help explain the age-related accumulation of iron in cortical and subcortical gray matter regions (Hallgren and Sourander 1958; Bartzokis et al. 2007d) and the higher vulnerability of gray matter myelin to Aβ toxicity (Desai et al. 2009; Mitew et al. 2010; Behrendt et al. 2013) (reviewed in Bartzokis et al. 2007b; Bartzokis 2011a).

Myelin breakdown and loss is associated with the release of iron (Izawa et al. 2010) which is highly concentrated in oligodendrocytes and their myelin (Todorich et al. 2009). When myelin and oligodendrocytes are damaged, astrocytes and especially microglia are activated and internalize the iron debris (Skripuletz et al. 2008; Izawa et al. 2010; Schulz et al. 2012; Norkute et al. 2009). Microglia may also have the capacity to subsequently recycle this iron by transferring iron loaded ferritin to oligodendrocyte precursors and thus promote their proliferation and differentiation into mature oligodendrocytes that can remyelinate lost sheaths (Schonberg et al. 2012; Schonberg and McTigue 2009) (Fig. 14.1). Consistent with a role in clearing myelin and iron debris (Bartzokis 2011a), microglia activation increases with age in these most vulnerable late-myelinating regions (Schuitemaker et al. 2012) which undergo the most extensive repair/remyelination (Peters 2007) (see Sect. 14.3.1).

14.3.1 Lifelong Myelination and Repair/Remyelination: The Consequence of Failure is Dysfunction

Myelin-based network plasticity is dependent, at least in part, on continued oligogenesis. Life-long oligogenesis is a distinctive oligodendrocyte feature that is central to brain development and plasticity throughout life. Unlike neurons, whose numbers are essentially established at birth, in healthy primates, vast numbers of progenitor (NG2) cells are produced to support the decades-long processes of postnatal myelination and repair/remyelination (Levine et al. 2001; Vinet et al. 2010). The NG2 cells comprise approximately 5 % of total adult brain cells and continue to divide with more than 80 % differentiating into oligodendrocytes whose numbers increase by as much as 50 % during adulthood (O'Kusky and Colonnier 1982; Peters and Sethares 2004; Peters et al. 2008; Vostrikov et al. 2007; Vostrikov and Uranova 2011) (reviewed in Peters 2009). NG2 cells can thus support both continued myelination of additional axons or portions thereof (e.g., intracortical) as well as remyelinate damaged or lost myelin sheaths (Figs. 14.1 and 14.2) (Peters and Sethares 2003; Peters et al. 2008) (Bartzokis et al. 2012).

Primate data suggests that during adult aging, repair/remyelination and plasticity processes result in oligodendrocyte numbers increasing substantially more in the cortex (50 %) than in subcortical white matter tracks (<25 %). Furthermore, associations between myelin damage and cognitive function are also strongest in cortex (Peters et al. 2010) which myelinates primarily after childhood (O'Kusky and Colonnier 1982) (Fig. 14.2). Repaired/remyelinated myelin segments are thinner and shorter and can thus be detected on electron microscopy by an increased number of paranodal myelin regions. There are substantial differences in age-related increase in paranodal myelin with a 90 % increase in later-myelinating prefrontal cortex compared to 57 % and 69 % in earlier myelinating visual cortex and in the anterior commissure respectively (Peters 2007).

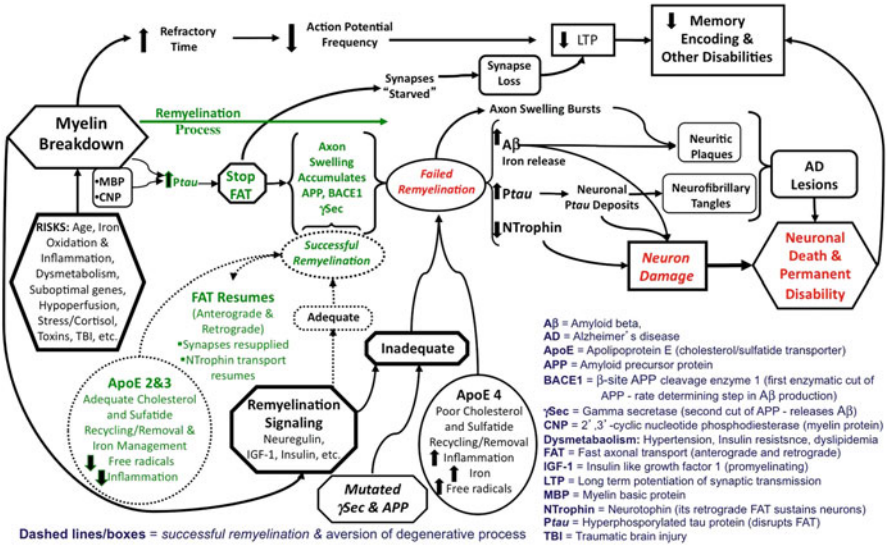


Fig. 14.3 Myelin breakdown-driven age-related cognitive decline into Alzheimer's disease with early loss of memory encoding. Risks of myelin breakdown and loss is multifactorial and similar to AD risks. The risks include age, suboptimal genes such as ApoE4, APP, and PS1, as well as iron accumulation and common age-related metabolic derangements such as hypertension, diabetes, and dyslipidemias (see Sect. 14.5). Myelin breakdown initially degrades network synchrony and cognitive functions through reductions in action potential refractory time which reduce higher frequency action potentials on which certain functions such as LTP and speed of movement are dependent. Loss of myelin segments can also reduce transmission speed and thus alter the network synchrony thus further degrading function. If myelin repair is slow, the support of distant synapses which are dependent on FAT is lost resulting in synapse loss and further cognitive decline. If repair/remyelination fails, neurotrophic support from synapses back to the neuronal body is reduced, which may result in loss of that particular axon. Eventually, toxic accumulations of tau, Aβ and loss of neurotrophic support could result in neuronal loss (adapted from and reviewed in Bartzokis 2011a). *Note*: Not all interactions are depicted including a possible direct toxic effect of Aβ on intracortical myelin (Mitew et al. 2010)

The remyelination process is complex and involves interactions between neurons (especially their axons and synapses) and oligodendrocytes (Fig. 14.3). Inefficient or disturbed neuron-glial communication can slow repair/remyelination and may have deleterious consequences. The organelles that coordinate protein synthesis are only present in the neuron body, rendering synapses and axons dependent on "supplies" delivered by fast axonal transport (FAT) from the neuron body. FAT is a bidirectional process powered by energy-requiring motors (kinesins for anterograde and dyneins for retrograde transport). Almost everything from mitochondria (for energy) to neurotransmitter vesicles must be anterogradely transported down axons to synapses. Conversely, damaged mitochondria destined for destruction, and products such as neurotrophin signaling molecules that are essential for neuronal survival, need to be retrogradely transported from synapses back to neuron body.

The dependence of synapses on FAT means that synaptic deficits, often observed with normal aging as well as very early in the process of several degenerative diseases including AD (DeKosky and Scheff 1990; Terry et al. 1991), could be *secondary to FAT disruption* (Fiala et al. 2002; Wishart et al. 2006) (reviewed in Bartzokis 2011a).

Amyloid precursor protein (APP), whose cleavage by β-site APP cleavage enzyme (BACE1 also known as β-secretase) and presenilin (PS1, also known as γ-Secretase) produces Aβ, is a key adhesion molecule for the FAT process itself. Thus APP adheres to the vesicles transported by FAT to the energy-requiring kinesin protein motors that propel them down axons on microtubule "tracks" towards the synapses. BACE1 and PS1 are also transported in these APP-anchored vesicles. Additional roles of APP in membrane adhesion may involve maintenance of axonal-myelin adherence at perinodal regions. Cellular signaling promoting myelination involves BACE1 and PS1 while adhering newly formed myelin onto axons depends partly on APP. In the context of the myelin model, the cotransport by FAT of three components of the myelination machinery (BACE1, PS1, and APP) is suggestive of a coordinated delivery to specific axonal myelin segment in need of repair/remyelination. The mechanism through which the myelin repair process is executed involves stopping/slowing axonal transport mechanisms and especially FAT which creates axonal swellings that contain the necessary signaling components to promote the repair/debris clearance and subsequent remyelination. Stopping FAT will also result in "starving" the synapses of the supplies delivered by FAT (Fig. 14.3). Thus, myelin receives the highest "repair priority" reinforcing the suggestion that myelin holds a preeminent role in information fidelity and functional connectivity of vertebrate CNS (reviewed in Bartzokis 2011a).

14.4 Complex Evolution of Glycogen Synthase Kinase-3 (GSK3) Myelination Mechanism and Its Integration with Metabolism and Inflammation

As summarized above, the human species' exceptional myelination is supported by recent evolutionary changes that may have evolved in part to support the extremely expensive metabolic processes of creating and maintaining a highly myelinated CNS. Given the very recent evolution of myelinating oligodendrocytes (in vertebrates), myelination's exceptional metabolic requirements had to be integrated with the many metabolic and developmental processes that predated its evolution. Glycogen synthase kinase-3 (GSK3) as well as other kinases that have similar/overlapping functions is highly conserved from sponges, through insects and vertebrates and is a central control mechanism that coordinates metabolism, inflammation, as well as myelination (reviewed in Bartzokis 2011b).

By the time myelin evolved, many processes were already modulated by GSK3 through its >40 substrates that include metabolic and signaling proteins, structural proteins, and transcription factors in different cellular components within the

cytoplasm but also in nucleus and mitochondria where GSK3 is highly activated (Bijur and Jope 2003) (Jope and Johnson 2004; Sutherland 2011). These multiple GSK3 metabolic and signaling functions could thus be integrated with the negative control GSK3 exerts on myelination. Given the complexity of GSK3 actions, the plethora of pharmacologic and non-pharmacologic interventions that can impact the myelination process are not entirely unexpected (reviewed in Bartzokis 2012). In this context it should also not be surprising that metabolic abnormalities such as insulin resistance and dyslipidemias that interact with GSK3 would impact myelination. These metabolic abnormalities not only increase the risk of AD and other degenerative brain disorders (see Sect. 14.5) but also predate the onset of psychiatric disease such as schizophrenia and bipolar disorder and are associated with worse outcomes (reviewed in Bartzokis 2011b).

14.4.1 Coordination of Myelination and Inflammation Through Glycogen Synthase Kinase 3 (GSK3)

Cholesterol and its derivatives such as sulfatide (a myelin-specific lipid that in brain is almost exclusively produced by oligodendrocytes) are indispensable for myelin formation and stability (Saher et al. 2005; Marcus et al. 2006). However, when myelin breaks down, *repair* is initiated by the efficient removal of myelin and especially its sulfatide lipid debris which is highly inflammatory (Kanter et al. 2006; Halder et al. 2007). Myelin debris is also distinctive in its myelination-inhibiting properties and exceptionally high iron content (reviewed in Bartzokis 2011b). The repair processes that clear myelin debris is an essential permissive step for the subsequent remyelination and functional restoration to occur (Neumann et al. 2008; Baer et al. 2009) (reviewed in Pohl et al. 2011; Fancy et al. 2011). The importance of lipid debris removal in the highly myelinated human brain may have contributed to our species evolving novel, more efficient ApoE isoforms (the brain's principal lipid binding and transport protein) to speed up the clearance and recycling of those lipids (reviewed in Bartzokis 2011a).

Activation and proliferation of microglia and astrocytes also have essential repair functions in debris clearance (see below) as well as helping to meet the increased metabolic needs associated with such plastic reorganization and repair of the CNS by producing substrates such as lactate (see Sect. 14.2.1) (Gehrmann et al. 1995; Kolomeets and Uranova 2010) (reviewed in Pohl et al. 2011). The GSK3 signal transduction pathway is capable of altering DNA methylation of imprinted loci (Popkie et al. 2010). It could thus help coordinate epigenetic DNA changes to transform dormant to activated (phagocyte) microglia and astrocytes as well as helping trigger proliferation of microglia and oligodendrocyte precursors (Fig. 14.1) (Steiner et al. 2008; Niu et al. 2010). Activated microglia are especially avid in clearing interstitial iron (fivefold more than astrocytes) (Bishop et al. 2011) although astrocytes are larger and more numerous (Pelvig et al. 2007) especially in humans (Han et al. 2013) and therefore they also contribute significantly to debris clearance (Peters and Sethares 2003). Evidence of this function comes from accumulation

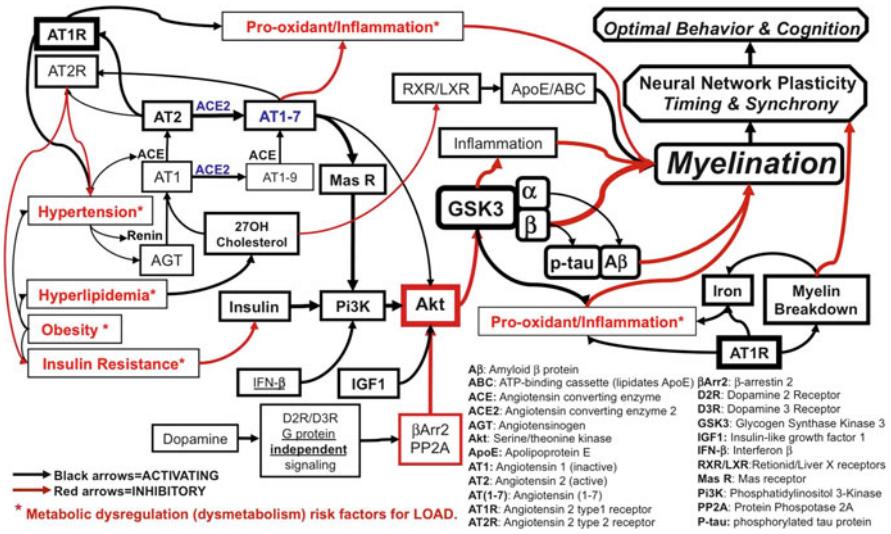


Fig. 14.4 Heuristic systems biology model of renin angiotensin system (RAS) and dysmetabolism. Hypertension, insulin resistance, hyperlipidemia, and obesity have complex interactions involving both the central and the peripheral RAS (Nakata et al. 1998; Brands et al. 1997; Mateos et al. 2011). Angiotensin 2 (AT2) promotes systemic hypertension as well as increased local tissue iron, oxidation, and inflammation. AT2 also interferes with insulin/Pi3K/Akt/GSK3 signaling and promotes insulin resistance (Saavedra 2012; Bartzokis 2012; Mak et al. 2012; Ishizaka et al. 2005), tau phosphorylation (Tian et al. 2012), and impairs cognitive function (Inaba et al. 2009; Kerr et al. 2005). Antihypertensives such as ACE inhibitors, AT2 blockers, and β-blockers (which reduce renin) oppose these effects directly. By blocking AT1R and shifting AT metabolism to ACE2, AT2 blockers promote formation of angiotensin 1-7 (AT1-7). AT1-7 generally opposes AT2 effects and is antihypertensive and antioxidant/anxiolitic (Bild and Ciobica 2012; Xia et al. 2012; Zhang et al. 2010; Bild et al. 2012) (reviewed in Zimmerman 2011), restores insulin sensitivity through Mas R/Pi3K/Akt/GSK3 signaling (Munoz et al. 2012; King et al. 2013) and AT2R activity on PPARγ (Ohshima et al. 2012), restores baroreceptor sensitivity (Arnold et al. 2013), and enhances LTP (Hellner et al. 2005; Staschewski et al. 2012; Bild et al. 2012). *Note*: Not all signaling pathways and their interactions are depicted. Adapted from Bartzokis (2012)

within astrocytes of an internal insoluble byproduct produced by the cumulative history of cellular oxidative peroxidation called lipofuscin which is strongly associated with iron accumulation and aging (Terman and Brunk 2004; Johnstone and Milward 2010). On the other hand, inhibiting GSK3 can limit microglia activation and inflammation (Yuskaitis and Jope 2009).

It is thus possible that GSK3 serves as a linchpin for coordinating the opposing processes of reparative inflammation/debris clearance as well as remyelination (Fig. 14.4). First, *upregulation* of GSK3 activity would *promote* inflammation and facilitate clearance of myelin debris while *inhibiting* myelin production during this process. Second, removal of pro-inflammatory debris such as sulfatide and iron should aid in the termination of inflammatory responses. Third, *downregulation* of GSK3 would serve to pivot the process towards *promoting* remyelination while *inhibiting* inflammation and microglial activation (Yuskaitis and Jope 2009) during this rebuilding process. Inefficient remyelination could increase oligomeric

(soluble) Aβ levels that may activate GSK3β (Jimenez et al. 2011), further inhibit remyelination (Mitew et al. 2010), promote inflammation, and thus propagate a vicious cycle that leads to formation of AD pathologic lesions and synaptic, axonal, and eventually neuronal cell loss (Figs. 14.3 and 14.4) (reviewed in Bartzokis 2011a). Failing to turn inflammatory pathways off, by delaying the clearance of inflammatory debris for example (see Sect. 14.5.1), could also contribute to triggering intrinsic GSK3-mediated apoptotic pathways and cell loss (Mines et al. 2011). Given the importance of these processes, it is likely that some signaling redundancy is provided by additional parallel pathways such as mammalian target of rapamycin complex (mTOR), mitogen-activated protein kinase (MAPK), and cyclin-dependant kinase (Cdk) (not depicted in Fig. 14.4) (reviewed in Bartzokis 2012).

14.5 Dysmetabolism and Modifiable Risk Factors for Age-Related Degenerative Brain Diseases

This final section will focus on risk factors for degenerative brain diseases that are modifiable. It will focus on LOAD, the most prevalent and best studied of these diseases. Modifiable risks include medical conditions subsumed under the rubric metabolic syndrome (central obesity, diabetes mellitus type 2 (DM2), hypertension (HTN)) and renal disease which are all strongly age-related (Franklin et al. 1997; Knopman 2010; National_Institute_of_Diabetes_and_Digestive_and_Kidney_Diseases 2008). Additional contributors are late-life depression, and environmental/lifestyle factors including dietary patterns, physical activity, education level, and head trauma (Yaffe et al. 2012; Craft et al. 2012; Knopman 2010; Whitmer et al. 2008; Barnes et al. 2012; Lee et al. 2012) that have all been shown to affect myelination (reviewed in Bartzokis 2004a, 2011a). These risk factors have produced calls for "eumetabolic" therapies dealing with multiple contributing biologic systems as a way to prevent and treat AD (Cai et al. 2012; Saavedra 2012).

In addition to increasing AD risk, some of these risk factors (HTN, DM2, as well as ApoE4) are associated with white matter damage through mechanisms that seem to be partially independent of each other (Knopman et al. 2011; Devisser et al. 2011; Raji et al. 2012; Kivipelto et al. 2002). These observations support the suggestion that with increasing age, metabolic deviations, epigenetic modifications, and suboptimal genetic endowment may *converge* to promote myelin damage and/or reduce repair/remyelination efficiency (Shen et al. 2008) in vulnerable late-myelinating regions (Marner et al. 2003; Murray et al. 2012) where the pathognomonic AD lesions first appear (Braak and Braak 1996; Braak and Del Tredici 2012; Desai et al. 2009; Mitew et al. 2010; Behrendt et al. 2013) (reviewed in Bartzokis 2004a, 2011a). Herein, we will refer to the metabolic deviations that occur in middle and old age and increase risk of LOAD as "dysmetabolism" to specify their detrimental impact on CNS/myelin and differentiate them from "metabolic syndrome", which will denote processes that damage peripheral organs and vasculature.

14.5.1 The Renin-Angiotensin System: Much More Than Hypertension

The potentially modifiable risk factors may account for as many as half of LOAD cases (Barnes and Yaffe 2011). They seem to accelerate cognitive aging (Yaffe et al. 2012; Craft et al. 2012; Knopman 2010; Whitmer et al. 2008) as well as exacerbate imaging biomarkers of brain aging (Goldstein et al. 2005; Bartzokis et al. 2007a; Gons et al. 2012; Debette et al. 2011; Lee et al. 2012; Geerlings et al. 2012). Recently, regional white matter hyperintensity (WMH) volume, which increases with age and is widely believed to be due to hypertension and vascular disease, was found to predict incident LOAD in the community while hippocampal or medial temporal lobe atrophy did not (Brickman et al. 2012; Poggesi et al. 2011) and reaffirmed earlier cross-sectional and prospective observations (Prins et al. 2004; van Dijk et al. 2008). Furthermore, WMH have been prospectively associated with AD pathology at postmortem (Moghekar et al. 2012) confirming an earlier association study (Polvikoski et al. 2010). Similarly, presence of WMH as well as reduced white matter integrity predicted (by as much as 10 years) subsequent decline into amnestic mild cognitive impairment (aMCI) while hippocampal volume did not (Silbert et al. 2012; Zhuang et al. 2012). Finally, both postmortem (Bronge 2002; de la Monte 1989) and imaging studies (Bartzokis et al. 2007a; Agosta et al. 2011; Zhuang et al. 2012; Silbert et al. 2012; Brickman et al. 2012) show substantial white matter alterations with relatively preserved gray matter in individuals at increased genetic risk or preclinical AD. These observations are consistent with a hypothesized model of AD that suggests myelin breakdown and repair/remyelination attempts are the initiator of the disease and that Aβ and tau lesions as well as synapse loss are byproducts of this process (Fig. 14.3) (Bartzokis 2004a, 2011a; Bartzokis et al. 2007b).

White matter lesions denoted as WMH on MRI are often presumed to be due to preexisting hypertension, dyslipidemias, and/or DM2 which all promote vascular dysfunction. Hypertension is estimated to affect 50 million Americans, accounts for approximately 50 % of population attributable risk for cerebrovascular and cardiovascular disease in individuals older than 50 years, and is the greatest risk factor for mortality (Chobanian et al. 2003). Like AD, hypertension is a strongly age-related disease. *Systolic blood pressure increases linearly between ages 30 and 84* (Franklin et al. 1997) driving the lifetime risk of hypertension to nearly 90 % and making its primary prevention a key public health goal (Vasan et al. 2002; Soros et al. 2012). Prospective data showed that even in very healthy normotensive (mean blood pressure 117/71) older individuals small increases in blood pressure that are considered "clinically insignificant" and do not cross the generally accepted 140/90 threshold for initiating treatment are associated with increases in WMH and brain atrophy (Goldstein et al. 2005) confirming meta-analyses that showed a progressively increasing risk of cerebrovascular accidents as blood pressure increases above a threshold of 115/75 (Lewington et al. 2002). The low blood pressure at which these risks begin increasing is consistent with suggestions that factors underlying

hypertension, but not necessarily blood pressure elevation itself, are associated with white matter damage and increased risk of LOAD (Fig. 14.4) (Prins et al. 2004; van Dijk et al. 2008 #17616; Moghekar et al. 2012) (reviewed in Saavedra 2012; Arnold et al. 2013; Jennings and Zanstra 2009).

The assumption that hypertension causes brain damage has been recently reconceptualized as the reverse. Considerable data support the suggestion that brain changes may be involved in promoting the imbalance in CNS sympathetic/parasympathetic outflow that eventually degrades cardiovagal baroreceptor reflex function during aging and ultimately promotes hypertension as well as insulin resistance and weight gain (e.g., metabolic syndrome). The age-related increase in the brain's *local renin-angiotensin system (RAS)* has been proposed to be the nexus of hypertension and aging (Jennings and Zanstra 2009; Arnold et al. 2013). Within this framework, a reassessment of the apparent coincidence of myelin breakdown and hypertension is worthwhile. In healthy humans age-related myelin breakdown of the frontal lobe begins in the early 30s (Bartzokis et al. 2012) which is also the beginning of age-related increase in blood pressure (Franklin et al. 1997). Combined autopsy-MRI studies confirm that WMH are in large part imaging manifestations of myelin damage and loss (Takao et al. 1999; Bronge et al. 2002; Ihara et al. 2010) (Murray et al. 2012; Castano et al. 2012). The age-related decline in myelin integrity and increasing WMH that begin at the same time as the beginning of blood pressure increases support the hypothesis that these brain changes may be an important cause of rising blood pressure as opposed to vice versa.

The RAS becomes more sensitive with age (Thompson et al. 2000) however, peripheral angiotensin cannot pass the blood brain barrier (Harding et al. 1988). Nevertheless, many organs including the brain have *their own local RAS*. Production of angiotensin 2 (AT2) is *higher* in brain than peripheral levels and is upregulated in hypertension (Hermann et al. 1984). In brain, angiotensin 2 receptor type 1 (AT1R) is present on both astrocytes and oligodendrocytes (Fogarty and Matute 2001) while astrocytes and microglia are the primary source of angiotensinogen, the precursor of angiotensin (Fig. 14.4) (Stornetta et al. 1988; Garrido-Gil et al. 2012) (reviewed in Arnold et al. 2013).

Myelin breakdown could therefore be the initiator of increased brain RAS activity. The breakdown triggers microglia and astrocyte activation necessary for removal of lipid and iron debris (see Sect. 14.4.1) (Izawa et al. 2010; Schulz et al. 2012; Norkute et al. 2009; Bishop et al. 2011) and could therefore increase angiotensinogen levels (Stornetta et al. 1988; Garrido-Gil et al. 2012) (reviewed in Arnold et al. 2013). Not surprisingly, in certain progressively debilitating forms of the canonical myelin-destructive disease (MS), elevated levels of CNS angiotensinogen are observed (Ottervald et al. 2013). During normal aging, the large amount of myelin of the frontal lobe begins breaking down at an accelerating rate in the fourth decade (Bartzokis et al. 2012). It is thus possible that the resulting increased activation of microglia and astrocytes may drive increased angiotensinogen levels and RAS activation and help trigger the peripheral triad of increased adiposity, hypertension, and insulin resistance (Arnold et al. 2013) as well as a decline in mitochondrial function (Nautiyal et al. 2013). Support for this possibility comes

from transgenic rodent models with reduced endogenous glial RAS that have reduced blood pressure, improved baroreflex function, and longer lifespans (reviewed in Arnold et al. 2013) as well as improved mitochondrial function (Nautiyal et al. 2013). Extreme human longevity has also been associated with variations in the AT1R gene (Benigni et al. 2012; Zajc Petranovic et al. 2012). Finally, age-related cognitive decline as well as AD pathology seems to be modified by the use of brain-penetrant angiotensin converting enzyme inhibitors (ACEis) (Sink et al. 2009) and especially angiotensin receptor blockers (ARBs) (Li et al. 2010; Davies et al. 2011; Hajjar et al. 2012, 2013). This protective effect on brain may also be shared in part by beta blockers (White et al. 2013; Gelber et al. 2013; Dobarro et al. 2013) that, like ACEis and ARBs, can also mitigate RAS activation by lowering renin (Morton et al. 1995) (Fig. 14.4).

The observations of reduced AD pathology with the use of such antihypertensives (Hajjar et al. 2012; Hoffman et al. 2009; Petrovitch et al. 2000; White et al. 2013) suggest that reducing brain RAS activity could modify the trajectory of age-related decline into AD. This possibility is supported by data from studies of DM2. Like AD and hypertension, aging is a key risk factor shared with DM2. Approximately 23 % of individuals over the age of 60 were afflicted as of 2007 (National_Institute_of_Diabetes_and_Digestive_and_Kidney_Diseases 2008). Antihypertensive use in DM2 patients is associated with decreased risk of developing AD. Amongst the classes of antihypertensive treatments, AD risk mitigation was most robust with ARB use (24 % risk reduction versus 14 %, 11 %, 7 %, and 4 % for diuretics, ACEis, calcium channel blockers, and beta-blockers respectively). For the RAS antihypertensives, risk reduction was also observed in DM2 patients that did *not* have hypertension (12.5 % risk reduction with ARBs and 5.9 % reduction with ACEis) reinforcing the idea that these particular medications could have a protective/reparative CNS effect that is independent of their antihypertensive effects (Johnson et al. 2012).

More subtle changes in WM microstructural integrity can be detected with diffusion weighted imaging (DTI) as well as transverse relaxation rate (R_2), a related nondiffusion MRI biomarker that has the advantage of helping quantify WM myelin content (Bartzokis et al. 2012). Studies using these biomarkers suggest hypertension or its underlying causes (Saavedra 2012) can promote white matter/myelin damage, especially in the later-myelinating anterior brain regions (Kennedy and Raz 2009; Salat et al. 2012; Gons et al. 2012; Maillard et al. 2012; Raji et al. 2012; Bendlin et al. 2012) and this damage is associated with declines in cognitive performance (Gons et al. 2012; Debette et al. 2011; Bartzokis et al. 2007a; Raji et al. 2012; Jacobs et al. 2011; Bendlin et al. 2012).

These deleterious effects were confirmed in recent studies which showed that radial diffusivity, the DTI parameter most influenced by myelin breakdown (Bartzokis et al. 2012), is associated with increased blood pressure as well as reduced cognitive function (Salat et al. 2010). The association between cognitive function and radial diffusivity was also present in normotensive subjects and was independent of age, once again supporting the suggestion that the underlying mechanisms may directly damage myelin in the absence of elevated blood pressure (Fig. 14.4). Notably, this association was reduced in a group of hypertensive subjects receiving

antihypertensive treatment (Salat et al. 2012). These data support the hypothesis that treatment of the underlying causes of hypertension, such as brain RAS activation, may modify age-related myelin breakdown and repair processes and thus mitigate cognitive decline and LOAD (Fig. 14.4) (Li et al. 2010; Davies et al. 2011; Hajjar et al. 2012, 2013; Johnson et al. 2012; Hoffman et al. 2009; Petrovitch et al. 2000; White et al. 2013; Furiya et al. 2012; Imabayashi et al. 2011; Kume et al. 2012; Mak et al. 2012) (reviewed in Fournier et al. 2009; Saavedra 2012).

14.5.2 Glucose Regulation, Insulin Resistance, and Glycogen Synthase Kinase 3 (GSK3)

Insulin signaling negatively regulates GSK3 and promotes myelination (Fig. 14.4). Conversely, insulin resistance with decreased brain insulin/Pi3K/Akt signaling result in GSK3 *activation* and have been reported in AD with or without diabetes mellitus (Steen et al. 2005) (reviewed in Liu et al. 2011). The reduced insulin signaling is expected to contribute to myelination/remyelination deficits since myelination is also under negative control by GSK3 (Azim and Butt 2011) (reviewed in Bartzokis 2011a) (Fig. 14.4). These signaling abnormalities could therefore help explain why metabolic disorders such as diabetes mellitus and metabolic syndrome, both of which are characterized by increase in prevalence with age and reduced insulin signaling (e.g., insulin resistance), have been noted to double the risk of developing AD (reviewed in Liu et al. 2011) (Fig. 14.4).

Activation of GSK3 seems to directly increase Aβ production and tau phosphorylation resulting in the proteinopathies that define the pathology of AD and other age-related degenerative brain diseases (reviewed in Zhao and Townsend 2009; Lei et al. 2011; Mines et al. 2011). Myelin repair needs increase with age and ordinarily require oligogenesis and remyelination of axons however, GSK3 activation inhibits remyelination (Azim and Butt 2011). Inhibited or inefficient remyelination is hypothesized to drive Aβ as well as tau production that secondarily lead to AD pathology (reviewed in Bartzokis 2011a) (Figs. 14.3 and 14.4).

14.5.3 Cortical Microinfarcts (CMIs) and White Matter Hyperintensities (WMHs)

Several studies observed that hypertension was associated with WMH as well as gray matter volume reductions (reviewed in Raji et al. 2012). These associations are evident even in *younger* middle-aged individuals whose higher systolic blood pressure is associated with DTI evidence of WM injury in late-myelinating subcortical regions as well as reductions in cortical gray matter (Maillard et al. 2012). Subcortical WMH can be explained, at least in part, by myelin damage/loss in vulnerable later-myelinating subcortical fibers of the anterior brain (Marner et al. 2003; Murray et al. 2012;

Bartzokis et al. 2012). Gray matter losses could also be partially due to losses in *intracortical* myelin (ICM, see Fig. 14.2) which is also laid down late in development (Miller et al. 2012) (reviewed in Bartzokis 2012) and damaged in AD (Desai et al. 2009; Mitew et al. 2010; Behrendt et al. 2013). Nevertheless, successful treatment of hypertension with an ACEi (lisinopril) or beta blocker (atenolol) (both with low lipophilicity suggesting poor brain penetrance (Ranadive et al. 1992; Yang et al. 2007; Cruickshank 1980)) does not seem to reduce the decline in cortical volume (Jennings et al. 2012). This result can be reinterpreted to suggest that the more vulnerable ICM continued to be lost despite lowering of blood pressure. Focal loss of ICM associated with Aβ plaque deposits (Mitew et al. 2010), cortical microinfarcts (CMI, see below), as well as other factors (Fig. 14.4) could all contribute to gray matter deficits in AD.

Both DM2 and hypertension can increase infarct risk. Until recently cortical microinfarcts (CMI) have been underappreciated since by definition CMIs can only be observed with a microscope as they are too small to be detected on gross anatomy and, unlike subcortical WMH, are also difficult to identify on MRI. Nevertheless, recent data shows that they have a disproportionate impact on brain function and especially cognitive function (Kovari et al. 2007) (reviewed in Costanza et al. 2012; Arvanitakis et al. 2011; Smith et al. 2012; Brundel et al. 2012). Studies examining CMIs suggest that they explain most of the functional effects (on cognition especially) that are usually attributed to WMH (reviewed in Costanza et al. 2012; Smith et al. 2012; Brundel et al. 2012). When quantified separately and entered into logistic regression analyses, the impact of WMH is reduced compared to CMIs (Sonnen et al. 2007; Sinka et al. 2010; Arvanitakis et al. 2011) (reviewed in Costanza et al. 2012). CMIs are present in approximately half of AD patients (43-57 %) compared to less than one-third (24–31 %) of those without dementia at death (reviewed in Smith et al. 2012; Brundel et al. 2012). It thus appears that the recently developed appreciation of the paramount importance of cortical compared to visible subcortical lesions in MS (Fig. 14.2) may also apply in the new context of vascular damage. Macroscopic lesions such as WMH that are readily detectable on MRI may be "markers" for more numerous and widespread microinfarcts (50 % of individuals with WMH had CMIs and vice versa) (reviewed in Smith et al. 2012). Importantly, as many as 50 % of individuals with microinfarcts may *not* have macroscopic infarcts (Arvanitakis et al. 2011) and thus vascular contributions may not be fully accounted for by routine MRI. Nevertheless CMIs have important and possibly independent effects on cognition (reviewed in Smith et al. 2012).

A rodent model has shown that even a single or very few CMIs can produce cognitive deficits however, the model also demonstrated that reducing excitotoxicity through pharmacologic intervention may reduce these deficits (Shih et al. 2013). It thus appears that consistent with the MS data (Fig. 14.2), ICMs can have a very disruptive effect on cognitive processing. The loss of the compensatory effects of ICM, the "last line of defense" against the loss of network timing and synchrony (Fig. 14.2), could unmask subcortical transmission deficits and reveal cognitive and behavioral symptoms early in the process before neuronal death and irreversible loss of function (Fig. 14.3). Similar focal ICM losses associated with Aβ plaques has also been recently documented in AD (Desai et al. 2009; Mitew et al. 2010;

Behrendt et al. 2013). Age-related global declines in ICM as well as focal loses from CMIs and Aβ plaques may also significantly contribute to the trajectory of cognitive and behavioral function decline observed in aging and the transition into AD.

14.6 Conclusions

This chapter provided an overview of dynamic and complex interdependence of all the brain's cellular elements with a special focus on the role of glia and especially oligodendrocytes in the generation of degenerative brain disease. We have attempted to rebalance the historic bias that has focused attention primarily on neurons and gray matter. In doing so we clarify the importance of a systems-level understanding of healthy brain functioning and the age-related interdependent shifts in *both* central and peripheral homeostatic mechanisms that can lead to remarkably prevalent age-related degenerative disease states such as AD (Figs. 14.1–14.4). The integration of cellular, molecular, network, and systems-based definitions of "health" and "disease" that includes the interplay between CNS and peripheral components (Figs. 14.1 and 14.4) is essential for identifying, validating, targeting, and optimally timing an array of possible novel therapeutic interventions.

The emerging evidence suggests that the common assumption that mid-life peripheral metabolic changes such as hypertension, DM2/insulin resistance, hyperlipidemia, and obesity (e.g., metabolic syndrome) are the drivers of brain damage may not be entirely correct. Recent evidence suggests that vulnerable aspects of the brain such as myelin (Figs. 14.1 and 14.2) that have evolved most recently may substantially contribute to creating some of these peripheral metabolic derangements (Fig. 14.4). Furthermore, the vulnerability of myelin and the homeostatic repair mechanisms triggered by its breakdown may also drive the slow and continually progressive processes that result in cognitive decline as well as the pathology that define the prevalent degenerative diseases of the brain (Fig. 14.3). The looming medical and financial crisis that will result from the exponential increase in degenerative brain diseases of the aging baby boomer generation has become an immediate threat to the fabric of our society. Safe therapeutic interventions that could mitigate the catastrophic age-driven exponential increase in degenerative brain diseases are urgently needed. Pharmacologic interventions that could mitigate this trajectory may already be in wide use and the epidemiologic and postmortem evidence suggests that such interventions could have disease-modifying effects.

Imaging, blood, and genetic biomarkers have emerged that make it possible to track the complex, dynamic, nonlinear, and progressive trajectories from optimal function to dysfunction and into degenerative brain disease states. These developments provide unprecedented opportunities to prospectively examine the interactions between changes in disease state and environmental (including medications), genetic, and epigenetic factors (Figs. 14.1 and 14.4). Disease classification based on integrated molecular, cell function, neural network, and systems levels should lead to improved management and prevention of degenerative brain disease.

Such an integrated approach can potentially replicate advances made in preventing peripheral diseases (e.g., cardiovascular disease) and targeting treatments based on etiologic as opposed to symptomatic stratification (e.g., latest cancer therapeutics). Discovering, developing, validating, and standardizing the most specific and informative biomarkers and utilizing them to track the pathology of common degenerative brain diseases and the impact of treatment and preventive interventions is a fundamental and acute clinical challenge.

Acknowledgment This work was supported in part by NIH grants (MH066029; AG027342), Research and Psychiatry Services of the Department of Veterans Affairs, and the Litwin and RCS Foundations.

References

Agosta F, Pievani M, Sala S, Geroldi C, Galluzzi S, Frisoni GB, Filippi M (2011) White matter damage in Alzheimer disease and its relationship to gray matter atrophy. Radiology 258(3): 853–863. doi:10.1148/radiol.10101284

Alba-Ferrara LM, de Erausquin GA (2013) What does anisotropy measure? Insights from increased and decreased anisotropy in selective fiber tracts in schizophrenia. Front Integr Neurosci 7:9. doi:10.3389/fnint.2013.00009

Als H, Duffy FH, McAnulty GB, Rivkin MJ, Vajapeyam S, Mulkern RV, Warfield SK, Huppi PS, Butler SC, Conneman N, Fischer C, Eichenwald EC (2004) Early experience alters brain function and structure. Pediatrics 113(4):846–857

Ames A 3rd (2000) CNS energy metabolism as related to function. Brain Res Brain Res Rev 34(1–2):42–68

Arnold AC, Gallagher PE, Diz DI (2013) Brain renin-angiotensin system in the nexus of hypertension and aging. Hypertens Res 36(1):5–13

Arvanitakis Z, Leurgans SE, Barnes LL, Bennett DA, Schneider JA (2011) Microinfarct pathology, dementia, and cognitive systems. Stroke 42(3):722–727. doi:10.1161/strokeaha.110.595082

Ashford JW (2004) APOE genotype effects on alzheimer's disease onset and epidemiology. J Mol Neurosci 23(3):157–166

Azim K, Butt AM (2011) GSK3beta negatively regulates oligodendrocyte differentiation and myelination in vivo. Glia 59(4):540–553

Baer AS, Syed YA, Kang SU, Mitteregger D, Vig R, Ffrench-Constant C, Franklin RJ, Altmann F, Lubec G, Kotter MR (2009) Myelin-mediated inhibition of oligodendrocyte precursor differentiation can be overcome by pharmacological modulation of Fyn-RhoA and protein kinase C signalling. Brain 132(Pt 2):465–481

Barnes DE, Yaffe K (2011) The projected effect of risk factor reduction on Alzheimer's disease prevalence. Lancet Neurol 10(9):819–828

Barnes DE, Yaffe K, Byers AL, McCormick M, Schaefer C, Whitmer RA (2012) Midlife vs late-life depressive symptoms and risk of dementia: differential effects for Alzheimer disease and vascular dementia. Arch Gen Psychiatry 69(5):493–498

Barton RA, Harvey PH (2000) Mosaic evolution of brain structure in mammals. Nature 405(6790): 1055–1058

Bartzokis G (2002) Schizophrenia: breakdown in the well-regulated lifelong process of brain development and maturation. Neuropsychopharmacology 27(4):672–683. doi:10.1016/S0893-133X(02)00364-0

Bartzokis G (2004a) Age-related myelin breakdown: a developmental model of cognitive decline and Alzheimer's disease. Neurobiol Aging 25(1):5–18. doi:10.1016/j.neurobiolaging.2003.03.001, Author reply 25:49–62

Bartzokis G (2004b) Quadratic trajectories of brain myelin content: unifying construct for neuropsychiatric disorders. Neurobiol Aging 25(1):49–62. doi:10.1016/j.neurobiolaging.2003.08.001

Bartzokis G (2005) Brain myelination in prevalent neuropsychiatric developmental disorders: Primary and comorbid addiction. Adolesc Psychiatry 29:55–96

Bartzokis G (2011a) Alzheimer's disease as homeostatic responses to age-related myelin breakdown. Neurobiol Aging 32(8):1341–1371. doi:10.1016/j.neurobiolaging.2009.08.007

Bartzokis G (2011b) Neuroglialpharmacology: white matter pathophysiologies and psychiatric treatments. Front Biosci 16:2695–2733. doi:10.2741/3881

Bartzokis G (2012) Neuroglialpharmacology: myelination as a shared mechanism of action of psychotropic treatments. Neuropharmacology 62(7):2137–2153. doi:10.1016/j.neuropharm.2012.01.015

Bartzokis G, Beckson M, Lu PH, Nuechterlein KH, Edwards N, Mintz J (2001) Age-related changes in frontal and temporal lobe volumes in men: a magnetic resonance imaging study. Arch Gen Psychiatry 58:461–465. doi:10.1001/archpsyc.58.5.461

Bartzokis G, Lu PH (2009) Brain volume: age-related changes. In: Squire LR (ed) Encyclopedia of neuroscience, vol 2. Academic Press, Oxford, pp 417–447. doi:10.1016/B978-008045046-9.00107-8

Bartzokis G, Lu PH, Geschwind D, Tingus K, Huang D, Mendez MF, Edwards N, Mintz J (2007a) Apolipoprotein E affects both myelin breakdown and cognition: implications for age-related trajectories of decline into dementia. Biol Psychiatry 62(12):1380–1387. doi:10.1016/j.biopsych. 2007.03.024

Bartzokis G, Lu PH, Heydari P, Couvrette A, Lee GJ, Kalashyan G, Freeman F, Grinstead JW, Villablanca P, Finn JP, Mintz J, Alger JR, Altshuler LL (2012) Multimodal MRI assessment of white matter aging trajectories over the lifespan of healthy individuals. Biol Psychiatry 72(12):1026–1034. doi:10.1016/j.biopsych.2012.07.010

Bartzokis G, Lu PH, Mintz J (2007b) Human brain myelination and amyloid beta deposition in Alzheimer's disease. Alzheimers Dement 3(2):122–125

Bartzokis G, Lu PH, Tingus K, Peters DG, Amar CP, Tishler TA, Finn JP, Villablanca P, Altshuler LL, Mintz J, Neely E, Connor JR (2011) Gender and iron genes may modify associations between brain iron and memory in healthy aging. Neuropsychopharmacology 36(7):1375–1384. doi:10.1038/npp.2011.22

Bartzokis G, Lu PH, Tishler TA, Fong SM, Oluwadara B, Finn JP, Huang D, Bordelon Y, Mintz J, Perlman S (2007c) Myelin breakdown and iron changes in Huntington's disease: pathogenesis and treatment implications. Neurochem Res 32(10):1655–1664. doi:10.1007/s11064-007-9352-7

Bartzokis G, Sultzer D, Lu PH, Nuechterlein KH, Mintz J, Cummings J (2004) Heterogeneous age-related breakdown of white matter structural integrity: implications for cortical "disconnection" in aging and Alzheimer's disease. Neurobiol Aging 25(7):843–851. doi:10.1016/j.neurobiolaging.2003.09.005

Bartzokis G, Tishler TA, Lu PH, Villablanca P, Altshuler LL, Carter M, Huang D, Edwards N, Mintz J (2007d) Brain ferritin iron may influence age- and gender-related risks of neurodegeneration. Neurobiol Aging 28(3):414–423. doi:10.1016/j.neurobiolaging.2006.02.005

Bauer J, Bradl M, Klein M, Leisser M, Deckwerth TL, Wekerle H, Lassmann H (2002) Endoplasmic reticulum stress in PLP-overexpressing transgenic rats: gray matter oligodendrocytes are more vulnerable than white matter oligodendrocytes. J Neuropathol Exp Neurol 61(1):12–22

Behrendt G, Baer K, Buffo A, Curtis MA, Faull RL, Rees MI, Gotz M, Dimou L (2013) Dynamic changes in myelin aberrations and oligodendrocyte generation in chronic amyloidosis in mice and men. Glia 61(2):273–286

Bendlin BB, Carlsson CM, Johnson SC, Zetterberg H, Blennow K, Willette AA, Okonkwo OC, Sodhi A, Ries ML, Birdsill AC, Alexander AL, Rowley HA, Puglielli L, Asthana S, Sager MA

(2012) CSF T-Tau/Abeta42 predicts white matter microstructure in healthy adults at risk for Alzheimer's disease. PLoS One 7(6):e37720
Bengtsson SL, Nagy Z, Skare S, Forsman L, Forssberg H, Ullen F (2005) Extensive piano practicing has regionally specific effects on white matter development. Nat Neurosci 8(9):1148–1150
Benigni A, Orisio S, Noris M, Iatropoulos P, Castaldi D, Kamide K, Rakugi H, Arai Y, Todeschini M, Ogliari G, Imai E, Gondo Y, Hirose N, Mari D, Remuzzi G (2012) Variations of the angiotensin II type 1 receptor gene are associated with extreme human longevity. Age (Dordr) 35(3): 993–1005
Bijur GN, Jope RS (2003) Glycogen synthase kinase-3 beta is highly activated in nuclei and mitochondria. Neuroreport 14(18):2415–2419
Bild W, Ciobica A (2012) Angiotensin-(1-7) central administration induces anxiolytic-like effects in elevated plus maze and decreased oxidative stress in the amygdala. J Affect Disord 145(2): 165–171
Bild W, Hritcu L, Stefanescu C, Ciobica A (2012) Inhibition of central angiotensin II enhances memory function and reduces oxidative stress status in rat hippocampus. Prog Neuropsychopharmacol Biol Psychiatry 43:79–88. doi:10.1016/j.pnpbp.2012.12.009
Bishop GM, Dang TN, Dringen R, Robinson SR (2011) Accumulation of non-transferrin-bound iron by neurons, astrocytes, and microglia. Neurotox Res 19(3):443–451
Bjorkhem I (2006) Crossing the barrier: oxysterols as cholesterol transporters and metabolic modulators in the brain. J Intern Med 260(6):493–508
Braak E, Braak H (1996) Development of Alzheimer-related neurofibrillary changes in the neocortex inversely recapitulates cortical myelogenesis. Acta Neuropathol 92:197–201
Braak H, Del Tredici K (2012) Alzheimer's disease: pathogenesis and prevention. Alzheimers Dement 8(3):227–233
Brands MW, Harrison DL, Keen HL, Gardner A, Shek EW, Hall JE (1997) Insulin-induced hypertension in rats depends on an intact renin-angiotensin system. Hypertension 29(4): 1014–1019
Brickman AM, Provenzano FA, Muraskin J, Manly JJ, Blum S, Apa Z, Stern Y, Brown TR, Luchsinger JA, Mayeux R (2012) Regional white matter hyperintensity volume, not hippocampal atrophy, predicts incident Alzheimer disease in the community. Arch Neurol 69(12): 1621–1627
Bronge L (2002) Magnetic resonance imaging in dementia. A study of brain white matter changes. Acta Radiol Suppl 428:1–32
Bronge L, Bogdanovic N, Wahlund LO (2002) Postmortem MRI and histopathology of white matter changes in Alzheimer brains. A quantitative, comparative study. Dement Geriatr Cogn Disord 13(4):205–212
Brundel M, de Bresser J, van Dillen JJ, Kappelle LJ, Biessels GJ (2012) Cerebral microinfarcts: a systematic review of neuropathological studies. J Cereb Blood Flow Metab 32(3):425–436. doi:10.1038/jcbfm.2011.200
Butt AM (2011) ATP: a ubiquitous gliotransmitter integrating neuron-glial networks. Semin Cell Dev Biol 22(2):205–213
Butt AM, Berry M (2000) Oligodendrocytes and the control of myelination in vivo: new insights from the rat anterior medullary velum. J Neurosci Res 59(4):477–488
Cai H, Cong WN, Ji S, Rothman S, Maudsley S, Martin B (2012) Metabolic dysfunction in Alzheimer's disease and related neurodegenerative disorders. Curr Alzheimer Res 9(1):5–17
Calabrese M, Filippi M, Rovaris M, Bernardi V, Atzori M, Mattisi I, Favaretto A, Grossi P, Barachino L, Rinaldi L, Romualdi C, Perini P, Gallo P (2009) Evidence for relative cortical sparing in benign multiple sclerosis: a longitudinal magnetic resonance imaging study. Mult Scler 15(1):36–41
Castano EM, Maarouf CL, Wu T, Leal MC, Whiteside CM, Lue LF, Kokjohn TA, Sabbagh MN, Beach TG, Roher AE (2012) Alzheimer disease periventricular white matter lesions exhibit specific proteomic profile alterations. Neurochem Int 62(2):145–156. doi:10.1016/j.neuint.2012.12.001

Castellani RJ, Moreira PI, Perry G, Zhu X (2012) The role of iron as a mediator of oxidative stress in Alzheimer disease. BioFactors 38(2):133–138

Chen H, Lukas TJ, Du N, Suyeoka G, Neufeld AH (2009) Dysfunction of the retinal pigment epithelium with age: increased iron decreases phagocytosis and lysosomal activity. Invest Ophthalmol Vis Sci 50(4):1895–1902

Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, Jones DW, Materson BJ, Oparil S, Wright JT Jr, Roccella EJ (2003) The seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure: the JNC 7 report. JAMA 289(19):2560–2572

Connor JR, Menzies SL (1996) Relationship of iron to oligodendrocytes and myelination. Glia 17:83–93

Corneveaux JJ, Myers AJ, Allen AN, Pruzin JJ, Ramirez M, Engel A, Nalls MA, Chen K, Lee W, Chewning K, Villa SE, Meechoovet HB, Gerber JD, Frost D, Benson HL, O'Reilly S, Chibnik LB, Shulman JM, Singleton AB, Craig DW, Van Keuren-Jensen KR, Dunckley T, Bennett DA, De Jager PL, Heward C, Hardy J, Reiman EM, Huentelman MJ (2010) Association of CR1, CLU and PICALM with Alzheimer's disease in a cohort of clinically characterized and neuropathologically verified individuals. Hum Mol Genet 19(16): 3295–3301

Costanza A, Xekardaki A, Kovari E, Gold G, Bouras C, Giannakopoulos P (2012) Microvascular burden and Alzheimer-type lesions across the age spectrum. J Alzheimers Dis 32(3):643–652. doi:10.3233/jad-2012-120835

Craft S, Foster TC, Landfield PW, Maier SF, Resnick SM, Yaffe K (2012) Session III: mechanisms of age-related cognitive change and targets for intervention: inflammatory, oxidative, and metabolic processes. J Gerontol A Biol Sci Med Sci 67(7):754–759

Cruickshank JM (1980) The clinical importance of cardioselectivity and lipophilicity in beta blockers. Am Heart J 100(2):160–178

Davies NM, Kehoe PG, Ben-Shlomo Y, Martin RM (2011) Associations of anti-hypertensive treatments with Alzheimer's disease, vascular dementia, and other dementias. J Alzheimers Dis 26(4):699–708

de la Monte SM (1989) Quantitation of cerebral atrophy in preclinical and end-stage Alzheimer's disease. Ann Neurol 25(5):450–459

de los Monteros AE, Korsak RA, Tran T, Vu D, de Vellis J, Edmond J (2000) Dietary iron and the integrity of the developing rat brain: a study with the artificially-reared rat pup. Cell Mol Biol (Noisy-le-grand) 46(3):501–515

Debette S, Seshadri S, Beiser A, Au R, Himali JJ, Palumbo C, Wolf PA, DeCarli C (2011) Midlife vascular risk factor exposure accelerates structural brain aging and cognitive decline. Neurology 77(5):461–468

DeKosky ST, Scheff SW (1990) Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. Ann Neurol 27:457–464

Desai MK, Sudol KL, Janelsins MC, Mastrangelo MA, Frazer ME, Bowers WJ (2009) Triple-transgenic Alzheimer's disease mice exhibit region-specific abnormalities in brain myelination patterns prior to appearance of amyloid and tau pathology. Glia 57(1):54–65

Devisser A, Yang C, Herring A, Martinez JA, Rosales-Hernandez A, Poliakov I, Ayer A, Garven A, Zaver S, Rincon N, Xu K, Tuor UI, Schmidt AM, Toth C (2011) Differential impact of diabetes and hypertension in the brain: adverse effects in grey matter. Neurobiol Dis 44(2): 161–173

Diamond MC, Rosenzweig MR, Bennett EL, Lindner B, Lyon L (1972) Effects of environmental enrichment and impoverishment on rat cerebral cortex. J Neurobiol 3(1):47–64

Dobarro M, Orejana L, Aguirre N, Ramirez MJ (2013) Propranolol restores cognitive deficits and improves amyloid and Tau pathologies in a senescence-accelerated mouse model. Neuropharmacology 64:137–144. doi:10.1016/j.neuropharm.2012.06.047

Fancy SP, Chan JR, Baranzini SE, Franklin RJ, Rowitch DH (2011) Myelin regeneration: a recapitulation of development? Annu Rev Neurosci 34:21–43

Fiala JC, Spacek J, Harris KM (2002) Dendritic spine pathology: cause or consequence of neurological disorders? Brain Res Brain Res Rev 39(1):29–54
Fields RD, Burnstock G (2006) Purinergic signalling in neuron-glia interactions. Nat Rev Neurosci 7(6):423–436
Fogarty DJ, Matute C (2001) Angiotensin receptor-like immunoreactivity in adult brain white matter astrocytes and oligodendrocytes. Glia 35(2):131–146
Fournier A, Oprisiu-Fournier R, Serot JM, Godefroy O, Achard JM, Faure S, Mazouz H, Temmar M, Albu A, Bordet R, Hanon O, Gueyffier F, Wang J, Black S, Sato N (2009) Prevention of dementia by antihypertensive drugs: how AT1-receptor-blockers and dihydropyridines better prevent dementia in hypertensive patients than thiazides and ACE-inhibitors. Expert Rev Neurother 9(9):1413–1431
Franklin RJ, Ffrench-Constant C (2008) Remyelination in the CNS: from biology to therapy. Nat Rev Neurosci 9(11):839–855
Franklin SS, Wt G, Wong ND, Larson MG, Weber MA, Kannel WB, Levy D (1997) Hemodynamic patterns of age-related changes in blood pressure. The Framingham Heart Study. Circulation 96(1):308–315
Furiya Y, Ryo M, Kawahara M, Kiriyama T, Morikawa M, Ueno S (2012) Renin-angiotensin system blockers affect cognitive decline and serum adipocytokines in Alzheimer's disease. Alzheimers Dement 9(5):512–518
Garrido-Gil P, Valenzuela R, Villar-Cheda B, Lanciego JL, Labandeira-Garcia JL (2012) Expression of angiotensinogen and receptors for angiotensin and prorenin in the monkey and human substantia nigra: an intracellular renin-angiotensin system in the nigra. Brain Struct Funct 218(2):373–388. doi:10.1007/s00429-012-0402-9
Geerlings MI, Brickman AM, Schupf N, Devanand DP, Luchsinger JA, Mayeux R, Small SA (2012) Depressive symptoms, antidepressant use, and brain volumes on MRI in a population-based cohort of old persons without dementia. J Alzheimers Dis 30(1):75–82
Gehrmann J, Matsumoto Y, Kreutzberg GW (1995) Microglia: intrinsic immuneffector cell of the brain. Brain Res Brain Res Rev 20(3):269–287
Gelber RP, Launer LJ, Petrovitch H, Masaki KH, Ross GW, White LR (2013) Beta-blocker treatment of hypertensive older persons decreases the risk of cognitive impairment: the Honolulu-Asia Aging Study. Paper presented at the Presented at the American Academy of Neurology 65th Annual meeting, San Diego, 16–23 March
Goldstein IB, Bartzokis G, Guthrie D, Shapiro D (2005) Ambulatory blood pressure and the brain. A 5-year follow-up. Neurology 64(11):1846–1852
Gons RA, van Oudheusden LJ, de Laat KF, van Norden AG, van Uden IW, Norris DG, Zwiers MP, van Dijk E, de Leeuw FE (2012) Hypertension is related to the microstructure of the corpus callosum: The RUN DMC study. J Alzheimers Dis 32(3):623–631
Grayson DR, Chen Y, Dong E, Kundakovic M, Guidotti A (2009) From trans-methylation to cytosine methylation: evolution of the methylation hypothesis of schizophrenia. Epigenetics 4(3):144–149
Guerreiro R, Wojtas A, Bras J, Carrasquillo M, Rogaeva E, Majounie E, Cruchaga C, Sassi C, Kauwe JS, Younkin S, Hazrati L, Collinge J, Pocock J, Lashley T, Williams J, Lambert JC, Amouyel P, Goate A, Rademakers R, Morgan K, Powell J, St George-Hyslop P, Singleton A, Hardy J (2012) TREM2 variants in Alzheimer's disease. N Engl J Med 368(2):117–127
Hajjar I, Brown L, Mack WJ, Chui H (2012) Impact of angiotensin receptor blockers on Alzheimer disease neuropathology in a large brain autopsy series. Arch Neurol 69(12):1632–1637
Hajjar I, Hart M, Chen YL, Mack W, Novak V, Chui CH, Lipsitz L (2013) Antihypertensive therapy and cerebral hemodynamics in executive mild cognitive impairment: results of a pilot randomized clinical trial. J Am Geriatr Soc 61(2):194–201. doi:10.1111/jgs.12100
Halder RC, Jahng A, Maricic I, Kumar V (2007) Mini review: immune response to myelin-derived sulfatide and CNS-demyelination. Neurochem Res 32(2):257–262
Hallgren B, Sourander P (1958) The effect of age on the non-haemin iron in the human brain. J Neurochem 3:41–51

Han X, Chen M, Wang F, Windrem M, Wang S, Shanz S, Xu Q, Oberheim NA, Bekar L, Betstadt S, Silva AJ, Takano T, Goldman SA, Nedergaard M (2013) Forebrain engraftment by human glial progenitor cells enhances synaptic plasticity and learning in adult mice. Cell Stem Cell 12(3):342–353. doi:10.1016/j.stem.2012.12.015

Harding JW, Sullivan MJ, Hanesworth JM, Cushing LL, Wright JW (1988) Inability of [125I] Sar1, Ile8-angiotensin II to move between the blood and cerebrospinal fluid compartments. J Neurochem 50(2):554–557

Harold D, Abraham R, Hollingworth P, Sims R, Gerrish A, Hamshere ML, Pahwa JS, Moskvina V, Dowzell K, Williams A, Jones N, Thomas C, Stretton A, Morgan AR, Lovestone S, Powell J, Proitsi P, Lupton MK, Brayne C, Rubinsztein DC, Gill M, Lawlor B, Lynch A, Morgan K, Brown KS, Passmore PA, Craig D, McGuinness B, Todd S, Holmes C, Mann D, Smith AD, Love S, Kehoe PG, Hardy J, Mead S, Fox N, Rossor M, Collinge J, Maier W, Jessen F, Schurmann B, van den Bussche H, Heuser I, Kornhuber J, Wiltfang J, Dichgans M, Frolich L, Hampel H, Hull M, Rujescu D, Goate AM, Kauwe JS, Cruchaga C, Nowotny P, Morris JC, Mayo K, Sleegers K, Bettens K, Engelborghs S, De Deyn PP, Van Broeckhoven C, Livingston G, Bass NJ, Gurling H, McQuillin A, Gwilliam R, Deloukas P, Al-Chalabi A, Shaw CE, Tsolaki M, Singleton AB, Guerreiro R, Muhleisen TW, Nothen MM, Moebus S, Jockel KH, Klopp N, Wichmann HE, Carrasquillo MM, Pankratz VS, Younkin SG, Holmans PA, O'Donovan M, Owen MJ, Williams J (2009) Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. Nat Genet 10(10):1088–1093

Harris JJ, Attwell D (2012) The energetics of CNS white matter. J Neurosci 32(1):356–371

Hartman BK, Agrawal HC, Agrawal D, Kalmbach S (1982) Development and maturation of central nervous system myelin: comparison of immunohistochemical localization of proteolipid protein and basic protein in myelin and oligodendrocytes. Proc Natl Acad Sci U S A 79(13):4217–4220

Hellner K, Walther T, Schubert M, Albrecht D (2005) Angiotensin-(1-7) enhances LTP in the hippocampus through the G-protein-coupled receptor Mas. Mol Cell Neurosci 29(3):427–435. doi:10.1016/j.mcn.2005.03.012

Hermann K, McDonald W, Unger T, Lang RE, Ganten D (1984) Angiotensin biosynthesis and concentrations in brain of normotensive and hypertensive rats. J Physiol (Paris) 79(6): 471–480

Hoffman LB, Schmeidler J, Lesser GT, Beeri MS, Purohit DP, Grossman HT, Haroutunian V (2009) Less Alzheimer disease neuropathology in medicated hypertensive than nonhypertensive persons. Neurology 72(20):1720–1726

Ihara M, Polvikoski TM, Hall R, Slade JY, Perry RH, Oakley AE, Englund E, O'Brien JT, Ince PG, Kalaria RN (2010) Quantification of myelin loss in frontal lobe white matter in vascular dementia, Alzheimer's disease, and dementia with Lewy bodies. Acta Neuropathol 119(5): 579–589

Imabayashi E, Matsuda H, Yoshimaru K, Kuji I, Seto A, Shimano Y, Ito K, Kikuta D, Shimazu T, Araki N (2011) Pilot data on telmisartan short-term effects on glucose metabolism in the olfactory tract in Alzheimer's disease. Brain Behav 1(2):63–69

Inaba S, Iwai M, Furuno M, Tomono Y, Kanno H, Senba I, Okayama H, Mogi M, Higaki J, Horiuchi M (2009) Continuous activation of renin-angiotensin system impairs cognitive function in renin/angiotensinogen transgenic mice. Hypertension 53(2):356–362. doi:10.1161/hypertensionaha.108.123612

Ishizaka N, Saito K, Mori I, Matsuzaki G, Ohno M, Nagai R (2005) Iron chelation suppresses ferritin upregulation and attenuates vascular dysfunction in the aorta of angiotensin II-infused rats. Arterioscler Thromb Vasc Biol 25(11):2282–2288

Izawa T, Yamate J, Franklin RJ, Kuwamura M (2010) Abnormal iron accumulation is involved in the pathogenesis of the demyelinating dmy rat but not in the hypomyelinating mv rat. Brain Res 1349:105–114

Jacobs HI, Leritz EC, Williams VJ, Van Boxtel MP, Elst WV, Jolles J, Verhey FR, McGlinchey RE, Milberg WP, Salat DH (2011) Association between white matter microstructure, executive

functions, and processing speed in older adults: the impact of vascular health. Hum Brain Mapp 34(1):77–95

Jennings JR, Mendelson DN, Muldoon MF, Ryan CM, Gianaros PJ, Raz N, Aizenstein H (2012) Regional grey matter shrinks in hypertensive individuals despite successful lowering of blood pressure. J Hum Hypertens 26(5):295–305. doi:10.1038/jhh.2011.31

Jennings JR, Zanstra Y (2009) Is the brain the essential in hypertension? Neuroimage 47(3): 914–921. doi:10.1016/j.neuroimage.2009.04.072

Jimenez S, Torres M, Vizuete M, Sanchez-Varo R, Sanchez-Mejias E, Trujillo-Estrada L, Carmona-Cuenca I, Caballero C, Ruano D, Gutierrez A, Vitorica J (2011) Age-dependent accumulation of soluble amyloid beta (A{beta}) oligomers reverses the neuroprotective effect of soluble amyloid precursor protein-{alpha} (sAPP{alpha}) by modulating phosphatidylinositol 3-kinase (PI3K)/Akt-GSK-3{beta} pathway in Alzheimer mouse model. J Biol Chem 286(21):18414–18425

Johnson ML, Parikh N, Kunik ME, Schulz PE, Patel JG, Chen H, Aparasu RR, Morgan RO (2012) Antihypertensive drug use and the risk of dementia in patients with diabetes mellitus. Alzheimers Dement 8(5):437–444. doi:10.1016/j.jalz.2011.05.2414

Johnstone D, Milward EA (2010) Genome-wide microarray analysis of brain gene expression in mice on a short-term high iron diet. Neurochem Int 56(6–7):856–863

Jonsson T, Stefansson H, Ph DS, Jonsdottir I, Jonsson PV, Snaedal J, Bjornsson S, Huttenlocher J, Levey AI, Lah JJ, Rujescu D, Hampel H, Giegling I, Andreassen OA, Engedal K, Ulstein I, Djurovic S, Ibrahim-Verbaas C, Hofman A, Ikram MA, van Duijn CM, Thorsteinsdottir U, Kong A, Stefansson K (2012) Variant of TREM2 associated with the risk of Alzheimer's disease. N Engl J Med 368(2):107–116

Jope RS, Johnson GV (2004) The glamour and gloom of glycogen synthase kinase-3. Trends Biochem Sci 29(2):95–102

Kang SH, Li Y, Fukaya M, Lorenzini I, Cleveland DW, Ostrow LW, Rothstein JD, Bergles DE (2013) Degeneration and impaired regeneration of gray matter oligodendrocytes in amyotrophic lateral sclerosis. Nat Neurosci 65(5):571–579. doi:10.1038/nn.3357

Kanter JL, Narayana S, Ho PP, Catz I, Warren KG, Sobel RA, Steinman L, Robinson WH (2006) Lipid microarrays identify key mediators of autoimmune brain inflammation. Nat Med 12(1):138–143

Kemper T (1994) Neuroanatomical and neuropathological changes during aging and dementia. In: Albert M, Knoefel J (eds) Clinical neurology of aging, 2nd edn. Oxford University Press, New York, pp 3–67

Kennedy KM, Raz N (2009) Pattern of normal age-related regional differences in white matter microstructure is modified by vascular risk. Brain Res 1297:41–56

Kerr DS, Bevilaqua LR, Bonini JS, Rossato JI, Kohler CA, Medina JH, Izquierdo I, Cammarota M (2005) Angiotensin II blocks memory consolidation through an AT2 receptor-dependent mechanism. Psychopharmacology (Berl) 179(3):529–535. doi:10.1007/s00213-004-2074-5

Kessaris N, Fogarty M, Iannarelli P, Grist M, Wegner M, Richardson WD (2006) Competing waves of oligodendrocytes in the forebrain and postnatal elimination of an embryonic lineage. Nat Neurosci 9(2):173–179

Kim JK, Samaranayake M, Pradhan S (2008) Epigenetic mechanisms in mammals. Cell Mol Life Sci 66(4):596–612

Kimura F, Itami C (2009) Myelination and isochronicity in neural networks. Front Neuroanat 3:12

King MR, Anderson NJ, Guernsey LS, Jolivalt CG (2013) Glycogen synthase kinase-3 inhibition prevents learning deficits in diabetic mice. J Neurosci Res 91(4):506–514. doi:10.1002/jnr.23192

Kivipelto M, Helkala EL, Laakso MP, Hanninen T, Hallikainen M, Alhainen K, Iivonen S, Mannermaa A, Tuomilehto J, Nissinen A, Soininen H (2002) Apolipoprotein E epsilon4 allele, elevated midlife total cholesterol level, and high midlife systolic blood pressure are independent risk factors for late-life Alzheimer disease. Ann Intern Med 137(3):149–155

Klausberger T, Somogyi P (2008) Neuronal diversity and temporal dynamics: the unity of hippocampal circuit operations. Science 321(5885):53–57

Knopman DS (2010) Invited commentary: albuminuria and microvascular disease of the brain—a shared pathophysiology. Am J Epidemiol 171(3):287–289, author reply 290-281

Knopman DS, Penman AD, Catellier DJ, Coker LH, Shibata DK, Sharrett AR, Mosley TH Jr (2011) Vascular risk factors and longitudinal changes on brain MRI: the ARIC study. Neurology 76(22):1879–1885

Kolomeets NS, Uranova N (2010) Ultrastructural abnormalities of astrocytes in the hippocampus in schizophrenia and duration of illness: a postortem morphometric study. World J Biol Psychiatry 11(2 Pt 2):282–292

Kovari E, Gold G, Herrmann FR, Canuto A, Hof PR, Bouras C, Giannakopoulos P (2007) Cortical microinfarcts and demyelination affect cognition in cases at high risk for dementia. Neurology 68(12):927–931

Kume K, Hanyu H, Sakurai H, Takada Y, Onuma T, Iwamoto T (2012) Effects of telmisartan on cognition and regional cerebral blood flow in hypertensive patients with Alzheimer's disease. Geriatr Gerontol Int 12(2):207–214

Lahiri DK, Sambamurti K, Bennett DA (2004) Apolipoprotein gene and its interaction with the environmentally driven risk factors: molecular, genetic and epidemiological studies of Alzheimer's disease. Neurobiol Aging 25(5):651–660

Lambert JC, Heath S, Even G, Campion D, Sleegers K, Hiltunen M, Combarros O, Zelenika D, Bullido MJ, Tavernier B, Letenneur L, Bettens K, Berr C, Pasquier F, Fievet N, Barberger-Gateau P, Engelborghs S, De Deyn P, Mateo I, Franck A, Helisalmi S, Porcellini E, Hanon O, de Pancorbo MM, Lendon C, Dufouil C, Jaillard C, Leveillard T, Alvarez V, Bosco P, Mancuso M, Panza F, Nacmias B, Bossu P, Piccardi P, Annoni G, Seripa D, Galimberti D, Hannequin D, Licastro F, Soininen H, Ritchie K, Blanche H, Dartigues JF, Tzourio C, Gut I, Van Broeckhoven C, Alperovitch A, Lathrop M, Amouyel P (2009) Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. Nat Genet 41(10):1094–1099

Lassek WD, Gaulin SJ (2012) Sex differences in the relationship of dietary Fatty acids to cognitive measures in american children. Front Evol Neurosci 3:5

Lee GJ, Lu PH, Hua X, Lee S, Wu S, Nguyen K, Teng E, Leow AD, Jack CR Jr, Toga AW, Weiner MW, Bartzokis G, Thompson PM (2012) Depressive symptoms in mild cognitive impairment predict greater atrophy in Alzheimer's disease-related regions. Biol Psychiatry 71(9):814–821. doi:10.1016/j.biopsych.2011.12.024

Lehmann DJ, Schuur M, Warden DR, Hammond N, Belbin O, Kolsch H, Lehmann MG, Wilcock GK, Brown K, Kehoe PG, Morris CM, Barker R, Coto E, Alvarez V, Deloukas P, Mateo I, Gwilliam R, Combarros O, Arias-Vasquez A, Aulchenko YS, Ikram MA, Breteler MM, van Duijn CM, Oulhaj A, Heun R, Cortina-Borja M, Morgan K, Robson K, Smith AD (2010) Transferrin and HFE genes interact in Alzheimer's disease risk: the Epistasis Project. Neurobiol Aging 33(1):202e.1–202e.13. doi:10.1016/j.neurobiolaging.2010.07.018

Lei P, Ayton S, Bush AI, Adlard PA (2011) GSK-3 in neurodegenerative diseases. Int J Alzheimers Dis 2011:189246

Leonard WR, Snodgrass JJ, Robertson ML (2007) Effects of brain evolution on human nutrition and metabolism. Annu Rev Nutr 27:311–327

Levine JM, Reynolds R, Fawcett JW (2001) The oligodendrocyte precursor cell in health and disease. Trends Neurosci 24(1):39–47

Lewington S, Clarke R, Qizilbash N, Peto R, Collins R (2002) Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. Lancet 360(9349):1903–1913

Li N-C, Lee A, Whitmer RA, Kivipelto M, Lawler E, Kazis LE, Wolozin B (2010) Use of angiotensin receptor blockers and the risk of dementia in a predominantly male population: a prospective cohort analysis. BMJ (Br Med J) 340:b5465

Liu A, Han YR, Li J, Sun D, Ouyang M, Plummer MR, Casaccia-Bonnefil P (2007) The glial or neuronal fate choice of oligodendrocyte progenitors is modulated by their ability to acquire an epigenetic memory. J Neurosci 27(27):7339–7343

Liu Y, Liu F, Grundke-Iqbal I, Iqbal K, Gong CX (2011) Deficient brain insulin signalling pathway in Alzheimer's disease and diabetes. J Pathol 225(1):54–62

Maillard P, Seshadri S, Beiser A, Himali JJ, Au R, Fletcher E, Carmichael O, Wolf PA, Decarli C (2012) Effects of systolic blood pressure on white-matter integrity in young adults in the Framingham Heart Study: a cross-sectional study. Lancet Neurol 11(12):1039–1047

Mak IT, Landgraf KM, Chmielinska JJ, Weglicki WB (2012) Angiotensin II promotes iron accumulation and depresses PGI(2) and NO synthesis in endothelial cells: effects of losartan and propranolol analogs. Can J Physiol Pharmacol 90(10):1413–1418

Makinodan M, Rosen KM, Ito S, Corfas G (2012) A critical period for social experience-dependent oligodendrocyte maturation and myelination. Science 337(6100):1357–1360. doi:10.1126/science.1220845

Marcus J, Honigbaum S, Shroff S, Honke K, Rosenbluth J, Dupree JL (2006) Sulfatide is essential for the maintenance of CNS myelin and axon structure. Glia 53(4):372–381

Marner L, Nyengaard JR, Tang Y, Pakkenberg B (2003) Marked loss of myelinated nerve fibers in the human brain with age. J Comp Neurol 462(2):144–152

Mateos L, Ismail MA, Gil-Bea FJ, Leoni V, Winblad B, Bjorkhem I, Cedazo-Minguez A (2011) Upregulation of brain renin angiotensin system by 27-hydroxycholesterol in Alzheimer's disease. J Alzheimers Dis 24(4):669–679

Metzler MJ, Saucier DM, Metz GA (2013) Enriched childhood experiences moderate age-related motor and cognitive decline. Front Behav Neurosci 7:1. doi:10.3389/fnbeh.2013.00001

Miller DJ, Duka T, Stimpson CD, Schapiro SJ, Baze WB, McArthur MJ, Fobbs AJ, Sousa AM, Sestan N, Wildman DE, Lipovich L, Kuzawa CW, Hof PR, Sherwood CC (2012) Prolonged myelination in human neocortical evolution. Proc Natl Acad Sci U S A 109(41):16480–16485

Mines MA, Beurel E, Jope RS (2011) Regulation of cell survival mechanisms in Alzheimer's disease by glycogen synthase kinase-3. Int J Alzheimers Dis 2011:861072

Mitew S, Kirkcaldie MT, Halliday GM, Shepherd CE, Vickers JC, Dickson TC (2010) Focal demyelination in Alzheimer's disease and transgenic mouse models. Acta Neuropathol 119(5):567–577

Moghekar A, Kraut M, Elkins W, Troncoso J, Zonderman AB, Resnick SM, O'Brien RJ (2012) Cerebral white matter disease is associated with Alzheimer pathology in a prospective cohort. Alzheimers Dement 8(5 Suppl):S71–77. doi:10.1016/j.jalz.2012.04.006

Moll NM, Rietsch AM, Ransohoff AJ, Cossoy MB, Huang D, Eichler FS, Trapp BD, Ransohoff RM (2008) Cortical demyelination in PML and MS: similarities and differences. Neurology 70(5):336–343

Morell P, Toews AD (1984) In vivo metabolism of oligodendroglial lipids. In: Norton WT (ed) Oligodendroglia. Plenum, New York, pp 47–86

Morton KD, Johnson MD, Van de Kar LD (1995) Serotonin and stress-induced increases in renin secretion are not blocked by sympathectomy/adrenal medullectomy but are blocked by beta antagonists. Brain Res 698(1–2):185–192

Munoz MC, Giani JF, Dominici FP (2012) Angiotensin-(1-7) stimulates the phosphorylation of Akt in rat extracardiac tissues in vivo via receptor Mas. Regul Pept 161(1–3):1–7

Murray ME, Vemuri P, Preboske GM, Murphy MC, Schweitzer KJ, Parisi JE, Jack CR Jr, Dickson DW (2012) A quantitative postmortem MRI design sensitive to white matter hyperintensity differences and their relationship with underlying pathology. J Neuropathol Exp Neurol 71(12):1113–1122

Nakata T, Takeda K, Hatta T, Kiyama M, Moriguchi J, Miki S, Kawa T, Morimoto S, Nakamura K, Uchida A, Itoh H, Sasaki S, Nakagawa M (1998) Blockade of angiotensin II receptors inhibits the increase in blood pressure induced by insulin. J Cardiovasc Pharmacol 31(2):248–252

National_Institute_of_Diabetes_and_Digestive_and_Kidney_Diseases, (2008) National Diabetes Statistics, 2007 Fact Sheet. U.S. Department of Health and Human Services, National Institutes of Health, Bethesda, MD

Nautiyal M, Arnold AC, Chappell MC, Diz DI (2013) The brain renin-angiotensin system and mitochondrial function: influence on blood pressure and baroreflex in transgenic rat strains. Int J Hypertens 2013:136028. doi:10.1155/2013/136028

Neumann H, Kotter MR, Franklin RJ (2008) Debris clearance by microglia: an essential link between degeneration and regeneration. Brain 132(Pt 2):288–295

Niu J, Mei F, Li N, Wang H, Li X, Kong J, Xiao L (2010) Haloperidol promotes proliferation but inhibits differentiation in rat oligodendrocyte progenitor cell cultures. Biochem Cell Biol 88(4):611–620

Noble M, Arhin A, Gass D, Mayer-Proschel M (2003) The cortical ancestry of oligodendrocytes: common principles and novel features. Dev Neurosci 25(2–4):217–233

Norkute A, Hieble A, Braun A, Johann S, Clarner T, Baumgartner W, Beyer C, Kipp M (2009) Cuprizone treatment induces demyelination and astrocytosis in the mouse hippocampus. J Neurosci Res 87(6):1343–1355

O'Kusky J, Colonnier M (1982) Postnatal changes in the number of astrocytes, oligodendrocytes, and microglia in the visual cortex (area 17) of the macaque monkey: a stereological analysis in normal and monocularly deprived animals. J Comp Neurol 210(3):307–315

Oakley AE, Collingwood JF, Dobson J, Love G, Perrott HR, Edwardson JA, Elstner M, Morris CM (2007) Individual dopaminergic neurons show raised iron levels in Parkinson disease. Neurology 68(21):1820–1825

Ohshima K, Mogi M, Jing F, Iwanami J, Tsukuda K, Min LJ, Ogimoto A, Dahlof B, Steckelings UM, Unger T, Higaki J, Horiuchi M (2012) Direct angiotensin II type 2 receptor stimulation ameliorates insulin resistance in type 2 diabetes mice with PPARgamma activation. PLoS One 7(11):e48387. doi:10.1371/journal.pone.0048387

Ottervald J, Franzen B, Nilsson K, Andersson LI, Khademi M, Eriksson B, Kjellstrom S, Marko-Varga G, Vegvari A, Harris RA, Laurell T, Miliotis T, Matusevicius D, Salter H, Ferm M, Olsson T (2013) Multiple sclerosis: Identification and clinical evaluation of novel CSF biomarkers. J Proteomics 73(6):1117–1132

Pelvig DP, Pakkenberg H, Stark AK, Pakkenberg B (2007) Neocortical glial cell numbers in human brains. Neurobiol Aging 29(11):1754–1762

Petanjek Z, Judas M, Simic G, Rasin MR, Uylings HB, Rakic P, Kostovic I (2012) Extraordinary neoteny of synaptic spines in the human prefrontal cortex. Proc Natl Acad Sci U S A 108(32): 13281–13286

Peters A (2007) The effects of normal aging on nerve fibers and neuroglia in the central nervous system. In: Riddle DR (ed) Brain aging: models, methods, and mechanisms. Frontiers in neuroscience. CRC, Boca Raton, FL

Peters A (2009) The effects of normal aging on myelinated nerve fibers in monkey central nervous system. Front Neuroanat 3 (article 11): 10 p

Peters A, Kemper T (2012) A review of the structural alterations in the cerebral hemispheres of the aging rhesus monkey. Neurobiol Aging 33(10):2357–2372

Peters A, Sethares C (2003) Is there remyelination during aging of the primate central nervous system? J Comp Neurol 460(2):238–254

Peters A, Sethares C (2004) Oligodendrocytes, their progenitors and other neuroglial cells in the aging primate cerebral cortex. Cereb Cortex 14:995–1007

Peters A, Sethares C, Moss MB (2010) How the primate fornix is affected by age. J Comp Neurol 518(19):3962–3980

Peters A, Verderosa A, Sethares C (2008) The neuroglial population in the primary visual cortex of the aging rhesus monkey. Glia 56(11):1151–1161

Petrovitch H, White LR, Izmirilian G, Ross GW, Havlik RJ, Markesbery W, Nelson J, Davis DG, Hardman J, Foley DJ, Launer LJ (2000) Midlife blood pressure and neuritic plaques, neurofibrillary tangles, and brain weight at death: the HAAS. Honolulu-Asia aging Study. Neurobiol Aging 21(1):57–62

Poggesi A, Pantoni L, Inzitari D, Fazekas F, Ferro J, O'Brien J, Hennerici M, Scheltens P, Erkinjuntti T, Visser M, Langhorne P, Chabriat H, Waldemar G, Wallin A, Wahlund A (2011) 2001–2011: A decade of the LADIS (Leukoaraiosis And DISability) study: what have we learned about white matter changes and small-vessel disease? Cerebrovasc Dis 32(6):577–588. doi:10.1159/000334498

Pohl HB, Porcheri C, Mueggler T, Bachmann LC, Martino G, Riethmacher D, Franklin RJ, Rudin M, Suter U (2011) Genetically induced adult oligodendrocyte cell death is associated with poor myelin clearance, reduced remyelination, and axonal damage. J Neurosci 31(3):1069–1080

Polvikoski TM, van Straaten EC, Barkhof F, Sulkava R, Aronen HJ, Niinisto L, Oinas M, Scheltens P, Erkinjuntti T, Kalaria RN (2010) Frontal lobe white matter hyperintensities and neurofibrillary pathology in the oldest old. Neurology 75(23):2071–2078

Popkie AP, Zeidner LC, Albrecht AM, D'Ippolito A, Eckardt S, Newsom DE, Groden J, Doble BW, Aronow B, McLaughlin KJ, White P, Phiel CJ (2010) Phosphatidylinositol 3-kinase (PI3K) signaling via glycogen synthase kinase-3 (Gsk-3) regulates DNA methylation of imprinted loci. J Biol Chem 285(53):41337–41347

Popko B (2008) Epigenetic control of myelin repair. Nat Neurosci 11(9):987–988

Power J, Mayer-Proschel M, Smith J, Noble M (2002) Oligodendrocyte precursor cells from different brain regions express divergent properties consistent with the differing time courses of myelination in these regions. Dev Biol 245(2):362–375

Prins ND, van Dijk EJ, den Heijer T, Vermeer SE, Koudstaal PJ, Oudkerk M, Hofman A, Breteler MM (2004) Cerebral white matter lesions and the risk of dementia. Arch Neurol 61(10): 1531–1534. doi:10.1001/archneur.61.10.1531

Quintana C, Bellefqih S, Laval JY, Guerquin-Kern JL, Wu TD, Avila J, Ferrer I, Arranz R, Patino C (2006) Study of the localization of iron, ferritin, and hemosiderin in Alzheimer's disease hippocampus by analytical microscopy at the subcellular level. J Struct Biol 153(1):42–54

Raber J, Huang Y, Ashford JW (2004) ApoE genotype accounts for the vast majority of AD risk and AD pathology. Neurobiol Aging 25(5):641–650

Raji CA, Lopez OL, Kuller LH, Carmichael OT, Longstreth WT Jr, Gach HM, Boardman J, Bernick CB, Thompson PM, Becker JT (2012) White matter lesions and brain gray matter volume in cognitively normal elders. Neurobiol Aging 33(4):834 e837–834 e816

Rakic P (2002) Genesis of neocortex in human and nonhuman primates. In: Lewis M (ed) Child and adolescent psychiatry: a comprehensive textbook, 3rd edn. Lippincott Williams & Williams, Philadelphia, PA, pp 22–46

Ranadive SA, Chen AX, Serajuddin AT (1992) Relative lipophilicities and structural-pharmacological considerations of various angiotensin-converting enzyme (ACE) inhibitors. Pharm Res 9(11): 1480–1486

Rinholm JE, Hamilton NB, Kessaris N, Richardson WD, Bergersen LH, Attwell D (2011) Regulation of oligodendrocyte development and myelination by glucose and lactate. J Neurosci 31(2):538–548

Roosendaal SD, Moraal B, Pouwels PJ, Vrenken H, Castelijns JA, Barkhof F, Geurts JJ (2009) Accumulation of cortical lesions in MS: relation with cognitive impairment. Mult Scler 15(6): 708–714

Saavedra JM (2012) Angiotensin II AT1 receptor blockers as treatments for inflammatory brain disorders. Clin Sci (Lond) 123(10):567–590

Saher G, Brugger B, Lappe-Siefke C, Mobius W, Tozawa R, Wehr MC, Wieland F, Ishibashi S, Nave KA (2005) High cholesterol level is essential for myelin membrane growth. Nat Neurosci 8(4):468–475

Sakai T, Mikami A, Tomonaga M, Matsui M, Suzuki J, Hamada Y, Tanaka M, Miyabe-Nishiwaki T, Makishima H, Nakatsukasa M, Matsuzawa T (2011) Differential prefrontal white matter development in chimpanzees and humans. Curr Biol 21(16):1397–1402

Salami M, Itami C, Tsumoto T, Kimura F (2003) Change of conduction velocity by regional myelination yields constant latency irrespective of distance between thalamus and cortex. Proc Natl Acad Sci U S A 100(10):6174–6179

Salat DH, Tuch DS, van der Kouwe AJ, Greve DN, Pappu V, Lee SY, Hevelone ND, Zaleta AK, Growdon JH, Corkin S, Fischl B, Rosas HD (2010) White matter pathology isolates the hippocampal formation in Alzheimer's disease. Neurobiol Aging 31(2):244–256

Salat DH, Williams VJ, Leritz EC, Schnyer DM, Rudolph JL, Lipsitz LA, McGlinchey RE, Milberg WP (2012) Inter-individual variation in blood pressure is associated with regional white matter integrity in generally healthy older adults. Neuroimage 59(1):181–192

Sanchez-Abarca LI, Tabernero A, Medina JM (2001) Oligodendrocytes use lactate as a source of energy and as a precursor of lipids. Glia 36(3):321–329

Schoenemann PT, Sheehan MJ, Glotzer LD (2005) Prefrontal white matter volume is disproportionately larger in humans than in other primates. Nat Neurosci 8(2):242–252

Scholz J, Klein MC, Behrens TE, Johansen-Berg H (2009) Training induces changes in white-matter architecture. Nat Neurosci 12(11):1370–1371

Schonberg DL, Goldstein EZ, Sahinkaya FR, Wei P, Popovich PG, McTigue DM (2012) Ferritin stimulates oligodendrocyte genesis in the adult spinal cord and can be transferred from macrophages to NG2 cells in vivo. J Neurosci 32(16):5374–5384

Schonberg DL, McTigue DM (2009) Iron is essential for oligodendrocyte genesis following intraspinal macrophage activation. Exp Neurol 218(1):64–74

Schuitemaker A, van der Doef TF, Boellaard R, van der Flier WM, Yaqub M, Windhorst AD, Barkhof F, Jonker C, Kloet RW, Lammertsma AA, Scheltens P, van Berckel BN (2012) Microglial activation in healthy aging. Neurobiol Aging 33(6):1067–1072

Schulz K, Kroner A, David S (2012) Iron efflux from astrocytes plays a role in remyelination. J Neurosci 32(14):4841–4847. doi:10.1523/jneurosci.5328-11.2012

Schulz K, Vulpe CD, Harris LZ, David S (2011) Iron efflux from oligodendrocytes is differentially regulated in gray and white matter. J Neurosci 31(37):13301–13311. doi:10.1523/jneurosci.2838-11.2011

Scotland PB, Heath JL, Conway AE, Porter NB, Armstrong MB, Walker JA, Klebig ML, Lavau CP, Wechsler DS (2012) The PICALM protein plays a key role in iron homeostasis and cell proliferation. PLoS One 7(8):e44252

Shen S, Liu A, Li J, Wolubah C, Casaccia-Bonnefil P (2006) Epigenetic memory loss in aging oligodendrocytes in the corpus callosum. Neurobiol Aging 29(3):452–463

Shen S, Sandoval J, Swiss VA, Li J, Dupree J, Franklin RJ, Casaccia-Bonnefil P (2008) Age-dependent epigenetic control of differentiation inhibitors is critical for remyelination efficiency. Nat Neurosci 11(9):1024–1034

Shields SA, Gilson JM, Blakemore WF, Franklin RJ (1999) Remyelination occurs as extensively but more slowly in old rats compared to young rats following gliotoxin-induced CNS demyelination. Glia 28(1):77–83

Shih AY, Blinder P, Tsai PS, Friedman B, Stanley G, Lyden PD, Kleinfeld D (2013) The smallest stroke: occlusion of one penetrating vessel leads to infarction and a cognitive deficit. Nat Neurosci 16(1):55–63. doi:10.1038/nn.3278

Silbert LC, Dodge HH, Perkins LG, Sherbakov L, Lahna D, Erten-Lyons D, Woltjer R, Shinto L, Kaye JA (2012) Trajectory of white matter hyperintensity burden preceding mild cognitive impairment. Neurology 79(8):741–747. doi:10.1212/WNL.0b013e3182661f2b

Simon B, Schmidt S, Lukas C, Gieseke J, Traber F, Knol DL, Willinek WA, Geurts JJ, Schild HH, Barkhof F, Wattjes MP (2010) Improved in vivo detection of cortical lesions in multiple sclerosis using double inversion recovery MR imaging at 3 Tesla. Eur Radiol 20(7):1675–1683

Sink KM, Leng X, Williamson J, Kritchevsky SB, Yaffe K, Kuller L, Yasar S, Atkinson H, Robbins M, Psaty B, Goff DC Jr (2009) Angiotensin-converting enzyme inhibitors and cognitive decline in older adults with hypertension: results from the Cardiovascular Health Study. Arch Intern Med 169(13):1195–1202

Sinka L, Kovari E, Gold G, Hof PR, Herrmann FR, Bouras C, Giannakopoulos P (2010) Small vascular and Alzheimer disease-related pathologic determinants of dementia in the oldest-old. J Neuropathol Exp Neurol 69(12):1247–1255. doi:10.1097/NEN.0b013e3181ffc3b9

Skripuletz T, Lindner M, Kotsiari A, Garde N, Fokuhl J, Linsmeier F, Trebst C, Stangel M (2008) Cortical demyelination is prominent in the murine cuprizone model and is strain-dependent. Am J Pathol 172(4):1053–1061

Smaers JB, Schleicher A, Zilles K, Vinicius L (2010) Frontal white matter volume is associated with brain enlargement and higher structural connectivity in anthropoid primates. PLoS One 5(2):e9123

Smith EE, Schneider JA, Wardlaw JM, Greenberg SM (2012) Cerebral microinfarcts: the invisible lesions. Lancet Neurol 11(3):272–282. doi:10.1016/s1474-4422(11)70307-6

Sonnen JA, Larson EB, Crane PK, Haneuse S, Li G, Schellenberg GD, Craft S, Leverenz JB, Montine TJ (2007) Pathological correlates of dementia in a longitudinal, population-based sample of aging. Ann Neurol 62(4):406–413. doi:10.1002/ana.21208

Soros P, Whitehead S, Spence JD, Hachinski V (2012) Antihypertensive treatment can prevent stroke and cognitive decline. Nat Rev Neurol 9(3):174–178. doi:10.1038/nrneurol.2012.255

Sow A, Lamant M, Bonny JM, Larvaron P, Piaud O, Lecureuil C, Fontaine I, Saleh MC, Garcia Otin AL, Renou JP, Baron B, Zakin M, Guillou F (2006) Oligodendrocyte differentiation is increased in transferrin transgenic mice. J Neurosci Res 83(3):403–414

Stark AK, Uylings HB, Sanz-Arigita E, Pakkenberg B (2004) Glial cell loss in the anterior cingulate cortex, a subregion of the prefrontal cortex, in subjects with schizophrenia. Am J Psychiatry 161(5):882–888

Staschewski J, Kulisch C, Albrecht D (2012) Different isoforms of nitric oxide synthase are involved in angiotensin-(1-7)-mediated plasticity changes in the amygdala in a gender-dependent manner. Neuroendocrinology 94(3):191–199

Steen E, Terry BM, Rivera EJ, Cannon JL, Neely TR, Tavares R, Xu XJ, Wands JR, de la Monte SM (2005) Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease—is this type 3 diabetes? J Alzheimers Dis 7(1):63–80

Steiner J, Bielau H, Brisch R, Danos P, Ullrich O, Mawrin C, Bernstein HG, Bogerts B (2008) Immunological aspects in the neurobiology of suicide: elevated microglial density in schizophrenia and depression is associated with suicide. J Psychiatr Res 42(2):151–157

Stornetta RL, Hawelu-Johnson CL, Guyenet PG, Lynch KR (1988) Astrocytes synthesize angiotensinogen in brain. Science 242(4884):1444–1446

Sutherland C (2011) What are the bona fide GSK3 Substrates? Int J Alzheimers Dis 2011:505607

Syner FN, Goodman M (1966) Differences in the lactic dehydrogenases of primate brains. Nature 209(5021):426–428

Szeligo F, Leblond CP (1977) Response of the three main types of glial cells of cortex and corpus callosum in rats handled during suckling or exposed to enriched, control and impoverished environments following weaning. J Comp Neurol 172(2):247–263

Takao M, Koto A, Tanahashi N, Fukuuchi Y, Takagi M, Morinaga S (1999) Pathologic findings of silent hyperintense white matter lesions on MRI. J Neurol Sci 167:127–131

Tanzi RE (2012) The genetics of Alzheimer disease. Cold Spring Harb Perspect Med 2(10), doi:10.1101/cshperspect.a006296

Terman A, Brunk UT (2004) Lipofuscin. Int J Biochem Cell Biol 36(8):1400–1404. doi:10.1016/j.biocel.2003.08.009

Terry RD, Masliah E, Salmon DP, Butters N, DeTeresa R, Hill R, Hansen LA, Katzman R (1991) Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. AnnNeurol 30:572–580

Thompson MM, Oyama TT, Kelly FJ, Kennefick TM, Anderson S (2000) Activity and responsiveness of the renin-angiotensin system in the aging rat. Am J Physiol Regul Integr Comp Physiol 279(5):R1787–1794

Tian J, Shi J, Bailey K, Harris JM, Pritchard A, Lambert JC, Chartier-Harlin MC, Pickering-Brown SM, Lendon CL, Mann DM (2004) A polymorphism in the angiotensin 1-converting enzyme gene is associated with damage to cerebral cortical white matter in Alzheimer's disease. Neurosci Lett 354(2):103–106

Tian M, Zhu D, Xie W, Shi J (2012) Central angiotensin II-induced Alzheimer-like tau phosphorylation in normal rat brains. FEBS Lett 586(20):3737–3745

Todorich B, Pasquini JM, Garcia CI, Paez PM, Connor JR (2009) Oligodendrocytes and myelination: the role of iron. Glia 57(5):467–478

Tripathi RB, Clarke LE, Burzomato V, Kessaris N, Anderson PN, Attwell D, Richardson WD (2011) Dorsally and ventrally derived oligodendrocytes have similar electrical properties but myelinate preferred tracts. J Neurosci 31(18):6809–6819

Tsuang D, Leverenz JB, Lopez OL, Hamilton RL, Bennett DA, Schneider JA, Buchman AS, Larson EB, Crane PK, Kaye JA, Kramer P, Woltjer R, Trojanowski JQ, Weintraub D, Chen-Plotkin AS, Irwin DJ, Rick J, Schellenberg GD, Watson GS, Kukull W, Nelson PT, Jicha GA, Neltner JH, Galasko D, Masliah E, Quinn JF, Chung KA, Yearout D, Mata IF, Wan JY, Edwards KL, Montine TJ, Zabetian CP (2013) APOE 4 increases risk for dementia in pure synucleinopathies. JAMA Neurol 70(2):223–228. doi:10.1001/jamaneurol.2013.600

Uranova NA, Vostrikov VM, Orlovskaya DD, Rachmanova VI (2004) Oligodendroglial density in the prefrontal cortex in schizophrenia and mood disorders: a study from the Stanley Neuropathology Consortium. Schizophr Res 67(2–3):269–275

van Dijk EJ, Prins ND, Vrooman HA, Hofman A, Koudstaal PJ, Breteler MM (2008) Progression of cerebral small vessel disease in relation to risk factors and cognitive consequences: Rotterdam Scan study. Stroke 39(10):2712–2719. doi:10.1161/strokeaha.107.513176

Vasan RS, Beiser A, Seshadri S, Larson MG, Kannel WB, D'Agostino RB, Levy D (2002) Residual lifetime risk for developing hypertension in middle-aged women and men: the Framingham Heart Study. Jama 287(8):1003–1010

Versace A, Andreazza AC, Young LT, Fournier JC, Almeida JR, Stiffler RS, Lockovich JC, Aslam HA, Pollock MH, Park H, Nimgaonkar VL, Kupfer DJ, Phillips ML (2013) Elevated serum measures of lipid peroxidation and abnormal prefrontal white matter in euthymic bipolar adults: toward peripheral biomarkers of bipolar disorder. Mol Psychiatry. doi:10.1038/mp.2012.188

Vinet J, Lemieux P, Tamburri A, Tiesinga P, Scafidi J, Gallo V, Sik A (2010) Subclasses of oligodendrocytes populate the mouse hippocampus. Eur J Neurosci 31(3):425–438

Voineskos AN, Foussias G, Lerch J, Felsky D, Remington G, Rajji TK, Lobaugh N, Pollock BG, Mulsant BH (2013) Neuroimaging evidence for the deficit subtype of schizophrenia. JAMA Psychiatry 70(5):472–480. doi:10.1001/jamapsychiatry.2013.786

Vostrikov V, Uranova N (2011) Age-related increase in the number of oligodendrocytes is dysregulated in schizophrenia and mood disorders. Schizophr Res Treat (Article ID 174689P:10 pages. doi:10.1155/2011/174689

Vostrikov VM, Uranova NA, Orlovskaya DD (2007) Deficit of perineuronal oligodendrocytes in the prefrontal cortex in schizophrenia and mood disorders. Schizophr Res 94(1–3):273–280

Wegner C, Esiri MM, Chance SA, Palace J, Matthews PM (2006) Neocortical neuronal, synaptic, and glial loss in multiple sclerosis. Neurology 67(6):960–967

White LR, Gelber RP, Launer LJ, Zarow C, Sonnen J, Uyehara-Lock J, Masaki K, Ross GW, Petrovitch H (2013) Beta blocker treatment of hypertensive older persons ameliorates the brain lesions of dementia measured at autopsy: the Honolulu-Asia Aging Study. Paper presented at the Presented at the American Academy of Neurology 65th Annual meeting, San Diego, 16–23 March

Whitmer RA, Gustafson DR, Barrett-Connor E, Haan MN, Gunderson EP, Yaffe K (2008) Central obesity and increased risk of dementia more than three decades later. Neurology 71(14):1057–1064

Wiggins RC (1982) Myelin development and nutritional insufficiency. Brain Res 257(2):151–175

Wishart TM, Parson SH, Gillingwater TH (2006) Synaptic vulnerability in neurodegenerative disease. J Neuropathol Exp Neurol 65(8):733–739

Xia H, Suda S, Bindom S, Feng Y, Gurley SB, Seth D, Navar LG, Lazartigues E (2012) ACE2-mediated reduction of oxidative stress in the central nervous system is associated with improvement of autonomic function. PLoS One 6(7):e22682

Yaffe K, Falvey C, Hamilton N, Schwartz AV, Simonsick EM, Satterfield S, Cauley JA, Rosano C, Launer LJ, Strotmeyer ES, Harris TB (2012) Diabetes, glucose control, and 9-year cognitive decline among older adults without dementia. Arch Neurol 69(9):1170–1175

Yang S, Li C, Qiu X, Zhang L, Lu W, Chen L, Zhao YY, Shi XY, Huang CX, Cheng GH, Tang Y (2013) Effects of an enriched environment on myelin sheaths in the white matter of rats during normal aging: a stereological study. Neuroscience 234C:13–21. doi:10.1016/j.neuroscience.2013.01.003

Yang Y, Faustino PJ, Volpe DA, Ellison CD, Lyon RC, Yu LX (2007) Biopharmaceutics classification of selected beta-blockers: solubility and permeability class membership. Mol Pharm 4(4):608–614. doi:10.1021/mp070028i

Yuskaitis CJ, Jope RS (2009) Glycogen synthase kinase-3 regulates microglial migration, inflammation, and inflammation-induced neurotoxicity. Cell Signal 21(2):264–273

Zajc Petranovic M, Skaric-Juric T, Smolej Narancic N, Tomas Z, Krajacic P, Milicic J, Barbalic M, Tomek-Roksandic S (2012) Angiotensin-converting enzyme deletion allele is beneficial for the longevity of Europeans. Age (Dordr) 34(3):583–595

Zhang K, Sejnowski TJ (2000) A universal scaling law between gray matter and white matter of cerebral cortex. Proc Natl Acad Sci U S A 97(10):5621–5626

Zhang M, Mao Y, Ramirez SH, Tuma RF, Chabrashvili T (2010) Angiotensin II induced cerebral microvascular inflammation and increased blood-brain barrier permeability via oxidative stress. Neuroscience 171(3):852–858

Zhao WQ, Townsend M (2009) Insulin resistance and amyloidogenesis as common molecular foundation for type 2 diabetes and Alzheimer's disease. Biochim Biophys Acta 1792(5):482–496

Zhuang L, Sachdev PS, Trollor JN, Kochan NA, Reppermund S, Brodaty H, Wen W (2012) Microstructural white matter changes in cognitively normal individuals at risk of amnestic MCI. Neurology 79(8):748–754. doi:10.1212/WNL.0b013e3182661f4d

Zimmerman MC (2011) Angiotensin II and angiotensin-1-7 redox signaling in the central nervous system. Curr Opin Pharmacol 11(2):138–143

Ziskin JL, Nishiyama A, Rubio M, Fukaya M, Bergles DE (2007) Vesicular release of glutamate from unmyelinated axons in white matter. Nat Neurosci 10(3):321–330

Chapter 15
Unmyelinated and Myelinated Axons Exhibit Differential Injury and Treatment Responses Following Traumatic Injury

Thomas M. Reeves, Adele E. Doperalski, and Linda L. Phillips

Abbreviations

ACSF	Artificial cerebrospinal fluid
APP	Amyloid precursor protein
BBB	Blood–brain barrier
CAP	Compound action potential
CC	Corpus callosum
CsA	Cyclosporin-A
DAI	Diffuse axonal injury
DTI	Diffusion tensor imaging
FPI	Fluid percussion injury
HRP	Horseradish peroxidase
IC	Internal capsule
IPI	Interpulse interval
MMP	Matrix metalloproteinase
MS	Multiple sclerosis
N1	CAP generated by myelinated axons
N2	CAP generated by unmyelinated axons
TAI	Traumatic axonal injury
TBI	Traumatic brain injury

Over 1.5 million new traumatic brain injury (TBI) cases are reported annually in the U.S. alone (Rutland-Brown et al. 2006), leading to an enduring public health problem and persistent challenges in patients' lives. Experimental and clinical evidence

T.M. Reeves, Ph.D. (✉) • A.E. Doperalski, Ph.D. • L.L. Phillips
Department of Anatomy and Neurobiology, Virginia Commonwealth University,
P.O. Box 980709, Richmond, VA 23298, USA
e-mail: tmreeves@vcu.edu

S. Baltan et al. (eds.), *White Matter Injury in Stroke and CNS Disease*, Springer Series in Translational Stroke Research 4, DOI 10.1007/978-1-4614-9123-1_15,

reveals TBI as a complex and multifaceted pathology, affecting widespread cellular and vascular systems (for reviews: Graham et al. 2000; Phillips and Reeves 2001; Leker and Shohami 2002; Povlishock and Katz 2005; Park et al. 2008). Despite these complexities, it has become clear that axons are among the most vulnerable, and the most commonly injured, cellular components in the nervous system (Adams et al. 1989; Maxwell et al. 1997; Povlishock 1992; Smith and Meaney 2000). Axonal injury is a concomitant of most TBIs requiring hospitalization. Diffuse axonal injury (DAI) was recently observed to occur in 72 % of patients with moderate or severe TBI, and was associated with worse outcome (Skandsen et al. 2010). This diffuse pathology is also associated with posttraumatic coma, and persistent memory deficits with impaired information processing capacity (Meythaler et al. 2001).

Research has identified key aspects of traumatic axonal injury and established that the pathology is multiphasic, with a rapid primary injury phase, including a failure of ionic homeostasis, evolving over hours and days to a more protracted secondary injury phase involving aberrant biochemical cascades and proteolysis. Research findings in recent years have led to significant advances towards an understanding of the pathogenetic mechanisms in traumatic axonal injury, but many questions remain unanswered.

A fundamental gap, in the knowledge-base pertaining to traumatic axonal injury, is uncertainty regarding whether all axons respond to injury in the same way. Currently there is only sparse information regarding how axon phenotype influences the extent of injury and the capacity for recovery, and this impedes progress towards a comprehensive understanding of the pathomechanisms of axonal injury while limiting the search for new therapeutic strategies. The following discussion reviews evidence, accumulated using experimental animal models of TBI, indicating that separate populations of axons undergo distinctive responses to injury, and to treatment with neuroprotective compounds. The presence or absence of myelin is one phenotypic dimension on which axons may be classified into discrete populations. As an initial approach to study how axon type influences the injury process, our laboratory has investigated how unmyelinated axons undergo an injury response distinct from that of myelinated axons.

This chapter first considers the conceptual basis for predicting an elevated risk to traumatic injury in the unmyelinated axon population. This is followed by a summary of quantitative ultrastructural evidence of fiber-type-specific changes in morphology observed in corpus callosum (CC) axons following TBI. Next, we describe electrophysiological findings which document dissimilar conduction deficits in the unmyelinated and myelinated axon populations, and differential degrees of responsiveness to neuroprotective compounds. Subsequently, we discuss a set of results which demonstrate the critical role of the extracellular matrix in axonal posttraumatic sequelae, and how the unmyelinated fiber interaction with matrix elements may diverge from that of myelinated fibers. Finally, we describe initial results in a new line of inquiry, recently undertaken in our laboratory, to contrast axonal injury in white matter having mixed unmyelinated and myelinated axons, the CC, with that of the internal capsule, which is predominantly composed of myelinated axons.

15.1 Intrinsic Axon Properties Influence Vulnerability

The conceptual framework, regarding mechanisms of axonal injury following closed head (nonpenetrating) injury, has evolved beyond the once prevalent notion that axon death is due mainly to shear and tensile forces acting at the moment of injury (Strich 1961). It is now well established that, at least for myelinated CNS axons, most injured fibers undergo a progressive secondary pathology involving cytoskeletal changes, impaired axoplasmic transport, and axonal swelling (Povlishock and Christman 1995; Maxwell et al. 1997; Saatman et al. 1998; Smith et al. 1998). However, more recent studies provided indirect evidence for subpopulations of axons which undergo different forms of the secondary injury response. Some injured axons exhibited neurofilament compaction independently of impaired fast transport (Stone et al. 2001; DiLeonardi et al. 2009). Moreover, the degree to which these two pathological components were both expressed in individual axons was determined, in part, by axon caliber and anatomical location. Larger medial lemniscal fibers exhibited both pathologies concurrently to a greater extent than was the case for smaller corticospinal axons (Stone et al. 2001), and treatment with the immunophilin ligand FK506 differentially attenuated these abnormalities (Marmarou and Povlishock 2006). Other workers, using an in vivo optic-nerve stretch model of injury, reported that caliber of the myelinated axons was associated with several pathological parameters of axonal injury, including the degree of neurofilament and microtubule damage (Jafari et al. 1997, 1998). In a morphological study of the CC of nonhuman primates, axolemmal tearing was observed to occur selectively at the nodes of small, thinly-myelinated axons (Maxwell et al. 1993). These diverse findings converge on the idea that intrinsic properties of axons, especially axon caliber, are important determinants of the severity of posttraumatic pathology. This presents a contrast to prevailing concepts of axonal injury as an undifferentiated pathology, present in varying degrees but generally affecting populations of axons indiscriminately.

15.1.1 Rationale for Predicting Elevated Risk to Unmyelinated Axons

There are both theoretical and empirical reasons for predicting that differences in fiber size, and the presence or absence of myelin, are likely to be critical factors in the injury processes affecting axons. Figure 15.1 suggests how fundamental physical differences between unmyelinated vs. myelinated axons are predicted to affect the course of pathological events following TBI. Myelin itself may exert a protective benefit in at least two ways. First, layers of myelin may provide physical support and function as a protective cushion at the instant of injury, reducing the mechanical forces acting on underlying axolemma. Secondly, myelin may shield underlying axolemmal membrane from the aberrant extracellular milieu known to

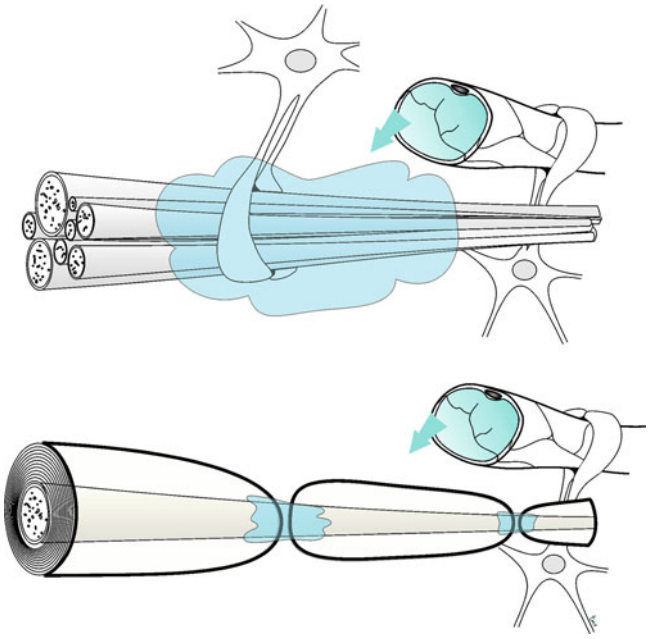


Fig. 15.1 Morphological properties of unmyelinated axons may place them at elevated risk to TBI. Diagram of unmyelinated axons, and a myelinated axon, in relation to nonneuronal elements (blood vessels, astrocytes). Unmyelinated axolemma is more exposed, than myelinated, to destructive extracellular influences (*shading*) arising locally (e.g., ionic imbalances and activated proteases), or from blood-borne factors infiltrating through a compromised blood–brain barrier (*arrows*). These structural differences are likely to contribute to a greater vulnerability of unmyelinated axons in some injury conditions. From Reeves et al. 2012, with permission

prevail after neurotrauma, including ionic imbalances (Katayama et al. 1990), and destructive proteolysis (Hall et al. 2005; Saatman et al. 2010; Reeves et al. 2010). Figure 15.1 also conveys that these two types of fibers present strikingly different degrees of axolemmal exposure to deleterious blood-borne factors, known to gain parenchymal access subsequent to TBI-induced failure of the blood–brain barrier (Kelley et al. 2007; Morganti-Kossmann et al. 2007; Cederberg and Siesjo 2010). While the lack of myelin may constitute a risk factor for the unmyelinated fibers, it may, paradoxically, provide a treatment benefit to those same axons as neuroprotective drugs are developed and applied, because these compounds will have more access to the full length of axolemma in the unmyelinated population.

Based on their biophysical properties, we hypothesized that unmyelinated axons are at an elevated risk to TBI. Among all components of neuronal cytoarchitecture, axons are the subcellular compartment with the highest membrane-to-cytoplasm ratio, which likely renders them vulnerable to membrane-targeting pathomechanisms of TBI, including lipid peroxidation (Evans 1993; Xiong et al. 2007), rapid proteolysis of voltage-gated sodium channels (Iwata et al. 2004; von Reyn et al. 2009), and more protracted proteolytic events attacking submembrane ankyrin

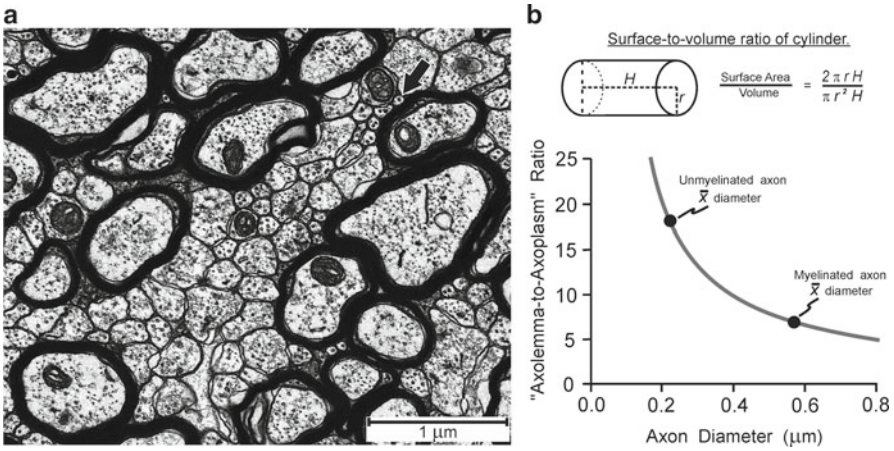


Fig. 15.2 Small diameter axons have a proportionately high membrane content. (**a**) Sample electron micrograph of CC axons in cross section, illustrating striking differences in size of unmyelinated and myelinated axons, with some unmyelinated diameters below 0.1 µm (*arrow*). (**b**) Modeling axons as uniform cylinders of arbitrary length, a decrease in diameter corresponds to an increase in surface-to-volume ratio. In cellular terms, an increased ratio of axolemma area to axoplasm volume, probably constitutes a risk to membrane-targeting TBI pathologies. In the *lower panel*, the mean diameters for unmyelinated and myelinated axons, measured in our laboratory, are plotted on the *curve* relating diameter to the surface-to-volume ratio. The mean diameter of unmyelinated axons is about 60 % smaller than the mean myelinated diameter, but this corresponds to an approximate 160 % increase in the surface-to-volume ratio. Panel B from Reeves et al. 2012, with permission

(Reeves et al. 2010) and spectrin (Hall et al. 2005; Saatman et al. 2003, 2010). In ultrastructural examination of CNS axons, the striking differences in scale of the unmyelinated and myelinated axons is readily apparent. Most of our recent observations in this regard have been directed at the CC of adult rats, and Fig. 15.2a shows an example micrograph of callosal axons in sagittal section, as they cross the midline. In an extensive stereological examination of these fibers (described below), we measured the mean diameter of the unmyelinated axons as 0.22 µm, and myelinated axons as 0.57 µm (axolemmal-bound region only, not including myelin layers). Unmyelinated axons at the smallest end of the size distribution present a cross-sectional appearance which is little more than a single microtubule with only a minimal volume of cytoplasm, corresponding to an extremely high membrane fraction. To quantify this relationship, Fig. 15.2b models axons as uniform cylinders of arbitrary length, and plots our measured values of mean axon diameter on the curve relating surface-to-volume ratio to diameter. It is notable that while the mean diameter of unmyelinated axons is about 60 % smaller than the mean myelinated diameter, this corresponds to a 160 % increase in the surface-to-volume ratio, or "axolemma-to-axoplasm" ratio as depicted in the graphic. There is reason to expect this substantial difference in cellular geometry affects the course of postinjury axonal pathology. Excessive elevation of intracellular calcium is widespread after TBI, and

this pathomechanism may especially challenge small axons with less cytoplasmic volume and calcium buffering/sequestration capacity, which is known to be critical in white matter injury (Stys 2004). These concepts formed the basis for our hypothesis that unmyelinated and myelinated axons would exhibit a differential vulnerability following an experimental TBI. The following material summarizes the methods and key results from those series of experiments.

15.2 Description of Injury Model

15.2.1 Midline Fluid Percussion Injury Model

The experimental TBI model selected for these studies was fluid percussion injury (FPI) in adult rats. This model was originally developed at our institution (Dixon et al. 1987), and has subsequently been extensively characterized and applied to a wide range of cellular pathologies initiated by neurotrauma (for recent reviews, Thompson et al. 2005; O'Connor et al. 2011; Reeves and Colley 2012). All procedures followed national guidelines for the care and use of experimental animals, and experimental protocols were approved by our institutional Animal Research Committee. For details of the FPI methodology readers are referred to the original reports summarized in this chapter (Colley et al. 2010; Reeves and Colley 2012; Reeves et al. 2005, 2007, 2010, 2012). In brief, FPI is induced by transiently injecting a small volume of saline into the epidural space through a 4.5 mm craniotomy, which deforms the brain tissue for a duration of approximately 20 ms. Injury severity is controlled by varying the pressure pulse magnitude, and the technique allows flexible craniotomy placement, to locate the pressure pulse over the cortical region of interest. In all experiments described here, FPI was applied at a moderate intensity (~2.0 atm.) to the brain midline, equidistant between bregma and lambda.

Our laboratory has consistently focused on TBI pathomechanisms related to sublethal changes in neurons. Cells which survive the primary-phase trauma will form the basis for subsequent functional recovery. In the case of axons, degenerating axons are not salvageable, but fibers which undergo time-dependent changes in structure and function form valid targets for intervention. The specific FPI parameters used for our studies (moderate intensity, midline location) induce a diffuse brain injury without contusion or hematoma, and without significant Wallerian degeneration. However, this injury does produce functional impairments, including suppression of axonal excitability (Baker et al. 2002; Reeves et al. 2005, 2007; Ai et al. 2007; Colley et al. 2010), and deficits in spatial cognition without hippocampal cell death (Lyeth et al. 1990). For these reasons, this injury model was well suited to investigate how unmyelinated axons exhibit postinjury structural and functional changes distinct from those seen in myelinated axons.

It must be emphasized that the changes we observe in axon function and structure are not an experimental form of the clinical condition of DAI. While the

most severe cases of DAI may be detected by computerized tomography and conventional magnetic resonance imaging, growing evidence indicates newer technologies, especially diffusion tensor imaging (DTI), provide a more accurate assessment of the pathology (reviewed by Li and Feng 2009). The microscopic features of DAI correspond to cytoskeletal and membrane disruptions, culminating in Wallerian-type axonal degeneration (Arfanakis et al. 2002). These dying axons do not participate in time-dependent functional recovery of white matter which we and others have documented to occur in animal TBI models. As detailed below, we observe TBI-induced axonal changes along multiple functional, structural, and molecular dimensions, which do not necessarily involve degeneration. Accordingly, to differentiate these observations from the more narrowly defined clinical DAI, the following discussion uses the term traumatic axonal injury (TAI) to indicate the full spectrum of structural and functional alterations affecting axons after TBI.

15.3 Quantitative Ultrastructure of Axonal Changes in Corpus Callosum Following TBI

15.3.1 Most Forebrain Axons are Unmyelinated

The vast majority of previous laboratory studies of TAI have focused on myelinated axons. This emphasis is understandable and reasonable from two perspectives. First, the existing knowledge-base pertaining to axonal injury is based largely on observations from heavily myelinated brainstem fiber tracts. Secondly, it was not recognized until recently that a surprisingly large proportion of cerebral axons are unmyelinated, and quantification of injury phenomena in these diminutive fibers presents technical challenges. In rodent injury models using impact acceleration or fluid percussion, TAI is most strongly expressed in brainstem fiber tracts such as the corticospinal tact and the medial longitudinal fasciculus, and is observed less frequently in cerebral sites. The brainstem tracts are predominantly comprised of comparatively large myelinated axons, and the proportion of pyramidal tract fibers which are unmyelinated was estimated as only ~20 % in earlier quantitative ultrastructural work (Samorajski and Friede 1968). No definitive explanation has been advanced to account for why the rodent TBI models more severely injure brainstem axons than more rostrally positioned axons. We speculate that mechanical forces may come to a focus as the rodent cranial vault narrows ventrally and posteriorly, and brainstem axons are compressed against bone rather than underlying soft tissue. Nevertheless, a rich body of knowledge has been generated through observations of posttraumatic sequential changes manifested in these fibers, usually evoked using impact acceleration injury models of TBI (e.g., Pettus et al. 1994; Povlishock 1992; Povlishock et al. 1997; Okonkwo et al. 1999; Buki et al. 1999, 2003; Singleton et al. 2001; Stone et al. 2001, 2002; Marmarou and Povlishock 2006).

These reports reveal time-dependent axon pathology beginning with focal axolemmal perturbations, probably mechanically induced at the instant of injury and associated with aberrant permeability. Subsequent stages involve microtubule loss, neurofilament alterations, axonal swelling, and scattered degenerating fibers.

Until 2003, quantitative data pertaining to the caliber and type of axons in the forebrain was only available for the CC. A study by Partadiredja and colleagues (2003) provided rigorous ultrastructural stereological assessments of fiber type and caliber in adult rat cerebral white matter, sampling frontal, parietal, and occipital regions at parasagittal sites chosen to minimize overlap with fibers of the CC (approximately 4.2 mm lateral from midline). Their results, based on systematic random sampling methods, showed that unmyelinated axons made up 76–90 % of axons, depending upon region sampled. Prior estimates of fiber composition in the rat CC have shown a historical trend. Earlier studies confined to the rodent splenium portion of the callosum estimated that about 45 % of axons were unmyelinated (e.g., Seggie and Berry 1972). More recent studies have consistently estimated the proportion of splenial axons which are unmyelinated to be about 88 % (Juraska and Kopcik 1988; Gravel et al. 1990; Kim et al. 1996). This recent consensus probably reflects sampling strategies which span the dorsal-to-ventral extent of the callosum, and thus avoid samples restricted to strata containing primarily a single fiber type.

The fact that the majority of forebrain axons are unmyelinated has ramifications for the field of TBI. If laboratory results point to distinctive pathophysiological responses in the unmyelinated population, then these should be assimilated into any comprehensive conceptual models of TAI. Equally important, if preclinical findings show unmyelinated and myelinated axons to have different responses to neuroprotective compounds (or different therapeutic temporal windows), then this information may usefully inform clinical trials targeting DAI.

15.3.2 A Measurement Focus on the Corpus Callosum

For both theoretical and practical reasons, we initially focused our studies of white matter injury on the CC. Clinical studies indicate the CC is particularly vulnerable to TBI, and some have attributed this vulnerability to the structure's unique anatomic location and composition (King et al. 1995; Gorrie et al. 2001). The presence of DAI in the CC was found to be more strongly predictive of poor outcome than even brainstem DAI (Rosa et al. 2011). The grave consequences of CC damage would be expected considering the multiple functions involving the structure, including transfer of lateralized verbal information, coordination of bilateral movements, and participation in cognitive and executive processes (Pandya et al. 1971; Geffen et al. 1994; Bhadelia et al. 2009). In the context of research using experimental rodent models of TBI, observations of the CC confer practical advantages. Because the CC is the largest white matter tract in the mammalian CNS, it presents a favorable site for replicable electrophysiological electrode placement, and selective dissections of the callosum provide sufficient tissue volume for molecular assays.

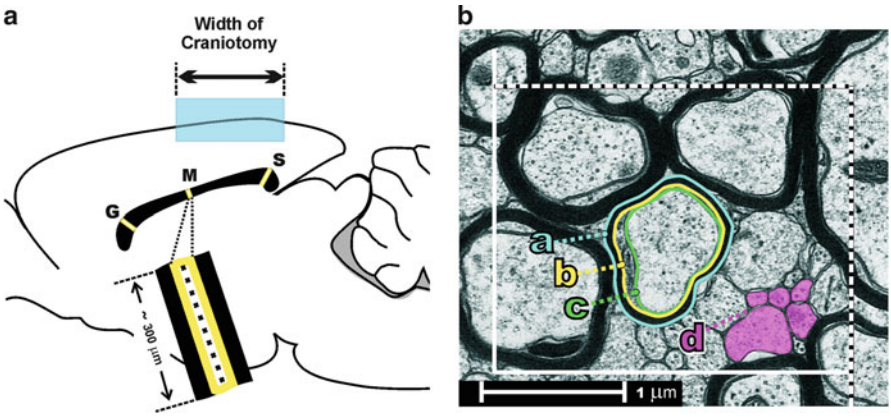


Fig. 15.3 (**a**) Midsagittal diagram of rat brain, showing stereological sampling scheme covering the dorsal-to-ventral extent of the CC at genu (G), mid-callosum (M), and splenium (S). *Shaded region* shows width and location of craniotomy for midline FPI. (**b**) Example of an unbiased stereological counting frame. *Shaded profiles* (*d*) show example unmyelinated axon profiles. Representative myelinated profile shows three contours were digitally traced for areal measurements: *a*, outer myelin border; *b*, inner myelin border; *c*, axolemma. From Reeves et al. 2012, with permission

15.3.3 Ultrastructural Stereology of Corpus Callosum Following FPI

Our laboratory assessed ultrastructural changes in CC of adult rats ($N=54$) at survival intervals ranging from 3 h to 15 days following midline FPI (Reeves et al. 2012). Some rats were given sham injuries, which involved all preparatory surgeries, but no pressure pulse was applied. Brains were processed for standard transmission electron microscopy, and stereological sampling conducted on genu, mid-callosal, and splenium regions. Because our primary objective was to monitor changes in surviving axons, the measurement variables were density and caliber (fiber cross-sectional area) of axons which met an operational definition of "intact." Specifically, axons were counted which met three criteria: (1) the membrane exhibited a continuous profile, (2) axoplasm contained at least one microtubule, and (3) axoplasm showed no degenerative debris. The sampling scheme, and an example of the unbiased counting frame, are shown in Fig. 15.3. This process yielded a total of 17,075 axonal profiles (6,659 from sham injured rats, and 10,416 from FPI rats), which were digitally traced and cross-sectional areas computed. Broken down by fiber type, 13,797 unmyelinated, and 3,278 myelinated, axons were measured. We predicted that axon caliber and density in our sham injured rats would be equivalent to that in naïve rats, because the sham procedure dissected only the midline scalp and cranium, without disturbing underlying tissue. Indeed, frequency distributions based on the 6,659 axons measured from our sham animals (Fig. 15.4a) demonstrated a close agreement with previous rodent studies (Waxman and Swadlow

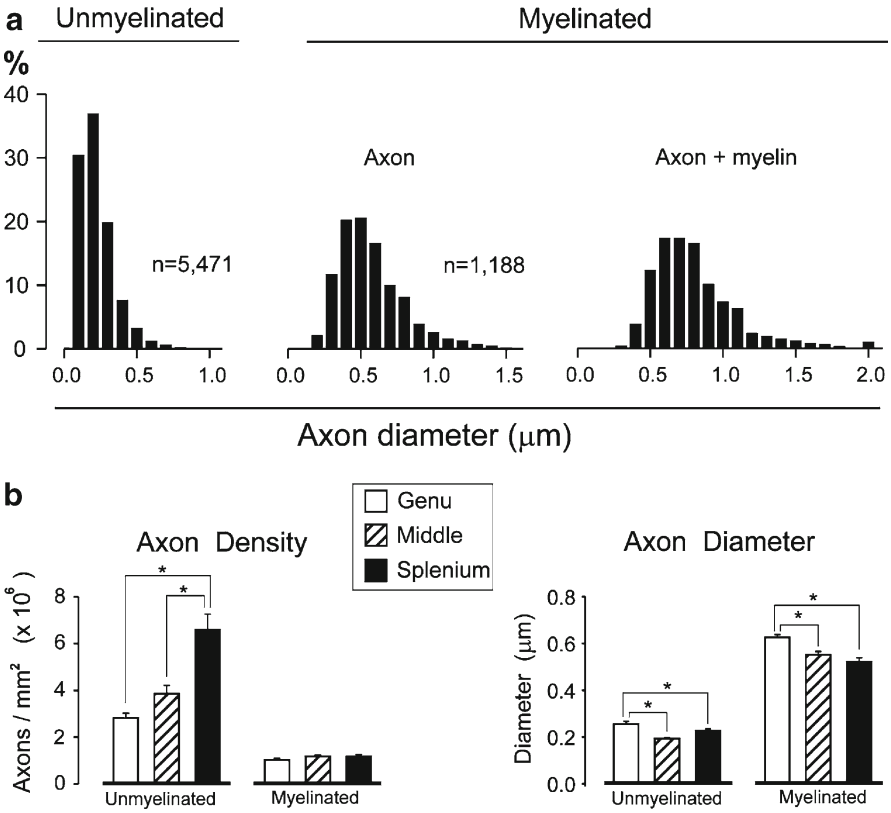


Fig. 15.4 Characterization of axon populations in sham lesion control rats. (**a**) Distributions of axon diameters for myelinated and unmyelinated axons. (**b**) (*left panel*) Separate analyses at CC regions revealed a rostral-to-caudal (genu-to-splenium) increase in the density of unmyelinated axons, but no gradient for myelinated axons. (*right panel*) Mean axon diameter was significantly greater in the genu than in either posterior region. *$p<0.05$. From Reeves et al. 2012, with permission

1976; Sturrock 1980; Gravel et al. 1990), the mean diameter of unmyelinated axons being 0.223 ± 0.002 μm, and myelinated 0.568 ± 0.006 μm. It was also notable that the sham material showed fiber caliber and density to differ significantly among the callosal regions. Unmyelinated axons exhibited a striking rostrocaudal gradient, being most numerous in the splenium (mean $= 6.2 \times 10^6/\text{mm}^2$) and falling off by 38.7 % in the mid-callosum, and by 54.8 % in the genu (Fig. 15.4b). In contrast, myelinated axon density in the various callosal regions did not differ from an overall mean of $1.1 \times 10^6/\text{mm}^2$. Axon diameters in sham rats also showed significant spatial variation, with genu fibers being largest for both unmyelinated (mean = 0.26 μm), and myelinated (mean = 0.63 μm) axon populations. At more caudal locations, mean diameter decreased for both fiber types (Fig. 15.4b).

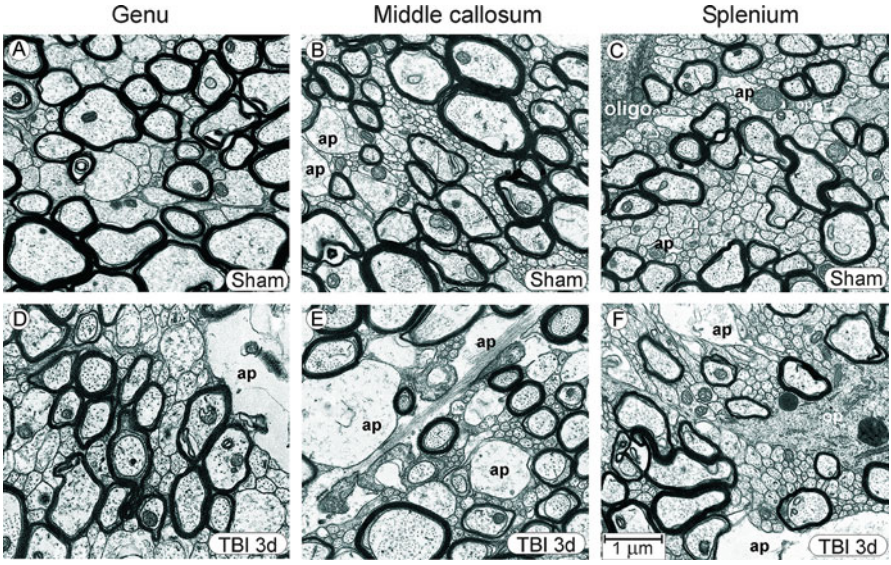


Fig. 15.5 Representative micrographs, showing genu, mid-callosum, and splenium regions in sham injured control rats (*top row*) and during the postinjury period when FPI-induced change in the unmyelinated axons was well developed (3 days postinjury) (*bottom row*). Even though quantitative analyses revealed significant morphometric changes to axonal dimensions at 3 days postinjury, the general architecture of injured tissue was similar to sham control tissue, although in some injured rats astrocyte processes were larger and encountered more frequently. *ap* astrocyte process, *oligo* oligodendrocyte cell body, *op* oligodendrocyte process. From Reeves et al. 2012, with permission

Representative micrographs from each region of the callosum in sham injured rats are shown in the top row of Fig. 15.5.

The moderate midline FPI induced a diffuse injury. No contused areas were observed during dissection, and the ultrastructural appearance of injured CC was typically quite similar to sham material, except that most injured cases exhibited more astrocyte profiles, consistent with reactive astrogliosis. Representative micrographs, showing genu, mid-callosum, and splenium regions at 3 days postinjury, are shown in the bottom row of Fig. 15.5. The following quantitative analysis first summarizes postinjury changes to the whole CC (pooling results from genu, middle, and splenium), and then considers injury effects in the specific callosal regions.

Analyses of axonal cross-sectional area, revealed a transient injury-induced decrease in unmyelinated axon area, with this effect reaching significance at 3 days (20.0 % below sham levels) and 7 days (22.8 % below sham levels) following injury. However, by 15 days postinjury mean unmyelinated axon area was not different from sham-operated rats (Fig. 15.6a). For the myelinated axons, there was a suggestion of an initial postinjury swelling at 3 h and 1 day, but these increases did not reach significance (Fig. 15.6a). Overall, myelinated fiber caliber did not significantly differ from sham control levels at any postinjury time point. Areal measures

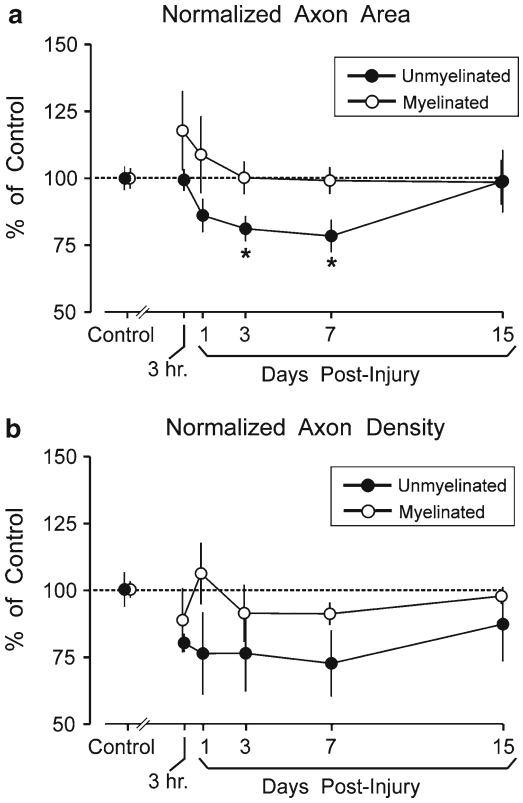


Fig. 15.6 Effect of FPI on cross-sectional area of axons and axon density. (**a**) Mean cross-sectional area of axons, averaged across all CC regions (genu, mid-callosum, splenium). Results are normalized to the mean axonal area measured in sham injured control rats. Time-dependent axonal shrinkage of unmyelinated fibers was significant at 3 and 7 days postinjury, but recovered to control levels by 15 days. FPI did not significantly alter cross-sectional areas of myelinated axons. (**b**) Mean normalized axon density, averaged across all CC regions. FPI effects on axonal density were more variable, and reductions in unmyelinated fiber density did not reach significance at any single survival interval. However, analyses which pooled together all postinjury time points (3 h to 15 days), providing a single injury group, indicated significant injury-related reductions in unmyelinated fiber density. FPI did not significantly alter density of myelinated axons. From Reeves et al. 2012, with permission

which included the axon plus myelin (contour "a" in Fig. 15.3) also did not significantly vary from sham levels at any time. These data allowed an evaluation of whether FPI altered the ratio of the inner axonal diameter to the total outer diameter (contours "c" to "a" in Fig. 15.3): the so-called g-ratio, which has been widely employed as a structural index of optimal axonal myelination since the historical work of Rushton (1951). Although the g-ratio has been reported to change in some

injury models (e.g., myelin thinning following spinal cord injury [Nashmi and Fehlings 2001]), the FPI did not induce a shift in this ratio from the sham mean of 0.708 ± 0.008, which approximates the optimal ratio for conduction velocity predicted by computer simulation (Smith and Koles 1970).

Compared to the substantial postinjury decline in mean diameter of unmyelinated axons, postinjury changes in the density of intact CC fibers were more variable. The mean density of CC unmyelinated axons was below the sham control level at all postinjury time points. However, this reduction did not reach significance in a statistical design with survival-interval as a factor, and which could detect time-dependent changes in density (Fig. 15.6b). However, analyses which pooled all postinjury time points together did indicate a significant injury-related reduction in unmyelinated fiber density ($F_{(1,28)} = 5.855$; $p = 0.022$). The density of myelinated axons was not significantly altered by the FPI (Fig. 15.6b). Ultrastructural analyses showed unmyelinated axons to exhibit abnormalities which were related to their decreased density, including membrane discontinuities or cytoplasmic abnormalities which prevented them from being scored as intact. This often took the form of atrophic changes where the axoplasm appeared to constrict, producing empty membranous folds and aberrant spaces in the adjacent extracellular compartment (examples in Fig. 15.7a). In other cases, degenerative changes affected clusters of unmyelinated axons, preventing their designation as intact. An additional pathological pattern, affecting unmyelinated fibers, was an apparent selective vulnerability of some relatively large axons, which often demonstrated axoplasmic constriction and membrane foldings. These malformed large axons were sometimes observed within fields of intact small unmyelinated axons, as illustrated in Fig. 15.7c. Although the unmyelinated axons, as a class, were more vulnerable to these injury conditions, these scattered instances of aberrant large unmyelinated fibers were exceptions to the general tendency for small diameter to comprise a risk factor after TBI.

Because diffusion tensor imaging in TBI patients commonly reveals a reduction in fractional anisotropy in the CC (reviewed by Maller et al. 2010), the finding of changes in axon morphology may have implications for the current theories of DAI. Following severe TBI, major white matter tracts, including the CC, have been reported to decrease axial, and increase radial, diffusivity (Arfanakis et al. 2002; Sidaros et al. 2008). These patterns of postinjury DTI alterations have been interpreted as consistent with Wallerian-like degeneration (Pierpaoli et al. 2001; Thomalla et al. 2004). However, the possibility that other axon pathologies may accompany degenerative changes, and further exacerbate the changes in diffusivity, have not previously been considered. It is conceivable that a reduction in unmyelinated axon caliber could also contribute to radial diffusion of water, and, in the context of clinical DAI, summate with Wallerian degeneration to produce the observed diffusivity alterations. Our observation, that the apparent shrinkage of unmyelinated axon diameters recovered to control levels over time, may be relevant to the fact that DTI abnormalities also improve in patients with good recovery. For example, diffusivity changes in the CC and internal capsule appeared to normalize by 1 year among patients with good outcome scores (Sidaros et al. 2008).

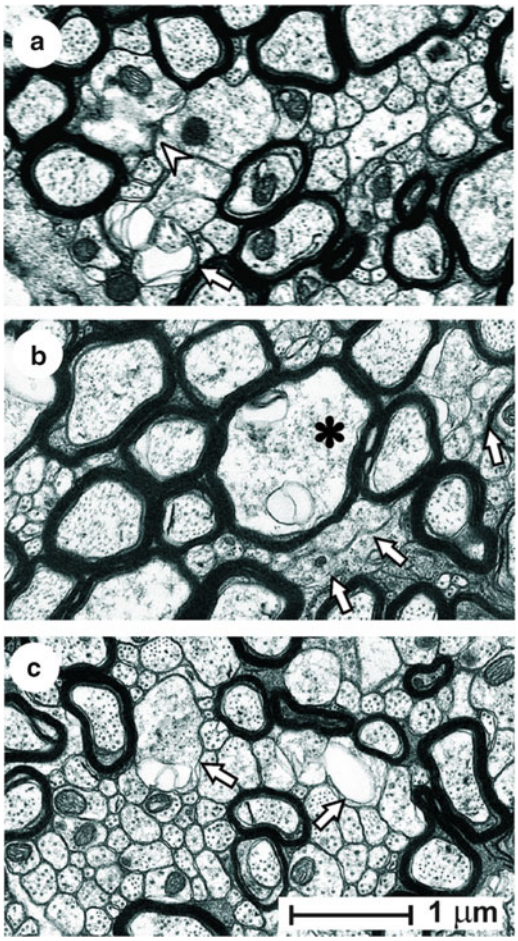


Fig. 15.7 Representative axonal profiles failing to meet the operational definition of intact, all from splenium at 3 days postinjury. (**a**) Isolated unmyelinated axons exhibiting membrane discontinuities (*arrowhead*) and membranous foldings apposed to aberrant extracellular spaces (*arrow*). (**b**) Clusters of unmyelinated fibers lacking distinct membranes (*arrows*), along with a myelinated axon (*asterisk*) with cytoplasmic abnormalities. (**c**) Example of vulnerability of relatively large unmyelinated axons (*arrows*) juxtaposed to intact small unmyelinated axons. From Reeves et al. 2012, with permission

Turning to a consideration of how injury effects varied along the rostro-caudal axis of the CC, our analyses of unmyelinated axons revealed a reduction in fiber caliber initially affecting the splenium at 1 day, and then progressing rostrally to impact the mid-callosal region at 3 days, and finally involving the genu at 7 days postinjury (Fig. 15.8 (*line graphs*)). The fact that changes to CC axons displayed a caudal-to-rostral temporal gradient may be related to heterogeneity in fiber composition along this axis. It has long been appreciated that the distributions of axon type and caliber are not continuous along the rostro-caudal axis in the CC of multiple species, from rodents to man (Gravel et al. 1990; Lamantia and Rakic 1990; Aboitiz et al. 1992, 2003). The high density of unmyelinated splenial axons, with decreasing numbers rostrally, was also confirmed in the present results, and these data are

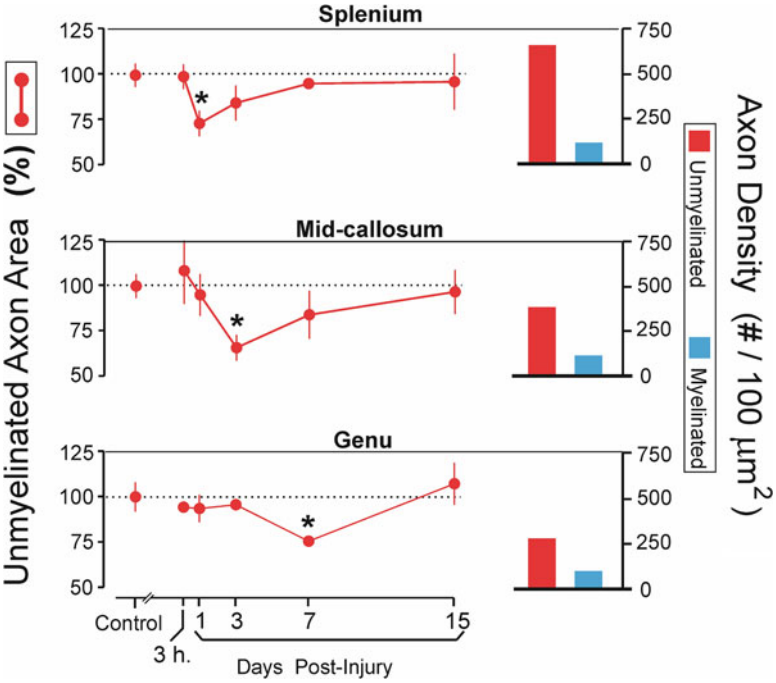


Fig. 15.8 Relationship between unmyelinated axon shrinkage in CC subregions and local density of unmyelinated axons. Effect of FPI on cross-sectional area of unmyelinated axons in the splenium, middle, and genu regions of the CC, normalized to sham levels (*line graphs*). FPI produced a caudal-to-rostral sequence of significant axonal shrinkage expressed at 1 day in the splenium, 3 days in the mid-callosum, and 7 days in the genu. Each region showed a recovery to sham control levels following the shrinkage. The temporal sequence of regional changes may be related to local density of unmyelinated axons (*histograms*). Line graphs from Reeves et al. 2012, with permission

reorganized into the histograms of Fig. 15.8. Juxtaposed to the splenium-to-genu temporal progression of TBI-induced reductions in axon caliber, the variation in unmyelinated axon density (Fig. 15.8 histograms) suggests the local proportion of these fibers may influence the onset of postinjury morphological changes. Large concentrations of unmyelinated fibers may, in itself, constitute a risk factor. Our laboratory is currently conducting electrophysiological recordings at the various CC regions, to assess the interaction of intrinsic fiber composition and post-TBI functional deficits. An additional motivation to evaluate injury effects, at CC subregions, is a number of clinical neuroimaging reports showing evidence for local differences in trauma vulnerability within the CC (Gentry et al. 1988; Rutgers et al. 2008; Matsukawa et al. 2011).

15.4 Electrophysiological Evidence for Differential Injury Responses in Unmyelinated and Myelinated Axons

15.4.1 The Compound Action Potential (CAP) as a Functional Assessment in Experimental TBI

Due to the diffuse nature of axonal damage in closed head injuries, the sparse distribution of injured axons may be difficult to detect even with modern imaging technology. As regards electrophysiological assessments in experimental animal TBI models, the diffuse pathology presents methodological challenges, especially approaches based on single unit recording or individual axons. A creative approach to this issue was reported by Andrew Baker and colleagues at the University of Toronto, who were the first to apply the technique of compound action potential (CAP) recording from CNS axons, as a functional assessment of axonal injury in experimental TBI. Because CAPs are a large amplitude field potential, representing the summation of individual action potentials in a population of axons, each CAP is already a sample of multiple axons. It is reasonable to assume that contributing to each CAP waveform are axons ranging from severely injured to uninjured, and thus CAP amplitude is a valid measure of TAI severity. Baker et al. (2002) reported that FPI in adult rats produced a significant suppression of CAPs evoked in the CC.

Our laboratory modified the methods of Baker et al. (2002) to enable separate quantification of CAPs generated by myelinated vs. unmyelinated axons. Using conventional brain slice recording technique, stimulating and recording electrodes were positioned into the CC of 450 μm thick coronal slices (Fig. 15.9a, b), at an inter-electrode distance of approximately 1.0 mm. With this electrode arrangement, each pulse evokes a waveform which is partially obscured by the stimulus artifact. However, we observed that lowering the temperature of the recording chamber slowed conduction sufficiently to allow a short-latency CAP waveform ("N1"), generated by fast-conducting myelinated axons, to be quantified separately from "N2," a CAP component generated by slower unmyelinated axons (Fig. 15.9c). It should be pointed out that there may be some minimal overlap of these distributions, i.e., a small number of especially large diameter unmyelinated axons may contribute to the early N1 CAP wave, and the smallest of the myelinated axons may be sufficiently slow conducting as to contribute to N2. However, work in multiple laboratories has converged on the conclusion that the clear majority of the distributions are nonoverlapping, and N1 and N2 are generated predominantly by myelinated and unmyelinated axons, respectfully. First, diameters of the ultrastructurally observed populations of myelinated and unmyelinated callosal fibers agree well with recorded conduction velocities. Specifically, Waxman and Swadlow (1976) noted that the diameters of myelinated (0.3–1.85 μm in diameter) and unmyelinated (0.08–0.6 μm) fibers, which they measured in the posterior callosum, corresponded well with the distribution of observed conduction velocities (0.3–12.9 m/s). Those same authors interpreted refractoriness and threshold results to suggest that the N1 and N2 field

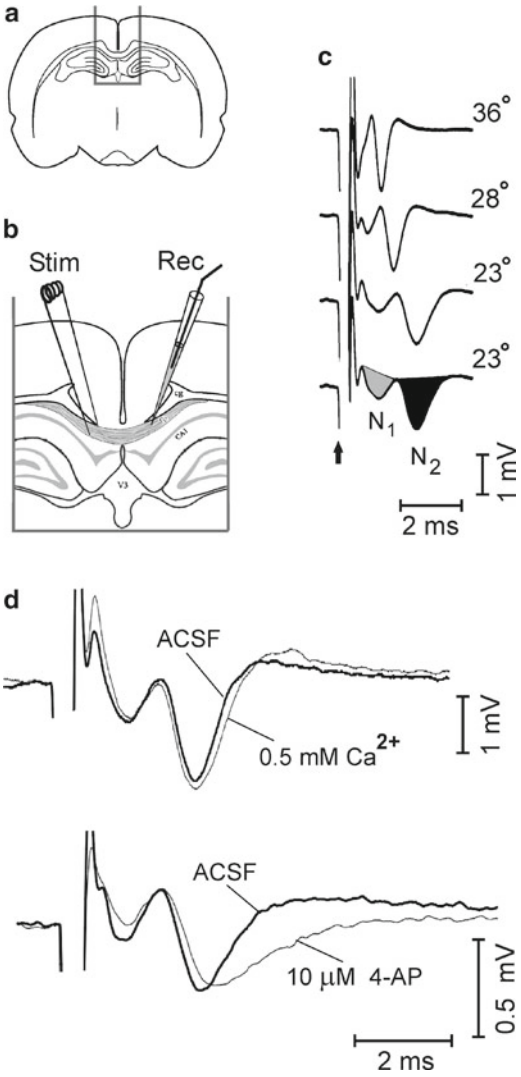


Fig. 15.9 Placement of electrodes for callosal CAP recording, and responses of N1 and N2 CAP components to temperature and ionic manipulations. (**a**, **b**) Stimulating and recording electrodes were positioned in midline CC separated by approximately 1.0 mm. (**c**) Decreasing bath temperature slowed conduction time, enabling quantification of N1 (*gray shading*) and N2 (*black shading*) waveforms. (**d**) Recording in low $[Ca2^{+}]_{o}$ did not significantly alter evoked CAPs, while bath application of the K^{+} channel blocker (4-AP) selectively prolonged the N2 CAP component. From Reeves et al. 2005, with permission

components were produced by fibers that did not vary simply along a single continuous dimension, but more likely by the presence or absence of myelin. Our laboratory observed that externally applied potassium channel blocker (4-AP) prolonged only the N2 CAP component, leaving N1 unchanged (Fig. 15.9d). This is consistent with an N2 generated by unmyelinated axons, having 4-AP-sensitive K^{+} channels along the entire axolemma, whereas in myelinated axons the channels are located along the internodal axolemma, beneath the myelin sheath and "masked" from externally applied 4-AP. These results are in agreement with earlier work in dorsal column axons (Kocsis and Waxman 1980) as well as CC (Swanson et al. 1998).

Figure 15.9d also shows that recording CAPs after lowering the perfusate to a low-calcium condition (0.5 mM) did not alter the waveform from that seen in normal artificial cerebrospinal fluid (ACSF), consistent with the expectation that callosal CAPs are a purely axonal phenomena, and do not reflect any synaptic events.

15.4.2 Unmyelinated and Myelinated Axons Exhibit Differential Conduction Deficits After TBI

Analysis of CAPs following FPI revealed that the myelinated and unmyelinated waveform components were differentially affected by the injury, and showed different courses of postinjury recovery (Reeves et al. 2005). We routinely evaluate CAPs using an "input–output" function: a graduated series of stimulus intensities, ranging from near-threshold to maximum response. Figure 15.10a illustrates representative CAPs from input–output series (superimposed) from a sham control rat and from a rat recorded at 1 day post-TBI. Suppression of both N1 and N2 was typical at that survival period. The curves in Fig. 15.10b, c illustrate the mean injury-induced suppression of N1 and N2 amplitude, respectively, over the full range of stimulation current used. For both N1 and N2, this injury effect was greatest at 1 day postinjury. Statistical analyses indicated that the N1 amplitudes were significantly depressed at postinjury times 3 h, 1 day, and 3 days, but were no longer significantly different from control levels at 7 days postinjury. N2 amplitudes remained significantly below control levels at all postinjury survival intervals (3 h to 7 days), and no significant time-dependent recovery was observed for N2 amplitudes.

Postinjury decreases in CAP amplitude, such as those noted here, may result from fewer axons recruited into the CAP waveform; for example, due to dead axons, or inactivation of axons through a depolarization block. Alternatively, the injury process may not alter the number of responsive fibers, but instead alter conduction properties and reduce amplitudes of action potentials in individual axons, which would also reduce the amplitude of the overall CAP signal. Thus, amplitude measures of the CAP waveform, alone, cannot distinguish between these alternative pathological processes. This issue may be addressed using stimulation protocols which assess the intrinsic functional properties of individual axons, and are less sensitive to the absolute numbers of recruited fibers. For example, refractoriness of callosal fibers may be analyzed by quantifying the suppression of the second CAP response in paired stimulus trials. Figure 15.11 shows example series of the second response evoked in paired stimulus presentations, at the indicated interpulse intervals (IPI, 1.5–6.0 ms), after subtracting out the responses to the conditioning pulse, for a control slice (Fig. 15.11a) and for a slice recorded at 1 day postinjury (Fig. 15.11b). Figure 15.11c plots the CAP amplitude elicited by the second pulse in each paired stimulation (C2) divided by the CAP amplitude to single pulse stimulation (C1). These C2/C1 ratios were averaged for each survival group and plotted for N1 and N2. Rightward shifts in these curves indicate increases in the refractory recovery cycle in the CC axons, consistent with axonal damage. For example, these

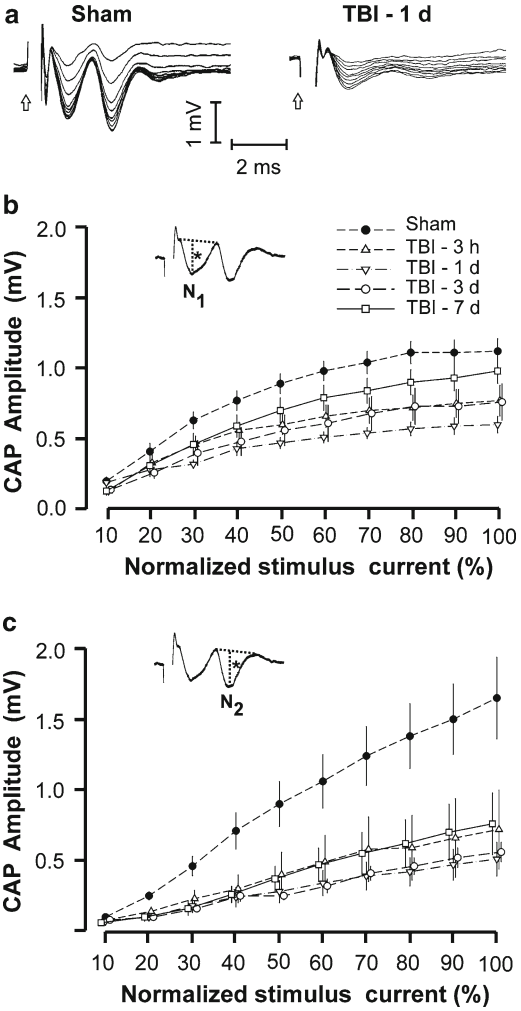


Fig. 15.10 Differential effects of TBI on N1 and N2 CAPs. (**a**) Recruitment of field potential with graduated series of stimulus pulses (10–100 % of maximum) for a sham control rat and for a rat recorded at 1 day postinjury. (**b**) Average amplitude of N1 over the full range of stimulation current used, for sham and TBI groups. N1 amplitude was significantly decreased by TBI at 3 h, 1 day, and 3 days, but was not at 7 days. (**c**) Average amplitude of N2 over the full stimulus range, for sham and TBI groups. More persistent injury-induced suppression of average response amplitude was observed for the N2, which remained significantly below control levels at all postinjury survival intervals (3 h, 1 day, 3 days, and 7 days). Inserts in panels (**b**) and (**c**) show CAP amplitude measurement technique for N1 and N2 components (height of vertical line *asterisk*). From Reeves et al. 2005, with permission

curves show the sham N1 waveform, evoked by the second of a pair of pulses, achieved 50 % of the amplitude of a single pulse presentation, when the interpulse interval was approximately 3.7 ms, and this parameter for the N2 field component was 4.5 ms. Averaged over all postinjury groups, the fluid percussion injury degraded the refractory performance for these fibers by approximately 0.5 ms for both N1 and N2 field components. The refractory curves were significantly right-shifted at 3 h, 1 day, and 3 days, for N1, and at 3 h and 1 day for N2. The refractory curves showed evidence of recovery (left-shifts), and by 7 days were not statistically different from control curves for either the N1 or N2 wave component.

The refractoriness results suggested that postinjury CAPs reflected, at least in part, injury-induced alterations in the functional properties of individual axons, such

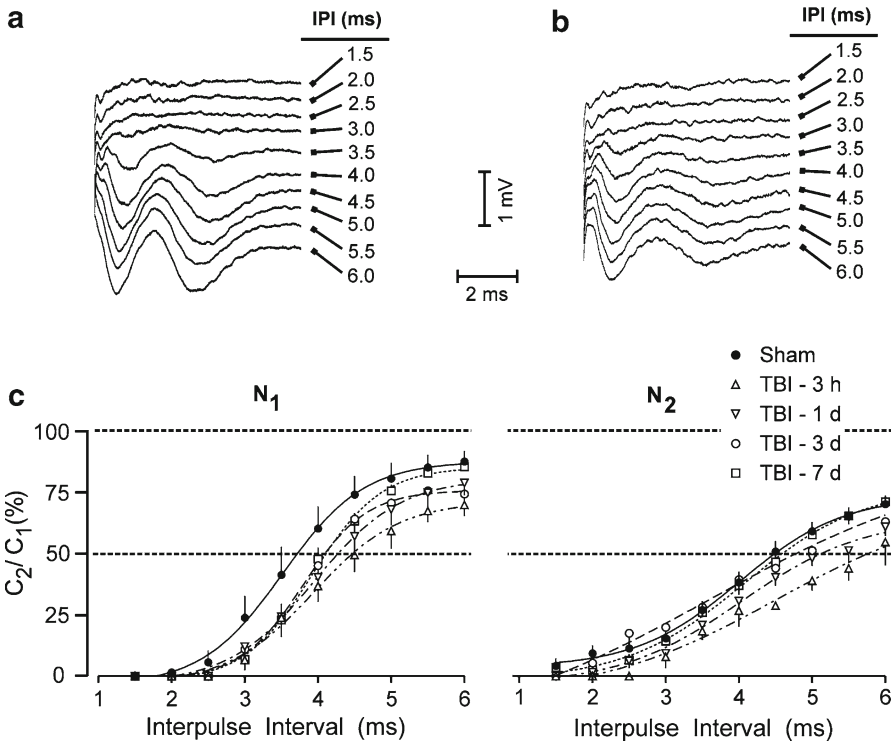


Fig. 15.11 Effect of TBI on callosal CAP refractoriness. Examples waveforms at top show second potentials in paired responses, after subtraction of response to the conditioning pulse, from a sham control rat (**a**) and from a rat recorded at 1 day post-TBI (**b**), at indicated interpulse intervals from 1.5 to 6.0 ms. (**c**) Plots of mean CAP amplitude elicited by the second pulse in each paired stimulation (C2) divided by the CAP amplitude to single pulse stimulation (C1), for control and injured groups. Average C2/C1 ratios were fitted to Boltzmann sigmoid curves, and showed significant increases in refractoriness (*rightward curve shifts*) at 3 h, 1 day, and 3 days for N1 (*left panel*) and at 3 h and 1 day N2 (*right panel*). From Reeves et al. 2005, with permission

as level of depolarization or pathological changes to Na^+ channels. Refractoriness has long been recognized to depend largely on recovery of Na^+ channels from inactivation (Hodgkin and Huxley 1952), and is sensitive to changes in membrane potential (Burke et al. 1998). A depolarizing shift will increase the extent of Na^+ channel inactivation. The observed refractory deficits are consistent with injury-induced axonal depolarization or alterations in the function of Na^+ channels: changes which may not be lethal but recover over time. A tetrodotoxin (TTX)-sensitive Na^+ influx has been reported as an initiating pathology in axonal injury, and was associated with proteolytic damage to Na^+ channel α-subunits (Iwata et al. 2004). The primary ionic event, generating the callosal CAP, is influx through voltage gated Na^+ channels, and TTX has been reported to entirely suppress callosal CAPs in brain slices (Swanson et al. 1998).

It is useful to compare the CAP recording results with the preceding ultrastructural analyses. In this regard, the most robust ultrastructural finding was a time-dependent reduction in the average diameter (cross-sectional area) of unmyelinated CC axons, which had recovered to control levels by 15 days postinjury. While the ultrastructural results also indicated a post-TBI reduction in the number of unmyelinated axonal profiles which could be categorized as "intact," this experimental effect was more variable than caliber changes, but also appeared to recover by 15 days. Our study of post-TBI CAP suppression, which examined survival periods up to 7 days, was conducted prior to the finding of ultrastructural recovery at 15 days. This, of course, is an interpretive limitation of this CAP dataset. However, it is notable that the FPI affected the unmyelinated axon population to a much greater extent than the myelinated fibers: both ultrastructurally and with regard to CAP amplitude. Moreover, the primary ultrastructural finding was a transient reduction in unmyelinated axon caliber, and not irreversible loss of axons. While we have not extended CAP recording to 15 days postinjury, to confirm a functional recovery in the unmyelinated population to match the ultrastructural results, certain electrophysiological findings were consistent with reversible changes in axons rather than with axon death. The refractoriness findings demonstrated a cycle of deficit and recovery. A similar interpretation was offered for analyses which assessed threshold and excitability properties using a "strength-duration" stimulus protocol [not illustrated here], which indicated a significant suppression of excitability at 3 h postinjury for both N1 and N2, with subsequent recovery (Reeves et al. 2005). The finding of posttraumatic structural changes preferentially affecting unmyelinated axons of the brain is novel, and quite dissimilar from the well characterized cytoskeletal breakdown and swellings noted in heavily myelinated brainstem tracts (Pettus et al. 1994; Povlishock 1992; Povlishock et al. 1997; Okonkwo et al. 1999; Buki et al. 1999, 2003; Singleton et al. 2001; Stone et al. 2001, 2002; Marmarou and Povlishock 2006). It must also be stressed that the pathological changes observed in the brainstem tracts were usually elicited with the impact acceleration injury model, and this involves injury forces greater than applied in the FPIs described here. However, we hypothesize that aberrant changes in the unmyelinated axon population, including the reduction in caliber, likely coexist with focal myelinated pathology in models of more severe TBI, at all levels of the neuraxis.

15.5 Unmyelinated and Myelinated Axons Exhibit Differential Responses to Neuroprotective Compounds

To date, no effective treatment for DAI has been demonstrated for head injured patients. However, studies using rodent models of TBI have demonstrated promising reductions in axonal injury using treatment with immunosuppressant drugs, especially FK506 and Cyclosporin-A (CsA). These compounds reduce axonal damage in multiple ways following TBI: attenuating cytoskeletal compaction (Buki et al. 1999; Okonkwo et al. 1999), and reducing impairments to axonal transport (Singleton et al. 2001; Marmarou and Povlishock 2006). Here, we discuss how

these compounds also benefit axonal conduction properties following FPI (Reeves et al. 2007; Colley et al. 2010). CsA and FK506 are widely used clinically to reduce activation of the immune system in organ transplantation, by inhibiting the enzyme calcineurin, resulting in decreased interleukin (IL-2) production and T lymphocyte activation. However, neuroprotective effects of CsA and FK506 do not depend on immunosuppression, and involve calcineurin pathways separate from interleukin production. For example, axonal neuroprotection may result when calcineurin is prevented from acting on known axonal substrates: MAP2, tubulin, and tau protein (Goto et al. 1985), and neurofilament proteins (Eyer and Leterrier 1988).

15.5.1 Preinjury Administration of FK506

As a first approach to evaluating the neuroprotective efficacy of FK506 in alleviating TBI-induced impairments in the function of CC axons, we selected a preinjury treatment paradigm: administering FK506 at 30 min prior to FPI. This strategy was not intended to have a direct clinical relevance; for obvious reasons, in any practical application a drug cannot be administered prior to an injury. Instead, our objective was to examine the protective potential of the compound, where conditions are optimal to detect treatment effects on the earliest injury phases, including primary axonal damage occurring during the mechanical trauma. This was the first study (Reeves et al. 2007) to address the specific issue of differential therapeutic efficacy in unmyelinated and myelinated axons, in response to a neuroprotectant administration.

The study methodology, in brief, was to administer 3 mg/kg FK506 intravenously at 30 min prior to FPI in adult rats, using injury parameters identical to the above ultrastructural and electrophysiological experiments. Previous work had demonstrated that this intravenous dosage crosses the blood–brain barrier and establishes therapeutic levels of brain parenchymal concentration (Singleton et al. 2001) which correlate with near maximum inhibition of calcineurin (Ochiai et al. 1989; Butcher et al. 1997) consistent with neuroprotection. CAP recording was conducted in vitro, as described above, at 24 h following the FPI. A subset of rats were used to evaluate FK506 efficacy in reducing the prevalence of amyloid precursor protein (APP)-labeled axons, which is a commonly used marker for impaired axonal transport (Stone et al. 2000).

Consistent with our earlier study, FPI suppressed callosal CAP amplitudes when measured at 24 h postinjury. Representative CAP waveforms, evoked using graduated stimulus levels in input–output testing, are shown in Fig. 15.12a for vehicle- and FK506-treated sham and injured rats. CAPs from a vehicle-treated sham and TBI rat (left side of Fig. 15.12a) are representative of the profound response reductions observed postinjury. Mean CAP amplitudes are plotted for each analytic group in Fig. 15.12b, as curves relating evoked CAP amplitude to normalized stimulus current. Statistical analysis of these curves showed that TBI significantly suppressed both myelinated (N1) and unmyelinated (N2) CAP components.

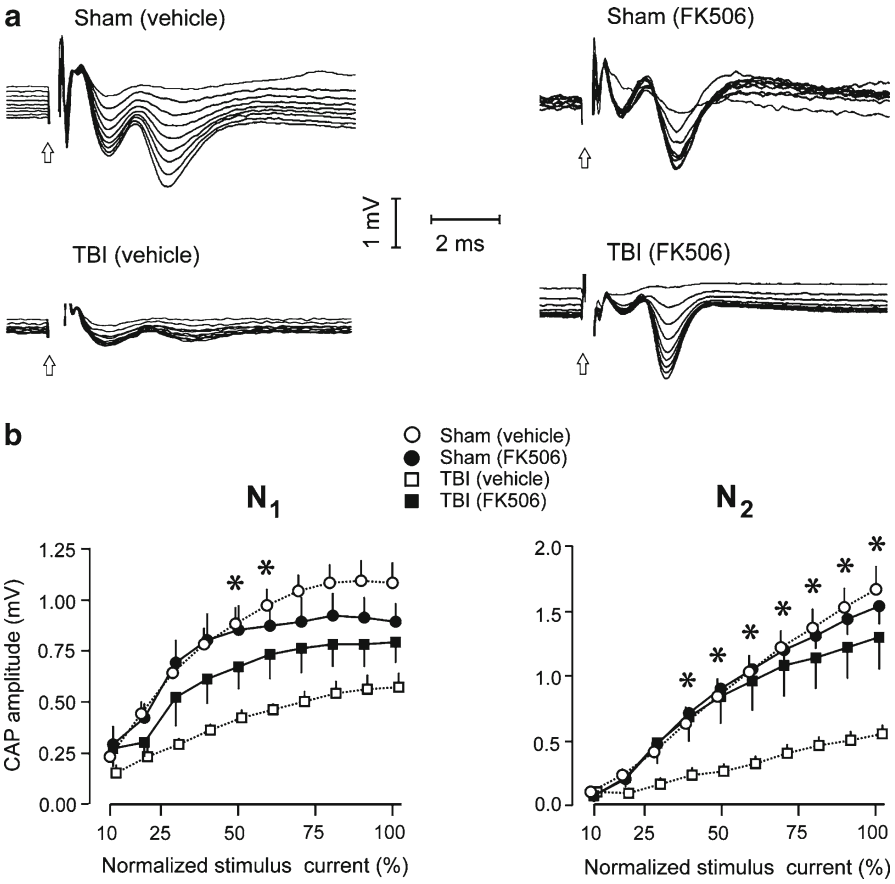


Fig. 15.12 Differential effects of FK506 treatment on postinjury suppression of callosal N1 and N2 CAP amplitudes. (**a**) Samples of CAP field potentials generated in input–output testing. (**b**) (*Left panel*) Average amplitude of N1 over the full range of stimulation current used, for all groups. N1 amplitude was significantly decreased by TBI, but FK506 treatment provided significant neuroprotection only at selective stimulus intensities (50–60 % of maximum). (*Right panel*) Average amplitude of N2 over the full stimulus range, for all groups. TBI produced a more severe suppression of the N2 CAP amplitude, at a greater range of stimulus intensities (40–100 %). From Reeves et al. 2007, with permission

The FK506 pretreatment produced a marked protection against postinjury decreases in unmyelinated CAP amplitude, and a more moderate level of protection for myelinated amplitudes. FK506 effects were dependent on the level of stimulus current, and the drug effects were evaluated at specific levels of normalized stimulus intensity. N2 CAP amplitudes recorded in FK506-treated TBI rats were significantly elevated above those in vehicle-treated TBI rats. Comparisons at specific levels of stimulus intensity showed the FK506 protection of the N2 CAPs to be significant at stimulus levels of 40–100 % (asterisks in Fig. 15.12b). In contrast,

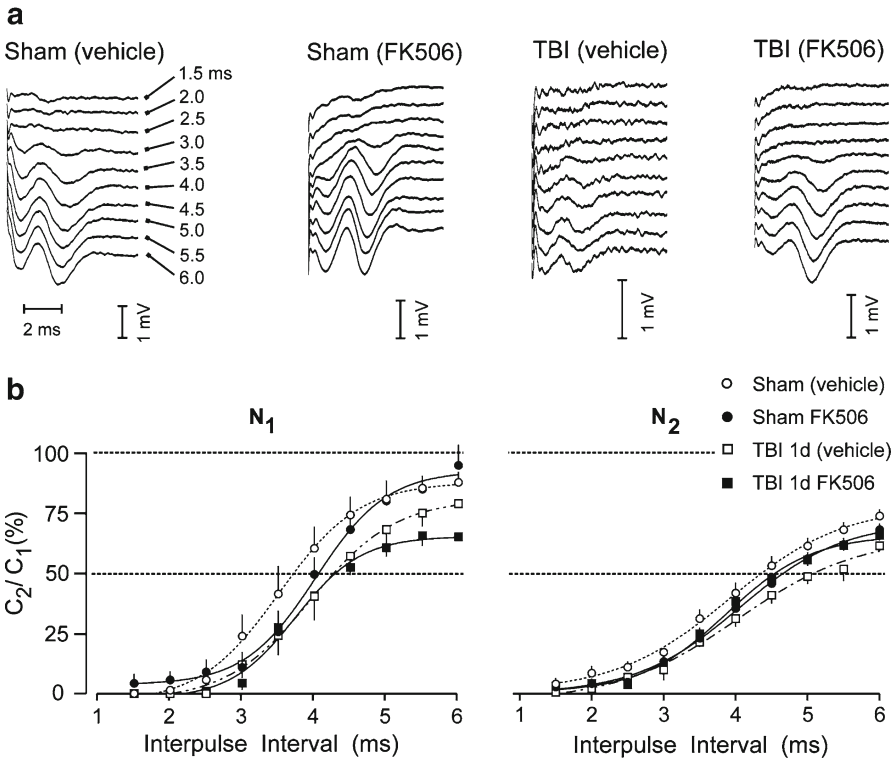


Fig. 15.13 Effect of TBI and FK506 treatment on callosal CAP refractoriness. (**a**) Example waveforms show second potentials in paired responses (interpulse intervals = 1.5–6.0 ms), after subtraction of response to the conditioning pulse, of representative cases from each experimental group. (**b**) Plots of mean CAP amplitude elicited by the second pulse in each paired stimulation (C2) divided by the CAP amplitude to single pulse stimulation (C1), for all groups. Average C2/C1 ratios were fitted to Boltzmann sigmoid curves, and showed TBI-induced increases in refractoriness (*rightward curve shifts*) which was significant for N1 and N2. FK506 prevented this injury effect only for the N2 CAP component. From Reeves et al. 2007, with permission

FK506 treatment led to significant N1 CAP protection only at two stimulus intensities (50 and 60 %). These results, and additional statistical analyses contained in Reeves et al. (2007) were consistent with a larger degree of FK506 protection for unmyelinated axons than for myelinated axons.

Refractoriness of the callosal fibers was analyzed, using methods described above, in the evaluation of FK506 neuroprotection. Figure 15.13a shows representative series of the second response evoked in paired stimulus presentations, at the indicated interpulse intervals (IPI, 1.5–6.0 ms), and Fig. 15.13b plots CAP amplitudes elicited by the second pulse in each paired stimulation (C2) divided by the CAP amplitude to single pulse stimulation (C1). Refractory results showed that FPI produced significant rightward shifts for both N1 and N2. However, FK506 treatment significantly ameliorated this deficit only for the N2 CAP, the refractory curve for which was not different from that of vehicle-treated sham rats. In TBI rats treated with FK506 the refractory curve

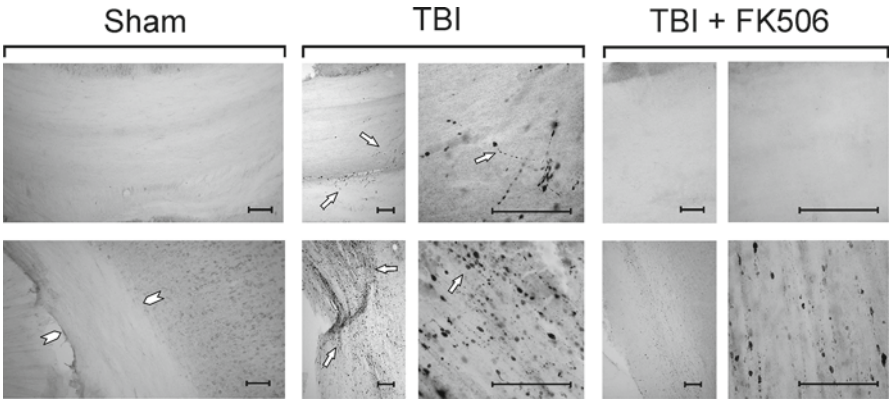


Fig. 15.14 FK506 effects on postinjury increases in APP labeled axons within the CC and subcortical white matter adjacent to electrophysiological recording at 24 h postinjury. *Top row*: Representative sections show immunohistochemical detection of APP labeled damaged axons in mid-dorsal corpus callosum of sham-injured, vehicle- and FK506-treated TBI conditions. As previously observed, APP staining of injured axons was absent in the sham control CC, but visible at discrete foci after TBI (*arrows*). With FK506 treatment APP profile was similar to that of sham control. *Bottom row*: Representative sections show APP label in subcortical white matter of sham-injured, vehicle- and FK506-treated TBI conditions. As in CC, sham control cases showed no APP staining of white matter axons (*arrowheads*). After TBI, significant axonal damage is detected with APP in the white matter (*arrows*). FK506 treatment reduced APP staining. Calibration bars = 50 μm. From Reeves et al. 2007, with permission

for the N1 CAP remained significantly right-shifted relative to vehicle-treated sham rats. While the absolute magnitudes of these injury-induced rightward shifts were modest (0.55 ms for N1 and 0.73 ms for N2), the significance of the curve shifts suggested that TBI altered fundamental activation properties of the axons, slowing the recovery cycle times of both the myelinated and unmyelinated axon populations. FK506 treatment reduced refractory changes only for the N2 CAP, consistent with a differential therapeutic effect for the unmyelinated axon population.

In the histological component of the study, APP immunoreactive swellings were observed in the CC and adjacent subcortical white matter at 24 h postinjury (Fig. 15.14). Qualitative comparisons between the FK506-treated and vehicle groups revealed consistent and striking differences. FK506-treated animals showed dramatic reduction of immunoreactive axonal swellings in all regions sampled, although these immunocytochemical observations could not discriminate between unmyelinated and myelinated damage.

15.5.2 Postinjury Administration of Cyclosporin-A

In a subsequent study (Colley et al. 2010) we further pursued the issue of neuroprotection of immunophilin ligands, but now investigating CsA using a more clinically relevant postinjury administration paradigm. While FK506 neuroprotection is

attributed to calcineurin inhibition, recent evidence indicates that CsA protects against TBI primarily by preventing mitochondrial permeability transition pore (MPTP) formation. The CsA derivative NIM811, which inhibits MPTP formation but not calcineurin activity, was reported to retain the same neuroprotective potency as CsA in reducing injury-induced spectrin proteolysis, axonal degeneration, and neurologic dysfunction (Mbye et al. 2008, 2009).

In the CsA study, methods for injury production and electrophysiological assessments were identical to those of the FK506 study above. Rats received a single 20 mg/Kg bolus of CsA, or cremaphor vehicle, at either 15 m or 1 h following a moderate midline fluid percussion injury. Figure 15.15 summarizes effects of injury and CsA treatment on CAP amplitude. In our assessments of CsA efficacy, we reported on CAP area as the primary dependent variable, although it is important to note that we obtain essentially identical results using either waveform peak or area-under-the-curve as endpoints. Figure 15.15b shows CAPs collected at maximum stimulus intensities, representative of vehicle-treated sham and TBI rats, as well as TBI rats given CsA at 15 m and 1 h postinjury. These sample CAPs typify the injury-induced suppression observed in both N1 and N2 CAPs (compare Sham + veh and TBI + veh waveforms), and the attenuation of this deficit in rats administered CsA at 15 m postinjury (compare TBI + veh and TBI + CsA (15 m) waveforms).

Input–output curves, averaged for each experimental group, are shown in Fig. 15.15c. Assessing the effects of injury in this way, across the full input–output range, revealed a greater injury deficit, and a larger degree of CsA neuroprotection, for the myelinated than for the unmyelinated CAPs. In TBI rats treated with CsA at 15 min postinjury the myelinated CAP amplitude was no longer suppressed below the Sham + veh level, and was significantly elevated above the TBI + veh group, indicating a significant neuroprotection for the myelinated fibers. Delaying CsA treatment until 1 h postinjury entirely eliminated the neuroprotection for the N1 CAP area.

In contrast to the significant injury and drug effects seen for the myelinated CAP amplitude, the input–output curve measurements for the unmyelinated CAP did not reveal significant injury or treatment effects (right panel in Fig. 15.15c). However, inspection of the N2 input–output curves suggested that the TBI + CsA (15 m) group tended to elevate above the TBI + veh group as stimulus intensity was increased. Thus, it is possible that the detection of a statistically significant degree of CsA neuroprotection, in the case of the N2 CAP, may depend in part on stimulus conditions. One factor underlying dissimilar N1 and N2 area results may be a difference in how myelinated and unmyelinated axons are recruited into the CAP response as stimulus current is increased from threshold to higher levels. To address this issue, the effects of injury and CsA treatment were also examined at a static level of stimulus intensity. Figure 15.16 shows mean CAP amplitude measured at the maximum current level used during input–output testing (100 % level in Fig. 15.15c). This approach continued to show a neuroprotection afforded to the myelinated axons when CsA was delivered at 15 m postinjury, as indicated by the fact that N1 amplitude in TBI + veh rats fell below sham levels, while N1 amplitude in TBI + CsA(15 m) rats was not different from sham cases. Importantly, this analysis also revealed a significant increase in N2 CAPs in TBI rats administered CsA at 15 m after injury, which may reflect a beneficial effect of CsA, although the TBI + vehicle group

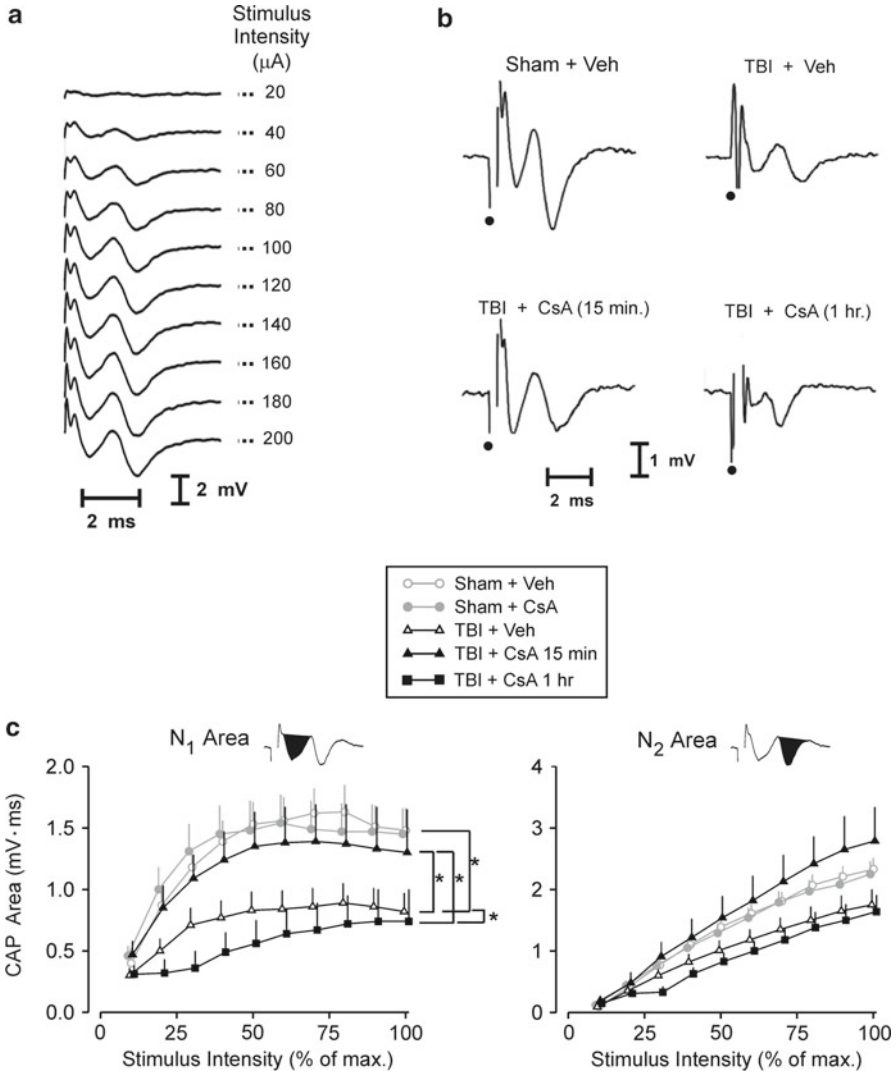


Fig. 15.15 Effects of injury and CsA treatment on CAP area. (**a**) Input–output series evoked with stimulus currents ranging from threshold to CAP maximum. (**b**) Representative CAPs evoked at the maximum stimulus level for vehicle treated sham and TBI rats, and for TBI rats given CsA at 15 m and 1 h. (**c**) Input–output curves, showing N1 and N2 CAP area plotted as a function of normalized stimulus intensity. The average input–output function of the N1 CAP area was protected in TBI rats by treatment with CsA at 15 m, but not at 1 h, postinjury. Average N2 area input–output functions were not significantly shifted by injury or CsA treatment. From Colley et al. 2010, with permission

narrowly failed to show a statistically significant injury effect. This instance of a mild injury effect to unmyelinated CAPs contrasted with patterns of injury observed previously (Ai et al. 2007; Reeves et al. 2005), where unmyelinated deficits were equal to, or greater than, those in the myelinated axons. We attributed this to a

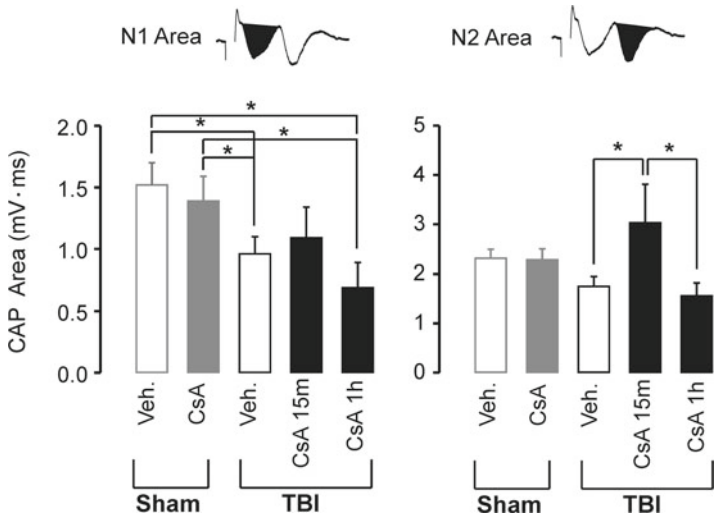


Fig. 15.16 Effects of injury and CsA on area of CAPs evoked with maximum stimulation. Mean N1 and N2 areas, evoked using the 100 % stimulation intensity, are plotted for each analytic group. N1 areas in TBI + veh rats were significantly suppressed below sham levels, but N1 areas in TBI + CsA (15 m) rats were not different from those of sham rats. For the N2 CAP areas evoked with maximum stimuli, there was not a significant injury effect (Sham + veh vs. TBI + veh), but CsA treatment administered at 15 m post-TBI significantly elevated the N2 area above vehicle-treated rats. This drug effect was not present when CsA was given at 1 h postinjury. From Colley et al. 2010, with permission

neuroprotective effect reported for the cremaphor vehicle (Setkowicz and Guzik 2007), and used in the present study. It is possible that the cremaphor-based vehicle exerted a protection selective to the unmyelinated axons, underlying the mild injury and drug effects observed for these fibers. Related to this issue, some aspects of the CsA evaluation also suggested modest detrimental effects when CsA was administered at 1 h postinjury. For example, the myelinated input–output function of the TBI + CsA (1 h) group was shifted below that of the TBI + veh group. This result, along with abnormalities affecting CAP durations and refractoriness, suggested CsA action led to fewer fibers being recruited into the aggregate CAP response (see detailed analyses in Colley et al. 2010). However, it was concluded that the CsA treatment conferred an overall protective effect, providing a benefit to CAP amplitude and improved high-frequency responding in myelinated axons when CsA was administered at 15 min postinjury (Fig. 15.17).

Although the analyses of CsA neuroprotection revealed a complex set of experimental effects, it was clear that the postinjury administration of the drug conferred greater benefit to the myelinated than to the unmyelinated axon population. Our laboratory has completed parallel experiments, not yet submitted for publication, evaluating the postinjury administration of FK506, which also showed a disproportionate benefit for myelinated axons. Our overall findings regarding functional neuroprotection with immunophilin ligands, to date, continue to underscore how very differently the unmyelinated fiber population responds to TBI, and evidently to this

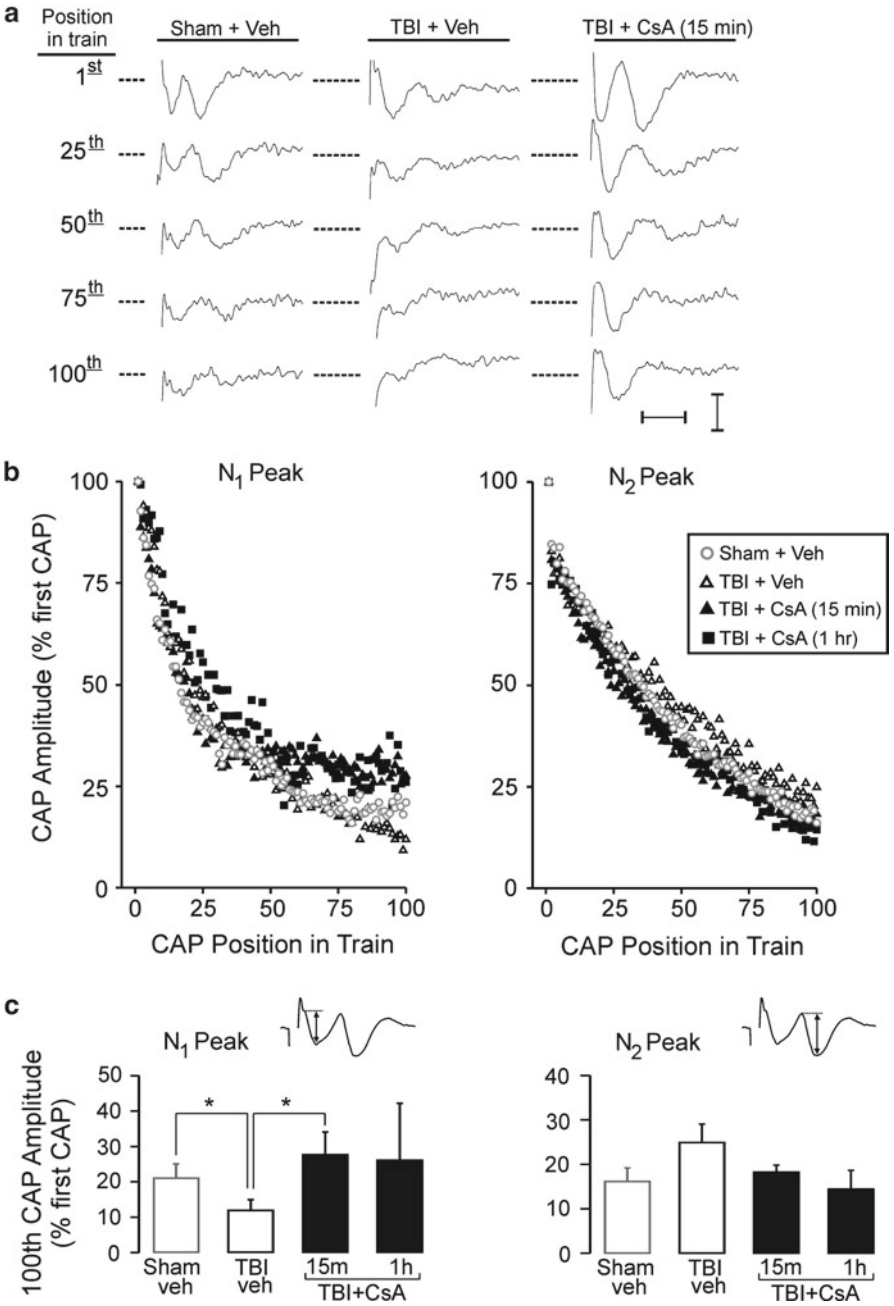


Fig. 15.17 Effects of injury and CsA treatment on high frequency responding. (**a**) Example evoked CAPs (single, nonaveraged) at serial position 1, 25, 50, 75, and 100 in response to a 100 Hz stimulus train of 1 s duration. Calibration: 1 mV×2 ms. (**b**) Scatterplot showing mean of CAP peaks for each 100 responses in high frequency train, with each data point normalized to the first CAP in the series. In N1 scatterplot, note fatigue of TBI+veh responses and preservation of CsA-treated responses between CAP position 75–100. (**c**) Group analysis of the N1 CAP number 100, expressed as a percentage of CAP number 1, showed TBI+veh condition to be significantly depressed by the injury, but CsA treatment at 15 m provided significant protection of this function. From Colley et al. 2010, with permission

class of compounds as well. The potent neuroprotective benefits for unmyelinated axon conduction, observed after a preinjury treatment with FK506, were absent when the immunophilin ligands were administered after the injury. In contrast, functioning of the myelinated axon improved with either preinjury or postinjury drug treatment. This pattern of results would be expected if unmyelinated callosal axons are, in fact, more vulnerable to the FPI than are the myelinated axons. At least for the two test drugs, FK506 and CsA, it would appear that the unmyelinated fibers receive a benefit if the drug is present at the time of injury, but these small axons may sustain sufficient damage that a treatment delayed by only 15 min may not prevent TBI-induced functional compromises. Our working interpretation of these results, taken together, is that structural disparities discussed in the introduction to this chapter (refer back to Fig. 15.1) place unmyelinated axons at a disproportionate risk to TBI. The high membrane content, small cytoplasmic buffering volume and axolemmal exposure, of unmyelinated axons, may present special challenges as drug treatments and interventions are developed and tested.

15.6 Fiber Type as a Factor in Posttraumatic Degradation of the Membrane Skeleton

To this point in the discussion, the presence or absence of myelin has been suggested as a fundamental factor in determining the axonal response to injury. Closely allied to this concept is the idea that myelinated axons may be most vulnerable to TBI at nodal regions that lack myelin. Nodes of Ranvier may be susceptible to injury for reasons beyond the simple lack of structural support and cushioning otherwise provided by myelin. Nodes are characterized by complex and spatially organized molecular domains that regulate axo–glial interactions (reviews: Scherer 1999; Bhat 2003; Susuki and Rasband 2008a). Damage at the paranodal loops, or the cell adhesion molecules that stabilize these contacts, would be predicted to elevate the risk of myelinated axons to the deleterious effects of secondary axonal injury, by exposing internodal axolemma to the aberrant extracellular environment which arises after TBI.

Molecular domains at nodes interact with the "membrane skeleton," a network of spectrin and related molecules located on the cytoplasmic surface of the axolemma. Multiple laboratories have identified a consistent feature of traumatic axonal injury to be the proteolysis of sub-axolemmal spectrin, mediated by the calpain family of calcium-dependent neutral proteases (Newcomb et al. 1997; Saatman et al. 2003; Hall et al. 2005; Park et al. 2007). Spectrin is attached to the plasma membrane through interactions with ankyrin. However, injury-induced changes in ankyrin have not, until recently, been systematically investigated. Although ankyrin proteins were once regarded as passive "linker" molecules, mounting evidence demonstrates they have diverse binding partners and complex roles which may involve them in the deleterious molecular breakdowns occurring after TBI. Ankyrins interact with the cytoplasmic domains of ion channels (Wood and Slater 1998; Malhotra et al. 2002),

transporters (Li et al. 1993; Michaely and Bennett 1995), Na+K+-ATPase (Davis and Bennett 1990; Devarajan et al. 1994), cell adhesion molecules (Dubreuil et al. 1996), and some classes of receptors (Bourguignon and Jin 1995; Hayashi and Su 2001). In the mammalian CNS, there is evidence that ankyrins stabilize the nodal and paranodal structure of myelinated axons. This stabilizing role is implemented not only through spectrin, but also through interactions with transmembrane neurofascins (reviewed in Susuki and Rasband 2008a). Ankyrins are directly involved with the clustering of voltage-gated sodium channels (NaVs) within axonal initial segments and at Nodes of Ranvier (Kordeli et al. 1995; Davis et al. 1996; Zhou et al. 1998; Rasband et al. 1999). With this increasing appreciation for the multifunctional nature of ankyrin proteins, came the need to examine the ankyrin response during the pathogenesis of TBI.

15.6.1 Ankyrin-G and αII-Spectrin Breakdown: A Comparison in Gray vs. White Matter

Our laboratory examined changes in ankyrin-G and αII-spectrin expression following FPI in adult rats (Reeves et al. 2010), assessing these proteins in CC and in cerebral cortex during the postinjury time interval of 3 h to 7 days. Our analyses were directed at whether ankyrin-G undergoes a proteolytic fragmentation comparable to that observed for spectrin. This study was also the first to explicitly compare the injury response of these membrane cytoskeletal proteins in white versus gray matter.

Our Western blot analyses of ankyrin-G focused on TBI-induced changes to three immunopositive bands (220, 212, 75 kDa), corresponding to proteolyzed fragments of the intact protein. These changes were strikingly restricted to the CC, with little or no change observed in the cortical samples. Callosal ankyrin-G alterations elicited by the FPI followed a consistent pattern of early (3 h to 1 day) surges in protein levels, and then resolving to control levels at 3–7 days. The 75 kDa band departed from this general profile, evolving from early postinjury increases to significantly depressed levels at 3–7 days (Fig. 15.18a). In cortical samples, the major pattern for all ankyrin bands was to remain near control levels (Fig. 15.18b), with a possible exception being a 61 % increase in the 220 kDa band at 1 day, although this was not statistically significant.

Western blot analyses of αII-spectrin showed antibody recognition of five major bands, migrating at 280, 180, 150, 145, and 120 kDa. Although alterations in spectrin protein after TBI were relatively larger in cortex than in CC, the time course of these changes was quite similar in the two regions (Fig. 15.19a, b). The most prominent feature was a marked upsurge in the calpain-derived 145 kDa αII-spectrin fragment, peaking at 3 days, but declining by 7 days to a level not significantly different from controls. A comparable time course was observed for the 150 kDa αII-spectrin fragment, which may represent a mix of fragments from calpain and caspase-3 activity (Wang 2000; Aikman et al. 2006). Notably, the 120 kDa caspase-3 derived breakdown product was essentially unchanged after TBI.

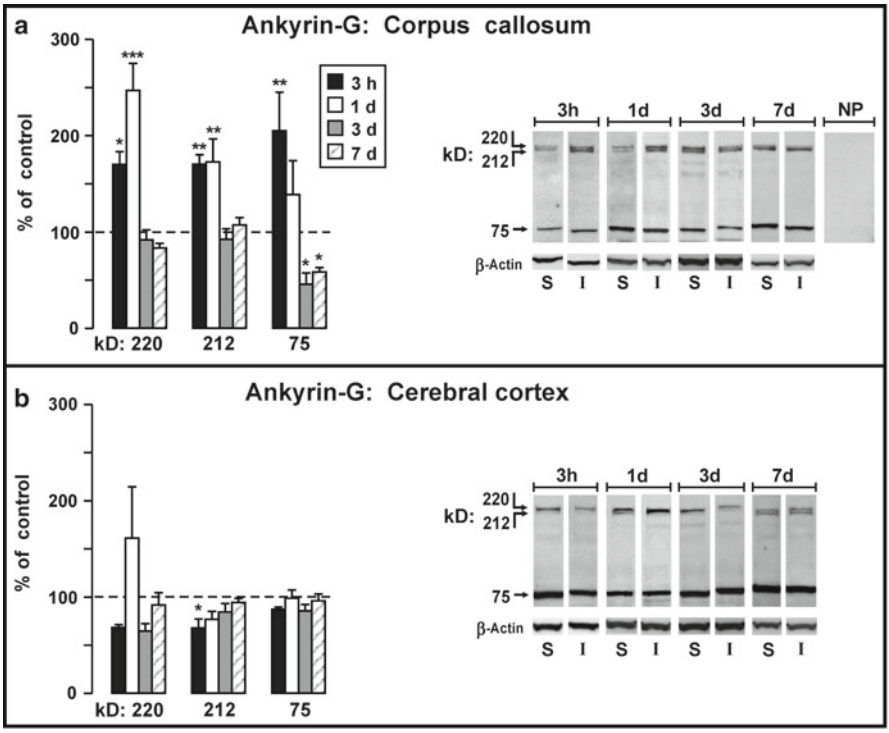


Fig. 15.18 Western blot analysis of ankyrin-G fragments following FPI. Data are plotted as percent change (mean +/− SEM) from sham-injured control rats at survival intervals 3 h, 1 day, 3 days, and 7 days. (**a**) In the CC, a significant surge was observed in 220 and 212 kDa ankyrin-G, which recovered to control levels on 3 and 7 days. The 75 kDa ankyrin-G product was significantly elevated at 3 h postinjury, but decreased to levels significantly below controls at 3 and 7 days. (**b**) The injury produced relatively minor changes in levels of ankyrin-G fragments in the parieto-temporal cortex, with the singular significant change being a 32 % decrease noted for the 212 kDa band measured at 3 h postinjury. From Reeves et al. 2010, with permission

An overall comparison, of ankyrin and spectrin modifications after TBI, suggests a surprising divergence in the response of these proteins to injury. Despite the well-established molecular binding of ankyrin and spectrin (Srinirasan et al. 1988; Kordeli and Bennett 1991; Kordeli et al. 1995; Susuki and Rasband 2008b), TBI produced dissimilar changes in these two proteins along several different dimensions: time course, magnitude of effect, and white matter specificity. First, changes in ankyrin-G showed a comparatively rapid time course, with significant increases confined to 3 h to 1 day, whereas alterations in αII-spectrin levels developed more slowly, and peaked at 3 days. Interestingly, among all immunopositive bands in the study, only the 75 kDa ankyrin-G fragment evolved from an early upsurge to a late (3–7 days) suppression significantly below controls. Secondly, the magnitude of changes in these proteins was quite disparate. The largest increase in αII-spectrin

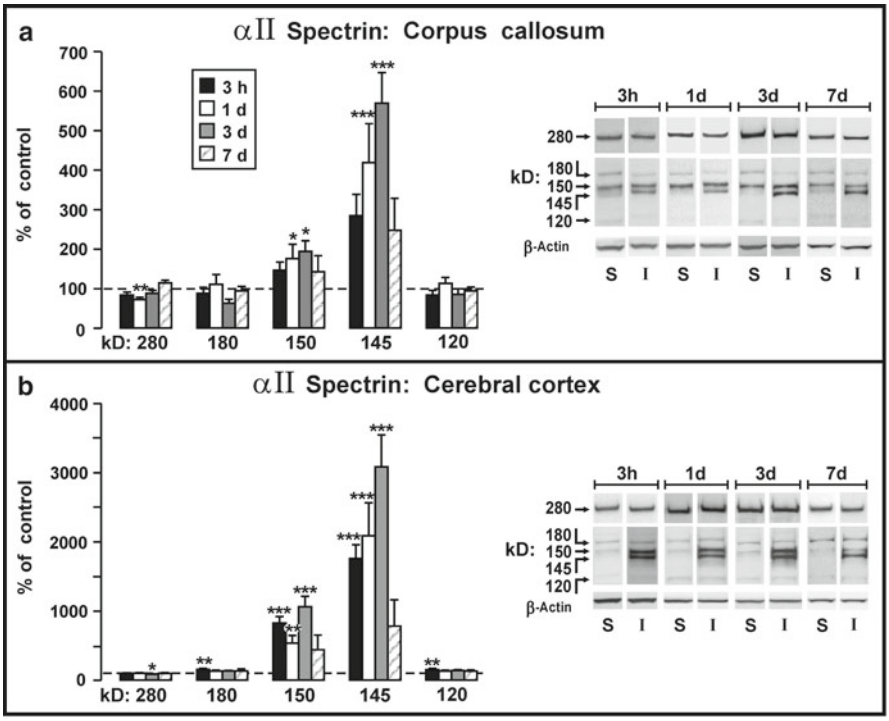


Fig. 15.19 Western blot analysis of αII-spectrin following FPI. Data are plotted as percent change from sham-injured control rats at survival intervals 3 h, 1 day, 3 days, and 7 days. (**a**) Analysis of αII-spectrin and fragments in the CC indicated an injury response primarily mediated by calpain proteolysis, with significant increases at 1 and 3 days in the 145/150 kDa products. No significant injury related changes were found for the 120 kDa fragment associated with caspase-3 activity. (**b**) Analysis of αII-spectrin and fragments in the parieto-temporal cortex revealed massive increases in the 145/150 kDa fragments, exceeding control levels by approximately 30-fold at 3 days postinjury. From Reeves et al. 2010, with permission

levels (+30-fold in 145 kDa) far exceeded the most sizeable change in ankyrin-G (+2.5-fold in 220 kDa). Thirdly, injury-induced changes in spectrin were clearly expressed in both cerebral cortex and CC, whereas the ankyrin response was a singularly white matter phenomenon.

The ankyrin-G protein is likely to be critical to axonal electrophysiology, through its role in spatially organizing ion channels and transporters essential for bioelectric function, and securing diverse proteins integral to the axolemma. For these reasons, and in view of the white matter specificity of post-TBI ankyrin changes, it was reasonable to consider if postinjury alterations to ankyrin proteins could be related to changes in CAP function previously observed to follow TBI (Reeves et al. 2005). Related to the issue of conduction deficits disproportionately affecting unmyelinated axons, there were reports which suggested the expression of distinct ankyrin isoforms within unmyelinated axons (Kordeli and Bennett 1991; Kordeli et al. 1995;

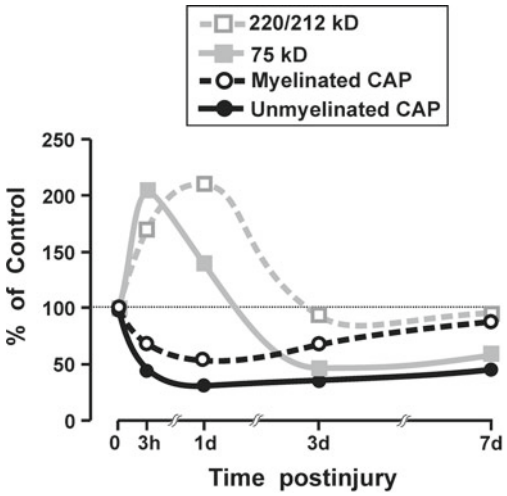


Fig. 15.20 Comparison of ankyrin-G Western blot results with prior measurements (Reeves et al. 2005) of CAP amplitudes recorded from the CC. All data are normalized as percent of sham-injured control levels. Data for the 220 and 212 kDa ankyrin-G fragments are pooled together. Several features of this graphical comparison suggest an association of the 220/212 kDa fragments with myelinated axons, and the 75 kDa product with unmyelinated axons. From Reeves et al. 2010, with permission

Peters et al. 1995; Rubtsov and Lopina 2000). To explore these issues, it was useful to replot the CAP amplitude data along with the measurements of ankyrin-G fragments, with all data normalized to uninjured control values (Fig. 15.20). For this comparison, the results for the 220 and 212 kDa ankyrin-G bands were averaged together, because they migrated in the gel as a doublet, and exhibited similar profiles of postinjury change. These plots suggested the hypothesis that there may be an association of the ankyrin fragments with postinjury CAP alterations. Injury effects reached a peak, for both protein levels and evoked CAPs, at the earlier (3 h to 1 day) time points. However, at 3 and 7 days the myelinated CAP component and the 220/212 kDa ankyrin level exhibit a return to control levels. A contrasting pattern was seen for the unmyelinated CAP signal and levels of the 75 kDa ankyrin, both of which remained significantly below control levels. It is conceivable that the upsurge and resolution of ankyrin 220/212 represents breakdown and clearance of higher isoforms of ankyrin, specifically the 270-kDa and 480-kDa isoforms of ankyrin-G which are highly localized to myelinated axons. In contrast, time-dependent changes in levels of the 75-kDa form may represent primarily a breakdown of the 190-kDa ankyrin-G, which has been associated with unmyelinated axons (reviewed in Rubtsov and Lopina 2000), although the possibility that some of the 75-kDa surge reflects residual fragments of the 270/480 kDa forms cannot be eliminated based on existing data. The processes leading to the rapid postinjury increase at 3 h in the 75 kDa fragment may be distinct from the reductions below control levels observed at 3 and 7 days. Specifically, the 3–7 days decreases indicate some constitutive

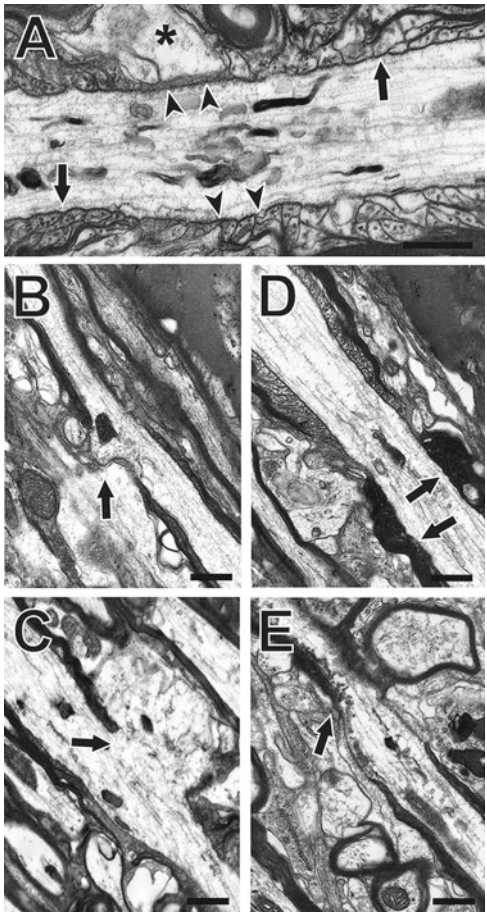


Fig. 15.21 Effect of FPI on Node of Ranvier ultrastructure in CC: (**a**), sham-injured control, (**b**–**e**), injured cases. Sham controls show typical nodal cytoarchitecture, with intact axolemmal membranes (*arrowheads* in (**a**)), normal profiles of adjacent paranodal loops (*arrows* in (**a**)) and axonal/glial interfaces (*asterisk* in (**a**)). Following injury, the axolemma is disrupted, with sites of extruded axonal cytoplasm visible (*arrows* in (**b**, **c**)), in some cases showing shifts of axial cytoskeleton into these regions. Further, paranodal loops of injured cases may appear abnormally electron dense (*arrows* in (**d**)), or show clear membrane disorganization (*arrows* in (**e**)). Bars = 0.5 μm. From Reeves et al. 2010, with permission

presence for 75 kDa ankyrin-G, and the loss of this protein may contribute to the persistent suppression of conduction in unmyelinated axons.

Concurrent with our evaluations of the membrane skeleton proteins, we also identified ultrastructural changes after FPI indicative of axonal pathology, characterized by compaction of axonal cytoskeleton, and damage to Nodes of Ranvier (Reeves et al. 2010). This approach was different from the ultrastructural stereology, conducted on axons in cross-section, as described above. Here, fibers viewed in longitudinal section exhibited nodal abnormalities appearing as cytoplasmic extravasations, or nodal blebs, as previously described in an optic nerve model of axonal injury (Maxwell et al. 1991). In control CC the axolemma was continuous across the node, exhibiting intact paranodal loops and normal nodal interface with glial processes (Fig. 15.21a). This contrasted with injured axonal profiles, which showed evidence of disrupted sub-membrane cytoarchitecture and axolemmal disorganization at the node, often presenting with expansion of axoplasm into the extracellular space (Fig. 15.21b, c). In some cases paranodal loops exhibited

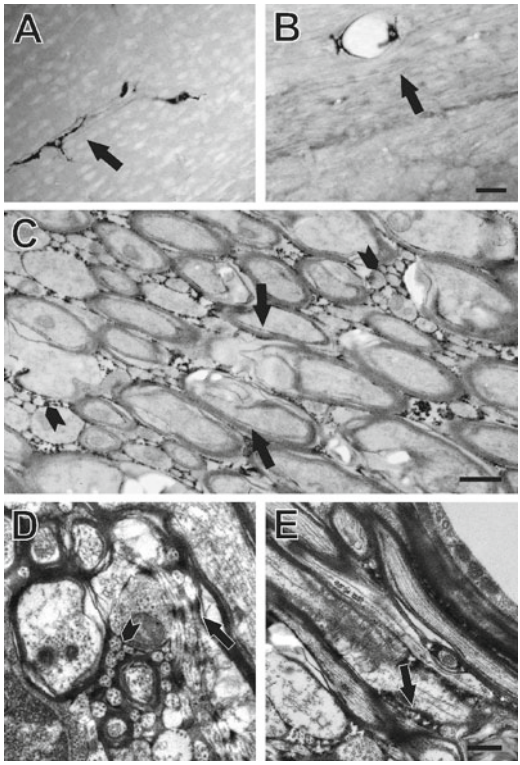


Fig. 15.22 Effects of FPI on white matter BBB disruption and nodal integrity. (**a**) Low magnification view of CC from control case infused with HRP 1 h before sham-injury. Intact BBB retards extravasation of HRP into neuropil, with tracer restricted to the callosal vasculature (*arrow*). (**b**) CC from animal administered HRP 1 h prior to FPI and sacrificed 24 h postinjury. Movement of HRP from vascular bed into space around axons is evident throughout the white matter (*arrow*). (**c**) Ultrastructure of noncounterstained injured case showing HRP diffusion into extracellular space around small unmyelinated fibers (*arrowheads*) and within the submyelin space between axolemma and inner myelin sheath (*arrows*). (**d**, **e**) Higher magnification of counterstained ultrastructure from an injured case. In D, HRP localization is seen around axon bundles (*arrowhead*) and along the surface of glial membranes (*arrows*) and infiltrating paranodal/submyelin space at *arrow* in (**e**) Bar = 30 μm in (**a**, **b**); 10 μm in (**c**); 0.5 μm in (**d**, **e**). From Reeves et al. 2010, with permission

darkened, floccular cytoplasm (Fig. 15.21d), while other injured axons displayed paranodal membrane degeneration (Fig. 15.21e).

Throughout our investigations, of fiber type as a factor in injury outcome, it has been useful to consider if axon populations differ with respect to the degree of axolemmal exposure to aberrant ionic and proteolytic conditions prevailing in the extracellular compartment after injury. This includes extravasating factors that gain parenchymal access in blood–brain barrier (BBB) failures that often accompany TBI. We examined BBB disruption in CC at 1 day postinjury, showing that horseradish peroxidase (HRP), normally retained within brain vasculature, had passed through the vessel walls and entered into the extracellular space around myelinated and unmyelinated fibers (Fig. 15.22). Movement of HRP into the submyelin space, adjacent to

damaged axolemma of myelinated axons, was also evident, and comparable to a pathological pattern previously reported in brainstem white matter tracts after impact acceleration injury (Pettus et al. 1994). At low magnification, the HRP tracer was observed confined within CC vasculature in control tissue, but extruded into the parenchyma of injured animals (Fig. 15.22a, b). When unstained thin sections were examined ultrastructurally, HRP was extensively distributed within the spaces surrounding myelinated axons and outlining bundles of small unmyelinated fibers (Fig. 15.22c). This view also revealed a thin band of tracer between myelin sheath and the axolemma of some fibers (the "submyelin space"). In counterstained thin sections, HRP was observed reaching the extracellular interface between myelinated and unmyelinated axons, and spaces surrounding glial processes (Fig. 15.22d). At injured nodes, HRP appears to fill the space around paranodal loops and extends further along the juxtaparanodal axon/glial interface (Fig. 15.22e). We concluded from these observations, that damaged nodes exhibiting compromised cytoskeletal integrity and membrane adhesion may permit exposure to molecules normally excluded from the internodal axolemma.

15.7 Extracellular Matrix Molecules are Mediators of Axonal Injury and Recovery

Given that our HRP studies in the injured CC revealed the extracellular matrix as a conduit for molecules mediating axonal pathology, we have begun to explore the potential for secreted matrix metalloproteinases (MMPs) to regulate the evolution of this pathology through their matrix/axolemma interaction. These Ca^{++}/Zn^{++} activated enzymes control the organization of the protein matrix around axons, and they are activated by molecules like tissue plasminogen activator (tPA) and circulating cytokines, which are released into that matrix with BBB disruption (Gurney et al. 2006 ; Seo et al. 2012). Prior studies of CNS insult point to MMPs as having the potential to generate axon pathology, not only in TBI (Truettner et al. 2005; Vilalta et al. 2008; Li et al. 2009), but after stroke and ischemia (Suzuki et al. 2007; Walker and Rosenberg 2009; Park et al. 2009), as well as with multiple sclerosis (MS) white matter degeneration (Rosenberg et al. 2001; Kanesaka et al. 2006; Yong et al. 2007) and spinal cord injury (Noble et al. 2002; Hsu et al. 2006). Further, studies of tissue disruption and repair following CNS trauma support a critical role for MMP substrates such as agrin (Solé et al. 2004; Falo et al. 2008), tenascin (Zhang et al. 1997; Hausmann and Betz 2001), phosphacan (Jones et al. 2003; Harris et al. 2011), versican (Asher et al. 2002; Harris et al. 2009) and neurofascin (Mathey et al. 2007; Reeves et al. 2010; Pomicter et al. 2010), each of which is well positioned to influence axolemma stability and axonal integrity. These matrix proteases are not only associated with axonal pathology, but also contribute to the extent of postinjury recovery (Yong 2005; Ahmed et al. 2005; Hsu et al. 2006). Moreover, their targeted inhibition can attenuate both structural and functional damage after CNS trauma, facilitating improved recovery (Falo et al. 2006; Homsi et al. 2009; Warren et al. 2012). In the context of brain injury, MMPs also have the potential to alter BBB stability (Lin et al. 2012; Higashida et al. 2011), as well as influence immune

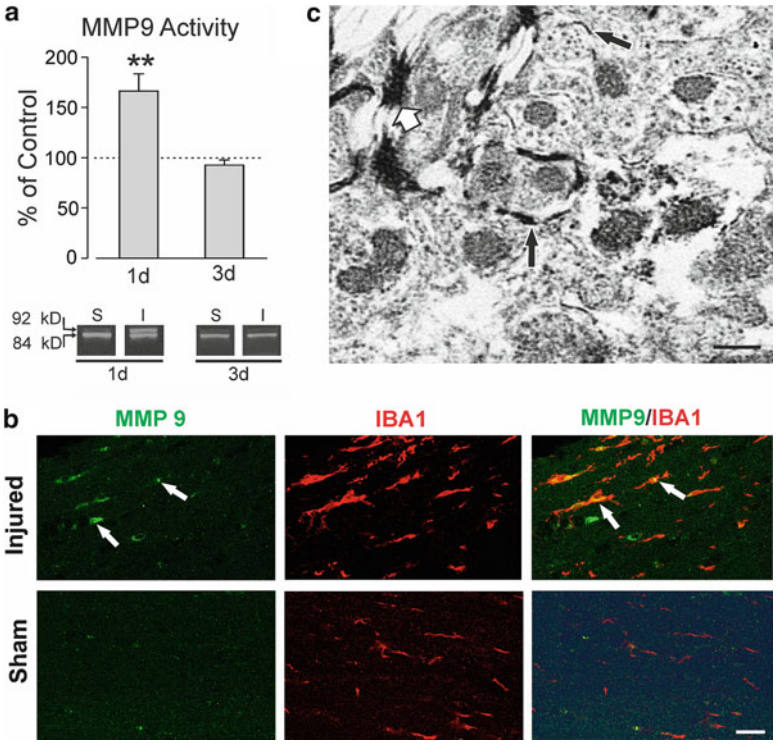


Fig. 15.23 MMP expression and distribution in CC following FPI. (**a**) Gelatin zymography of CC extracts after FPI showed significant acute elevation in 92 kDa pro MMP9 at 1 day postinjury and a return to sham control levels by 3 days ($n = 7–15$/group). (**b**) Confocal co-label experiments illustrate increased CC MMP9 at 1 day postinjury relative to sham controls, consistent with elevated pro MMP9 activity detected by zymography. Principal MMP9 tissue signal was found within reactive microglia (*arrows in overlay*). (**c**) Ultrastructural immunocytochemistry for CC MMP3 at 3 days after FPI revealed enzyme distribution among damaged axons. Noncounterstained sections showed MMP3 reaction product around unmyelinated axons (*black arrows*) and within disrupted myelin sheaths (*white arrowhead*). This MMP distribution is consistent with HRP identified sites where extravasated molecules were redistributed among CC axons following FPI. $*p<0.01$; Bar = 10 μm in (**b**), 0.5 μm in (**c**)

signaling between neurons and neuroglia (Agrawal et al. 2006; Csuka et al. 2000; Morganti-Kossmann et al. 2007).

Based upon our prior studies with injured gray matter (Falo et al. 2006; Phillips and Reeves 2001), we first tested gelatinase B (MMP9) and stromelysin-1 (MMP3) as candidates for callosal matrix activation after TBI. Zymographic analysis (Fig. 15.23a) showed a significant 66.3 % increase of 92 kDa pro MMP9 activity over sham controls at 1 day, but no change at 3 days postinjury. Confocal labeling experiments support elevated callosal MMP9 at 1 day after TBI, with colocalization of the enzyme in IAB1+ microglia (Fig. 15.23b) and GFAP + astrocytes (data not shown). Acute rise in MMP9 is consistent with published elevation of gelatinase

activity in gray matter and plasma after TBI (Muir et al. 2002; Grossetete et al. 2009), and is thought to underlie the onset of BBB permeability in models of stroke, TBI and spinal cord injury (Seo et al. 2012; Candelario-Jalil et al. 2011; Shigemori et al. 2006). Notably, white matter degeneration produced by MS and experimental autoimmune encephalomyelitis (EAE) display the common phenotype of exacerbated MMP9 proteolysis (Leppert et al. 1998; Gijbels et al. 1993).

A similar up regulation of MMP3 was reported in degenerative white matter tracts of patients with vascular dementia (Rosenberg et al. 2001) and MS (Cuzner et al. 1996; Anthony et al. 1997), and in a mouse model of spontaneous demyelination (D'Souza et al. 2002). Injury-induced increase of MMP3 is described following transection of optic axons (Agapova et al. 2003), and the enzyme is elevated in both rodent cortical contusion models (Li et al. 2009), as well as in the serum of MS patients with relapsing-remitting demyelination (Kanesaka et al. 2006). In preliminary Western blot studies, CC MMP3 protein expression was higher after injury, but this change did not reach significance when compared to sham controls (data not shown). Although the time course of MMP3 activity following TAI is not yet documented, we have applied ultrastructural immunocytochemistry to track its distribution in the CC, and our results demonstrate that MMP3 is localized among disrupted axonal profiles 3 days after callosal injury (Fig. 15.23c). Together, these data support an active role for secreted MMPs in the evolution of TAI and point to the potential benefit of MMP inhibition during the early phases of axonal injury.

While MMP inhibition has been demonstrated to affect recovery after vascular insult (Romanic et al. 1998; Asahi et al. 2000; Zhao et al. 2006), spinal cord or sciatic nerve injury (Wells et al. 2003; Busch et al. 2009; Liu et al. 2010), neurodegenerative disease (Yong et al. 2007), and axotomy induced deafferentation (Reeves et al., 2003; Falo et al. 2006; Warren et al. 2012), the specific effects of blocking MMP activity during the evolution of white matter pathobiology are not well understood. Recently we have used literature supported strategies to inhibit MMP9 and MMP3 following FPI in order to test the effect of this inhibition on callosal MMP activity and CAP deficits. One of the most efficient modulators of MMP9 activity for both stroke and MS has been the tricyclic antibiotic minocycline (Murata et al. 2008; Yenari et al. 2006; Giuliani et al. 2005). Although the drug is pleiotropic, it clearly targets MMP9 functional activity (Romero-Perez et al. 2008; Murata et al. 2008; Homsi et al. 2009). In the context of TBI, reports exploring minocycline efficacy have been mixed. Postinjury minocycline dosing after both weight drop (Bye et al. 2007) and impact acceleration (Homsi et al. 2009) TBI does appear to reduce microglial inflammatory response and cell death, as well as improve neurological outcome. By contrast, in the controlled cortical impact model, minocycline failed to improve cognitive deficits (Kelso et al. 2011), suggesting that minocycline protective effect may be complex and model dependent. Interestingly, when minocycline was delivered in combination with N-actyelcysteine in the latter model, a selective neuroprotection of white matter was observed (Baki et al. 2010). This beneficial effect of minocycline was in direct contrast to that reported by Homsi et al. (2010) after impact acceleration, where the drug did not alter APP deposition in large caliber myelinated axons exhibiting TAI. We hypothesized that these discrepancies in

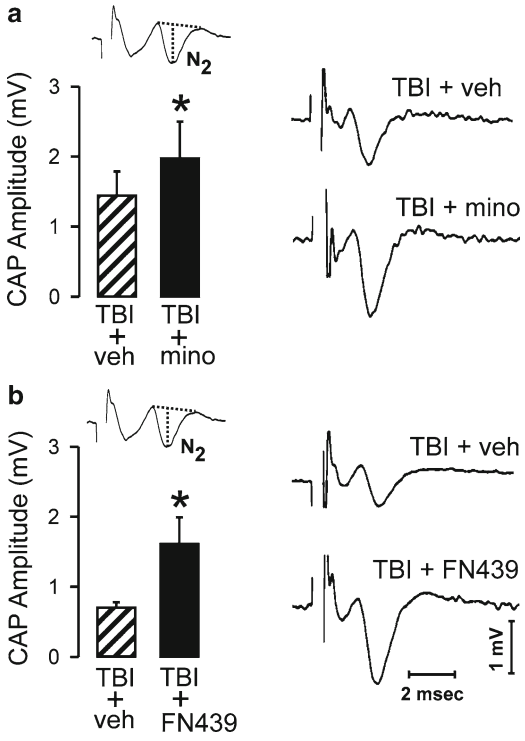


Fig. 15.24 Effect of MMP inhibitors (minocycline and FN439) on mean CAP amplitudes measured following FPI. Injury-induced suppression of CC unmyelinated CAP amplitude (mean post-TBI CAP amplitude shown as TBI + veh bars in (**a**, **b**)) was significantly reversed by either minocycline treatment (TBI + mino in (**a**)) or FN439 treatment (TBI + FN439 in (**b**)). Representative CAPs are shown at *right*. Neither MMP inhibitor significantly altered the amplitude of the mean myelinated CAP signal (N1) following TBI (not shown). *$p<0.05$, $n = 4–6$/group

minocycline efficacy may be due to the fact that the drug preferentially protects smaller caliber unmyelinated fibers through inhibition of matrix MMP9 pathways.

To test this hypothesis we first applied minocycline (45 mg/kg, i.p.; 30 min, 6 h) after moderate midline FPI and probed for effect on callosal gelatinase activity at 1 and 3 days postinjury. While minocycline failed to alter 92 kDa pro MMP activity, the drug significantly reduced proteolysis in the tightly regulated 84 kDa active form of MMP9 by 23.6 ± 8.7 % at 1 day. We observed no effect of minocycline treatment on MMP9 activity at 3 days survival. Next we examined CAP response in myelinated and unmyelinated fiber populations of the CC following minocycline treatment, increasing the dosing to 90 mg/kg i.p., delivered again at 30 min, 6 h postinjury. As for FK506 and CsA experiments, CAP amplitude was used to determine injury and drug effect. Figure 15.24a shows aggregate histograms of N2 callosal CAP response at maximal stimulus intensities, where we found that minocycline significantly reduced injury-induced CAP deficits in the unmyelinated fiber population, an effect present at 1 day, but not at 3 days survival. Minocycline had no effect on callosal myelinated fiber CAP deficits (data not shown). These CAP data are consistent with our zymographic results showing minocycline reduction of callosal MMP9 activity at 1 day but not 3 days after TBI.

In a second set of experiments we tested the effect of targeted MMP3 inhibition on callosal fiber function using MMP inhibitor I (FN439). While available MMP

inhibitors are not entirely selective, we have shown that FN439 can attenuate MMP3 functional activity by 40 % in gray matter deafferented by axonal lesion (Kim et al. 2005). We also know that FN439 attenuates synaptic degeneration resulting from this axonal lesion (Falo et al. 2006). Other published applications of FN439 to brain injury have been limited to models of physiological synaptic plasticity (Reeves et al., 2003; Meighan et al. 2007; Wójtowicz and Mozrzymas 2010) or behavioral assessments of spatial memory (Wright et al. 2007). To our knowledge, no studies have utilized FN439 to manipulate MMP3 within the injured CC, nor has the effect of FN439 on CAP deficits been tested. In pilot plate assays we found that lytic activity attributable to MMP3 within injured callosal extracts was blocked with exposure to FN439 (unpublished observations), supporting its application to inhibit MMP3 in white matter tracts. When rats were subjected to midline moderate FPI and treated 30 min postinjury with FN439 (10 mg/kg, i.v.), deficits in N2 CAP amplitude of unmyelinated axons were significantly reduced at 3 days survival (Fig. 15.24b). As with minocycline, myelinated CAP signal was not altered by FN439 exposure (data not shown). Given that MMP3 was pervasive among degraded unmyelinated axons at 3 days postinjury in our ultrastructural studies, FN439 would be predicted to significantly reduce 3 days N2 CAP deficits if MMP3 is involved in the axonal pathophysiology.

From these initial studies of MMP response following moderate FPI we conclude that both MMP9 and MMP3 contribute to the evolution of axonal pathology with DAI. The acute postinjury application of MMP inhibiting drugs can provide functional neuroprotection for injured unmyelinated axons, a response partially mediated through these two proteases. Finally, the fact that the beneficial effects of MMP inhibition target the fiber population with more exposed axonal membrane adds further support to the hypothesis that the interface between axons and extracellular matrix is a critical site for the maintenance of fiber integrity after TBI.

15.8 TBI Effects in a Predominantly Myelinated Fiber Tract: The Internal Capsule

On the basis of ultrastructural, electrophysiological, and molecular evidence, obtained from observations in CC, we conclude that fiber type is a significant determinant of outcome in axonal injury. However, it is essential to test the generality of this principle by examining how fiber type affects the properties of TAI in forebrain white matter other than the CC. One useful strategy will be to assess TAI in anatomical regions which are enriched in myelinated axons, with minimal unmyelinated fiber contribution. These types of comparative observations should help clarify the degree to which injury severity is related to the proportion of unmyelinated axons present. Although myelinated axons are susceptible to TAI, at all levels of the neuraxis, injury to white matter regions which are highly enriched in myelinated fibers should have a relatively small burden of dysfunctional, or structurally altered, unmyelinated axons. To address these issues our laboratory has initiated studies to

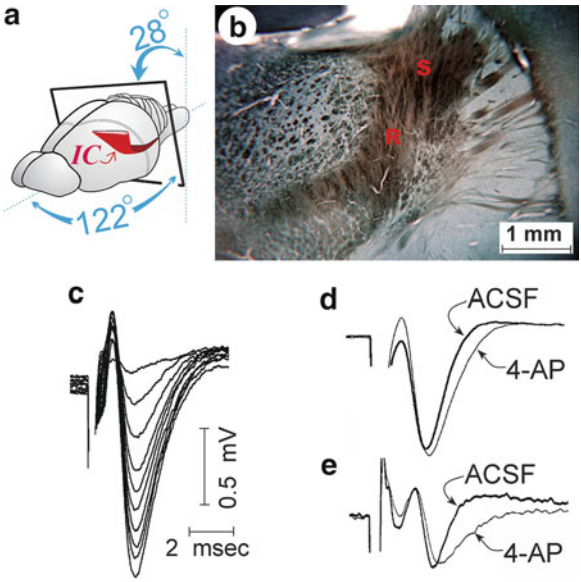


Fig. 15.25 CAP recording in internal capsule (IC). (**a**) Blocking angle for IC slice preparation. (**b**) Sudan Black-stained section showing location of stimulating "S" and recording "R" electrodes. (**c**) Graded stimulation generated family of monophasic CAPs. (**d**, **e**) Blockade of K^+ channels (4-AP), produced large increases in duration of unmyelinated CAPs in corpus callosum (**e**), but comparatively small increases in IC CAPs (**d**), consistent with preponderance of myelinated fibers in the IC

assess TAI in the internal capsule (IC), which is composed principally of large caliber myelinated axons. While posttraumatic damage to the brainstem portion of these fibers (Pettus et al. 1994; Povlishock 1992; Povlishock et al. 1997; Okonkwo et al. 1999; Buki et al. 1999, 2003; Singleton et al. 2001; Stone et al. 2001, 2002; Marmarou and Povlishock 2006), as well as thalamic branches (Singleton et al. 2002) have been well documented, studies examining structural and functional changes in the fibers further upstream have not been conducted.

In head injured patients, DAI lesions in the IC have been implicated in motor weakness following TBI (Choi et al. 2011). The diffusivity abnormalities, detected using DTI in CC of TBI patients, are also observed for the IC. Both regions exhibit reduced axial diffusivity, and increased radial diffusivity, following severe head injury (Sidaros et al. 2008; Betz et al. 2012). Injury also significantly reduced fractional anisotropy values in the IC and splenium (Arfanakis et al. 2002).

We have begun our investigation of functional changes in IC axons following TBI. An early step in this process was the development of IC brain slices for CAP recording. Due to the trajectory of the capsular axons, it was necessary to block the brains using a compound angle which maximally preserved the numbers of continuous longitudinal fibers within a slice (Fig. 15.25a). Using recording conditions identical to those of our callosal recording, we observed that stimulation of IC axons produced CAPs with a single negative-going phase (Fig. 15.25c). This was consistent with a largely homogeneous myelinated fiber population, and contrasted with the biphasic CAPs generated by the mixed myelinated and unmyelinated axons in CC. Further evidence confirming the predominant myelinated composition of the IC

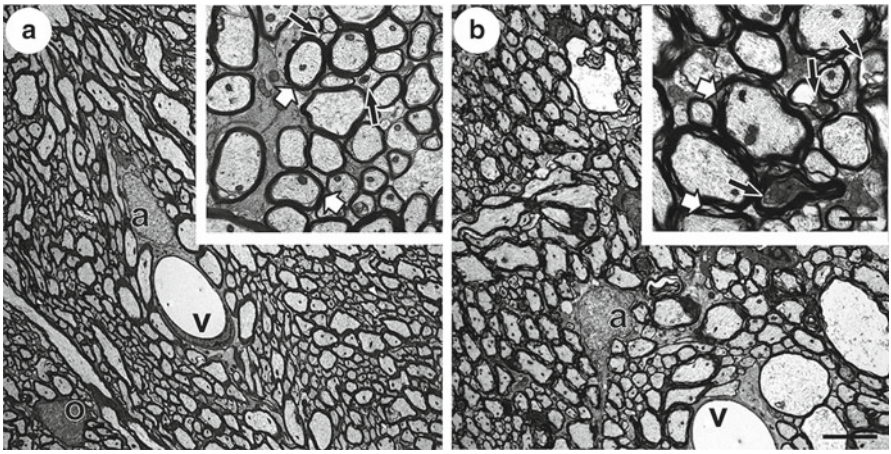


Fig. 15.26 Internal capsule axonal pathology at 3 days following FPI. (**a**) Ultrastructure of sham control IC showed intact axons, predominantly medium to large myelinated fibers. At higher magnification (inset in (**a**)) these fibers have intact myelin sheath and axoplasmic structure (*arrowheads*), with associated glial processes and interspersed unmyelinated axons (*arrows*). (**b**) At 3 days after FPI axonal pathology is observed in the myelinated population, with collapse of the axonal cytoplasm and myelin disruption. Under higher magnification (inset in (**b**)), injured axons showed a striking myelin sheath disruption, particularly in large caliber fibers (*arrowheads*). Axoplasm of injured axons was dark, collapsed and degraded (*arrows*). *a* astrocyte, *o* oligodendrocyte, *v* vessel; Bar = 5 μm in ((**a**, **b**) low magnification), 1 μm in each inset

was obtained in experiments where the slices were recorded after bath application of the K^+ channel blocker 4-AP. K^+ channels exposed on unmyelinated axolemma are readily affected by the 4-AP, which acts to prolong CAPs in unmyelinated axons. In the IC slices, 4-AP produced relatively small changes to CAP duration (Fig. 15.25d), especially compared to the broadening of the unmyelinated CAP observed in CC (Fig. 15.25e). Further, our initial stereological estimates of the relative proportions of myelinated and unmyelinated fibers in this region also indicate a chiefly myelinated tract. Specifically, using unbiased counting frames and sampling rules similar to those described above (Reeves et al. 2012), estimates in a preliminary set of rats ($n = 4$) indicated that 89.0 ± 5.2 % of the IC axons were myelinated. Example electron micrographs of the IC illustrate the fiber composition as principally medium to large diameter myelinated axons (Fig. 15.26a). An example of IC ultrastructure at 3 days following FPI suggests pathological cytoplasmic and myelin changes (Fig. 15.26b). In this case, FPI produced disruptions of the myelin sheath and dark collapsed cytoplasm (inset in Fig. 15.26b). Concurrent with our ongoing ultrastructural evaluation of TBI effects in the IC, we are in parallel assessing CAPs in this tract following midline FPI. In a pilot set of five rats (two sham injured, three TBI recorded at 3 days postinjury), we observed the average maximum CAP amplitude in TBI rats (0.56 ± 0.28 mV) to be suppressed relative to the sham level

(1.46 ± 0.08 mV; $p < 0.05$). We are confident that additional data acquisition using this slice model will lead to a greater understanding of how fiber composition influences functional changes after TBI.

15.9 Looking Forward: How Fiber-Type-Specific Vulnerability May Translate to Clinical Practice

From the clinical perspective, it is useful to consider if behavioral deficits, observed in head injured patients, may reflect damage sustained by specific populations of axons. While sensory and/or motor impairments may exert a devastating toll on some victims of TBI, it has long been recognized (e.g., Levin 1996) that cognitive and memory deficits are the most common, and enduring, problems facing head injury patients. Therefore, it is reasonable to examine evidence that axon populations subserving neocortical association areas may not be identical to those in pathways which are dedicated specifically to sensory or motor systems. In this context, morphometric studies of the CC in primates and humans have indicated that the composition of axon types varies among different callosal subregions. Specifically, the distribution of axon subtype, along the anteroposterior axis of the CC, is in register with projections from functionally and cytoarchitectonically distinct neocortical areas in rhesus monkey (Lamantia and Rakic 1990) and human (Aboitiz et al. 1992, 2003), and, notably, the callosal regions with the largest proportion of unmyelinated axons interconnect association cortex. It is conceivable that a postinjury loss of unmyelinated axons disproportionately impacts fibers subserving associative memory functions, with a relative sparing of sensorimotor-dedicated axons. In this scenario, damage to unmyelinated fibers may result in a transient functional deficit, or an actual numerical loss if they are not recoverable. In our studies, the primary morphometric parameter, axon caliber, transiently decreased only in unmyelinated axons after experimental TBI. A significant caliber reduction would be predicted to slow the velocity of axonal conduction, and correspondingly degrade information processing. A reduced unmyelinated axon caliber, followed by a recovery to normal dimensions, may significantly contribute to the common pattern of initial posttraumatic cognitive impairment, followed by some degree of improvement with the passage of time. We are confident that an increased understanding of the unmyelinated axon vulnerability to TBI, the evolution of their pathological features, and the unique response of these fibers to neuroprotective compounds, will facilitate the development of new therapeutic strategies.

Acknowledgements The authors wish to thank Terry Smith, Judy Williamson, Lesley Harris, Raiford Black, and Nancy Lee for their excellent technical assistance in this work. Supported by the National Institutes of Health (grants NS057758, NS056247, NS047463) and Virginia Commonwealth Neurotrauma Initiative 07–302 F. Microscopy was performed at VCU, Department of Anatomy and Neurobiology Microscopy Facility which is supported, in part, with funding from NIH-NINDS Center Core Grant NS047463.

References

Aboitiz F, Scheibel AB, Fisher RS et al (1992) Fiber composition of the human corpus callosum. Brain Res 598:143–153

Aboitiz F, Lopez J, Montiel J (2003) Long distance communication in the human brain: timing constraints for inter-hemispheric synchrony and the origin of brain lateralization. Biol Res 36:89–99

Adams JH, Doyle D, Ford I et al (1989) Diffuse axonal injury in head injury: definition, diagnosis and grading. Histopathology 15:49–59

Agapova OA, Kaufman PL, Lucarelli MJ et al (2003) Differential expression of matrix metalloproteinases in monkey eyes with experimental glaucoma or optic nerve transection. Brain Res 967:132–143

Agrawal S, Anderson P, Durbeej M et al (2006) Dystroglycan is selectively cleaved at the parenchymal basement membrane at sites of leukocyte extravasation in experimental autoimmune encephalomyelitis. J Exp Med 203:1007–1019

Ahmed Z, Dent RG, Leadbeater WE et al (2005) Matrix metalloproteases: degradation of the inhibitory environment of the transected optic nerve and the scar by regenerating axons. Mol Cell Neurosci 28:64–78

Ai J, Liu E, Wang J et al (2007) Calpain inhibitor MDL-28170 reduces the functional and structural deterioration of corpus callosum following fluid percussion injury. J Neurotrauma 24:960–978

Aikman J, O'Steen B, Silver X et al (2006) Alpha-II-spectrin after controlled cortial impact in the immature rat brain. Dev Neurosci 28:457–465

Anthony DC, Ferguson B, Matyzak MK et al (1997) Differential matrix metalloproteinase expression in cases of multiple sclerosis and stroke. Neuropathol Appl Neurobiol 23:406–415

Arfanakis K, Haughton VM, Carew JD et al (2002) Diffusion tensor MR imaging in diffuse axonal injury. Am J Neuroradiol 23:794–802

Asahi M, Asahi K, Jung JC et al (2000) Role for matrix metalloproteinase 9 after focal cerebral ischemia: effects of gene knockout and enzyme inhibition with BB-94. J Cereb Blood Flow Metab 20:1681–1689

Asher RA, Morgenstern DA, Shearer MC et al (2002) Versican is upregulated in CNS injury and is a product of oligodendrocyte lineage cells. J Neurosci 22:2225–2236

Baker AJ, Phan N, Moulton RJ et al (2002) Attenuation of the electrophysiological function of the corpus callosum after fluid percussion injury in the rat. J Neurotrauma 19:587–599

Baki SGA, Schwab B, Haber M et al (2010) Minocycline synergizes with N-acetylcysteine and improves cognition and memory following traumatic brain injury in rats. PLoS One 5:12490

Betz J, Zhou J, Roy A et al (2012) Prognostic value of diffusion tensor imaging parameters in severe traumatic brain injury. J Neurotrauma 29:1292–1305

Bhadelia RA, Price LL, Tedesco KL et al (2009) Diffusion tensor imaging, white matter lesions, the corpus callosum, and gait in the elderly. Stroke 40:3816–3820

Bhat MA (2003) Molecular organization of axo-glial junctions. Curr Opin Neurobiol 13:552–559

Bourguignon LY, Jin H (1995) Identification of the ankyrin-binding domain of the mouse T-lymphoma cell inositol 1,4,5-trisphosphate (IP3) receptor and its role in the regulation of IP3-mediated internal Ca2+ release. J Biol Chem 270:7257–7260

Buki A, Okonkwo DO, Povlishock JT (1999) Postinjury cyclosporin A administration limits axonal damage and disconnection in traumatic brain injury. J Neurotrauma 58:365–375

Buki A, Farkas O, Doczi T et al (2003) Preinjury administration of the calpain inhibitor MDL-28170 attenuates traumatically induced axonal injury. J Neurotrauma 20:261–268

Burke D, Mogyoros I, Vagg R et al (1998) Quantitative description of the voltage dependence of axonal excitability in human cutaneous afferents. Brain 121:1975–1983

Busch SA, Horn KP, Silver DJ et al (2009) Overcoming macrophage-mediated axonal dieback following CNS injury. J Neurosci 29:9967–9976

Butcher SP, Henshall DC, Teramura Y et al (1997) Neuroprotective actions of FK506 in experimental stroke: in vivo evidence against an antiexcitotoxic mechanism. J Neurosci 17:6939–6946

Bye N, Habgood MD, Callaway JK et al (2007) Transient neuroprotection by minocycline following traumatic brain injury is associated with attenuated microglial activation but no changes in cell apoptosis or neutrophil infiltration. Exp Neurol 204:220–233

Candelario-Jalil E, Thompson J, Taheri S et al (2011) Matrix metalloproteinases are associated with increased blood–brain barrier opening in vascular cognitive impairment. Stroke 42: 1345–1350

Cederberg D, Siesjo P (2010) What has inflammation to do with traumatic brain injury? Childs Nerv Syst 26:221–226

Choi GS, Kim OL, Kim SH et al (2011) Classification of cause of motor weakness in traumatic brain injury using diffusion tensor imaging. Arch Neurol 69:363–367

Colley BS, Phillips LL, Reeves TM (2010) The effects of cyclosporin-A on axonal conduction deficits following traumatic brain injury in adult rats. Exp Neurol 224:241–251

Csuka E, Hans VH, Ammann E et al (2000) Cell activation and inflammatory response following traumatic axonal injury in the rat. Neuroreport 11:2587–2590

Cuzner ML, Gveric D, Strand C et al (1996) The expression of tissue-type plasminogen activator, matrix metalloproteases and endogenous inhibitors in the central nervous system in multiple sclerosis: comparison of stages in lesion evolution. J Neuropathol Exp Neurol 55:1194–1204

Davis JQ, Bennett V (1990) The anion exchanger and Na+K(+)-ATPase interact with distinct sites on ankyrin in in vitro assays. J Biol Chem 265:17252–17256

Davis JQ, Lambert S, Bennett V (1996) Molecular composition of the node of Ranvier: identification of ankyrin-binding cell adhesion molecules neurofascin (mucin+/third FNIII domain-) and NrCAM at nodal axon segments. J Cell Biol 135:1355–1367

Devarajan P, Scaramuzzino DA, Morrow JS (1994) Ankyrin binds to two distinct cytoplasmic domains of Na, K-ATPase alpha subunit. Proc Natl Acad Sci U S A 91:2965–2969

DiLeonardi AM, Huh HW, Raghupathi R (2009) Impaired axonal transport and neurofilament compaction occur in separate populations of injured axons following diffuse brain injury in the immature rat. Brain Res 1263:174–182

Dixon CE, Lyeth BG, Povlishock JT et al (1987) A fluid percussion model of experimental brain injury in the rat. J Neurosurg 67:110–119

D'Souza CA, Mak B, Moscarello MA (2002) The up-regulation of stromelysin-1 (MMP-3) in a spontaneously demyelinating transgenic mouse precedes onset of disease. J Biol Chem 277: 13589–13596

Dubreuil RR, MacVicar G, Dissanayake S et al (1996) Neuroglian-mediated cell adhesion induces assembly of the membrane skeleton at cell contact sites. J Cell Biol 133:647–655

Evans PH (1993) Free radicals in brain metabolism and pathology. Br Med Bull 49:577–587

Eyer J, Leterrier JF (1988) Influence of the phosphorylation state of neurofilament proteins on the interactions between purified filaments in vitro. Biochem J 252:655–660

Falo MC, Fillmore HL, Reeves TM et al (2006) MMP3 expression profile differentiates adaptive and maladaptive synaptic plasticity induced by brain injury. J Neurosci Res 84:768–781

Falo MC, Reeves TM, Phillips LL (2008) Agrin expression during synaptogenesis induced by traumatic brain injury. J Neurotrauma 25:769–783

Geffen GM, Jones DL, Geffen LB (1994) Interhemispheric control of manual motor activity. Behav Brain Res 64:131–140

Gentry LR, Thompson B, Godersky JC (1988) Trauma to the corpus callosum: MR features. AJNR Am J Neuroradiol 9:1129–1138

Gijbels K, Proost P, Masure S et al (1993) Gelatinase B is present in the cerebrospinal fluid during experimental autoimmune encephalomyelitis and cleaves myelin basic protein. J Neurosci Res 36:432–440

Giuliani F, Metz LM, Wilson T et al (2005) Additive effect of the combination of glatiramer acetate and minocycline in a model of MS. J Neuroimmunol 158:213–221

Gorrie C, Duflou J, Brown J et al (2001) Extent and distribution of vascular brain injury in pediatric road fatalities. J Neurotrauma 18:849–860

Goto S, Yamamoto H, Fukunaga K et al (1985) Dephosphorylation of microtubule-associated protein 2, tau factor, and tubulin by calcineurin. J Neurochem 45:276–283
Graham DI, McIntosh TK, Maxwell WL et al (2000) Recent advances in neurotrauma. J Neuropathol Exp Neurol 59:641–651
Gravel C, Sasseville R, Hawkes R (1990) Maturation of the corpus callosum of the rat: II. Influence of thyroid hormones on the number and maturation of axons. J Comp Neurol 291:147–161
Grossetete M, Phelps J, Arko L et al (2009) Elevation of matrix metalloproteinases 3 and 9 in cerebrospinal fluid and blood in patients with severe traumatic brain injury. Neurosurgery 65:702–708
Gurney KJ, Estrada EY, Rosenberg GA (2006) Blood–brain barrier disruption by stromelysin-1 facilitates neutrophil infiltration in neuroinflammation. Neurobiol Dis 23:87–96
Hall ED, Sullivan PG, Gibson TR et al (2005) Spatial and temporal characteristics of neurodegeneration after controlled cortical impact in mice: more than a focal brain injury. J Neurotrauma 22:252–265
Harris NG, Carmichael ST, Hovda DA et al (2009) Traumatic brain injury result in disparate regions of chondroitin sulfate proteoglycan expression that are temporally limited. J Neurosci Res 87:2937–2950
Harris JL, Reeves TM, Phillips LL (2011) Phosphacan and receptor protein tyrosine Phosphatase β expression mediates deafferentation-induced synaptogenesis. Hippocampus 21:81–92
Hausmann R, Betz P (2001) Course of glial immunoreactivity for vimentin, tenascin and alpha1-antichymotrypsin after traumatic injury to human brain. Int J Legal Med 114:338–342
Hayashi T, Su TP (2001) Regulating ankyrin dynamics: roles of sigma-1 receptors. Proc Natl Acad Sci U S A 98:491–496
Higashida T, Kreipke CW, Rafols JA et al (2011) The role ofh y poxia-inducible factor-1α, aquaporin-4, and matrix metalloproteinase-9 in blood-brain barrier disruption and brain edema after traumatic brain injury. J Neurosurg 114:92–101
Hodgkin AL, Huxley AF (1952) A quantitative description of membrane current and its application to conduction and excitation in nerve. J Physiol 117:500–544
Homsi S, Federico F, Croci N et al (2009) Minocycline effects on cerebral edema: relations with inflammatory and oxidative stress markers following traumatic brain injury in mice. Brain Res 1291:122–132
Homsi S, Piaggio T, Croci N et al (2010) Blockade of acute microglial activation by minocycline promotes neuroprotection and reduces locomotor hyperactivity after closed head injury in mice: a twelve-week follow-up study. J Neurotrauma 27:911–921
Hsu JY, McKeon R, Goussev S et al (2006) Matrix metalloproteinase-2 facilitates wound healing events that promote functional recovery after spinal cord injury. J Neurosci 26:9841–9850
Iwata A, Stys PK, Wolf JA et al (2004) Traumatic axonal injury induces proteolytic cleavage of the voltage-gated sodium channels modulated by tetrodotoxin and protease inhibitors. J Neurosci 24:4605–4613
Jafari SS, Maxwell WL, Neilson M et al (1997) Axonal cytoskeletal changes after non-disruptive axonal injury. J Neurocytol 26:207–221
Jafari SS, Maxwell WL, Neilson M et al (1998) Axonal cytoskeletal changes after non-disruptive axonal injury: II. Intermediate sized axons. J Neurotrauma 15:955–966
Jones LL, Margolis RU, Tuszynski MH (2003) The chondroitin sulfate proteoglycans neurocan, brevican, phosphacan, and versican are differentially regulated following spinal cord injury. Exp Neurol 182:399–411
Juraska JM, Kopcik JR (1988) Sex and environmental influences on the size and ultrastructure of the rat corpus callosum. Brain Res 450:1–8
Kanesaka T, Mori M, Hattori T et al (2006) Serum matrix metalloproteinase-3 levels correlate with disease activity in relapsing-remitting multiple sclerosis. J Neurol Neurosurg Psychiatry 77: 185–188
Katayama Y, Becker DP, Tamura T et al (1990) Massive increases in extracellular potassium and the indiscriminate release of glutamate following concussive brain injury. J Neurosurg 73: 889–900

Kelley BJ, Lifshitz J, Povlishock JT (2007) Neuroinflammatory responses after experimental diffuse traumatic brain injury. J Neuropath Exp Neurol 66:989–1001
Kelso ML, Scheff NN, Scheff SW et al (2011) Melatonin and minocycline for combinatorial therapy to improve functional and histopathological deficits following traumatic brain injury. Neurosci Let 488:60–64
Kim JHY, Ellman A, Juraska JM (1996) A re-examination of sex differences in axon density and number in the splenium of the rat corpus callosum. Brain Res 740:47–56
Kim HJ, Fillmore HL, Reeves TM et al (2005) Elevation of hippocampal MMP-3 expression and activity during trauma-induced synaptogenesis. Exp Neurol 192:60–72
King AI, Ruan JS, Zhou C et al (1995) Recent advances in biomechanics of brain injury research: a review. J Neurotrauma 12:651–658
Kocsis JD, Waxman SG (1980) Absence of potassium conductance in central myelinated axons. Nature 287:348–349
Kordeli E, Bennett V (1991) Distinct ankyrin isoforms at neuron cell bodies and nodes of Ranvier resolved using erythrocyte ankyrin-deficient mice. J Cell Biol 114:1243–1259
Kordeli E, Lambert S, Bennett V (1995) AnkyrinG. A new ankyrin gene with neural-specific isoforms localized at the axonal initial segment and node of Ranvier. J Biol Chem 270: 2352–2359
Lamantia AS, Rakic P (1990) Cytological and quantitative characteristics of four cerebral commissures in the rhesus monkey. J Comp Neurol 291:520–537
Leker RR, Shohami E (2002) Cerebral ischemia and trauma: different etiologies yet similar mechanisms—neuroprotective opportunities. Brain Res Rev 39:55–73
Leppert D, Ford J, Stabler G et al (1998) Matrix metalloproteinase-9 (gelatinase B) is selectively elevated in CSF during relapses and stable phases of multiple sclerosis. Brain 121:2327–2334
Levin HS (1996) Neurobehavioral outcome of closed head injury: implications for clinical trials. In: Bandak FA, Eppinger RH, Ommaya AK (eds) Traumatic brain injury: bioscience and mechanics. Mary Ann Liebert, Larchmont, New York, pp 105–114
Li X-Y, Feng D-F (2009) Diffuse axonal injury: novel insights into detection and treatment. J Clin Neurosci 16:614–619
Li ZP, Burke EP, Frank JS et al (1993) The cardiac Na+-Ca2+ exchanger binds to the cytoskeletal protein ankyrin. J Biol Chem 268:11489–11491
Li RB, Guo XC, Liang HX et al (2009) Study on changes of MMP-3 expression after brain contusion in rats. Leg Med (Tokyo) 11(Suppl 1):S176–S179
Lin Y, Pan Y, Wang M et al (2012) Blood–brain barrier permeability is positively correlated with cerebral microvascular perfusion in the early fluid percussion-injured brain of the rat. Lab Invest 10:1038 [Epub ahead of print]
Liu H, Kim Y, Chattopadhyay S et al (2010) Matrix metalloproteinase inhibition enhances the rate of nerve regeneration in vivo by promoting dedifferentiation and mitosis of supporting schwann cells. J Neuropathol Exp Neurol 69:386–395
Lyeth BG, Jenkins LW, Hamm RJ et al (1990) Prolonged memory impairment in the absence of hippocampal cell death following traumatic brain injury in the rat. Brain Res 526:249–258
Malhotra JD, Koopmann MC, Kazen-Gillespie KA et al (2002) Structural requirements for interaction of sodium channel beta-1 subunits with ankyrin. J Biol Chem 277:26681–26688
Maller JJ, Thomson RH, Lewis PM et al (2010) Traumatic brain injury, major depression, and diffusion tensor imaging: making connections. Brain Res Rev 64:213–240
Marmarou CR, Povlishock JT (2006) Administration of the immunophilin ligand FK506 differentially attenuates neurofilament compaction and impaired axonal transport in injured axons following diffuse traumatic brain injury. Exp Neurol 197:353–362
Mathey EK, Derfuss T, Storch MK et al (2007) Neurofascin as a novel target for autoantibody-mediated axonal injury. J Exp Med 204:2363–2372
Matsukawa H, Shinoda M, Fujii M et al (2011) Genu of corpus callosum as a prognostic factor in diffuse axonal injury. J Neurosurg 115:1019–1024
Maxwell WL, Irvine A, Graham DI et al (1991) Focal axonal injury: the early axonal response to stretch. J Neurocytol 20:157–164

Maxwell WL, Watt C, Graham DI et al (1993) Ultrastructural evidence of axonal shearing as a result of lateral acceleration of the head in non-human primates. Acta Neuropathol 86: 136–144

Maxwell WL, Povlishock JT, Graham DL (1997) A mechanistic analysis of nondisruptive axonal injury: a review. J Neurotrauma 14:419–440

Mbye LH, Singh IN, Sullivan PG et al (2008) Attenuation of acute mitochondrial dysfunction after traumatic brain injury in mice by NIM811, a nonimmunosuppressive cyclosporin A analog. Exp Neurol 209:243–253

Mbye LH, Singh IN, Carrico KM et al (2009) Comparative neuroprotective effects of cyclosporin A and NIM811, a nonimmunosuppressive cyclosporin A analog, following traumatic brain injury. J Cereb Blood Flow Metab 29:87–97

Meighan PC, Meighan SE, Davis CJ et al (2007) Effects of matrix metalloproteinase inhibition on short- and long-term plasticity of schaffer collateral/CA1 synapses. J Neurochem 102: 2085–2096

Meythaler JM, Peduzzi JD, Eleftheriou E et al (2001) Current concepts: diffuse axonal injury-associated traumatic brain injury. Arch Phys Med Rehabil 82:1461–1471

Michaely P, Bennett V (1995) Mechanism for binding site diversity on ankyrin. Comparison of binding sites on ankyrin for neurofascin and the Cl−/HCO3− anion exchanger. J Biol Chem 270:31298–31302

Morganti-Kossmann MC, Satgunaseelan L, Bye N et al (2007) Modulation of immune response by head injury. Injury 38:1392–1400

Muir EM, Adcock KH, Morgenstern DA et al (2002) Matrix metalloproteases and their inhibitors are produced by overlapping populations of activated astrocytes. Brain Res Mol Brain Res 100:103–117

Murata Y, Rosell A, Scannevin RH et al (2008) Extension of the thrombolytic time window with minocycline in experimental stroke. Stroke 39:3372–3377

Nashmi R, Fehlings MG (2001) Changes in axonal physiology and morphology after chronic compressive injury of the thoracic spinal cord. Neuroscience 104:235–251

Newcomb JK, Kampfl A, Posmantur RM et al (1997) Immunohistochemical study of calpain-mediated breakdown products to a-spectrin following controlled cortical impact injury in the rat. J Neurotrauma 14:369–383

Noble LJ, Donovan F, Igarashi T et al (2002) Matrix metalloproteinases limit functional recovery after spinal cord injury by modulation of early vascular events. J Neurosci 22:7526–7535

Ochiai T, Nakajima K, Sakamoto K et al (1989) Comparative studies on the immunosuppressive activity of FK506, 15-deoxyspergualin, and cyclosporine. Transplant Proc 21:829–832

O'Connor WT, Smyth A, Gilchrist MD (2011) Animal models of traumatic brain injury: a critical evaluation. Pharmacol Ther 130:106–113

Okonkwo DO, Buki A, Siman R et al (1999) Cyclosporin A limits calcium-induced axonal damage following traumatic brain injury. Neuroreport 10:353–358

Pandya DN, Karol EA, Heilbronn D (1971) The topographical distribution of interhemispheric projections in the corpus callosum of the rhesus monkey. Brain Res 32:31–43

Park E, Liu E, Shek M et al (2007) Heavy neurofilament accumulation and alpha-spectrin degradation accompany cerebellar white matter functional deficits following forebrain fluid percussion injury. Exp Neurol 204:49–57

Park E, Bell JD, Baker AJ (2008) Traumatic brain injury: can the consequences be stopped? Canadian Med Assoc J 178:1163–1170

Park KP, Rosell A, Foerch C et al (2009) Plasma and brain matrix metalloproteinase-9 after acute focal cerebral ischemia in rats. Stroke 40:2836–2842

Partadiredja G, Miller R, Oorschot DE (2003) The number, size, and type of axons in rat subcortical white matter on left and right sides: a stereological, ultrastructural study. J Neurocytol 32:1165–1179

Peters LL, John KM, Lu FM et al (1995) Ank3 (epithelial ankyrin), a widely distributed new member of the ankyrin gene family and the major ankyrin in kidney, is expressed in alternatively spliced forms, including forms that lack the repeat domain. J Cell Biol 130:313–330

Pettus EH, Christman CW, Giebel ML et al (1994) Traumatically induced altered membrane permeability: its relationship to traumatically induced reactive axonal change. J Neurotrauma 11:507–522

Phillips LL, Reeves TM (2001) Interactive pathology following traumatic brain injury modifies hippocampal plasticity. Restor Neurol Neurosci 19:213–235

Pierpaoli C, Barnett A, Pajevic S et al (2001) Water diffusion changes in Wallerian degeneration and their dependence on white matter architecture. Neuroimage 13:1174–1185

Pomicter AD, Shroff SM, Fuss B et al (2010) Novel forms of neurofascin 155 in the central nervous system: alterations in paranodal disruption models and multiple sclerosis. Brain 133: 389–405

Povlishock JT (1992) Traumatically induced axonal injury: pathogenesis and pathobiological implications. Brain Pathol 2:1–12

Povlishock JT, Christman CW (1995) The pathobiology of traumatically induced axonal injury in animals and humans: a review of current thoughts. J Neurotrauma 12:555–564

Povlishock JT, Katz DI (2005) Update of neuropathology and neurological recovery after traumatic brain injury. J Head Trauma Rehabil 20:76–94

Povlishock JT, Marmarou T, McIntosh T et al (1997) Impact acceleration injury in the rat: evidence for focal axolemmal change and related neurofilament sidearm alteration. J Neuropathol Exp Neurol 56:347–359

Rasband MN, Peles E, Trimmer JS et al (1999) Dependence of nodal sodium channel clustering on paranodal axoglial contact in the developing CNS. J Neurosci 19:7516–7528

Reeves TM, Colley BS (2012) Electrophysiological approaches in traumatic brain injury. In: Chen J, Xu Z, Xu X-M, Zheng J (eds) Animal models of acute neurological injuries II: injury and mechanistic assessments. Humana Press, New York, pp 313–330

Reeves TM, Phillips LL, Povlishock JT (2005) Myelinated and unmyelinated axons of the corpus callosum differ in vulnerability and functional recovery following traumatic brain injury. Exp Neurol 196:126–139

Reeves TM, Phillips LL, Lee NN et al (2007) Preferential neuroprotective effect of tacrolimus (FK506) on unmyelinated axons following traumatic brain injury. Brain Res 1154:225–236

Reeves TM, Prins ML, Zhu J-P, Povlishock JT, Phillips LL (2003) Matrix metalloproteinase inhibition alters functional and structural correlates of deafferentation-induced sprouting in the dentate gyrus. J Neurosci 23:10182–10189

Reeves TM, Greer JE, Vanderveer AS et al (2010) Proteolysis of submembrane cytoskeletal proteins ankyrin-G and αII-spectrin following diffuse brain injury: a role in white matter vulnerability at Nodes of Ranvier. Brain Pathol 20:1055–1068

Reeves TM, Smith TL, Williamson JC et al (2012) Unmyelinated axons show selective rostrocaudal pathology in the corpus callosum following traumatic brain injury. J Neuropathol Exp Neurol 71:198–210

Romanic AM, White RF, Arleth AJ et al (1998) Matrix metalloproteinase expression increases after cerebral focal ischemia in rats: inhibition of matrix metalloproteinase-9 reduces infarct size. Stroke 29:1020–1030

Romero-Perez D, Fricovsky E, Yamasaki KG et al (2008) Cardiac uptake of minocycline and mechanisms for in vivo cardioprotection. J Am Coll Cardiol 52:1086–1094

Rosa CM, Luigi B, Antonio D et al (2011) Early prognosis after severe traumatic brain injury with minor or absent computed tomography scan lesions. J Trauma 70:447–451

Rosenberg GA, Sullivan N, Esiri MM (2001) White matter damage is associated with matrix metalloproteinases in vascular dementia. Stroke 32:1162–1168

Rubtsov AM, Lopina OD (2000) Ankyrins. FEBS Lett 482:1–5

Rushton WA (1951) A theory of the effects of fibre size in medullated nerve. J Physiol 115:101–122

Rutgers DR, Fillard P, Paradot G et al (2008) Diffusion tensor imaging characteristics of the corpus callosum in mild, moderate, and severe traumatic brain injury. AJNR Am J Neuroradiol 29:1730–1735

Rutland-Brown W, Langlois JA, Thomas KE et al (2006) Incidence of traumatic brain injury in the United States, 2003. J Head Trauma Rehabil 21:544–548
Saatman KE, Graham DI, McIntosh TK (1998) The neuronal cytoskeleton is at risk after mild and moderate brain injury. J Neurotrauma 15:1047–1058
Saatman KE, Abai B, Grosvenor A et al (2003) Traumatic axonal injury results in biphasic calpain activation and retrograde transport impairment in mice. J Cereb Blood Flow Metab 23:34–42
Saatman KE, Creed J, Raghupathi R (2010) Calpain as a therapeutic target in traumatic brain injury. Neurotherapeutics 7:31–42
Samorajski T, Friede RL (1968) A quantitative electron microscopic study of myelination in the pyramidal tract of rat. J Comp Neur 134:323–338
Scherer SS (1999) Nodes, paranodes, and incisures: from form to function. Ann N Y Acad Sci 883:131–142
Seggie J, Berry M (1972) Ontogeny of interhemispheric evoked potentials in the rat: significance of myelination of the corpus callosmn. Exp Neurol 35:215–232
Seo JH, Guo S, Lok J et al (2012) Neurovascular matrix metalloproteinases and the blood–brain barrier. Curr Pharm Des 18:3645–3648
Setkowicz Z, Guzik R (2007) Injections of vehicle, but not cyclosporin A or tacrolimus (FK506), afford neuroprotection following injury in the developing rat brain. Acta Neurobiol Exp 67:399–409
Shigemori Y, Katayama Y, Mori T et al (2006) Matrix metalloproteinase-9 is associated with blood–brain barrier opening and brain edema formation after cortical contusion in rats. Acta Neurochir Suppl 96:130–133
Sidaros A, Engberg AW, Sidaros K et al (2008) Diffusion tensor imaging during recovery from severe traumatic brain injury and relation to clinical outcome: a longitudinal study. Brain 131:559–572
Singleton RH, Stone JR, Okonkwo DO et al (2001) The immunophilin ligand FK506 attenuates axonal injury in an impact-acceleration model of traumatic brain injury. J Neurotrauma 18:607–614
Singleton RH, Zhu J, Stone JR et al (2002) Traumatically induced axotomy adjacent to the soma does not result in acute neuronal death. J Neurosci 22:791–802
Skandsen T, Kvistad KA, Solheim O et al (2010) Prevalence and impact of diffuse axonal injury in patients with moderate and severe head injury: a cohort study of early magnetic resonance imaging findings and 1-year outcome. J Neurosurg 113:556–563
Smith RS, Koles ZJ (1970) Myelinated nerve fibers: computed effect of myelin thickness on conduction velocity. Am J Physiol 219:1256–1258
Smith DH, Meaney DF (2000) Axonal damage in traumatic brain injury. Neuroscientist 6:483–495
Smith DH, Chen XH, Nonaka M et al (1998) Accumulation of amyloid beta and tau and the formation of neurofilament inclusions following diffuse brain injury in the pig. J Neuropathol Exp Neurol 58:982–992
Solé S, Petegnief V, Gorina R et al (2004) Activation of matrix metalloproteinase-3 and agrin cleavage in cerebral ischemia/reperfusion. J Neuropathol Exp Neurol 63:338–349
Srinirasan Y, Elmer L, Davis J et al (1988) Ankyrin and spectrin associate with voltage-dependent sodium channels in brain. Nature 333:177–180
Stone JR, Singleton RH, Povlishock JT (2000) Antibodies to the C-terminus of the beta-amyloid precursor protein (APP): a site specific marker for the detection of traumatic axonal injury. Brain Res 871:288–302
Stone JR, Singleton RH, Povlishock JT (2001) Intra-axonal neurofilament compaction does not evoke local axonal swelling in all traumatically injured axons. Exp Neurol 172:320–331
Stone JR, Okonkwo DO, Singleton RH et al (2002) Caspase-3-mediated cleavage of amyloid precursor protein and formation of amyloid Beta peptide in traumatic axonal injury. J Neurotrauma 19:601–614
Strich SJ (1961) Shearing of nerve fibers as a cause of brain damage due to head injury: a pathological study of twenty cases. Lancet 2:443–448

Sturrock RR (1980) Myelination of the mouse corpus callosum. Neuropathol Appl Neurobiol 6:415–420
Stys PK (2004) White matter injury mechanisms. Cur Mol Med 4:113–130
Susuki K, Rasband MN (2008a) Molecular mechanisms of node of Ranvier formation. Curr Opin Cell Biol 20:616–623
Susuki K, Rasband MN (2008b) Spectrin and ankyrin-based cytoskeletons at polarized domains in myelinated axons. Exp Biol Med 233:394–400
Suzuki Y, Nagai N, Umemura K et al (2007) Stromelysin-1 (MMP-3) is critical for intracranial bleeding after t-PA treatment of stroke in mice. J Thromb Haemost 5:1732–1739
Swanson TH, Krahl SE, Liu Y-Z et al (1998) Evidence for physiologically active axonal adenosine receptors in the rat corpus callosum. Brain Res 784:188–198
Thomalla G, Glauche V, Koch MA et al (2004) Diffusion tensor imaging detects early Wallerian degeneration of the pyramidal tract after ischemic stroke. Neuroimage 22:1767–1774
Thompson HJ, Lifshitz J, Marklund N et al (2005) Lateral fluid percussion brain injury: a 15-year review and evaluation. J Neurotrauma 22:42–75
Truettner JS, Alonso OF, Dietrich DW (2005) Influence of therapeutic hypothermia on matrix metalloproteinase activity after traumatic brain injury in rats. J Cereb Blood Flow Metab 25: 1505–1516
Vilalta A, Sahuquillo J, Rosell A et al (2008) Moderate and severe traumatic brain injury induce early overexpression of systemic and brain gelatinases. Intensive Care Med 34:1384–1392
von Reyn CR, Spaethling JM, Mesfin MN et al (2009) Calpain mediates proteolysis of the voltage-gated sodium channel alpha-subunit. J Neurosci 29:10350–10356
Walker EJ, Rosenberg GA (2009) TIMP-3 and MMP-3 contribute to delayed inflammation and hippocampal neuronal death following global ischemia. Exp Neurol 216:122–131
Wang KK (2000) Calpain and caspase: can you tell the difference? Trends Neurosci 23:20–26
Warren KM, Reeves TM, Phillips LL (2012) MT5-MMP, ADAM-10 and N-Cadherin act in concert to facilitate synapse reorganization following traumatic brain injury. J Neurotrauma 29:1922–1940
Waxman SG, Swadlow HA (1976) Ultrastructure of visual callosal axons in the rabbit. Exp Neurol 53:115–127
Wells JEA, Hurlbert RJ, Fehlings MG et al (2003) Neuroprotection by minocycline facilitates significant recovery from spinal cord injury in mice. Brain 126:1628–1637
Wójtowicz T, Mozrzymas JW (2010) Late phase of long-term potentiation in the mossy fiber-CA3 hippocampal pathway is critically dependent on metalloproteinases activity. Hippocampus 20:917–921
Wood SJ, Slater CR (1998) beta-Spectrin is colocalized with both voltage-gated sodium channels and ankyrin G at the adult rat neuromuscular junction. J Cell Biol 140:675–684
Wright JW, Brown TE, Harding JW (2007) Inhibition of hippocampal matrix metalloproteinase-3 and -9 disrupts spatial memory. Neural Plast 2007:73813
Xiong Y, Rabchevsky AG, Hall ED (2007) Role of peroxynitrite in secondary oxidative damage after spinal cord injury. J Neurochem 100:639–649
Yenari MA, Xu L, Tang XN et al (2006) Microglia potentiate damage to blood–brain barrier constituents. Stroke 37:1087–1093
Yong VW (2005) Metalloproteinases: mediators of pathology and regeneration in the CNS. Nat Rev Neurosci 6:931–944
Yong VW, Zabad RK, Agrawal S et al (2007) Elevation of matrix metalloproteinases (MMPs) in multiple sclerosis and impact of immunomodulators. J Neurol Sci 259:79–84
Zhang Y, Winterbottom JK, Schachner M et al (1997) Tenascin-C expression and axonal sprouting following injury to the spinal dorsal columns in the adult rat. J Neurosci Res 49:433–450
Zhao BQ, Wang S, Kim HY et al (2006) Role of matrix metalloproteinases in delayed cortical responses after stroke. Nat Med 12:441–445
Zhou D, Lambert S, Malen PL et al (1998) AnkyrinG is required for clustering of voltage-gated Na channels at axon initial segments and for normal action potential firing. J Cell Biol 143:1

Chapter 16
Age-Dependent Mechanisms of White Matter Injury After Stroke

Selva Baltan

16.1 Introduction

The significance of white matter injury in the clinical manifestations of stroke has been underestimated in experimental animal models. Rodents are the most commonly used animals for the study of stroke but ironically rodent brain constitutes only ~10 % white matter by volume (Fig. 16.1; Zhang and Sejnowski 2000). In addition, the most commonly used stroke model in rodents, the middle cerebral artery occlusion (MCAO) model consistently spares corpus callosum, the white matter tract in rodents (as reviewed in Ginsberg and Busto 1989). This is mainly due to the fact that the middle cerebral is not the main arterial supply to the corpus callosum area. Rodent corpus callosum receives collaterals from deep penetrating pial arteries and striate arteries that arise from the circle of Willis. Conversely, human corpus callosum receives 80 % of its blood supply from the anterior cerebral artery and its branches, the middle cerebral artery being one of them (Wolfram-Gabel et al. 1987; Ture et al. 1996). Predictably, then the injured rodent brain after an ischemic attack is, essentially neuronal injury with minimal or no contribution from white matter. Human brain comprises equal percentages of gray and white matter by volume (Fig. 16.1; Zhang and Sejnowski 2000), which means that injuries sustained after a stroke in humans inevitably involve more white matter than in rodents. In fact, white matter is injured during most strokes, and even small lesions located in a strategic area of white matter can lead to drastic neurological dysfunction and deficits. It is conceivable that all these factors may have contributed to the failure of in clinical trial of drug candidates that selectively conferred protection to neurons in

S. Baltan (✉)
Department of Neurosciences, Cleveland Clinic Lerner Research Institute, Cleveland, OH, USA
e-mail: baltans@ccf.org

S. Baltan et al. (eds.), *White Matter Injury in Stroke and CNS Disease*, Springer Series in Translational Stroke Research 4, DOI 10.1007/978-1-4614-9123-1_16,

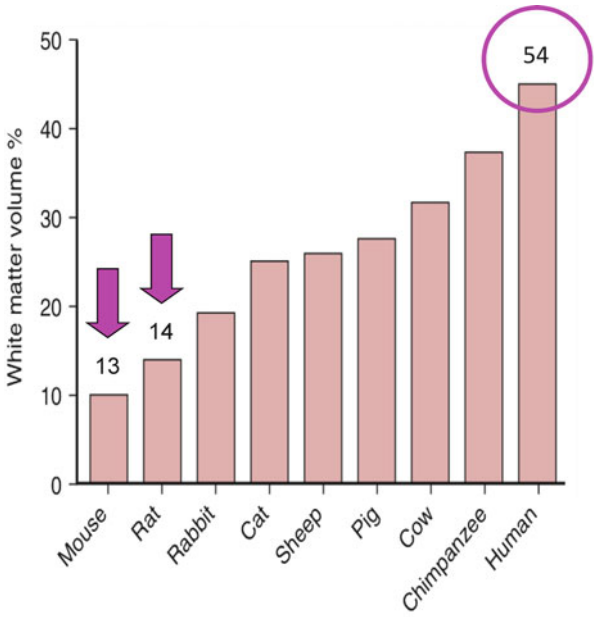


Fig. 16.1 Brain white matter volume expands as brain size enlarges. Histograms show the percentage of cerebral hemisphere volume composed of white matter in several mammals, ranging from mouse to human. (Figure is from Chap. 8 Molecular Pathophysiology of White Matter Anoxic Ischemic Injury by Ransom B, Goldberg MP and Baltan S; Stroke Edited by Mohr, Wolf, Grotta, Moskowitz, Mayberg, von Kummer, Elsevier 2011. Data calculated from Zhang K, Sejnowski TJ: A universal scaling law between gray matter and white matter of cerebral cortex. Proc Natl Sci U S A 97:5621–5626, 2000)

experimental stroke models (Del Zoppo 1995, 1998; Dirnagl et al. 1999; O'Collins et al. 2006). We suggest that failure to protect white matter is one of the principal reasons contributing to the lack of successful stroke therapy.

The risk of ischemic stroke increases drastically with age. A number of pathological changes occur in aging that preferentially influence brain structures and are candidates for causing age-associated neurological impairment, or increased susceptibility to impairment. The majority of studies of aging brain to date have focused on stereological estimates of total neuron numbers in gray matter. Interestingly, aging has global effects on white matter with relatively little effect on gray matter (Vincent et al. 1989; Peters and Sethares 1993). White matter appears to undergo generalized shrinkage in volume relative to gray matter (Albert 1993), due to the reduction in myelin content (Kemper 1994) and number of axons with age (Yamauchi et al. 2002). Myelin provides important insulating properties to axons allowing for propagation of action potentials over large distances at high velocity. Disruption of the myelin sheath could, therefore, contribute to cognitive impairment, as observed during aging (Peters and Sethares 1993; O'Sullivan et al. 2001; Ferro and Madureira 2002). Imaging studies in elderly people demonstrate

hypodense lesions in white matter, termed leukoaraiosis (Hachinski et al. 1987). The severity of leukoaraiosis may predict high risk for stroke (Yamauchi et al. 2002) and impaired cognitive function (Schmidt et al. 1991). Other imaging techniques (Basser et al. 1994; Pendlebury et al. 1999) show regional variability in white matter signal characteristics, reflecting age-related degenerative effects on myelination and white matter connectivity (Davatzikos and Resnick 2002). However, the cellular basis of these changes cannot be determined from clinical imaging protocols.

White matter is a target of hypoxic-ischemic injury throughout life, in clinical settings ranging from periventricular leukomalacia in neonates, stroke, and cardiac arrest in adults, to dementia in the aging brain (Goldberg and Ransom 2003). Although there is increasing evidence that the degenerative effects of aging on white matter contribute to cognitive impairment, dementia, and increased risk for stroke (Bonita et al. 1994; Kurtzke 1994), structural and functional changes in aging white matter axons and glial cells have been explored only recently (Baltan et al. 2008; Baltan 2009, 2012). With an increase in the elderly population around the world, there is an urgency to view successful brain aging as the goal of scientists in the broad field of neurobiology. Identification of risk factors, awareness of the relative importance of each factor, and knowledge of their interaction should facilitate maintenance of cognitive, emotive, motor, and sensory functions. The focus of this chapter is to summarize the current information on molecular and functional changes in axons and glial cells as a function of age and how these adaptive mechanisms transform the tissue into a state of increased vulnerability in the face of an ischemic attack.

16.2 Intact Optic Nerve and Corpus Callosum Slices as Models to Study White Matter

The optic nerve, a purely myelinated central nervous system (CNS) white matter tract, is sensitive to the aging process (Cavallotti et al. 2002, 2003) and offers several advantages to study the mechanisms of white matter injury: (a) tissue isolation does not require extensive surgical interventions so there is minimal preparation injury, (b) the isolated optic nerve is structurally and functionally stable in vitro for at least 18 h, (c) there are no neurons, therefore no synapses or synaptic machinery to indirectly contribute to white matter injury, (d) axon function can be monitored by recording evoked compound action potentials (CAPs) (Fig. 16.2a), (e) the cellular components of optic nerve can be identified using immunohistochemistry, isoform-specific antibodies and confocal imaging (Fig. 16.2b), (f) amino acid release such as glutamate can be quantitatively monitored (Fig. 16.13), (g) protein levels of interest can be quantified by western blots and (h) intravitreal injections enable a route of delivery to axons.

Rat optic nerve (RON) has been widely used to study white matter injury to determine the effects of anoxia (Stys et al. 1990, 1992), aglycemia (Brown et al. 2001) and ischemia (Garthwaite et al. 1999). Mouse optic nerve (MON), however, has important

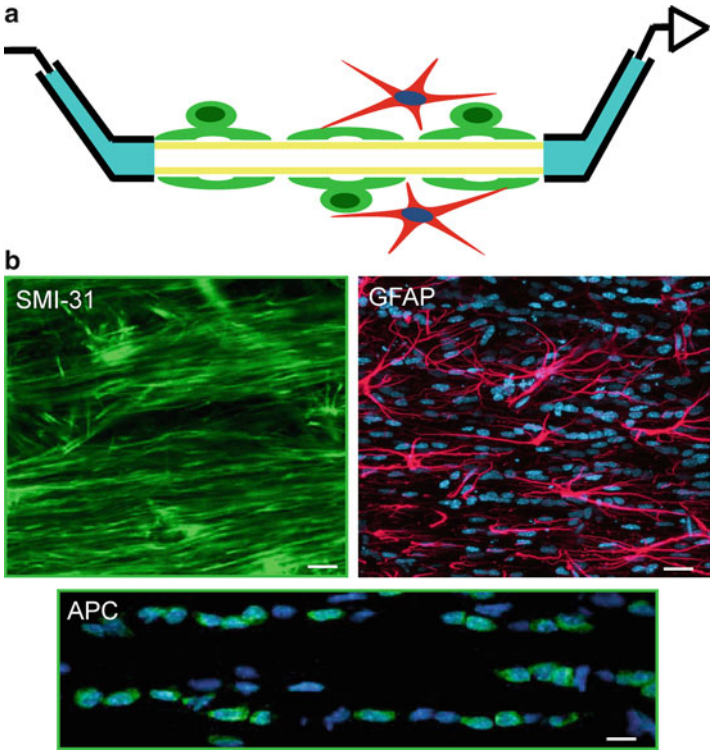


Fig. 16.2 Monitoring white matter function and architecture. (**a**) Use of suction electrodes allows all axons to be stimulated and a compound action potential (CAP) to be recorded. (**b**) Using cell-specific antibodies, white matter axons labeled with SMI-31 for neurofilament (*green*), GFAP for astrocytes (*magenta*) and APC for mature oligodendrocyte cell bodies (*green*). Sytox (+) glial nuclei are in *blue*. Scale bar = 50 μm for SMI-31 and, GFAP, 10 μm for APC. (Reproduced in part from Baltan 2009)

advantages over RON as an in vitro model for studying white matter injury. The diameter of the adult RON is about twice that of the adult MON and metabolism is limited by inadequate glucose diffusion into the RON, especially when glucose utilization is increased as during anoxia (Baltan Tekkök et al. 2002; Tekkok et al. 2003). Taking into account this technical point, and considering the promise of future transgenic mice to analyze specific injury pathways, we use MONs for our experiments.

On the other hand, corpus callosum (CC) slices offer important advantages for the investigation of injury in white matter with neighboring gray matter. Slices preserve the anatomical and structural integrity of the continuity of the axon with the cell body, allowing assessment of axons with or without myelin in the presence of fully differentiated oligodendrocytes. Use of these two in vitro white matter tracts allow exceptional combined function–structure analysis, where glial cells and axons have retained their native relationships to one another within a three-dimensional organization (Fig. 16.2).

An in vivo model that selectively causes ischemic injury to white matter tracts is necessary to test some important parameters of stroke. Several different approaches have been taken to address this issue. For instance, severe applications of the MCAO model cause injury in subcortical white matter in rodents (Pantoni et al. 1996). However, subcortical white matter axons are extensions of cortical neuronal cell bodies (mainly layers 3, 5 and 6). Therefore, subcortical white matter injury in stroke is not a selective white matter injury but is secondary to the injury of cortical neuronal cell bodies. Consequently, neither the injury mechanisms nor the protective approaches can be specified to white matter structures. The modified Rice Vanucci model (carotid artery ligation + recovery under hypoxic conditions) is used to mimic periventricular leukomalacia and/or prenatal hypoxia, and selectively injures immature oligodendrocytes and arrests myelin production in postnatal day 7 rodent brain without any axon damage or motor deficits (Follett et al. 2000; Jensen 2006). Current approaches to corpus callosum injury involve local injections of glutamate analogues (McDonald et al. 1998; Leroux et al. 2010), demyelinating substances (Gadea and Lopez-Colome 2001), or vasoconstrictive substances (Sozmen et al. 2009) to cause glial and/or axon injury. These models do not mimic ischemia and also cause comparable tissue injury due to trauma and volume effects. On the other hand, our earlier efforts to induce ischemic injury in corpus callosum or optic nerve using piglets resulted in inconsistent findings, despite initial promising results (Lee et al. 2006). Primate brains contain 35 % white matter by volume and MCA perfusion territory is closer to human brain, and therefore may be the most appropriate model (Frykholm et al. 2000; Enblad et al. 2001).

16.3 Architectural Organization of White Matter

Structure and function in white matter are integrated: one is a reflection of the other (Fig. 16.2). The challenge, therefore, is to decipher the meaning of molecular architecture of white matter components. Discovery of the structural design of white matter is an evolving process and has so far proved to be more complex than anticipated. Axons and their myelinating oligodendrocytes, together with nurturing astrocytes (Fig. 16.2b, c), defensive microglia (Fig. 16.4) and progenitor cells forming synapses onto axons form an intricate interactive environment. The morphological variability in these white matter components among different white matter tracts further attest to their sophisticated nature to adapt to their regional function. In particular, glutamate homeostasis of adult white matter and intricate distribution of glutamate receptors and glutamate transporters expressed by astrocytes, oligodendrocytes, axons and microglia in a region-specific manner further illustrate the sophistication of white matter architecture (Figs. 16.7 and 16.8). White matter is particularly vulnerable to excitotoxicity during ischemia (Fig. 16.11) and a detailed knowledge of structural elements is key to prevent or restore function after ischemic injury.

In optic nerve from rat, mouse, or rabbit oligodendrocytes express AMPA receptors that are composed of GluR1, GluR3, and GluR4, but GluR3 and GluR4 in spinal cord (rat) and corpus callosum (mouse). On the other hand, oligodendrocytes from optic nerve are rich in GluR6, GluR7, KA1, and KA2 subunits of kainate receptors while in spinal cord GluR5, GluR6, KA1 form the subunit composition of kainate receptors. Interestingly corpus callosum oligodendrocytes do not express kainate receptors (Baltan and Goldberg, unpublished data). The AMPA and kainate receptors are mainly located on oligodendrocyte cell bodies and are believed to be involved in axon–myelin signaling.

Oligodendrocytes express NMDA receptors that consist of NR1, NR2A-NR2C, and NR3A subunits. Remarkably, NMDAR are expressed in clusters on oligodendrocyte processes, whereas AMPA and kainate receptors are diffusely located on oligodendrocyte soma. In addition, immunogold electron microscopy revealed that NR1, NR2, and NR3 subunits are present in the myelin sheath. Functional aspects of NMDA receptors on oligodendrocytes were discovered by application of several approaches. NMDA-mediated currents were recorded in mature oligodendrocytes from corpus callosum and cerebellar white matter (Karadottir et al. 2005), influx of Ca was identified in myelin formed by oligodendrocytes in the RON (Micu et al. 2006) and the protein levels of NMDA receptor subunits NR1, NR2A-D, and NR3A were detected in different type of oligodendrocytes (Karadottir et al. 2005; Salter and Fern 2005; Micu et al. 2006).

Glutamate is found in ample amount in white matter (Fig. 16.7) and is released during oxygen and glucose deprivation (OGD) (Fig. 16.13), causing overactivation of AMPA/kainate receptors (Tekkok et al. 2007). Potential sources of glutamate include axons (Li et al. 1999), oligodendrocytes (Fern and Moller 2000), and astrocytes via the reversal of Na^+-dependent glutamate transporters (Li et al. 1999; Tekkok et al. 2007), Ca^{2+} increases (Parpura et al. 1994), or via release from swelling-activated anion channels and hemichannels (Ye et al. 2003). Vesicular glutamate release by vesicular glutamate transporter 1 (VGLUT1), 2 (VGLUT2) and 3 (VGLUT3) is Ca^{2+}-dependent and modulated by stimulation frequency in myelinated (Kukley et al. 2007) and in unmyelinated axons (Ziskin et al. 2007) of the corpus callosum. However Na^+-dependent glutamate transport inhibitors greatly diminish glutamate release in MONs during ischemia (Tekkok et al. 2007). Astrocytes express the greatest density of Na^+-dependent glutamate transporters in white matter; glutamate transporter-1 (GLT-1) and glutamate aspartate transporter (GLAST) (Figs. 16.7 and 16.8). Energy deprivation causes a gradual dissipation of the transmembrane Na^+ gradient in astrocytes setting up conditions for reverse exchange and glutamate release (Longuemare et al. 1999).

16.4 White Matter Components Reorganize with Aging

Across the age groups, there is no change in the proportion of total APC (+) oligodendrocytes, GFAP (+) astrocytes, NG2 (+) precursor cells (Fig. 16.3). Interestingly, microglial processes and the thickness and the length of these

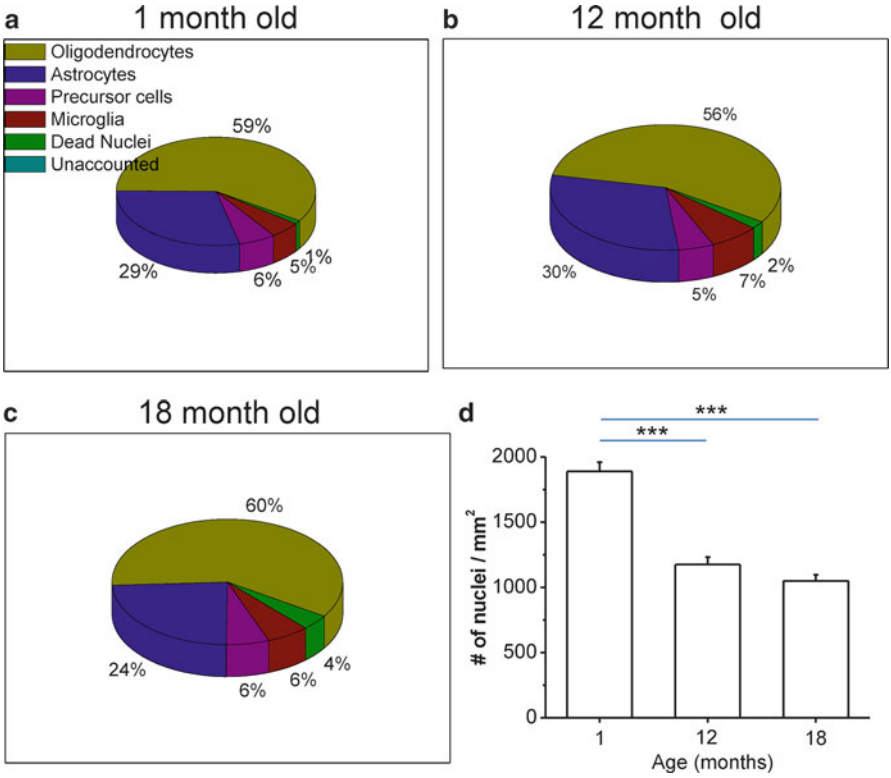


Fig. 16.3 Aging results in loss of cellularity but relative percentage of glial cells is preserved. (**a**–**c**) Pie charts summarize percentile of glial cells (percent of total nuclei) in white matter from 1-, 12-, and 18-month-old optic nerve. (**d**) Histograms demonstrate ~35 % fewer glial cells by 12 months of age. ***$p < 0.001$, one-way ANOVA

processes (ramification) increase three to fivefold in older white matter (Fig. 16.4), in agreement with age-related activation of white matter microglia in monkey (Sloane et al. 1999) and rats (Ogura et al. 1994; Morgan et al. 2004). Additionally, each microglial cell with its processes appears to occupy a distinct domain within the tissue, reminiscent of astrocytic domains (Oberheim et al. 2008) in young white matter. This territorial organization is lost with age as numerous processes reached out towards neighboring domains. Microglial activation is a pathological hallmark of stroke. Curiously, aging seems to unmask this capacity of microglia without any further insult. It is unclear whether the effect of activated microglia on white matter is beneficial, detrimental, or a combination of both.

Astrocytes exhibit thicker processes that change their orientation from transverse to a more longitudinal orientation with aging (Baltan et al. 2008, also see Fig. 16.8). However, the GFAP protein levels or the number of astrocytes do not change in older white matter (Baltan et al. 2008). Because astrocyte processes form the end-feet on the vascular wall and contribute to the neurovascular unit, the change in their direction raises the possibility that they may be adapting to vascular changes with aging.

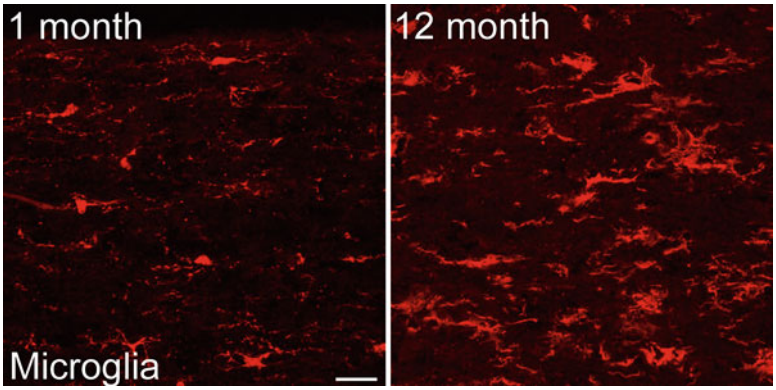


Fig. 16.4 Aging causes microglial activation. A few Iba (+) microglial cells (*red*) with their small cell bodies and few thin processes can be detected in 1-month-old MONs (*left*). Aging causes elaborate changes in their morphology including cell body size, and process ramification (*right*). Calibration bar = 20 μm

In addition, astrocyte processes elongate to the nodes of Ranvier and to sense the metabolic demands of axon function. Age-dependent reorganization of axonal architecture (nodal, paranodal, myelin content-see Figs. 16.5 and 16.6) may need expanded points of surveillance by astrocytes to maintain the metabolic burdens of aging axons.

Myelin provides a unique architecture to allow high-fidelity conduction along axons. Labeling studies for different myelin proteins reveal a significant loss of myelin basic protein (MBP) and myelin associated glycoprotein (MAG) as a function of age. On the other hand, and consistent with preserved oligodendrocyte numbers, CNPase levels show a small drop (Fig. 16.5). Oligodendrocytes and possibly myelin express AMPA/kainate and NMDA receptors (Micu et al. 2006; Baltan et al. 2008) which mediate excitotoxic injury during ischemia. Age-dependent changes in glutamate receptor type and subunit composition is of utmost interest, for they dictate the ensuing injury.

Clustering of Nav1.6, the predominant sodium channel at the nodes of Ranvier (Goldin et al. 2000) is an indicator of proper axo–glial contact dictated by myelin (Boiko et al. 2001) to support saltatory conduction. The contactin-associated protein (CASPR) rich paranodes are flanked by juxtaparanodal Kv1.1 or Kv1.2 potassium channels. Immunohistochemical analysis for CASPR and Nav1.6 indicate that CASPR (Fig. 16.6, green) is longer and thinner, while Nav1.6 clusters (Fig. 16.6, red) become shorter with aging. Although Nav1.6 is also found in unmyelinated axons (Black et al. 1999), it is not detectable in the internodal region of myelinated axons. However, aging causes ectopic regions of Nav1.6 immunoreactivity (Fig. 16.6, white arrows) which might lead to deleterious effects on axons especially if colocalized with the Na^+–Ca^{2+} exchanger (Craner et al. 2004). A majority of axon profiles with diffuse Na^+ channel immunoreactivity is associated with reduced MBP

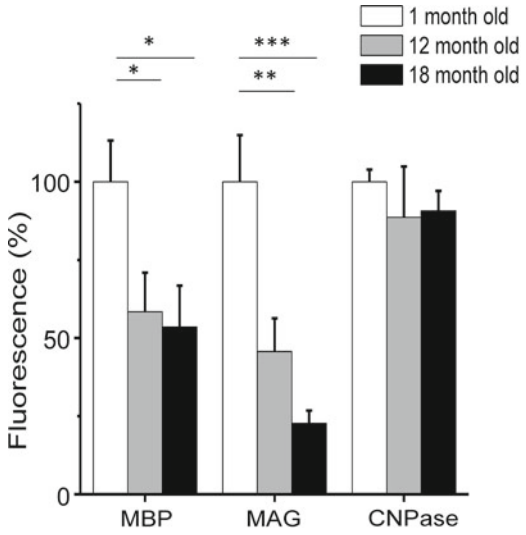


Fig. 16.5 MBP and MAG are down regulated with aging. Quantification of labeling intensity reveals that MBP and MAG decrease with age compared to 1 month old mice. *$p<0.05$, **$p<0.01$, ***$p<0.001$, one-way ANOVA

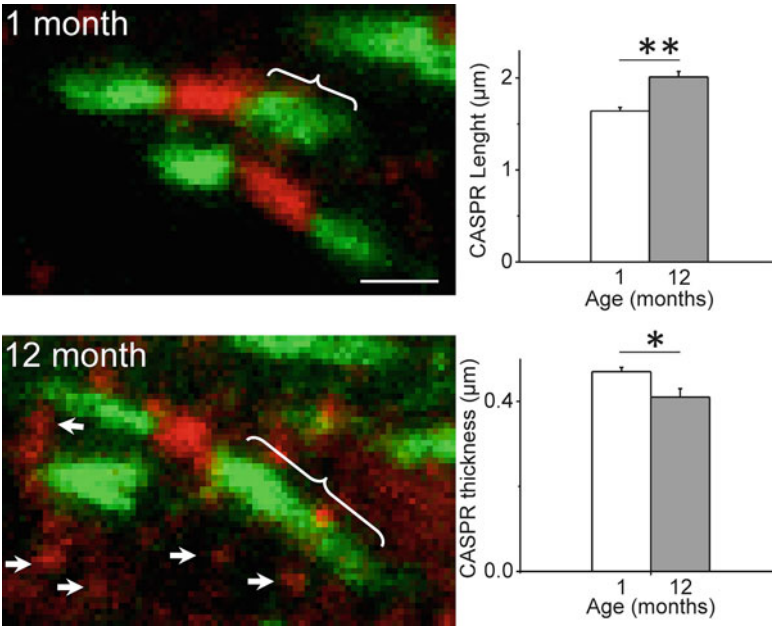


Fig. 16.6 Nodal and paranodal structures reorganize with aging. Immunolabeling of Nav1.6 sodium channels at the nodes of Ranvier show that sodium channel clusters (*red*) become smaller and assume an extranodal localization (*line with arrows*) in aging MONs. Paranodal CASPR (*green*) elongates (*brackets*) and becomes thinner, as summarized in the histograms. *$p=0.0317$, **$p=0.0019$, two-tailed Student's *t*-test. Calibration bar = 2 µM

immunolabeling in spinal cord experimental allergic encephalomyelitis (Craner et al. 2004) which paralleled structural changes in aging axons in optic nerve. In addition, Kv1.2 (+) potassium channels overlap with CASPR immunostaining indicating loss of the characteristic demarcation between paranodal and juxtaparanodal structures (Dupree et al. 1999; Bhat et al. 2001). Displacement or aberrant localization of nodal, paranodal, juxtaparanodal structures suggest that axonal function at the node may be compromised with age. Similar findings in the aging monkey and RON (e.g., Hinman et al. 2006) implicate age-related molecular reorganization at the nodes of Ranvier as intrinsic to white matter which crosses over species.

White matter glutamate homeostasis and the related machinery also go through a series of age-related restructuring (Figs. 16.7 and 16.8). The dominant glutamate transporter, GLT1, plays an essential role in removing glutamate from the extracellular space and maintaining glutamate below neurotoxic levels under normoxic conditions (Rothstein et al. 1996; Hazell et al. 2001). Although these transporters are predominantly expressed on astrocytes in young white matter, they extend to additional structures with aging (Fig. 16.8), implying that additional white matter constituents may contribute to toxic glutamate accumulation in aging white matter. In addition to GLT-1, the essential members to maintain glutamate homeostasis are GLAST, glutamate and glutamate synthatase (GS). White matter glutamate content increases considerably and in correlation with increased GS levels with age (Fig. 16.8). Together with a twofold increase in GLT-1 levels in older white matter (Baltan et al. 2008), these adjustments may infer an age-related adaptive mechanism in white matter to remove and to convert excessive glutamate to glutamine so as to maintain glutamate homeostasis. As a result glutamate levels under control conditions (Fig. 16.13, Baltan et al. 2008) and axon conduction across aging axons remains impressively stable over time under normoxic conditions. The number of GLT-1 transporters determines the capacity of the tissue to move glutamate between internal and external compartments (Fig. 16.13). However, it is the direction of the pump that acts to save or injure the tissue. During ischemia, these measures act against the tissue due to an accelerated Na^+ overload as a result of decreased tolerance to energy deprivation in aging white matter. Therefore, numerous GLT-1 transporters reverse and lead to early robust release of glutamate causing enhanced excitotoxicity (Fig. 16.13). Moreover, in young white matter glutamate resumes to baseline levels after the end of OGD, which is a sign of efficient astrocyte uptake of glutamate. However, glutamate levels remain elevated in old white matter, suggesting that aging astrocytes cannot take up excess glutamate and thereby expanding the excitotoxicity duration into the recovery period (Fig. 16.13). Despite the possibility that glutamate may be released from multiple sources in aging white matter, astrocytes are expected to remove and store glutamate efficiently. Therefore, these results suggest a prominent change in aging astrocyte capacity to remove glutamate.

On the other hand, GLAST expression in white matter falls with age, raising the possibility that, glutamate transporters can functionally substitute for one another with age (Fig. 16.7). It is well-known that the upregulation of GLT-1 participates in the induction of brain ischemic tolerance in gray matter (Romera et al. 2004; Kawahara et al. 2005; Zhang et al. 2007) and in reactive astrocytes in human periventricular leukomalacia (Desilva et al. 2008). Aging seems to saturate these

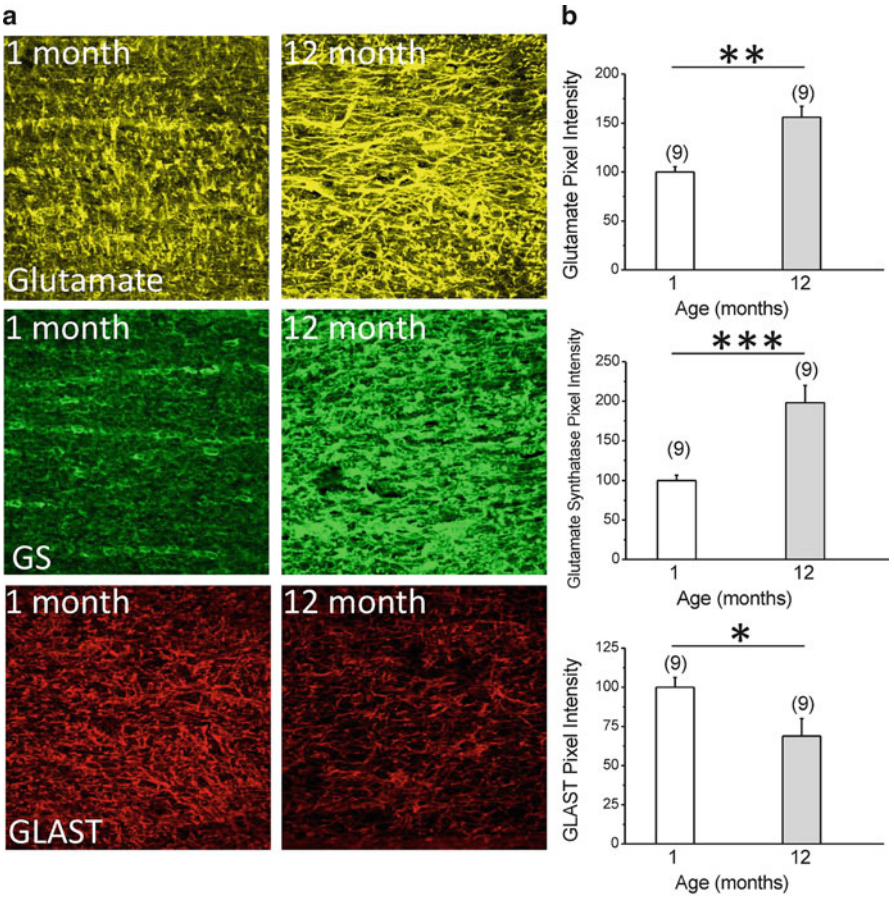


Fig. 16.7 Glutamate and glutamate synthatase (GS) expression increase in 12-month-old MONs. (**a**) Immunolabeling and (**b**) quantification of glutamate, GS and GLAST immunolabeling revealed that glutamate and GS labeling intensity increases by 156 ± 11.1 % and 198 ± 21.9 %, respectively, in 12 month old MONs. Note that GLAST labeling intensity decreased to 68.9 ± 11.2 % in 12 month old MONs. *$p = 0.0278$, **$p = 0.004$, ***$p = 0.0006$, two-tailed Student's *t*-test

mechanisms (Fig. 16.8), and it is intriguing whether preconditioning is impaired in white matter with aging. Unfortunately, these questions will remain unanswered until an in vivo model of adult white matter stroke is available.

16.5 Age-Dependent Mechanisms of Ischemic White Matter Injury

White matter axons are dependent on a constant supply of oxygen and glucose to faithfully transmit signals. We reported that CNS white matter function is exceptionally tolerant to a complete lack of oxygen (Tekkok et al. 2003) while there is

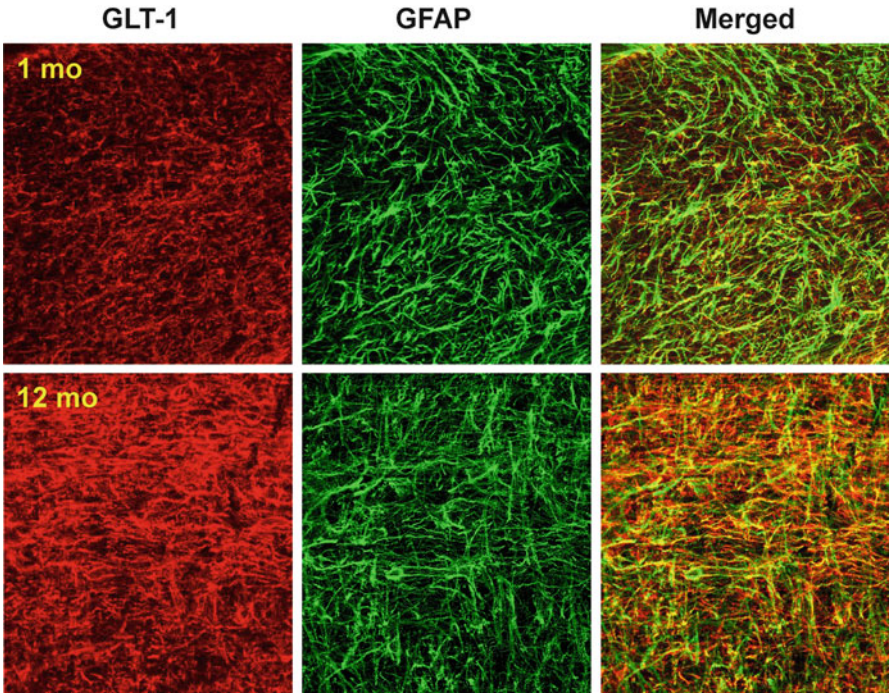


Fig. 16.8 Aging is correlated with upregulation of GLT-1. The overlap of GLT1 (*red*) and GFAP (*green*) labeling indicated that GLT-1 was mainly expressed in astrocytes (*merged*) in 1-month-old MONs. MONs. There was a twofold increase in GLT-1 pixel intensity (188.6 ± 18.5 %) with age. The pattern of GFAP expression in MONs changed with age but without an increase in GFAP pixel intensity. (Reproduced from Baltan et al. 2008)

regional variability in the ability to function and survive anoxia (Baltan 2006). However, young adult white matter is readily susceptible to ischemia induced by combined OGD (Fig. 16.9). Mechanisms underlying ischemic white matter injury prove to be unpredictably complex (Fig. 16.10) (Wrathall et al. 1994; Agrawal and Fehlings 1997; Fern and Ransom 1997; McDonald et al. 1998; Sanchez-Gomez and Matute 1999; Follett et al. 2000; Tekkök and Goldberg 2001; Stys 2004; Tekkok et al. 2007). White matter contains no neuronal soma but instead has myelinated axons, oligodendrocytes, and astrocytes. The cellular elements of white matter are individually under attack during ischemia, while they still remain interactive with each other in intricate mechanisms that are not well understood. Axons are injured (Fig. 16.10) directly by ionic mechanisms, resulting in accumulation of intracellular Na^+ and Ca^{2+} (Stys et al. 1990; Fern et al. 1995; Wolf et al. 2001; Ouardouz et al. 2003; Underhill and Goldberg 2007) while astrocytes, via the reversal of Na^+-dependent glutamate transporters (Li et al. 1999; Tekkok et al. 2007) initiate an excitotoxic path resulting in oligodendrocyte death and myelin damage (Fig. 16.11, Tekkök and Goldberg 2001; Tekkok et al. 2007). Glutamate accumulation concomitantly initiates the oxidative pathway attacking white matter constituents due to

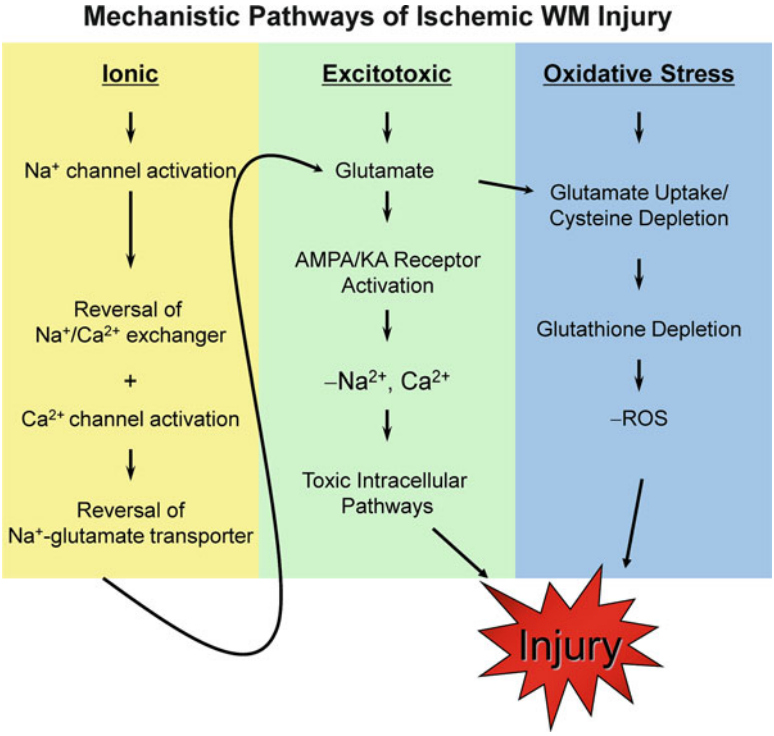


Fig. 16.9 Putative mechanisms of ischemic white matter injury. Ionic, excitotoxic, and oxidative stress converge in sequential order to cause irreversible injury in white matter during ischemia. Note that glutamate release, due to reverse Na^+-dependent transport dictates irreversible nature of the injury. (Reproduced from Baltan 2009)

formation of free radicals mediated by glutamate competing with cysteine at the glutamate-cysteine pump (Oka et al. 1993) and glutamate disrupting mitochondrial function (Chang and Reynolds 2006). Glutamate is necessary but not sufficient to explain ischemic injury in white matter. Consistent with this, exogenous application of glutamate or its agonists fails to initiate injury. Only a short and reversible OGD (15 min) combined with glutamate (or analogues) mimic ischemic injury (Tekkok et al. 2007). In accordance, glutamate levels remain very stable during the initial stage of OGD (25–30 min) and then steadily start to accumulate (Fig. 16.12). These results point to a sequential order of injury pathways converging in order for irreversible injury to ensue, such that an essential first stage of ionic dysfunction primes white matter for glutamate toxicity (Fig. 16.9; Tekkok et al. 2007). The sequential convergence of these pathways also manifests itself as duration-dependent injury (Tekkok et al. 2007; Baltan 2009). Predictably, removal of extracellular Ca^{2+}, blockade of AMPA/kainate receptors (Fig. 16.11), blockade of reverse glutamate transport (Tekkok et al. 2007), or prevention of reactive oxygen species (ROS) generation reduces ischemic white matter injury. On the other hand, although developing

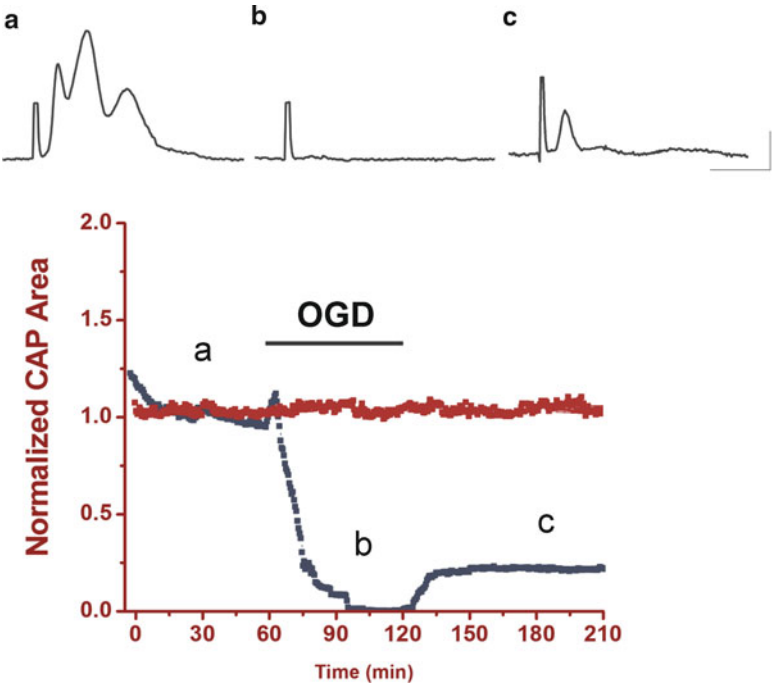


Fig. 16.10 White matter is susceptible to ischemic injury. Axon function is quantified as the area under the CAP, normalized to control, and plotted against time. Under normal conditions CAP area remains stable over time (*brown*). A 60 min period of oxygen glucose deprivation (OGD) depresses the CAP gradually until conduction along the axons is completely lost (*gray*) typically around 30 min. Restoring oxygen and glucose, axon function recovers to ~25 %. Sample traces from control (**a**), OGD (**b**) and recovery (**c**) periods are shown above the plot

oligodendrocyte processes (Salter and Fern 2005), mature oligodendrocyte cell bodies (Karadottir et al. 2005; Baltan et al. 2008), and myelin (Micu et al. 2006) express functional NMDARs, blockade of these receptors does not improve axon function after ischemia in young optic nerve (Tekkok et al. 2007) or corpus callosum (Tekkök and Goldberg 2001). These results do not negate the activation of NMDAR during ischemia but imply that their activation does not contribute to axonal damage and raises a caution for clinical implications of NMDAR antagonism during ischemia, particularly in aging white matter (see below). In fact, oligodendroglial NMDAR activation preserves axon function against ischemia by coupling axonal and glial energy metabolisms in developing and young optic nerves (Saab et al. 2012).

Although valid mechanisms, all of these studies were performed entirely on young animals. Because the risk for stroke increases with age, a thorough understanding of white matter ischemic injury in age-appropriate populations is of central importance to meet the challenge of developing effective stroke therapy. Indeed, CNS white matter becomes intrinsically more vulnerable to OGD in older animals and the mechanisms of white matter injury change as a function of age (Baltan et al. 2008). This increased sensitivity to OGD is due, in part, to increased susceptibility

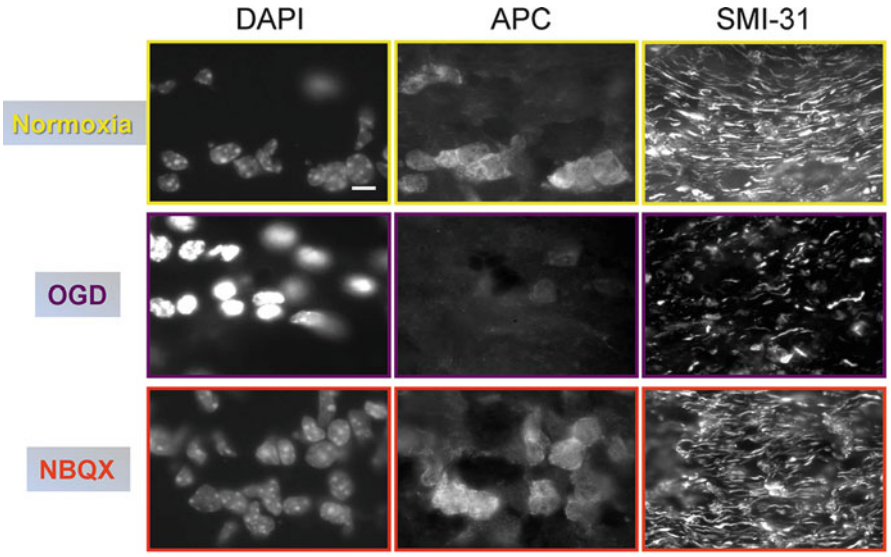


Fig. 16.11 Overactivation of AMPA/kainate receptors cause oligodendrocyte death and axon disruption. Under normoxic condition, DAPI (+) glial nuclei are mostly APC (+) oligodendrocytes among SMI-31 (+) labeled axons. A 30 min period of OGD in corpus callosum slices causes widespread loss of APC (+) oligodendrocytes and SMI-31 (+) axons with pyknotic bright DAPI (+) dead nuclei. Blockade of AMPA/kainate receptors with NBQX prevents oligodendrocyte death and axon disruption. Scale bar = 10 μm. (Reproduced from Tekkök and Goldberg 2001)

of aging axons to a lack of oxygen (Fig. 16.12). Mitochondrial dysfunction and disruption with age presumably underlie the loss of aerobic capacity of aging axons (Fig. 16.13, see mitochondria in insets). On the other hand, whether aging axons are less tolerant to removal of glucose is under investigation.

Accumulation of Ca^{2+} is an important step in the development of ischemic injury in young white matter, while Ca^{2+}, influx may be more vital to aging axons. The lack of protection by removal of extracellular Ca^{2+} or by blockade of Ca^{2+} entry secondary to reverse operation of the Na/Ca exchanger (NCX), denotes that preventing Ca^{2+} influx is not sufficient to preserve older axon function. Ironically, removal of extracellular Ca^{2+} worsens axon function recovery after OGD, a perplexing observation that implies Ca^{2+} entry during ischemia is a protective measure. It is possible that Ca^{2+} release from intracellular Ca^{2+} stores (ICS), and the interplay between intracellular and extracellular Ca^{2+}, is more critical during ischemia in aging axons. Although a role for Ca^{2+} release from endoplasmic reticulum via IP3 and ryanodine receptors is described in young white matter (Thorell et al. 2002), various Ca^{2+}-dependent neurophysiological (Landfield and Pitler 1984; Campbell et al. 1996) and signaling pathways (Verkhratsky et al. 1998) remain unexplored in white matter, particularly as these relate to ischemia and aging.

Because blockade of NMDARs cause a similar worsening of axon function recovery, it points to Ca^{2+} entry through NMDARs as an important element to protect aging axon function against ischemia. Alternatively the role of NMDAR as the axon–glia

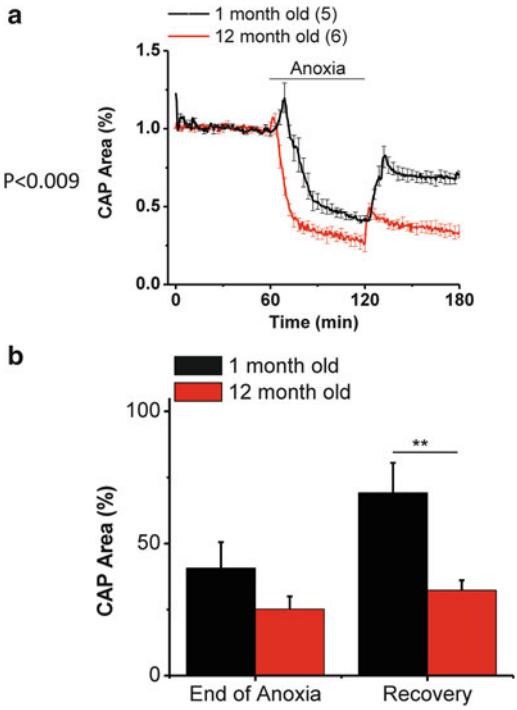


Fig. 16.12 Aging axons are more vulnerable to anoxia. Normalized data show that the CAP area rapidly falls and recovers less after 60 min of anoxia in 12-month-old (*red*) compared to 1-month-old (*black*) MONs. The CAP area and SEM are plotted every 3 min. $^{**}p<0.009$, one-way ANOVA

metabolic couplers becomes more critical to support the increased energy burden of aging axons. It is also plausible that removal of Ca^{2+} may lead to glutamate release via hemichannels from aging astrocytes thus exacerbating excitotoxic injury. These mechanisms remain to be explored.

The Ca^{2+}-independent nature of ischemic injury in aging white matter raises another important question as to how AMPA/kainate receptor activation mediates injury. Because blockade of AMPA/kainate receptors promotes axon function recovery across all age groups, it suggests that Na^+ entry, via AMPA/kainate receptors, rather than Ca^{2+}, mediates injury during ischemia in older white matter. Overload of Na^+ exhibits irreversible toxic swelling, even in the absence of extracellular Ca^{2+} (Rothman and Olney 1995) and a rise in intracellular Na^+ promotes reversal of the Na^+-dependent glutamate transporter, resulting in glutamate accumulation (Szatkowski et al. 1990). Together with the upregulation in GLT-1 expression, increases in intracellular Na^+ may be the leading cause of increased and early release of glutamate, overactivating AMPA/kainate receptors and creating a vicious cycle that underlies the vulnerability of aging white matter to ischemia (Fig. 16.13). Furthermore, an increase in Na^+ concentration interferes with maintenance of the transmembrane ion gradient. This challenges the Na–K ATPase pump, compromises the ability of aging axons to maintain membrane properties and axonal excitability and contributes to an increased vulnerability to ischemia (Fig. 16.17, Scavone et al. 2005).

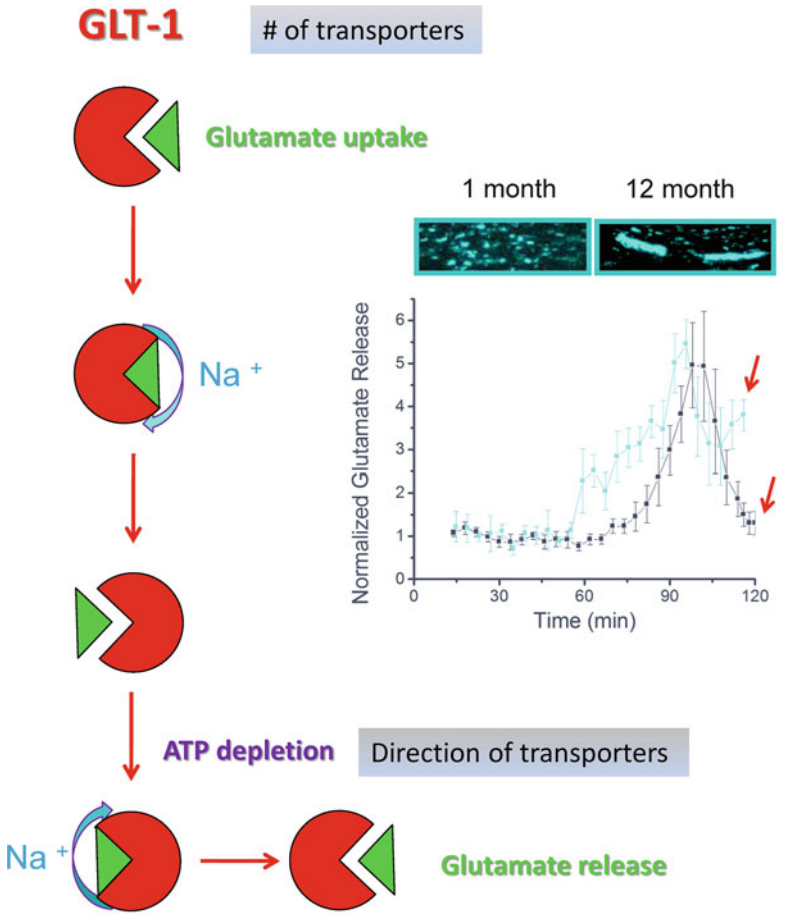


Fig. 16.13 Enhanced excitotoxicity due to impaired mitochondrial function leads to early and robust glutamate release in aging white matter. The principle Na^+-dependent glutamate transporter, GLT-1, typically takes up glutamate with co-transport of Na^+. During ATP depletion, due to increased intracellular Na^+ levels, the transporter reverses and releases glutamate. Therefore the number of transporters determines the capacity of the system for the amount of glutamate that can be transported, but it is the ATP levels that determine the direction of the transporter (to remove or release glutamate). Mitochondria in aging axons become longer and thicker compared to young axons, which may hinder ATP production and drive GLT-1 in reverse mode. Consistent with this, there is an early and robust glutamate release in aging white matter (*blue*) compared to optic nerves from young mice (*gray*). Note, that the glutamate levels return to baseline in young but remain elevated in aging white matter (*red arrows*). (Reproduced in part from Baltan et al. 2008)

The finding that blockade of NMDARs worsens outcome in older white matter (Baltan et al. 2008) has important clinical implications as it explains why NMDAR antagonists, that conferred protection to neuronal cell bodies in experimental stroke settings, failed to provide any benefit in clinical stroke trials. In this aspect, these results challenge the existing convention in stroke research that assumes a common

mechanism of injury in the brain across the life span. They also question the well-established agreement that glutamate receptor over-activation unequivocally causes injury. It is critical to consider that therapeutic options beneficial for white matter tracts may be ineffective for the gray matter and that therapeutic options proving successful in gray matter may be less useful or even harmful for white matter, especially when mechanisms of injury change as a function of age.

16.6 Mitochondrial Dysfunction Underlies Vulnerability of Aging Axons to Ischemia

The bioenergetics of mitochondria in neurons and their role in glutamate excitotoxicity are well described in gray matter (Nicholls et al. 2007). Mitochondrial dysfunction and excitotoxicity share common aspects and are believed to act synergistically by potentiating each other (Albin and Greenamyre 1992; Jacquard et al. 2006; Silva-Adaya et al. 2008). Mitochondria are dynamic organelles that travel along microtubules, using axonal transport to reach peripheral locations (Hollenbeck 2005; Hollenbeck and Saxton 2005) (Fig. 16.13). They constantly undergo fission and fusion events (Karbowski et al. 2004), and the relative rates of mitochondrial fusion and fission have been implicated in the regulation of their number, size and shape (Mozdy and Shaw 2003; Scott et al. 2003; Chen et al. 2007). The balanced delivery of mitochondria to cell body, dendrites and axons helps them serve multiple functions, including energy generation, regulation of Ca^{2+} homeostasis, cell death, synaptic transmission and plasticity (Chang and Reynolds 2006). Expectedly, an exciting link between a variety of neurological diseases, as well as aging, and defects in mitochondrial fusion and distribution is emerging (Karbowski and Youle 2003; Chen et al. 2007). Older neurons become more susceptible to glutamate excitotoxicity due to loss of mitochondrial membrane depolarization and increased ROS generation leading to reduced energy supply (Parihar and Brewer 2007). Mitochondria exhibit a cell-specific morphology; neuronal mitochondria are small and round as opposed to the longer tubular mitochondria in white matter axons (Fig. 16.14). Dynamin-related protein 1 (Drp-1) is known to affect the distribution of mitochondria (Otsuga et al. 1998; Smirnova et al. 1998), and Drp-1 protein levels show cell-specific variation, being expressed more in astrocytes compared to neurons (Uo et al. 2009). Mitochondrial function appears to decline in older animals, presumably causing reduced ATP production. This has been demonstrated in cardiac (Lesnefsky et al. 2001), liver (Selzner et al. 2007), and brain (Toescu 2005). Ion transport accounts for about 50 % of all ATP utilization and Na^+/K^+ ATPase activity alone is responsible for the majority of this consumption (Erecinska and Silver 1994). A loss of ATP reserve, diminishing the activity of this key enzyme with advanced age, is a plausible contributor to heightened white matter injury susceptibility (Scavone et al. 2005). Consistent with this axon function, when transiently challenged with OGD, *was* slower to restore normal ion gradients, permitting pathological processes related to ion derangement to operate for longer periods

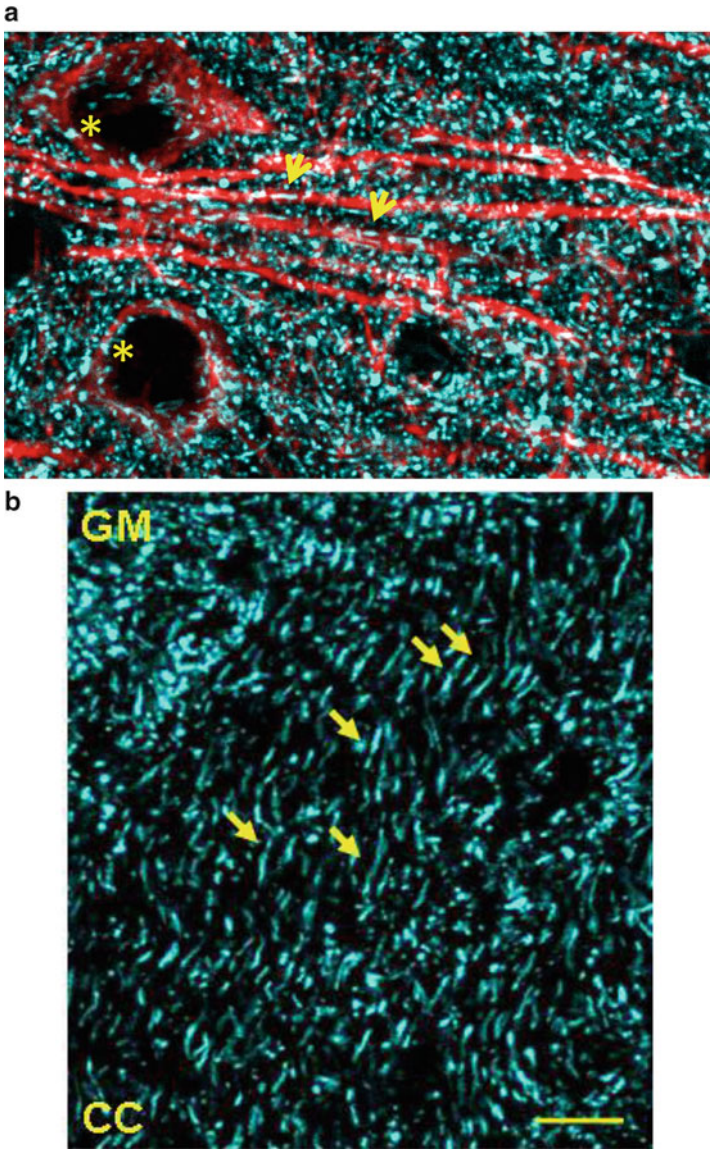


Fig. 16.14 CFP (+) somal and axonal mitochondria exhibit region-specific morphology. (**a**) Neuronal mitochondria are observed as CFP (+) structures in Thy-1 mito mice (Misgeld et al. 2007). CFP (+) mitochondria are small and round in neuronal cells bodies (*yellow asterisk*) labeled with MAP2 (*red*) but more linear and tubular in primary dendrites (*yellow arrows*). (**b**) Numerous long tubular mitochondria (*yellow arrows*) are evident in young corpus callosum. Note, the smaller round mitochondria in GM. Scale bar = 5 μm

(hence reversing Na^+-dependent glutamate transporter(s) earlier), and producing more injury in older MONs (Baltan et al. 2008). The disadvantage of compromised ATP levels in older animals was further verified by better recovery of white matter function in older animals when OGD was imposed at lower temperature (Baltan et al. 2008). Axons with high ATP requirements have many more mitochondria per unit length of process (Bristow et al. 2002); therefore, these axons would be preferentially targeted by low ATP conditions.

Excitotoxicity and elevated Ca^{2+} induce marked changes in mitochondrial morphology, arresting their motion (Rintoul et al. 2003; Barsoum et al. 2006; Chang and Reynolds 2006) and generating ROS in neurons (Nicholls et al. for review). In young white matter, activation of either AMPA or kainate receptors (Baltan et al. 2008) loads mitochondria with Ca^{2+} and fission is enhanced, associated with loss of fluorescence of mitochondria genetically tagged with CFP (Fig. 16.15a; Misgeld et al. 2007). A Ca^{2+} overload activates n-NOS to produce nitric oxide (NO) and ROS, which are proposed as diffusible second messengers linking oligodendrocyte excitotoxicity to axon injury (Matute et al. 2001; Ouardouz et al. 2006). Axon function directly correlates with tissue energy reserves, since Na^+–K^+ ATPase activity is intimately dependent on ATP levels. As a result, OGD causes a significant reduction in ATP levels and CFP (+) mitochondria, which could be prevented by AMPA/kainate receptor blockade (Fig. 16.14).

Aging stimulates mitochondrial fusion (Fig. 16.13) and this may be accompanied by a reduction in Drp-1 levels. The regulated process of mitochondrial fusion and fission controls the spatiotemporal properties of mitochondrial Ca^{2+} responses and the physiological and pathophysiological consequences of Ca^{2+} signals (Szabadkai and Rizzuto 2004; Szabadkai et al. 2004). By enhancing fusion or inhibiting fission, elongated mitochondria possibly absorbs Ca^{2+} efficiently preventing n-NOS activation and subsequent ROS production (Cheung et al. 2007, Fig. 16.17). However, this age-related adaptive reorganization of mitochondria becomes detrimental under ischemic conditions. Ischemia, in aging white matter, further enforces mitochondrial fusion as a result of the age-dependent drop in Drp-1 and age-dependent loss of mitochondrial motility with exposure to glutamate (Chang and Reynolds; Parihar and Brewer). Mitochondria fuse to collectively counteract the already increased excitotoxicity and Ca^{2+} load with aging, and this age-related change in mitochondrial dynamics could hinder ATP production. Because basal ROS generation is already elevated with aging, further increases in ROS accumulation under ischemic conditions result in increased vulnerability to ischemia (Fig. 16.17).

These results were further verified in a series of experiments investigating the protective effects of Class I HDAC inhibitors in young white matter (Baltan et al. 2011a, b). These HDAC inhibitors promoted functional recovery of axons and preserved white matter cellular architecture. This protection correlated with the upregulation of an astrocyte glutamate transporter, delayed and reduced glutamate accumulation during OGD, preservation of axonal mitochondria and oligodendrocytes, and maintained ATP levels in young optic nerves (Fig. 16.16) verifying the proof of principle that excitotoxic injury leads to mitochondrial dysfunction in white matter axons.

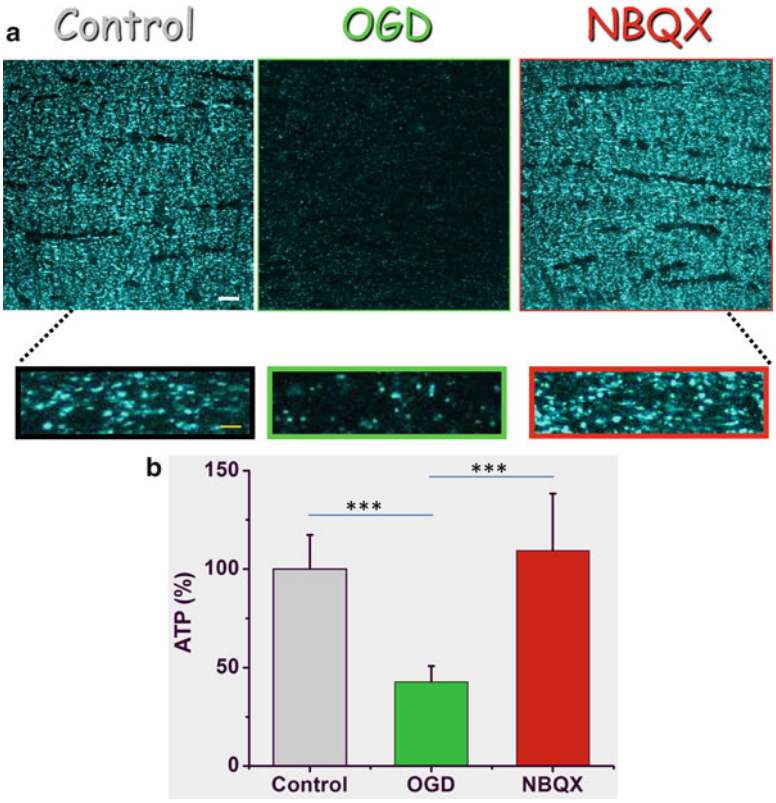


Fig. 16.15 Blockade of excitotoxicity preserves CFP (+) axonal mitochondria and ATP levels in response to OGD. (**a**) OGD drastically reduced CFP fluorescence in MONs from mito CFP (+) mice and pretreatment with NBQX (30 μM) protected against this loss. Note the change in mitochondrial morphology from small and tubular to tiny and punctuated form with OGD. Scale bar = 10 μm (insets = 2 μM) (**b**) Consistent with the preservation of CFP pixel intensity, NBQX pretreatment conserved ATP levels in MONs. ***$p < 0.0001$, one-way ANOVA. (Reproduced in part from Baltan et al. 2011b)

16.7 Region-Specific Mechanisms of Ischemic WM Injury

There is a growing sense that the mechanisms of white matter injury vary from one area of the brain to another (Tekkok et al. 2007). The explanations for regional differences in white matter injury are not yet understood at a cellular level. Regional differences in white matter oligodendrocytes and/or axons are logical possibilities but differences in astrocytes, cells rich in glutamate, should also be considered. Moreover, there may be regional differences in the glutamate receptors that

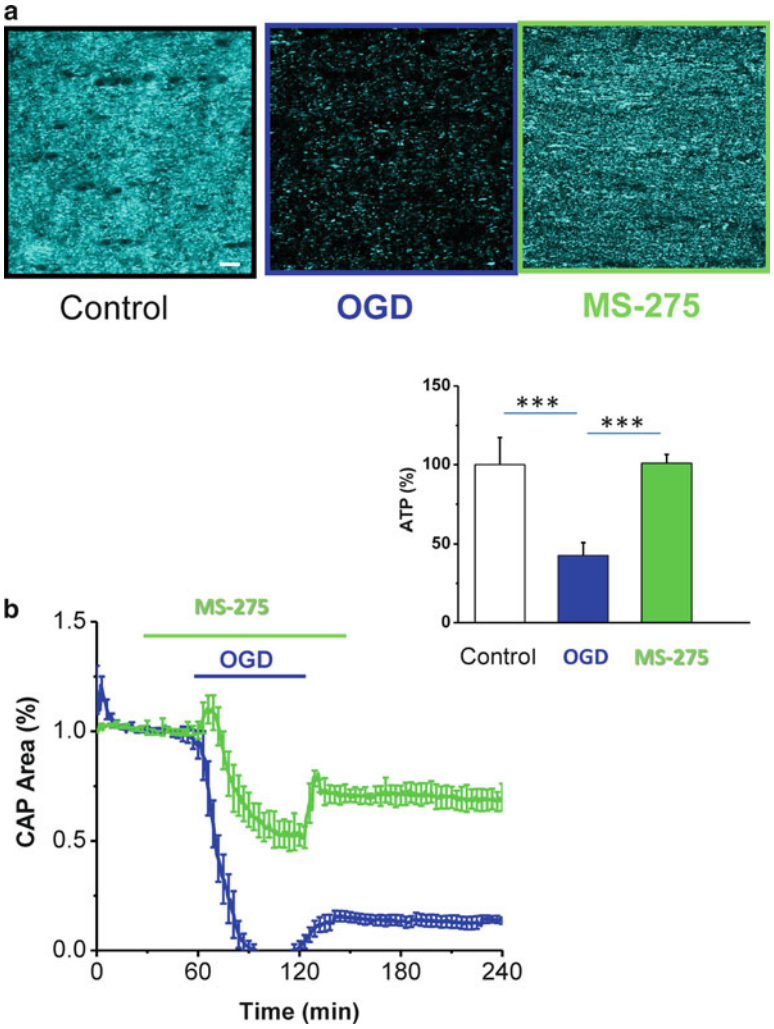


Fig. 16.16 The HDAC inhibitor MS-275 preserves CFP (+) axonal mitochondria and ATP levels in response to OGD. (**a**) OGD (*blue*) drastically reduced CFP fluorescence in MONs from mitoCFP (+) mice and pretreatment with MS-275 (1 μM) protected against this loss. (**b**) Consistent with the preservation of CFP pixel intensity, MS-275 pretreatment promoted axon function recovery (*green*) and conserved ATP levels in MONs. ***$p < 0.0001$, one-way ANOVA. Scale bar = 10 μm. (Reproduced in part from Baltan et al. 2011b)

participate in the injury process (Gallo and Russell 1995; Brand-Schieber and Werner 2003a, b; Tekkok et al. 2007). These differences in glutamate receptor pharmacology of white matter injury may reflect regional differences in the receptors themselves (e.g., degree of expression or subunit composition), regional variability in the amount or onset of glutamate release during ischemia, or the existence of regionally diverse oligodendrocytes. The latter point is particularly noteworthy,

given that the ratio of myelinating to non-myelinating oligodendrocytes vary between areas where all axons are myelinated, like the optic nerve (Foster et al. 1982), compared with areas containing many non-myelinated axons such as the corpus callosum (Olivares et al. 2001). These molecular properties indeed determine the mechanism of injury and the set of receptors that mediate that injury. For instance, activation of either AMPA or kainate receptors in MONs is sufficient to cause injury (Tekkok et al. 2007) while activation of Ca^{2+}-permeable AMPA receptors exclusively mediate the ischemic injury in corpus callosum slices (Tekkök and Goldberg 2001). Moreover, axon function is significantly more resilient to ischemia in optic nerve compared to corpus callosum while corpus callosum axons are more tolerant to anoxia compared to optic nerve. These findings point to a curious divergence between anoxic and ischemic injury mechanisms in white matter. Ischemia causes progressive injury as a function of glutamate accumulation. Anoxia does not cause glutamate release, therefore no region-specific glutamate receptors are involved in the injury process, implying a rather stagnant course for anoxic injury. It is conceivable that axons use energy from glycolysis to prevent Na+/K + pump failure during anoxia to suppress subsequent membrane depolarization and limit the rise in intracellular Ca^{2+} levels. Since more of the smaller diameter unmyelinated axons of corpus callosum survive anoxia, myelin may be the structural element underlying the vulnerability to anoxia, inferring that unmyelinated axons and/or the smallest axons with the thinnest myelin sheath are the resistant group (Baltan Tekkök and Ransom 2004). These results reveal that ischemia and anoxia are not interchangeable forms of injury in white matter and that CNS white matter is remarkably tolerant of anoxia although there is regional variability in their ability to function or survive (Baltan 2006).

16.8 Conclusions

The main goal of this review is to establish the proof of principle that CNS white matter becomes inherently more susceptible to an ischemic attack with age and that the molecular and cellular mechanisms of ischemic injury change as a function of age (Fig. 16.17). Predictably, age-related changes in the molecular architecture of white matter dictate the predominant injury mechanisms and determine the functional outcome. Consequently, protective interventions in young white matter such as removal of extracellular Ca^{2+} (Fig. 16.17a, black arrow), reduce functional recovery in aging axons (Fig. 16.17a, black dotted arrows). Together with the observation that blockade of reverse NCX fails to protect function in older mice (Fig. 16.17b, Baltan et al. 2008), these results propose a diminished role for the ionic pathway with aging. On the other hand, aging causes a prominent increase in the expression pattern of glutamate, GS and GLT-1 levels that extend to additional structures in white matter. These modifications may imply an age-related adaptive mechanism to maintain glutamate signaling and homeostasis. However, during an ischemic episode these adaptive changes act against the tissue and expedite and aggravate glutamate release (Fig. 16.17a, red dotted arrow) and expand the excitotoxic injury

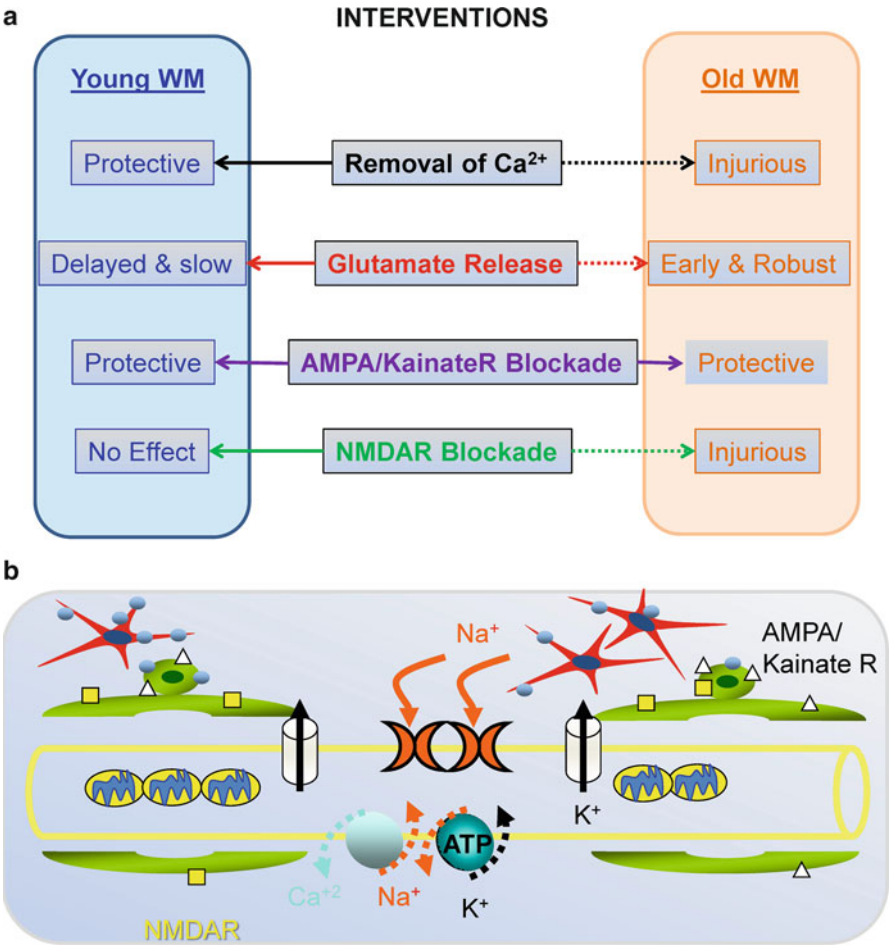


Fig. 16.17 Putative molecular and cellular mechanisms responsible for ischemic white matter injury. (**a**) Protective intervention in young white matter may become injurious or remain the same or become enhanced in aging white matter. (**b**) Age-related cellular reorganization of white matter components determines the injury mechanisms and functional outcome

into recovery period. Interestingly, AMPA/kainate receptors (Fig. 16.17b, white triangles) mediate the ischemic injury across age groups (Fig. 16.17a, purple arrows) indicating that certain steps of injury are preserved irrespective of age. Surprisingly, activation of NMDARs turns into an essential mode of protection for aging axons such that blockade of NMDARs impedes functional recovery after ischemia (Fig. 16.17a, green dotted arrow). NMDARs are expressed in oligodendrocyte cell bodies in young white matter but expand to myelin and to myelin processes with age (Fig. 16.17b, yellow squares; Baltan et al. 2008). Whether this modification of NMDA receptor expression enhances axon–glia metabolic coupling or mediates efficient Ca^{2+} influx to myelin (Micu et al. 2006) to promote aging axon function recovery remains to be explored.

Based on these findings, we propose that age-related upregulation of glutamate, GS and GLT-1 (Fig. 16.17b, blue circles) is not limited to astrocytes but extends to other white matter components (Baltan et al. 2008). Na^+-dependent and Ca^{2+}-dependent mechanisms involving astrocytes, oligodendrocytes expressing EAAC1 (Arranz et al. 2008), microglia expressing GLT-1 or axons with VGLUTs (Kukley et al. 2007; Ziskin et al. 2007) become additional sites of glutamate release with aging and contribute to increased excitotoxicity. In young white matter, activation of either AMPA or kainate receptors loads mitochondria with Ca^{2+} and fission is enhanced due to abundant Drp-1 levels. Ca^{2+} overload activates n-NOS to produce NO and ROS which are proposed as diffusible second messengers to link oligodendrocyte excitotoxicity to axon injury (Matute et al. 2001; Ouardouz et al. 2003, 2006). Aging leads to mitochondrial fusion (Fig. 16.17b, elongated mitochondria) due to a reduction in Drp-1 levels. The regulated process of mitochondrial fusion and fission controls the spatiotemporal properties of mitochondrial Ca^{2+} responses and the physiological and pathophysiological consequences of Ca^{2+} signals (Szabadkai and Rizzuto 2004; Szabadkai et al. 2004). By enhancing fusion or inhibiting fission, elongated mitochondria efficiently absorbs Ca^{2+} preventing n-NOS activation and subsequent ROS production (Cheung et al. 2007). However this age-related adaptive reorganization of mitochondria becomes detrimental under ischemic conditions. Ischemia, in aging white matter, further enforces mitochondrial fusion as a result of an age-dependent drop in Drp-1 and an age-dependent loss of mitochondrial motility with exposure to glutamate (Chang and Reynolds; Parihar and Brewer). Mitochondria fuse to collectively counteract the already increased excitotoxicity and Ca^{2+} load with aging, and this age-related change in mitochondrial dynamics could hinder ATP production. This challenges the Na^+/K^+ ATP pump to maintain axon excitability and the associated rise in Na^+ levels challenges the GLT-1 to function in forward a direction to take up glutamate.

An age-specific understanding of the mechanisms of injury processes in white matter is essential to design dynamic therapeutic approaches for stroke victims.

Therefore an age-dependent reduction in mitochondrial bioenergetics may underlie the increased vulnerability of aging axons to ischemia.

Acknowledgements The studies from the author's laboratory that are described in this chapter were supported by National Institutes of Health/National Institute of Aging grant R01AG033720.

References

Agrawal SK, Fehlings MG (1997) Role of NMDA and non-NMDA ionotropic glutamate receptors in traumatic spinal cord axonal injury. J Neurosci 17:1055–1063

Albert M (1993) Neuropsychological and neurophysiological changes in healthy adult humans across the age range. Neurobiol Aging 14:623–625

Albin RL, Greenamyre JT (1992) Alternative excitotoxic hypotheses. Neurology 42:733–738

Arranz AM, Hussein A, Alix JJ, Perez-Cerda F, Allcock N, Matute C, Fern R (2008) Functional glutamate transport in rodent optic nerve axons and glia. Glia 56:1353–1367

Baltan S (2006) Surviving anoxia: a tale of two white matter tracts. Crit Rev Neurobiol 18:95–103

Baltan S (2009) Ischemic injury to white matter: an age-dependent process. Neuroscientist 15: 126–133
Baltan S (2012) Histone deacetylase inhibitors preserve function in aging axons. J Neurochem 123(suppl 2):108–115
Baltan Tekkök SB, Ransom BR (2004) Anoxia effects on CNS function and survival: regional differences. Neurochem Res 29:2163–2169
Baltan Tekkök S, Brown AM, Ransom BR (2002) Persistence of axonal function during anoxia in mouse optic nerve. Glia S66
Baltan S, Besancon EF, Mbow B, Ye Z, Hamner MA, Ransom BR (2008) White matter vulnerability to ischemic injury increases with age because of enhanced excitotoxicity. J Neurosci 28: 1479–1489
Baltan S, Bachleda A, Morrison RS, Murphy SP (2011a) Expression of histone deacetylases in cellular compartments of the mouse brain and the effects of ischemia. Transl Stroke Res 2: 411–423
Baltan S, Murphy SP, Danilov CA, Bachleda A, Morrison RS (2011b) Histone deacetylase inhibitors preserve white matter structure and function during ischemia by conserving ATP and reducing excitotoxicity. J Neurosci 31:3990–3999
Barsoum MJ, Yuan H, Gerencser AA, Liot G, Kushnareva Y, Graber S, Kovacs I, Lee WD, Waggoner J, Cui J, White AD, Bossy B, Martinou JC, Youle RJ, Lipton SA, Ellisman MH, Perkins GA, Bossy-Wetzel E (2006) Nitric oxide-induced mitochondrial fission is regulated by dynamin-related GTPases in neurons. EMBO J 25:3900–3911
Basser PJ, Mattiello J, LeBihan D (1994) MR diffusion tensor spectroscopy and imaging. Biophys J 66:259–267
Bhat MA, Rios JC, Lu Y, Garcia-Fresco GP, Ching W, St Martin M, Li J, Einheber S, Chesler M, Rosenbluth J, Salzer JL, Bellen HJ (2001) Axon-glia interactions and the domain organization of myelinated axons requires neurexin IV/Caspr/Paranodin. Neuron 30:369–383
Black JA, Cummins TR, Plumpton C, Chen YH, Hormuzdiar W, Clare JJ, Waxman SG (1999) Upregulation of a silent sodium channel after peripheral, but not central, nerve injury in DRG neurons. J Neurophysiol 82:2776–2785
Boiko T, Rasband MN, Levinson SR, Caldwell JH, Mandel G, Trimmer JS, Matthews G (2001) Compact myelin dictates the differential targeting of two sodium channel isoforms in the same axon. Neuron 30:91–104
Bonita R, Beaglehole R, Asplund K (1994) The worldwide problem of stroke. Curr Opin Neurol 7:5–10
Brand-Schieber E, Werner P (2003a) AMPA/kainate receptors in mouse spinal cord cell-specific display of receptor subunits by oligodendrocytes and astrocytes and at the nodes of Ranvier. Glia 42:12–24
Brand-Schieber E, Werner P (2003b) (+/−)-Alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid and kainate receptor subunit expression in mouse versus rat spinal cord white matter: similarities in astrocytes but differences in oligodendrocytes. Neurosci Lett 345:126–130
Bristow EA, Griffiths PG, Andrews RM, Johnson MA, Turnbull DM (2002) The distribution of mitochondrial activity in relation to optic nerve structure. Arch Ophthalmol 120:791–796
Brown AM, Wender R, Ransom BR (2001) Metabolic substrates other than glucose support axon function in central white matter. J Neurosci Res 66:839–843
Campbell LW, Hao SY, Thibault O, Blalock EM, Landfield PW (1996) Aging changes in voltage-gated calcium currents in hippocampal CA1 neurons. J Neurosci 16:6286–6295
Cavallotti C, Pacella E, Pescosolido N, Tranquilli-Leali FM, Feher J (2002) Age-related changes in the human optic nerve. Can J Ophthalmol 37:389–394
Cavallotti C, Cavallotti D, Pescosolido N, Pacella E (2003) Age-related changes in rat optic nerve: morphological studies. Anat Histol Embryol 32:12–16
Chang DT, Reynolds IJ (2006) Differences in mitochondrial movement and morphology in young and mature primary cortical neurons in culture. Neuroscience 141:727–736
Chen H, McCaffery JM, Chan DC (2007) Mitochondrial fusion protects against neurodegeneration in the cerebellum. Cell 130:548–562

Cheung EC, McBride HM, Slack RS (2007) Mitochondrial dynamics in the regulation of neuronal cell death. Apoptosis 12:979–992
Craner MJ, Hains BC, Lo AC, Black JA, Waxman SG (2004) Co-localization of sodium channel Nav1.6 and the sodium-calcium exchanger at sites of axonal injury in the spinal cord in EAE. Brain 127:294–303
Davatzikos C, Resnick SM (2002) Degenerative age changes in white matter connectivity visualized in vivo using magnetic resonance imaging. Cereb Cortex 12:767–771
Del Zoppo GJ (1995) Why do all drugs work in animals but none in stroke patients? 1. Drugs promoting cerebral blood flow. J Intern Med 237:79–88
Del Zoppo GJ (1998) Clinical trials in acute stroke: why have they not been successful? Neurology 51:S59–S61
Desilva TM, Billiards SS, Borenstein NS, Trachtenberg FL, Volpe JJ, Kinney HC, Rosenberg PA (2008) Glutamate transporter EAAT2 expression is up-regulated in reactive astrocytes in human periventricular leukomalacia. J Comp Neurol 508:238–248
Dirnagl U, Iadecola C, Moskowitz MA (1999) Pathobiology of ischaemic stroke: an integrated view. Trends Neurosci 22:391–397
Dupree JL, Girault JA, Popko B (1999) Axo-glial interactions regulate the localization of axonal paranodal proteins. J Cell Biol 147:1145–1152
Enblad P, Frykholm P, Valtysson J, Silander HC, Andersson J, Fasth KJ, Watanabe Y, Langstrom B, Hillered L, Persson L (2001) Middle cerebral artery occlusion and reperfusion in primates monitored by microdialysis and sequential positron emission tomography. Stroke 32: 1574–1580
Erecinska M, Silver IA (1994) Ions and energy in mammalian brain. Prog Neurobiol 43:37–71
Fern R, Moller T (2000) Rapid ischemic cell death in immature oligodendrocytes: a fatal glutamate release feedback loop. J Neurosci 20:34–42
Fern R, Ransom BR (1997) Ischemic injury of optic nerve axons: the nuts and bolts. Clin Neurosci 4:246–250
Fern RJ, Hahm MS, Lu HK, Liu LP, Gorelick FS, Barrett PQ (1995) Ca2+/calmodulin-dependent protein kinase II activation and regulation of adrenal glomerulosa Ca2+ signaling. Am J Physiol 269:F751–F760
Ferro JM, Madureira S (2002) Age-related white matter changes and cognitive impairment. J Neurol Sci 203–204:221–225
Follett PL, Rosenberg PA, Volpe JJ, Jensen FE (2000) NBQX attenuates excitotoxic injury in developing white matter. J Neurosci 20:9235–9241
Foster RE, Connors BW, Waxman SG (1982) Rat optic nerve: electrophysiological, pharmacological and anatomical studies during development. Dev Brain Res 3:371–386
Frykholm P, Andersson JL, Valtysson J, Silander HC, Hillered L, Persson L, Olsson Y, Yu WR, Westerberg G, Watanabe Y, Langstrom B, Enblad P (2000) A metabolic threshold of irreversible ischemia demonstrated by PET in a middle cerebral artery occlusion-reperfusion primate model. Acta Neurol Scand 102:18–26
Gadea A, Lopez-Colome AM (2001) Glial transporters for glutamate, glycine and GABA I. Glutamate transporters. J Neurosci Res 63:453–460
Gallo V, Russell JT (1995) Excitatory amino acid receptors in glia: different subtypes for distinct functions? J Neurosci Res 42:1–8
Garthwaite G, Brown G, Batchelor AM, Goodwin DA, Garthwaite J (1999) Mechanisms of ischaemic damage to central white matter axons: a quantitative histological analysis using rat optic nerve. Neuroscience 94:1219–1230
Ginsberg MD, Busto R (1989) Rodent models of cerebral ischemia. Stroke 20:1627–1642
Goldberg MP, Ransom BR (2003) New light on white matter. Stroke 34:330–332
Goldin AL, Barchi RL, Caldwell JH, Hofmann F, Howe JR, Hunter JC, Kallen RG, Mandel G, Meisler MH, Netter YB, Noda M, Tamkun MM, Waxman SG, Wood JN, Catterall WA (2000) Nomenclature of voltage-gated sodium channels. Neuron 28:365–368
Hachinski VC, Potter P, Merskey H (1987) Leuko-araiosis. Arch Neurol 44:21–23
Hazell AS, Rao KV, Danbolt NC, Pow DV, Butterworth RF (2001) Selective down-regulation of the astrocyte glutamate transporters GLT-1 and GLAST within the medial thalamus in experimental Wernicke's encephalopathy. J Neurochem 78:560–568

Hinman JD, Peters A, Cabral H, Rosene DL, Hollander W, Rasband MN, Abraham CR (2006) Age-related molecular reorganization at the node of Ranvier. J Comp Neurol 495:351–362
Hollenbeck PJ (2005) Mitochondria and neurotransmission: evacuating the synapse. Neuron 47: 331–333
Hollenbeck PJ, Saxton WM (2005) The axonal transport of mitochondria. J Cell Sci 118: 5411–5419
Jacquard C, Trioulier Y, Cosker F, Escartin C, Bizat N, Hantraye P, Cancela JM, Bonvento G, Brouillet E (2006) Brain mitochondrial defects amplify intracellular [Ca2+] rise and neurodegeneration but not Ca2+ entry during NMDA receptor activation. FASEB J 20:1021–1023
Jensen FE (2006) Developmental factors regulating susceptibility to perinatal brain injury and seizures. Curr Opin Pediatr 18:628–633
Karadottir R, Cavelier P, Bergersen LH, Attwell D (2005) NMDA receptors are expressed in oligodendrocytes and activated in ischaemia. Nature 438:1162–1166
Karbowski M, Youle RJ (2003) Dynamics of mitochondrial morphology in healthy cells and during apoptosis. Cell Death Differ 10:870–880
Karbowski M, Arnoult D, Chen H, Chan DC, Smith CL, Youle RJ (2004) Quantitation of mitochondrial dynamics by photolabeling of individual organelles shows that mitochondrial fusion is blocked during the Bax activation phase of apoptosis. J Cell Biol 164:493–499
Kawahara K, Kosugi T, Tanaka M, Nakajima T, Yamada T (2005) Reversed operation of glutamate transporter GLT-1 is crucial to the development of preconditioning-induced ischemic tolerance of neurons in neuron/astrocyte co-cultures. Glia 49:349–359
Kemper TL (1994) Neuroanatomical and neuropathological changes during aging and dementia. Oxford University Press, New York
Kukley M, Capetillo-Zarate E, Dietrich D (2007) Vesicular glutamate release from axons in white matter. Nat Neurosci 10:311–320
Kurtzke JF (1994) Epidemiology of stroke: methods and trends. Health Rep 6:13–21
Landfield PW, Pitler TA (1984) Prolonged Ca2+–dependent after hyperpolarizations in hippocampal neurons of aged rats. Science 226:1089–1092
Lee L, Baltan Tekkok S, Deem S, Moulding J, Lam AM (2006) A novel model of in vivo pig posterior optic nerve ischemia. American Society of Anesthesiologists, Chicago, 14–17 Oct
Leroux P, Hennebert C, Catteau J, Legros H, Hennebert O, Laudenbach V, Marret S (2010) Neuroprotective effects vary across nonsteroidal antiinflammatory drugs in a mouse model of developing excitotoxic brain injury. Neuroscience 167:716–723
Lesnefsky EJ, Moghaddas S, Tandler B, Kerner J, Hoppel CL (2001) Mitochondrial dysfunction in cardiac disease: ischemia–reperfusion, aging, and heart failure. J Mol Cell Cardiol 33: 1065–1089
Li S, Mealing GA, Morley P, Stys PK (1999) Novel injury mechanism in anoxia and trauma of spinal cord white matter: glutamate release via reverse Na^+-dependent glutamate transport. J Neurosci 19:RC1–RC9
Longuemare MC, Rose CR, Farrell K, Ransom BR, Waxman SG, Swanson RA (1999) K(+)-induced reversal of astrocyte glutamate uptake is limited by compensatory changes in intracellular Na+. Neuroscience 93:285–292
Matute C, Alberdi E, Domercq M, Perez-Cerda F, Perez-Samartin A, Sanchez-Gomez MV (2001) The link between excitotoxic oligodendroglial death and demyelinating diseases. Trends Neurosci 24:224–230
McDonald JW, Althomsons SP, Hyrc KL, Choi DW, Goldberg MP (1998) Oligodendrocytes from forebrain are highly vulnerable to AMPA/kainate receptor-mediated excitotoxicity. Nat Med 4:291–297
Micu I, Jiang Q, Coderre E, Ridsdale A, Zhang L, Woulfe J, Yin X, Trapp BD, McRory JE, Rehak R, Zamponi GW, Wang W, Stys PK (2006) NMDA receptors mediate calcium accumulation in myelin during chemical ischaemia. Nature 439:988–992
Misgeld T, Kerschensteiner M, Bareyre FM, Burgess RW, Lichtman JW (2007) Imaging axonal transport of mitochondria in vivo. Nat Methods 4:559–561

Morgan SC, Taylor DL, Pocock JM (2004) Microglia release activators of neuronal proliferation mediated by activation of mitogen-activated protein kinase, phosphatidylinositol-3-kinase/Akt and delta-Notch signalling cascades. J Neurochem 90:89–101

Mozdy AD, Shaw JM (2003) A fuzzy mitochondrial fusion apparatus comes into focus. Nat Rev Mol Cell Biol 4:468–478

Nicholls DG, Johnson-Cadwell L, Vesce S, Jekabsons M, Yadava N (2007) Bioenergetics of mitochondria in cultured neurons and their role in glutamate excitotoxicity. J Neurosci Res 85:3206–3212

O'Collins VE, Macleod MR, Donnan GA, Horky LL, van der Worp BH, Howells DW (2006) 1,026 experimental treatments in acute stroke. Ann Neurol 59:467–477

O'Sullivan M, Jones DK, Summers PE, Morris RG, Williams SC, Markus HS (2001) Evidence for cortical "disconnection" as a mechanism of age-related cognitive decline. Neurology 57: 632–638

Oberheim NA, Tian GF, Han X, Peng W, Takano T, Ransom B, Nedergaard M (2008) Loss of astrocytic domain organization in the epileptic brain. J Neurosci 28:3264–3276

Ogura K, Ogawa M, Yoshida M (1994) Effects of ageing on microglia in the normal rat brain: immunohistochemical observations. Neuroreport 5:1224–1226

Oka A, Belliveau MJ, Rosenberg PA, Volpe JJ (1993) Vulnerability of oligodendroglia to glutamate: pharmacology, mechanisms, and prevention. J Neurosci 13:1441–1453

Olivares R, Montiel J, Aboitiz F (2001) Species differences and similarities in the fine structure of the mammalian corpus callosum. Brain Behav Evol 57:98–105

Otsuga D, Keegan BR, Brisch E, Thatcher JW, Hermann GJ, Bleazard W, Shaw JM (1998) The dynamin-related GTPase, Dnm1p, controls mitochondrial morphology in yeast. J Cell Biol 143:333–349

Ouardouz M, Nikolaeva MA, Coderre E, Zamponi GW, McRory JE, Trapp BD, Yin X, Wang W, Woulfe J, Stys PK (2003) Depolarization-induced Ca2+ release in ischemic spinal cord white matter involves L-type Ca2+ channel activation of ryanodine receptors. Neuron 40:53–63

Ouardouz M, Malek S, Coderre E, Stys PK (2006) Complex interplay between glutamate receptors and intracellular Ca2+ stores during ischaemia in rat spinal cord white matter. J Physiol 577:191–204

Pantoni L, Garcia JH, Gutierrez JA (1996) Cerebral white matter is highly vulnerable to ischemia. Stroke 27:1641–1646; discussion 1647

Parihar MS, Brewer GJ (2007) Simultaneous age-related depolarization of mitochondrial membrane potential and increased mitochondrial reactive oxygen species production correlate with age-related glutamate excitotoxicity in rat hippocampal neurons. J Neurosci Res 85: 1018–1032

Parpura V, Basarsky TA, Liu F, Jeftinija K, Jeftinija S, Haydon PG (1994) Glutamate-mediated astrocyte-neuron signalling. Nature 369:744–747

Pendlebury ST, Blamire AM, Lee MA, Styles P, Matthews PM (1999) Axonal injury in the internal capsule correlates with motor impairment after stroke. Stroke 30:956–962

Peters A, Sethares C (1993) Aging and the Meynert cells in rhesus monkey primary visual cortex. Anat Rec 236:721–729

Rintoul GL, Filiano AJ, Brocard JB, Kress GJ, Reynolds IJ (2003) Glutamate decreases mitochondrial size and movement in primary forebrain neurons. J Neurosci 23:7881–7888

Romera C, Hurtado O, Botella SH, Lizasoain I, Cardenas A, Fernandez-Tome P, Leza JC, Lorenzo P, Moro MA (2004) In vitro ischemic tolerance involves upregulation of glutamate transport partly mediated by the TACE/ADAM17-tumor necrosis factor-alpha pathway. J Neurosci 24: 1350–1357

Rothman SM, Olney JW (1995) Excitotoxicity and the NMDA receptor–still lethal after eight years. Trends Neurosci 18:57–58

Rothstein JD, Dykes-Hoberg M, Pardo CA, Bristol LA, Jin L, Kuncl RW, Kanai Y, Hediger MA, Wang Y, Schielke JP, Welty DF (1996) Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. Neuron 16:675–686

Saab A, Baltan S, Möbius I, Tzvetavona J, Hirrlinger B, Goetze HM, Jahn W, Huang A, Pérez-Samartín F, Pérez-Cerdá C, Matute S, Löwel K-A, Nave F, Kirchhoff (2012) Oligodendroglial NMDA receptor signalling in coupling axonal and glial energy metabolism. Society for Neuroscience Abstracts, New Orleans, LA

Salter MG, Fern R (2005) NMDA receptors are expressed in developing oligodendrocyte processes and mediate injury. Nature 438:1167–1171

Sanchez-Gomez MV, Matute C (1999) AMPA and kainate receptors each mediate excitotoxicity in oligodendroglial cultures. Neurobiol Dis 6:475–485

Scavone C, Munhoz CD, Kawamoto EM, Glezer I, de Sa LL, Marcourakis T, Markus RP (2005) Age-related changes in cyclic GMP and PKG-stimulated cerebellar Na, K-ATPase activity. Neurobiol Aging 26:907–916

Schmidt R, Fazekas F, Offenbacher H, Lytwyn H, Blematl B, Niederkorn K, Horner S, Payer F, Freidl W (1991) Magnetic resonance imaging white matter lesions and cognitive impairment in hypertensive individuals. Arch Neurol 48:417–420

Scott SV, Cassidy-Stone A, Meeusen SL, Nunnari J (2003) Staying in aerobic shape: how the structural integrity of mitochondria and mitochondrial DNA is maintained. Curr Opin Cell Biol 15:482–488

Selzner M, Selzner N, Jochum W, Graf R, Clavien PA (2007) Increased ischemic injury in old mouse liver: an ATP-dependent mechanism. Liver Transpl 13:382–390

Silva-Adaya D, Perez-De La Cruz V, Herrera-Mundo MN, Mendoza-Macedo K, Villeda-Hernandez J, Binienda Z, Ali SF, Santamaria A (2008) Excitotoxic damage, disrupted energy metabolism, and oxidative stress in the rat brain: antioxidant and neuroprotective effects of L-carnitine. J Neurochem 105:677–689

Sloane JA, Hollander W, Moss MB, Rosene DL, Abraham CR (1999) Increased microglial activation and protein nitration in white matter of the aging monkey. Neurobiol Aging 20:395–405

Smirnova E, Shurland DL, Ryazantsev SN, van der Bliek AM (1998) A human dynamin-related protein controls the distribution of mitochondria. J Cell Biol 143:351–358

Sozmen EG, Kolekar A, Havton LA, Carmichael ST (2009) A white matter stroke model in the mouse: axonal damage, progenitor responses and MRI correlates. J Neurosci Methods 180: 261–272

Stys PK (2004) White matter injury mechanisms. Curr Mol Med 4:113–130

Stys PK, Ransom BR, Waxman SG, Davis PK (1990) Role of extracellular calcium in anoxic injury of mammalian central white matter. Proc Natl Acad Sci U S A 87:4212–4216

Stys PK, Waxman SG, Ransom BR (1992) Ionic mechanisms of anoxic injury in mammalian CNS white matter: role of Na^+ channels and Na^+-Ca^{2+} exchanger. J Neurosci 12:430–439

Szabadkai G, Rizzuto R (2004) Participation of endoplasmic reticulum and mitochondrial calcium handling in apoptosis: more than just neighborhood? FEBS Lett 567:111–115

Szabadkai G, Simoni AM, Chami M, Wieckowski MR, Youle RJ, Rizzuto R (2004) Drp-1-dependent division of the mitochondrial network blocks intraorganellar Ca2+ waves and protects against Ca2+–mediated apoptosis. Mol Cell 16:59–68

Szatkowski M, Barbour B, Attwell D (1990) Non-vesicular release of glutamate from glial cells by reversed electrogenic glutamate uptake. Nature 348:443–446

Tekkök SB, Goldberg MP (2001) Ampa/kainate receptor activation mediates hypoxic oligodendrocyte death and axonal injury in cerebral white matter. J Neurosci 21:4237–4248

Tekkok SB, Brown AM, Ransom BR (2003) Axon function persists during anoxia in mammalian white matter. J Cereb Blood Flow Metab 23:1340–1347

Tekkok SB, Ye Z, Ransom BR (2007) Excitotoxic mechanisms of ischemic injury in myelinated white matter. J Cereb Blood Flow Metab 27:1540–1552

Thorell WE, Leibrock LG, Agrawal SK (2002) Role of RyRs and IP3 receptors after traumatic injury to spinal cord white matter. J Neurotrauma 19:335–342

Toescu EC (2005) Normal brain ageing: models and mechanisms. Philos Trans R Soc Lond B Biol Sci 360:2347–2354

Ture D, Yasargil MG, Krist AF (1996) The arteries of the corpus callosum: a microsurgical anatomic study. Neurosurgery 39:1075–1085

Underhill SM, Goldberg MP (2007) Hypoxic injury of isolated axons is independent of ionotropic glutamate receptors. Neurobiol Dis 25:284–290

Uo T, Dworzak J, Kinoshita C, Inman DM, Kinoshita Y, Horner PJ, Morrison RS (2009) Drp1 levels constitutively regulate mitochondrial dynamics and cell survival in cortical neurons. Exp Neurol 218:274–285

Verkhratsky A, Orkand RK, Kettenmann H (1998) Glial calcium: homeostasis and signaling function. Physiol Rev 78:99–141

Vincent SL, Peters A, Tigges J (1989) Effects of aging on the neurons within area 17 of rhesus monkey cerebral cortex. Anat Rec 223:329–341

Wolf JA, Stys PK, Lusardi T, Meaney D, Smith DH (2001) Traumatic axonal injury induces calcium influx modulated by tetrodotoxin-sensitive sodium channels. J Neurosci 21: 1923–1930

Wolfram-Gabel R, Maillot C, Koritke JG, Laude M (1987) [The vascularization of the human tela choroidea of the lateral ventricle]. Acta Anat (Basel) 128:301–321

Wrathall JR, Choiniere D, Teng YD (1994) Dose-dependent reduction of tissue loss and functional impairment after spinal cord trauma with the AMPA/kainate antagonist NBQX. J Neurosci 14:6598–6607

Yamauchi H, Fukuda H, Oyanagi C (2002) Significance of white matter high intensity lesions as a predictor of stroke from arteriolosclerosis. J Neurol Neurosurg Psychiatry 72:576–582

Ye ZC, Wyeth MS, Baltan-Tekkok S, Ransom BR (2003) Functional hemichannels in astrocytes: a novel mechanism of glutamate release. J Neurosci 23:3588–3596

Zhang K, Sejnowski TJ (2000) A universal scaling law between gray matter and white matter of cerebral cortex. Proc Natl Acad Sci U S A 97:5621–5626

Zhang M, Li WB, Geng JX, Li QJ, Sun XC, Xian XH, Qi J, Li SQ (2007) The upregulation of glial glutamate transporter-1 participates in the induction of brain ischemic tolerance in rats. J Cereb Blood Flow Metab 27:1352–1368

Ziskin JL, Nishiyama A, Rubio M, Fukaya M, Bergles DE (2007) Vesicular release of glutamate from unmyelinated axons in white matter. Nat Neurosci 10:321–330

Chapter 17
White Matter Damage in Multiple Sclerosis

María Victoria Sánchez-Gómez, Fernando Pérez-Cerdá, and Carlos Matute

17.1 Introduction

Multiple sclerosis (MS) is the leading cause of nontraumatic neurological disability in young adults in the United States and Europe. MS is an inflammatory demyelinating disease of the central nervous system (CNS) and generally considered a predominantly autoimmune disease, mediated by an autoreactive and aberrant T cell attack against CNS elements, particularly myelin. In line with this notion, immunomodulatory and anti-inflammatory therapies prove to be effective, especially early in the disease phase. However, the disease often progresses relentlessly in later disease stages without much evidence for acute inflammation and no obvious effect of anti-inflammatory therapies (Stadelmann et al. 2011).

MS initially presents as a relapsing-remitting disease in most patients, with immunological attacks (relapses) which may last from days to weeks with a posterior recovery phase. However, the antigenic stimuli that initiate or perpetuate this abnormal immune reactivity are still a matter of intense research and debate. Focal lesions showing perivascular inflammation, infiltration of immune cells, axonal degeneration, oligodendroglial death, and demyelination characterize this chronic and degenerative disease (Prineas et al. 2002). MS lesions can arise anywhere in the CNS but, they show a predilection for the optic nerve, spinal cord, brain stem, and periventricular areas. Furthermore, brain tissue immediately adjacent to the subarachnoid space, i.e. subpial gray matter, is especially vulnerable to demyelination (Bo et al. 2003), a fact that has only recently been appreciated. Mostly, within a patient, lesions of similar age

M.V. Sánchez-Gómez • F. Pérez-Cerdá • C. Matute (✉)
Departamento de Neurociencias, Achucarro Basque Center for Neuroscience, Universidad del País Vasco (UPV/EHU), Leioa 48940, Spain

Centro de Investigación Biomédica en Red en Enfermedades Neurodegenerativas (CIBERNED), Leioa 48940, Spain
e-mail: carlos.matute@ehu.es

S. Baltan et al. (eds.), *White Matter Injury in Stroke and CNS Disease*, Springer Series in Translational Stroke Research 4, DOI 10.1007/978-1-4614-9123-1_17,

resemble each other with respect to the extent and pattern of inflammation and remyelination (Lucchinetti et al. 1999, 2000; Patrikios et al. 2006).

Demyelination results in slower conduction or complete failure of transmission, leading to symptoms or neurological deficits associated with damage to the CNS, especially white matter tracks. Some of the most common findings are optic neuritis and visual impairment, weakness, sensory disturbances, lack of motor coordination, ataxia, nystagmus, and cognitive dysfunction. However, the clinical presentation of MS varies greatly and correlates well with the multiplicity of lesions and their distribution at various anatomical sites within the brain and spinal cord.

MS affects women more often than it does men. Its incidence is approximately 3.6 cases among women and 2 cases among men per 100,000 individuals per year. Its prevalence varies geographically: higher MS incidence is classically associated with countries with cold climates and at high latitudes (averaging 90–100 cases per 100,000 individuals), although recent studies report that this gradient has become attenuated after 1980, apparently due to increased incidence in lower latitudes (Alonso and Hernan 2008). Other factors than genetic determinants could also be influencing this observed change, such as infections or vitamin D and sun exposure, which are correlated with MS incidence (Giovannoni and Ebers 2007). Other environmental conditions, such as smoking and reproductive factors, are thought to be involved in the female-to-men incidence ratio for MS.

17.2 Characteristics of MS

17.2.1 Clinical Observations and Diagnostic Criteria for MS

Although MS starts with one attack, the profile of the disease is the multiplicity of relapses which underlies CNS dysfunction. Visual impairment to different degrees, orbital pain and frontal headaches are often the presenting symptoms, and disease diagnosis is based on clinical criteria supported by visualization of CNS white matter lesions by magnetic resonance imaging (MRI) following established standards, recompiled in the McDonald Criteria (Polman et al. 2011). MS lesions are typically disseminated in time and space. Dissemination in time involves more than one relapse, whereas dissemination in space implies involvement of more than one area of the CNS. The multiplicity of MS plaques and their location at various anatomic sites may account for the great variability of clinical symptoms and signs. Diagnostic complementary evidence to MRI scans includes analytical examination of cerebrospinal fluid (CSF), and evaluation of visual evoked potentials (VEP). CSF analysis can provide evidence of the immune and inflammatory nature of lesions, being the presence of different oligoclonal IgG bands and an elevated IgG index indicative of a CSF abnormality, whereas VEP typical of MS are delayed in time but with well-preserved wave form.

17.2.2 Types of MS

MS patients present several common patterns of symptoms, and each pattern is associated with variable intensities of inflammatory response. According symptoms and the course of disease, MS patients are classified into different subtypes. The most common form, affecting 85–90 % of newly diagnosed patients, is relapsing-remitting multiple sclerosis (RRMS), which is characterized by discrete clinical "attacks" or "relapses" followed by subsequent improvement. RRMS is the most typical presentation in younger patients with a mean age of onset of around 30 years. It is characterized by relapses of neurological dysfunction that last weeks to months and affect various locations of the brain, optic nerves and/or spinal cord. Multifocal areas of abnormality are found on magnetic resonance scanning, typically (but not exclusively) in the white matter. The MRI appearance of some lesions exhibit enhancement after intravenous administration of gadolinium, indicating breakdown of the blood–brain barrier as a result of active inflammation (reviewed in Stys et al. 2012). Within 10 years of disease onset, approximately 50 % of patients with RRMS will develop a slow, insidiously progressive, neurological deterioration and CNS atrophy (usually progressive gait impairment), with or without clinical attacks superimposed. This is termed secondary progressive multiple sclerosis (SPMS), and 90 % of RRMS patients advance into this stage within 30 years of disease onset. A minority of patients (approximately 10–15 %) have primary progressive multiple sclerosis (PPMS), which is characterized by a progressive course from onset, with occasional plateaus and absence of clinically evident relapses. In addition, PPMS presents less conspicuous inflammation on MRI. Due to its difference from RRMS, and particularly because of the absence of any relapse, it has been suggested that PPMS may represent a different disease (Thompson et al. 1997). However, although the initial courses of RRMS and PPMS are very different, the progressive phases of each proceed at remarkably similar rates (Scalfari et al. 2010) and, intriguingly, the conversion from RRMS to progressive MS tends to occur in a well-defined age window of 35–50 years, which is the same as the typical age of disease onset in patients with PPMS (Leray et al. 2010). Finally, approximately 5 % of the MS patients develop progressive relapsing multiple sclerosis (PRMS), which is characterized by worsening from onset with clear, acute relapses (with or without recovery), and with periods between relapses with continuing progression of deterioration (Lublin and Reingold 1996; Rovaris et al. 2006).

The prognosis of MS varies widely. The factors responsible for this variability are unclear and it therefore remains difficult to estimate prognosis in individual patients at the time of diagnosis. The outcome of an attack depends on the severity and extent of axonal injury, and this largely determines the degree of recovery or the persistence of neurological deficits. Usually, after inflammation has resolved and myelin debris has been removed, the conduction of the neural impulses is reestablished in the denuded nerve fibers. Although the resolution of inflammation allows a remission with full or partial recovery of neurologic deficits, the appearance of

new plaques, which may develop at any time throughout the course of the disease, would cause a relapse. Disability becomes permanent when the structural continuity of the nerve fibers is disrupted and wallerian degeneration develops (reviewed in Prineas et al. 2002).

Clinical indicators of a relatively good prognosis are female gender, younger age of onset, optic neuritis, sensory attacks, complete recovery from attacks, few attacks, and long inter-attack interval. Relatively poor prognostic factors include male gender, predominant cerebellar and motor involvement, incomplete resolution of attacks, progressive course from onset, frequent early attacks, and short inter-attack interval (Keegan and Noseworthy 2002).

In general, the average duration of the disease is 25–30 years. One-third of patients have a benign course, remain fully functional, and show little disability for 15 years after disease onset. In contrast, malignant MS is defined by a rapid, progressive course, leading to significant neurological deficits and even death (Lublin and Reingold 1996; Prineas et al. 2002).

17.2.3 Etiology of MS

MS is a complex and multifactorial disease which cannot be associated to a single genetic or environmental factor and, at least, interactions between a potential genetic susceptibility and environmental factors are implicated in the origin of MS (Zamvil and Steinman 2003). It is widely accepted that the etiology of this illness has autoimmune and inflammatory grounds, and that a derailment of the immune system leads to cell and antibody-mediated attacks on myelin.

17.2.3.1 Autoimmunity

The immune system plays an integral role in the initiation and progression of MS (Hemmer et al. 2002; Keegan and Noseworthy 2002). Thus, MS can be seen as a disease in which genetically susceptible individuals, upon encountering an environmental stimulus such as an infection, generate an autoimmune attack against CNS myelin based on molecular mimicry between infectious and myelin antigens.

Autoimmune-mediated reactions against myelin, which are based on the inappropriate immunological attack towards self-antigens, constitute the most widely accepted hypothesis for MS etiology, although the initial trigger of autoimmunity is still unknown. This autoimmune hypothesis is supported by similarities between the MS pathology and experimental allergic encephalomyelitis (EAE), the dominant MS animal model, which is induced by immunization with brain and spinal cord myelin extracts. In this model, myelin antigen-specific $CD4^+$ T cells can induce CNS inflammation, demyelination, and neurodegeneration, resulting in the loss of motor functions or paralysis (Brown and Sawchenko 2007).

17.2.3.2 Genetic Susceptibility

Familiar occurrence is recognized and the disease has been reported in monozygotic and dizygotic twins; however, the genes that contribute to MS susceptibility are difficult to identify because they exert a relatively modest effect on disease risk. In support of the genetic contribution, certain gene variants in the class II major histocompatibility complexes (MHCs) occur more frequently among MS patients than among the general population (Prineas et al. 2002). Thus, two large genetic studies have revealed an association of MS in a genetic region of the European and North American Caucasian population (Lincoln et al. 2005; Sawcer et al. 2005). Risk alleles are located in human leucocyte antigen (HLA) class II region. Specifically, HLA-DRB1 and HLA-DQB1 alleles (DR15 haplotype) are linked to MS susceptibility, with possible interactions between them and closing neighboring variants. This link was confirmed in the largest genome-wide association study conducted to date, which also revealed two other immune-related targets including the receptors for the interleukins IL-2 and IL-7 (Hafler et al. 2007; Lundmark et al. 2007). Some evidence indicate that the complexity of class II loci points to epistatic interactions at this locus, with interactions between susceptibility and resistance alleles (Giovannoni and Ebers 2007).

17.2.3.3 Environmental Factors

Increasing rate of MS in countries with cold climate and a high latitude, together with the higher incidence in women, suggest that early life events have an influence in MS development. Although, individuals who migrate from a cold climate to a warm climate after 15 years of age retain the higher risk associated with their native locality, high levels of vitamin D decrease the risk for MS. This suggests an immunomodulatory role of the vitamin D on T cells (Giovannoni and Ebers 2007). In addition, smoking before the onset of MS has a significant, although moderate, risk factor the subsequent development of MS.

17.2.3.4 Transmissible Agents

Aside from the genetic and environmental component, the role of transmissible agents as a cause of MS is a relatively popular hypothesis. It is possible that exposure to an unidentified infectious agent may occur during the early years of life. Several pathogens have been postulated to trigger the immune reaction: measles virus, Epstein-Barr virus (EBV), human herpes virus 6, retroviruses, and *Chlamidia pneumoniae*. Among them, data associating EBV infection with MS remains strong. Thus, people with symptomatic EBV infection are at increased risk of developing MS, similar to people with high titers of anti-EBV antibodies. There are also observations supporting EBV-induced molecular mimicry as an underlying mechanism in MS autoimmunity. This association may be causative, or it may simply be a phenomenon required for disease onset (Giovannoni and Ebers 2007).

17.3 Pathophysiology of MS

MS is a chronic, degenerative disease of the CNS which pathological hallmarks are inflammation, demyelination, neurodegeneration, oligodendroglial death, and axonal degeneration; these alterations occur either focally or diffusely throughout the white and gray matter in the brain and spinal cord.

Pathophysiological models of MS should reproduce the generation of acute demyelinating lesions and their evolution into chronic sclerotic plaques, as well as an unpredictable clinical course that is characterized initially by recurrent relapses and later by steady progression. Although MS is regarded as a white matter disease, and the principal features of the disease are demyelination, perivascular inflammation, and the presence of plaques within the white matter, the incidence of demyelination and oligodendrocyte or neuron/axon injury are also prominent and widespread in gray matter structures, such as the cerebral cortex, thalamus, and basal ganglia (Lassmann 2007; Stadelmann et al. 2008). Thus, a particularly high prevalence of plaques in the cerebral cortex has been observed in progressive stages of the disease, and constitutes a significant proportion of the overall pathology of the brains of MS patients.

The generally accepted pathophysiological model for MS is centered on an immune-mediated attack against CNS myelin antigens, in which several components of the immune system generate an inflammatory response that damages myelin and axons, leading to the formation of acute plaques (Frohman et al. 2006; Fontoura and Garren 2010). However, although MS is considered an autoimmune CNS inflammatory disease, it is widely accepted that neurodegeneration is the predominant pathophysiological substrate of disability. Indeed, there is a strong correlation between inflammation and neurodegeneration (Zipp and Atkas 2006; Franciotta et al. 2008; Frischer et al. 2009; Lee et al. 2011); however, debate remains regarding whether inflammation is a primary or secondary process during both the onset and the development of MS (Trapp and Nave 2008; Craner and Fugger 2011).

17.3.1 Inflammation in MS

Inflammatory events in MS could be due to an autoimmune reaction against myelin antigens, which is more profound in actively demyelinating lesions and involves both cellular and humoral immunity with the participation of macrophages, T lymphocytes, and B cells. In the early stages of the disease process, T cells are primed in the periphery by antigen-presenting dendritic cells, and activated $CD4^+$ T lymphocytes cross the blood–brain barrier and react with myelin and/or oligodendroglia antigens (Fig. 17.1). There is also evidence for humoral autoantibodies produced by local B cells binding myelin and other CNS components, as well as expression of immune-associated molecules, such as major histocompatibility antigens, adhesion molecules or pro-inflammatory cytokines, interleukin 12 (IL-12), and tumor necrosis factor-α (TNF-α). All of these factors contribute to the generation of a diffuse

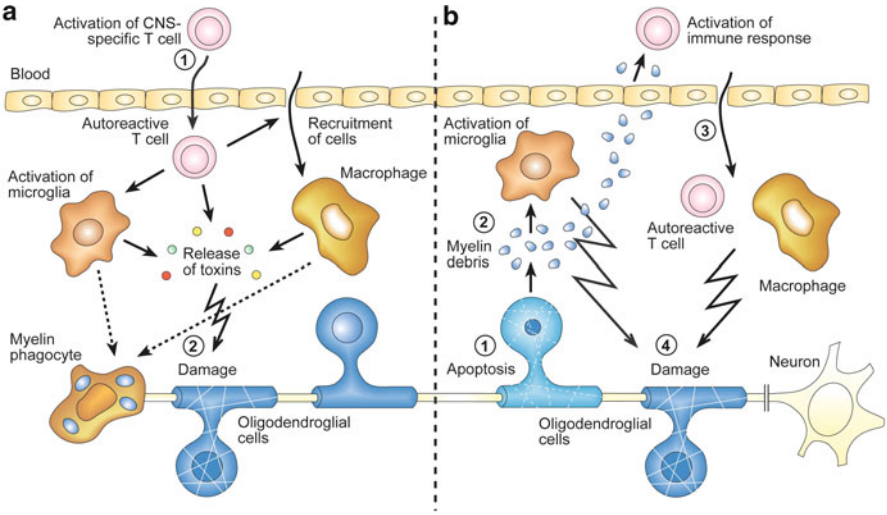


Fig. 17.1 Alternative views of the mechanisms of lesion formation in MS. (**a**) Activated T cells migrate into the CNS (1) and initiate inflammatory events including recruitment of blood macrophages, activation of local microglia, and the release of toxins. This leads to myelin destruction, oligodendrocyte death, and clearance of damaged tissue by phagocytes (2). (**b**) Viruses, glutamate, and other agents can cause extensive oligodendrocyte apoptosis in tissue foci (1). As a consequence, large amounts of myelin debris are generated (2) overwhelming the physiological mechanisms of elimination of apoptotic leftovers and thus triggering inflammation. Subsequently, T cells and macrophages invade the CNS (3) and initiate a stereotyped autoimmune attack of myelin (4) as described in (**a**). Reproduced from Matute and Pérez-Cerdá (2005)

inflammatory process, with infiltrates mainly composed by lymphocytes and macrophages and with additional activation of the resident microglia.

In general, the inflammation process occurs during all stages of MS, although it changes as the disease progresses. In acute MS, infiltrating macrophages and activated T cells are predominant in focal demyelinated lesions. In addition, in chronic MS there is a diffuse inflammatory/degenerative process supported by microglia and macrophages that may be more or less independent of current focal inflammation (Kerschensteiner et al. 2009; Edan and Leray 2010). In this chronic phase, particularly oligodendrocytes, myelin, and axons degenerate in the CNS, causing a wide variety of symptoms that often progress to physical and cognitive disabilities.

17.3.2 Neurodegeneration in MS

Although MS has been mainly considered an immune disease, there is an evolving concept regarding MS as a primary neurodegenerative disease with secondary inflammatory demyelination. Thus, early axonal damage can result from a direct interaction with immune cells, there is a positive correlation between axonal transection and the

degree of inflammation in white matter MS lesions undergoing demyelination (Zipp and Atkas 2006; Trapp and Nave 2008; Nikic et al. 2011). In addition, recent studies support the concept that neurodegeneration is an independent process in MS, which may explain why current disease-modifying therapies predominantly targeting immunomodulatory mechanisms have a reduced efficacy against the development of permanent physical disability in the later stages of MS (Craner and Fugger 2011).

In the vast majority of MS patients, disease develops into a progressive stage in which neurological deterioration continues in the absence of relapses (Dutta and Trapp 2011). Alternatively, others are diagnosed with PPMS, in which neurological dysfunction occurs without relapses from disease onset (Dutta and Trapp 2011). In all instances, imaging, neuropathological studies, and animal studies of MS show that markers for neurodegeneration (primarily axonal damage and atrophy) appear during the progressive phase of the illness and correlate with neurological disability. Although the mechanisms of axonal degeneration are uncertain, inflammation and demyelination appear to be major risk factors (Ferguson et al. 1997; Trapp et al. 1998; reviewed in Trapp and Nave 2008). With this in mind, numerous studies have focused on axonal protection as a major therapeutic goal in MS, both promoting remyelination and analyzing different aspects of spontaneous remyelination and axonal recovery (e.g., Patrikios et al. 2006; Nikic et al. 2011).

There is also evidence suggesting a link between autoimmunity, clinical phenotype, and neurodegeneration in MS. MS patients develop antibodies to oligodendrocytes, myelin, and other neuronal antigens that cause neurodegeneration and contribute to the pathogenesis of the disease (Franciotta et al. 2008; Lee et al. 2011).

These data strongly suggests that the molecular mechanisms responsible for axonal degeneration may differ between early and late stages of MS, and that there is an unclear link between the mechanisms of neurodegeneration and focal/diffuse inflammation and their relative contribution to the clinical deficits observed in different phases of the disease.

17.3.3 Oligodendrocyte Damage and Demyelination

The depletion of oligodendrocytes is a recognized feature of MS lesions, becoming more apparent as the disease evolves (Raine 1994). In compliance with this idea, Fas expression is elevated in oligodendrocytes in chronic active and chronic silent MS lesions. Fas is a cell surface receptor that transduces cell death signals, and its upregulation in oligodendrocytes suggests that Fas-mediated signaling might contribute to immune-mediated oligodendrocyte injury and subsequent demyelination in MS (D'Souza et al. 1996). In turn, treatment of oligodendrocytes with antibodies against myelin-oligodendrocyte glycoprotein (MOG) leads to an increase in Ca^{2+} influx and activation of the MAPK/Akt pathways, a signaling cascade relevant to the initial steps of MOG-mediated demyelination (Marta et al. 2005).

A number of experimental studies have demonstrated a strong positive correlation between oligodendrocyte susceptibility to injury and the extent of CNS

inflammation in EAE. In a knockout mouse system, absence of oligodendrocyte protective factors increases oligodendrocyte susceptibility to injury and augments the inflammatory reaction and the severity of symptoms (Butzkueven et al. 2002; Balabanov et al. 2007). In contrast, mice lacking proapoptotic genes or overexpressing antiapoptotic molecules, specifically in oligodendrocytes, display resistance to EAE and inflammatory demyelination (Hisahara et al. 2000; Hövelmeyer et al. 2005). A recent study has described that the oligodendrocytic overexpression of the dominant-negative form of interferon regulatory factor-1 (IRF-1), a severity factor for both MS and EAE, results in significant protection against EAE with a reduction of inflammatory demyelination and with oligodendrocyte and axonal preservation (Ren et al. 2011). These data suggest that oligodendrocytes are actively involved both in the regulation of EAE and in the network of neuroimmune responses. Therefore, exploring oligodendrocyte-related pathogenic mechanisms in addition to conventional immune-based mechanisms may have important therapeutic implications in MS.

On the other hand, early MS lesions have a prephagocytic nature and display primary oligodendrocyte injury in the absence of microglial activation and adaptive T cell or B cell responses (Barnett and Prineas 2004; reviewed in Matute and Pérez-Cerdá 2005; Fig. 17.1). The trigger for that selective and apparently primary oligodendrocyte damage is unknown; however, it may include viruses, glutamate, ATP, and other agents known to be oligotoxic (reviewed in Matute 2011; Matute and Cavaliere 2011). These data correlate with previous pathological characterizations of MS lesions, such that some plaques are highly suggestive of a primary oligodendrocyte dystrophy rather than an autoimmunity process (Lucchinetti et al. 2000). More recent studies on autopsy material from patients in early, active stages of MS show little evidence of T cell or B cell infiltration in areas of brisk demyelination and oligodendrocyte loss; they only show macrophage infiltration and microglial activation, which is evidence of an innate immune response that is triggered to clear debris (Henderson et al. 2009).

Likewise, lack of adaptive immune response occurs in multiple system atrophy, a degenerative disorder where the main target of the disease process is the oligodendrocyte, which shows prominent secondary myelin degeneration and a reactive microgliosis (Wenning et al. 2008). In addition, inducing primary death of oligodendrocytes per se does not engender an autoimmune reaction, despite causing robust demyelination, even in the case when they induced concomitant strong stimulation of the immune system (Locatelli et al. 2012).

These findings suggest that MS lesions may initiate with oligodendrocyte death of unknown origin in the absence of inflammatory activity, and that the heterogeneity observed in the neuropathology of the lesions within and among patients may be a reflection of the time point at which a given lesion is observed. Therefore, it is possible that primary oligodendrocyte injury leads to microglial activation, with the adaptive T cell and B cell response appearing as a secondary event. In this context, the innate immune system may play a much more fundamental role than previously thought in MS lesion pathogenesis, and therapies aimed at this side of the immune response will prove effective, including in progressive stages of the disease (Fontoura and Garren 2010).

17.4 Mechanisms Involved in White Matter Damage in MS

17.4.1 Microglial Activation

In addition to changes to oligodendrocytes and neurons, microglia are also relevant to MS pathophysiology (He and Sun 2007) and these glial cells play important roles in both the destructive and restorative phases of MS. Specifically, reactive microglia may be both deleterious or protective in MS pathogenesis (Muzzio et al. 2007; Sanders and De Keyser 2007).

MS is associated with the activation of immune cells, including macrophages, peripheral blood mononuclear cells and microglia. Microglial cells originate from monocyte/macrophage precursors and are regarded as the major immunocompetent cell type of the nervous system, constituting approximately 10 % of all cells in the brain. The immune response of the brain is spatially segregated from the peripheral immune response by the blood–brain barrier and, together with astrocytes and infiltrating peripheral immune cells, is predominantly executed by microglia. Thus, microglial cells, as brain-resident immune cells, are a sensor of pathological signals in the CNS and play a major role in host defence and tissue repair in the brain. They are rapidly activated and respond with morphological changes, transforming the resting ramified microglia into an amoeboid form with phagocytic activity, proliferation, and the production of a wide array of inflammatory mediators (Kreutzberg 1996; Cuadros and Navascués 1998). Past studies have shown that exposure to different factors, such as lipopolysaccharide, interferon-γ, or β-amyloid, leads to microglial activation and induces the production of various pro-inflammatory mediators that are potentially neurotoxic (Meda et al. 1995; Zielasek and Hartung 1996). These mediators include nitric oxide, prostaglandin E2, pro-inflammatory cytokines (e.g., TNF-α, IL-1β, and IL-6), and reactive oxygen species (reviewed in Bi et al. 2011).

There is evidence that the constant activation and release of pro-inflammatory factors promotes the development of neurodegenerative diseases and therefore, microglial activation plays an important role in the pathophysiology of these diseases (Bi et al. 2011). Therefore, inhibition of pro-inflammatory mediators in microglia attenuates the severity of Alzheimer's disease, Parkinson's disease, trauma, multiple sclerosis, and cerebral ischemia (Koning et al. 2007; Krause and Müller 2010; Qian et al. 2010).

In RRMS, as well as in progressive MS, active tissue injury is associated with microglial activation (Prineas et al. 2001). Activated microglia and microglial nodules are invariably seen in the normal-appearing white matter (NAWM) of patients with progressive MS. In addition, microglial activation is also seen in many other neuroinflammatory or neurodegenerative diseases in the absence of pathological changes resembling those in MS, such as selective primary demyelination (Czeh et al. 2011). Thus, microglial activation might contribute to neurodegeneration in MS, but additional mechanisms are required to trigger patterns of tissue damage that are specific to MS. Oxidative burst by activated microglia seems to have a major role in the induction of demyelination and progressive axonal injury in MS

lesions (Lassmann et al. 2012). Furthermore, microglia might also have neuroprotective functions depending on the types and triggers of activation, and could stimulate remyelination via removal of damaged tissue and secretion of neurotrophic molecules (Czeh et al. 2011).

17.4.2 Reactive Astrocytosis

Reactive astrocytes contribute to the glial scar that fills the demyelinated plaque in MS (Holley et al. 2003), however its role in disease progression is controversial. Astrocytes can support migration, proliferation and differentiation of oligodendrocyte progenitors, and at the same time they can also promote inflammation and damage to oligodendrocytes and axons (Williams et al. 2007).

Astrocytes can be targeted by the immune reaction, as in neuromyelitis optica (NMO), a disease closely resembling MS. NMO is characterized by demyelinating lesions of the optic nerve and spinal cord that are typically more destructive than MS lesions. This disease is characterized by antibodies against an antigen of astrocytic foot processes, aquaporin 4 (AQP4) (Lennon et al. 2005; Roemer et al. 2007). Recent data indicate that anti-AQP4 antibodies from NMO patients are able to induce selective astrocyte death in vivo and, at higher dosage, demyelination and axonal damage (Bradl et al. 2009; Bennett et al. 2009; Sharma et al. 2010.

This circumstance raises the question of whether the target structure in MS is necessarily the myelin sheath or oligodendrocyte. To date, no anti-AQP4 antibodies or astrocyte depletion have been observed in MS; however, the search for the antigen is still ongoing (Vyshkina and Kalman 2008; Stadelmann et al. 2011).

17.4.3 Axonal Damage and Loss

Degeneration of demyelinated CNS axons is increasingly recognized as a common accompaniment to inflammatory demyelination. Axons may be damaged by the same underlying primary degenerative processes that affect the myelinating unit, or they might undergo secondary degeneration by virtue of demyelination.

A number of studies highlight the emerging role of disturbed axonal ion homeostasis in the process of neurodegeneration. Internodal axons express glutamate receptors (Stirling and Stys 2010), and it is possible that these could be chronically overactivated, under pathological conditions, leading to primary axonal pathology. There is solid evidence that glutamate levels are increased in the human brain (Srinivasan et al. 2005) as a consequence of altered glutamate homeostasis (Vallejo-Illarramendi et al. 2006) and thus, trigger excitotoxic destruction of oligodendrocytes and myelin as well as of axons (Matute et al 2001; Domercq et al. 2005). AMPA and kainate axonal receptor activation can induces a small amount of Ca^{2+}

entry, which in turn releases further Ca^{2+} from the axoplasmic reticulum by opening intracellular calcium channels, known as ryanodine, or through of phospholipase C activation as well as L-type Ca^{2+} channels opening (Ouardouz et al. 2009a, b). The functional significance of these signaling mechanisms by glutamate receptors in axons is unknown but they may serve to amplify axonal Ca^{2+} signals which seem to be weak because of the limited quantity of cation available in the narrow space. In turn, high local concentrations of Ca^{2+} generated by these receptors may result in focal swellings and irreversible axonal transactions (Ouardouz et al. 2009b; Stirling and Stys 2010).

In addition, aberrant expression of Na^+ channels, acid-sensing Na^+ channels, glutamate receptors and voltage-gated Ca^{2+} channels has been detected in dystrophic or demyelinated axons. Alterations in the expression and/or activity of these ion channels could directly or indirectly lead to intra-axonal Ca^{2+} accumulation and concomitant axonal degeneration. As a consequence, such ion channels could be potential targets for neuroprotective pharmacological therapies in patients with MS (Lassmann et al. 2012).

Alternatively, axonal damage in MS might be a secondary phenomenon. For example, it could be damaged by the release of glutamate, reactive oxygen or nitric oxide species, and cytotoxic cytokines from immune cells in the vicinity of inflammatory plaques (Siffrin et al. 2010; Matute 2011) or could also occur through non-inflammatory mechanisms that cause disruptions of the close physical and biochemical relationship between axons and their myelin sheaths (Stys et al. 2012). The loss of myelin greatly enhances the propensity of axons for transport disturbance (Trapp and Stys 2009). Thereby, energy production may be compromised owing to mitochondrial disruption (Mahad et al. 2009) and Na^+-K^+-ATPase-mediated ion transport may be reduced in many demyelinated axons in the MS brain (Young et al. 2008), which could bias such an axon towards a state of virtual hypoxia. The resulting mismatch between energy supply and demand could culminate in degeneration (Stys 2004; Trapp and Stys 2009).

On the other hand, neuronal antigens have recently been identified as targets of the immune reaction. Specific immune reactions against neurofilament, beta-synuclein, contactin-2/TAG-1, and neurofascin lead to CNS inflammation (reviewed in Stadelmann et al. 2011). Importantly, anti-neurofascin antibodies have been identified in a proportion of MS patients and these antibodies have been shown to aggravate axonal damage and clinical disease in the EAE model (Mathey et al. 2007).

17.4.4 Oligodendroglial Injury

17.4.4.1 Excitotoxicity and Calcium Dysregulation

Primary and/or secondary alterations in glutamate signaling cause excitotoxicity that contribute to MS pathology. Thus, numerous studies carried out in cellular and animal models of MS as well as in postmortem brain and in patients indicate that

excitotoxicity mediated by Ca^{2+}-permeable glutamate receptors contributes to oligodendrocyte death, demyelination, and tissue damage in MS.

Glutamate dyshomeostasis results from primary and/or secondary inflammation as a consequence of the autoimmune attack to the CNS and/or resulting from ongoing cell damage within the brain and spinal cord. Thus, activated microglia releases cytokines and free radicals that diminish glutamate uptake. This in turn elevates the extracellular levels of this transmitter, resulting in overactivation of Ca^{2+}-permeable glutamate receptors which lead to oligodendrocyte excitotoxicity (Sánchez-Gómez et al. 2003; Domercq et al. 2007). Moreover, activated microglia increases their expression of the glutamate-cystine exchanger which contributes further to raising the levels of glutamate and its toxicity (Domercq et al. 2007). Other mechanisms accounting for glutamate dyshomeostasis include genetic variability in the promoter of the major glutamate transporter, EAAT2, which results in lower transporter expression (Pampliega et al. 2008). Finally, an additional component of the genetic background linking MS and deregulation of glutamate signaling and Ca^{2+}-dyshomeostasis may lie in a polymorphism in the Ca^{2+}-permeable AMPA receptor subunit GluR3, an abundantly expressed subunit in oligodendrocytes, which is associated with a subgroup of patients responding to interferon beta therapy in MS (Comabella et al. 2009).

In addition to glutamate, ATP signaling can trigger oligodendrocyte excitotoxicity via activation of Ca^{2+}-permeable P2X7 purinergic receptors expressed by these cells (Matute et al. 2007). Importantly, sustained activation of P2X7 receptors in vivo causes lesions which are reminiscent of the major features of MS plaques, and treatment with P2X7 antagonists of chronic EAE reduces demyelination and ameliorates the associated neurological symptoms. These results are in line with data in P2X7 null mice showing that this deficiency suppresses the development of EAE (Sharp et al. 2008), and at odds with earlier observations indicating that the lack of P2X7 receptors aggravates EAE (Chen and Brosnan 2006). These apparent discrepancies may be caused by the different strains of P2X7 KO mice used, and can be explained by compensatory mechanisms developed in the genetically modified animals during development and maturation. In this regard, pharmacological blockade of P2X7 receptors with selective antagonists after disease onset is probably a more relevant approach to study the importance of these receptors to MS than the use of P2X7 null mice.

In addition, P2X7 RNA and protein levels are elevated in normal appearing axon tracts in MS patients, suggesting that signaling through P2X7 receptors in oligodendroglia is enhanced in this disease which may render this cell type more vulnerable to ATP dysregulation (Matute et al. 2007). The increased expression of P2X7 receptors in axon tracts before lesions are formed indicates that this feature may constitute a risk factor associated with newly forming lesions in MS; this receptor subunit may thus prove to be a diagnostic and/or prognostic clinical biomarker for MS. On the other hand, blockade of P2X7 receptors protects oligodendrocyte from dying; this property has therapeutic potential for halting the progression of tissue damage in MS (Matute and Cavaliere 2011).

17.4.4.2 Mitochondrial Damage and Oxidative Stress

Current attention in the MS research is focused on the role of mitochondrial injury in demyelination and neurodegeneration (Trapp and Stys 2009; Witte et al. 2010). Mitochondrial injury induced by oxidative stress might underlie the pathological features of MS lesions, such as oligodendrocyte apoptosis, demyelination, destruction of thin-caliber axons, and lack of remyelination (Lassmann et al. 2012).

Evidence for mitochondrial damage in MS lesions was originally identified from biochemical analyses of impaired activity of mitochondrial enzymes in chronic active lesions in MS (Lu et al. 2000). Subsequent detailed immunohistochemical investigations of respiratory chain proteins revealed profound mitochondrial injury, possibly reflecting increased oxidative damage in areas of initial tissue injury within active MS lesions (Mahad et al. 2008). In general, the data indicate that active tissue damage in MS and key features of the pathology of MS lesions can be explained as a result of mitochondrial injury and oxidative stress.

In oligodendrocytes, mitochondrial injury results in the generation of oxygen free radicals and release of apoptotic factors leading activation of caspase-dependent death pathways (Sánchez-Gómez et al. 2003). In addition, dysfunction mitochondrial triggers release of apoptosis-inducing factor, its translocation into the nuclei, and activation of poly-ADP-ribose polymerase (PARP), a mechanism demonstrated in vivo in the experimental model of oligodendrocyte destruction and demyelination induced by cuprizone intoxication (Veto et al. 2010). Furthermore, in vitro oligodendrocyte progenitor cells are more resistant to mitochondrial injury than are mature oligodendrocytes, but are impaired in their ability to differentiate and form myelin sheaths (Ziabreva et al. 2010). This observation could explain the failure of remyelination in chronic MS plaques, despite the presence of oligodendrocyte progenitor cells (Chang et al. 2002).

The relationship between mitochondrial alterations and oxidative stress is complex. Mitochondrial dysfunction results in generation of free radical and conversely, oxidative stress also drives mitochondrial dysfunction by several different mechanisms. Free radicals disrupt mitochondrial enzyme function, modify mitochondrial proteins and accelerate their degradation, interfere with de novo synthesis of respiratory chain components, and can directly induce mitochondrial DNA damage (reviewed in Lassmann et al. 2012). In active MS lesions, the expression of enzymes involved in free radical production is markedly increased, most prominently in areas of initial tissue injury, and oxidized DNA and lipids are abundantly present (Van Horssen et al. 2011; Fischer et al. 2012). The signs of oxidative stress in MS lesions are concomitant with the upregulation of proteins involved in antioxidant defence mechanisms (van Horssen et al. 2010) and might explain the high levels of expression of molecules associated with endoplasmic reticulum stress, such as CHOP or BiP (Cunnea et al. 2011).

Oxidized DNA and lipids in apoptotic oligodendrocytes and dystrophic axons strongly supports the contribution of these alterations to demyelination and neurodegeneration (Haider et al. 2011). Moreover, it is known that iron accumulates in

the aging human brain where it is predominantly stored in oligodendrocytes and detoxified by its binding to ferritin. As shown in vitro, intracytoplasmic accumulation of Fe^{2+} in oligodendrocytes might in part explain the high susceptibility of these cells to degeneration under conditions of oxidative stress induced by inflammation and mitochondrial dysfunction (Zhang et al. 2005). Importantly, oligodendrocyte destruction releases accumulated Fe^{2+} into the extracellular space and, its uptake into cells within the lesions might further amplify oxidative damage and increase the susceptibility of the surrounding tissue to free-radical-driven demyelination and neurodegeneration.

17.5 Neuropathology of MS

Common histopathological hallmarks of MS is the presence of multifocal areas of inflammatory demyelination and axonal loss distributed over time and space within the brain and spinal cord white and gray matter. Although demyelination in MS is largely restricted to focal lesions, other aspects of its pathology are less confined (Prineas et al. 2002). Whether inflammatory demyelination is primary or secondary in the disease progression remains controversial (Trapp and Nave 2008). This is due to the difficulty of obtaining appropriate MS specimens to evaluate the temporal course of each individual lesion.

Classical active MS lesions are described as perivascular inflammatory infiltrates, consisting mainly of T cells and macrophages, together with myelin breakdown and degeneration of axons. These features led to a pathophysiological view in which the disease trigger is an immune dysregulation (Hu and Lucchinetti 2009; Lassmann 2011). However, recent neuropathological studies have provided evidence of primary oligodendropathy as a cause of demyelination (Barnett and Prineas 2004; Prineas and Parrat 2012). If this were the common mechanism of MS, the disorder would be regarded as a primarily degenerative disorder rather than an autoimmune disease (Nakahara et al. 2010; Stys et al. 2012).

17.5.1 Focal Lesions and Diffuse Damage in MS

Focal plaques of demyelination in white matter are the diagnostic hallmark of MS pathology at all stages of the disease. They are typically classified into four categories: classic active lesions, slowly expanding lesions, inactive lesions and remyelinated shadow plaques (Prineas et al. 2002). In addition, the so called NAWM may show some pathological changes (Allen et al. 2001), and fine evaluation of myelin in gray matter areas resulted in different types of cortical pathologies in MS brains (Kutzelnigg et al. 2005).

17.5.1.1 Classical Active Lesions

These lesions are the most abundantly found in patients with acute and relapsing disease. In contrast, they become rare during the progressive stage of the disease (Prineas et al. 2002; Lassmann 2011; Lassmann et al. 2012). Active MS lesions display activated macrophages which contain remnants of myelin sheaths taken up during the demyelinating process, reactive astrocytes forming a glial scar intermixed with variable T cell (both $CD4^+$ and $CD8^+$) and B cell perivascular and parenchymal infiltrates, and blood–brain barrier breakage. Axonal injury characterized by transections and swellings is also pronounced, and its extent correlates with the number of lymphocytes (Trapp and Nave 2008). In this highly inflammatory environment, macrophages can be densely packed either throughout the lesion (acute plaques) or at the periphery (chronic active plaques).

The architecture of a chronic active plaque varies with the different temporal developmental sequence of injury from the center (less recent) to the edges (more recent) where the lesions expand. Thus, lesions commonly have an onion-like shape with a layer of initial tissue injury ("prephagocytic" area characterized by activated microglia) surrounding a region of early myelin phagocytosis (early active), a zone of advanced myelin digestion (late active) and an inactive central area which often shows early but unstable remyelination while inflammation is active (Lassmann et al. 2012). Early and late active demyelination can be distinguished by the presence of minor myelin proteins (MOG and myelin-associated glycoprotein) or major myelin proteins including myelin basic protein and proteolipid protein as degradation products within macrophages (Hu and Lucchinetti 2009). Most probably, chronic active lesions recapitulate events encountered in acute lesions. However, the initial stages of MS lesion formation are not well characterized as a consequence of the limited availability of human samples with recent new lesions.

17.5.1.2 Slowly Expanding Lesions

This lesion type accounts for approximately half of the lesions in progressive MS. They probably reflect a gradual expansion of preexisting lesions in the absence of major blood–brain barrier disturbance (Prineas et al. 2002; Lassmann 2011). These lesions display at their edge a narrow zone of robustly activated microglia with prominent acute axonal injury and macrophages containing early myelin degradation products. In addition, they are surrounded by diffusely activated microglia, and their inner part shows a dense astrocytic scar, profound axonal loss, absence of myelin without signs of remyelination, and nearly complete loss of oligodendrocytes, macrophages and microglia. Little is known about the mechanisms leading to cell extinction in the core of these lesions. It is conceivable that the absence of inflammatory reaction may limit the local capacity of restoring damaged myelin (Gay 2007), since phagocytes support initiating proliferation and differentiation of oligodendrocyte progenitor cells (Zhao et al. 2005).

17.5.1.3 Inactive Lesions

Inactive lesions are the most frequent lesion type found at all stages of MS (Prineas et al. 2002). In particular, this type of lesion accounts for the great majority of plaques in patients with long-standing disease. They are hypocellular in nature, have a glial population composed largely of small fibrous astrocytes, are clearly demarcated from the surrounding NAWM, and display no demyelinating or neurodegenerative activity at the lesion border. The core of these lesions is devoid of myelin and oligodendrocytes and has no signs of remyelination. Usually axon densities are very reduced and embedded in astrocytic scar tissue; and infiltrates of T or B lymphocytes are rare. In addition, the appearance of microglial cells changes from a few ramified cells inside the lesion to a densely packed population of ramified cells immediately outside the plaque (Lassmann et al. 2012).

17.5.1.4 Remyelinated Shadow Plaques

This is a term traditionally used in MS for any extensive area of partial reduction in myelin density (Prineas et al. 2002). While many shadow plaques show stable remyelination and inflammation is reduced practically to control levels (Patrikios et al. 2006), others become the site of fresh activity, with new lesions forming within or overlapping previously remyelinated tissue (Prineas et al. 2002).

17.5.1.5 Normal-Appearing White Matter

Although demyelination in MS is largely restricted to focal lesions, other aspects of pathology are less confined. Thus, NAWM has been defined pathologically as macroscopically normal white matter at least 1 cm away from a plaque edge with histological abnormalities. They include astrogliosis, microglial activation, vascular hyalinization, blood–brain barrier disturbances, mild inflammation with little T cell infiltration, reduced myelin density, remyelination, axonal loss and damage, as well as microplaque formation (Allen et al. 2001; Kutzelnigg et al. 2005; Hu and Lucchinetti 2009). Interestingly, diffuse NAWM microglial activation and axonal injury are most prominent in patients with PPMS or SPMS. Moreover, the extent of NAWM pathology correlates with the extent of cortical lesions but not with focal white matter load. Indeed, if microglial activation represents early pathogenic signs in preactive lesions, microglia may play a more relevant role in the development of lesions than inflammatory cell infiltrates (Gay 2007).

17.5.1.6 Gray Matter Lesions

With the introduction of new and sensitive methods for staining myelin, it is apparent that gray matter lesions, and particularly cortical demyelination, is extensively

and significantly involved in MS pathology (Kutzelnigg et al. 2005; Trapp and Nave 2008; Hu and Lucchinetti 2009; Dutta and Trapp 2011; Lassmann 2011). Compared to white matter lesions, cortical lesions contain less blood–brain barrier breakdown, few lymphocytic and macrophage infiltration, and more efficient myelin repair, being ramified microglia the dominant population. Despite limited inflammation, demyelination is present and neuronal and axonal pathology (neuritic transection, neuronal apoptosis, synaptic loss) are prominent features of cortical lesions, questioning the basic premises of MS pathogenesis. Typically cortical lesions appear as contiguously leukocortical areas of demyelination (classified as type I lesions) or strips or bands of subpial demyelination (type III lesions) that can not be considered as focal lesions. In addition but less frequently, small perivascular areas of demyelination can be seen (type II lesions). While type I and II lesions are found at all stages of MS, including acute and relapsing MS, type III lesions are mainly seen in patients with progressive disease. In addition, cortical demyelination correlates in part with diffuse white matter but not with focal white matter lesions.

17.6 Neuroimaging of MS

Although pathological assessment is the gold standard to identify MS lesions, there are intrinsic limitations owing to the very limited availability of biopsy tissue and additionally, tissue evaluation only provides one snapshot in time, not allowing observation of the evolution of pathological changes over time. Because of that, MRI and related techniques with higher specificity are promising for a better understanding of the pathophysiology of the MS "in vivo" (Polman et al. 2011).

On conventional T2-weighted MRI images, MS lesions appear as nonspecific focal areas of signal increase and therefore, may resemble by themselves and in absence of clinical information, many other types of pathology. In MS, hyperintense signals result from confluent perivenular lesions. In contrast, T1-weighted images show less sensitivity but more specificity to MS pathology and probably T1 hypointense signals are related to edema, demyelination, axonal loss and gliosis (Filippi et al. 2012). The sensitivity of lesion detection in T1-weighted imaging can be increased selectively with the injection of gadolinium-diethylenetriaminepentacetate (Gd-DTPA), a quelated form of Gd. Normally, Gd-DTPA in serum does not cross the blood–brain barrier, so T1-weighted images obtained after its administration allow the MS enhancing lesions that have a blood–brain barrier breakdown associated with ongoing inflammation to be identified (Kermode et al. 1990). The use of Gd-DTPA is a very sensitive and reproducible way to assess MS activity in focal white matter lesions. In fact, using conventional MRI techniques is possible to distinguish between active, acute or chronic, and inactive lesions. However, an underestimation of the degree of disease activity must be taken in mind because a relatively large proportion of MS lesions have very short-lived enhancement (Cotton et al. 2003). Importantly, enhancement is probably absent in slowly spanding lesions (Hochmeister et al. 2006).

Conventional MRI approaches are basically unable to detect tissue damage in the so-called NAWM in MS characterized by diffuse microglial activation and axonal injury. Some recent techniques that are far away of being integrated into routine clinical practice at present, have shown initial correlation between dysfunction and imaging that still needs future research and validation (Moll et al. 2011; Filippi et al. 2012; Rocca et al. 2012). These include magnetization transfer (MT) imaging and its quantitative index MT ratio, diffusion tensor MRI tractography and proton magnetic resonance spectroscopy.

17.7 Conclusions

MS is a complex and heterogeneous disease with an ill defined etiology. Although it is thought that damage in MS occurs primarily in CNS myelin and in oligodendrocytes, injury to axons, and ultimately to neurons, determines disease progression. In addition, autoimmunity and neuroinflammation are crucial to fluctuating neurological deficits in RRMS and in accumulating CNS injury.

MS has been traditionally considered a white matter disease in which lesions in plaques are the major pathological hallmark. However, closer inspection of the pathology has recently revealed that neurons and synapses are also deeply altered, and that profound diffuse damage is present in normally-appearing white matter. Moreover, gray matter lesions are also present often in MS.

A key unanswered question to MS etiology is whether the disease initiates by cytodegeneration or by a primary autoimmune attack. This point is critical to drug development, since most strategies to find new treatments have relied on the use of EAE as an auto-immunity animal model of disease. As a consequence, most therapeutic agents of clinical application are anti-inflammatory in nature. Therefore, alternative disease models reproducing the neurodegenerative processes underlying MS are needed to advance in its understanding and treatment.

Acknowledgments Work in our laboratory is supported by CIBERNED and by grants from the Ministerio de Ciencia e Innovación, Gobierno Vasco, Ikerbasque and the Universidad del País Vasco.

References

Allen IV, McQuaid S, Mirakhur M, Nevin G (2001) Pathological abnormalities in the normal-appearing white matter in multiple sclerosis. Neurol Sci 22:141–144

Alonso A, Hernan MA (2008) Temporal trends in the incidence of multiple sclerosis: a systematic review. Neurology 71:129–135

Balabanov R, Strand K, Goswami R, McMahon E, Begolka W, Miller SD, Popko B (2007) Interferon-gamma-oligodendrocyte interactions in the regulation of experimental autoimmune encephalomyelitis. J Neurosci 27:2013–2024

Barnett MH, Prineas JW (2004) Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. Ann Neurol 55:458–468

Bennett JL, Lam C, Kalluri SR, Saikali P, Bautista K, Dupree C, Glogowska M, Case D, Antel JP, Owens GP, Gilden D, Nessler S, Stadelmann C, Hemmer B (2009) Intrathecal pathogenic anti-aquaporin-4 antibodies in early neuromyelitis optica. Ann Neurol 66:617–629

Bi W, Zhu L, Wang C, Liang Y, Liu J, Shi Q, Tao E (2011) Rifampicin inhibits microglial inflammation and improves neuron survival against inflammation. Brain Res 1995:12–20

Bo L, Vedeler CA, Nyland HI, Trapp BD, Mork SJ (2003) Subpial demyelination in the cerebral cortex of multiple sclerosis patients. J Neuropathol Exp Neurol 62:723–732

Bradl M, Misu T, Takahashi T, Watanabe M, Mader S, Reindl M, Adzemovic M, Bauer J, Berger T, Fujihara K, Itoyama Y, Lassmann H (2009) Neuromyelitis optica: pathogenicity of patient immunoglobulin in vivo. Ann Neurol 66:630–643

Brown DA, Sawchenko PE (2007) Time course and distribution of inflammatory and neurodegenerative events suggest structural bases for the pathogenesis of experimental autoimmune encephalomyelitis. J Comp Neurol 502:236–260

Butzkueven H, Zhang JG, Soilu-Hanninen M, Hochrein H, Chionh F, Shipham KA, Emery B, Turnley AM, Petratos S, Ernst M et al (2002) LIF receptor signaling limits immune mediated demyelination by enhancing oligodendrocyte survival. Nat Med 8:613–619

Chang A, Tourtelotte WW, Rudick RA, Trapp BD (2002) Premyelinating oligodendrocytes in chronic lesions of multiple sclerosis. N Engl J Med 346:165–200

Chen L, Brosnan CF (2006) Exacerbation of experimental autoimmune encephalomyelitis in P2X7R−/− mice: evidence for loss of apoptotic activity in lymphocytes. J Immunol 176: 3115–3126

Comabella M, Craig DW, Morcillo-Suárez C, Río J, Navarro A, Fernández M, Martín R, Montalban X (2009) Genome-wide scan of 500,000 single-nucleotide polymorphisms among responders and nonresponders to interferon beta therapy in multiple sclerosis. Arch Neurol 66:972–978

Cotton F, Weiner HL, Jolesz FA, Guttmann CR (2003) MRI contrast uptake in new lesions in relapsing-remitting MS followed at weekly intervals. Neurology 60:640–646

Craner MJ, Fugger L (2011) Axonal injury in reverse. Nat Med 17:423–425

Cuadros MA, Navascués J (1998) The origin and differentiation of microglial cells during development. Prog Neurobiol 56:173–189

Cunnea P, Mháille AN, McQuaid S, Farrell M, McMahon J, FitzGerald U (2011) Expression profiles of endoplasmic reticulum stress-related molecules in demyelinating lesions and multiple sclerosis. Mult Scler 17:808–818

Czeh M, Gressens P, Kaindl AM (2011) The yin and yang of microglia. Dev Neurosci 33:199–201

D'Souza SD, Bonetti B, Balasingam V, Cashman NR, Barker PA, Troutt AB, Raine CS, Antel JP (1996) Multiple sclerosis: Fas signaling in oligodendrocyte cell death. J Exp Med 184: 2361–2370

Domercq M, Etxebarria E, Pérez-Samartín A, Matute C (2005) Excitotoxic oligodendrocyte death and axonal damage induced by glutamate transporter inhibition. Glia 52:36–46

Domercq M, Sánchez-Gómez MV, Sherwin C, Etxebarria E, Fern R, Matute C (2007) System xc- and glutamate transporter inhibition mediates microglial toxicity to oligodendrocytes. J Immunol 178:6549–6556

Dutta R, Trapp BD (2011) Mechanisms of neuronal dysfunction and degeneration in multiple sclerosis. Prog Neurobiol 93:1–12

Edan G, Leray E (2010) A new treatment era in multiple sclerosis: clinical applications of new concepts. J Neurol Sci 30:170–172

Ferguson B, Matyszak MK, Esiri MM, Perry VH (1997) Axonal damage in acute multiple sclerosis lesions. Brain 120:393–399

Filippi M, Rocca MA, Barkhof F, Brück W, Chen JT, Comi G, DeLuca G, DeStefano N, Erickson BJ, Evangelou N, Fazekas F, Geurts JJG, Lucchinetti C, Miller DH, Pelletier D, Popescu BFG, Lassmann H (2012) Association between pathological and MRI findings in multiple sclerosis. Lancet Neurol 11:349–359

Fischer MT, Sharma R, Lim JL, Haider L, Frischer JM, Drexhage J, Mahad D, Bradl M, van Horssen J, Lassmann H (2012) NADPH oxidase expression in active multiple sclerosis lesions in relation to oxidative tissue damage and mitochondrial injury brain. Brain 135:886–899

Fontoura P, Garren H (2010) Molecular basis of multiple sclerosis. In: Martin R, Lutterotti A (eds) Results and problems in cell differentiation, vol 51. Springer, Berlin, pp 259–285

Franciotta D, Salvetti M, Lolli F, Serafini B, Aloisi F (2008) B cells and multiple sclerosis. Lancet Neurol 7:852–858

Frischer JM, Bramow S, Dal-Bianco A, Lucchinetti CF, Rauschka H, Schmidbauer M, Laursen H, Sorensen PS, Lassmann H (2009) The relation between inflammation and neurodegeneration in multiple sclerosis brains. Brain 132:1175–1189

Frohman EM, Racke MK, Raine CS (2006) Multiple sclerosis—the plaque and its pathogenesis. N Engl J Med 354:942–955

Gay F (2007) Activated microglia in primary MS lesions: defenders or aggressors. Int MS J 14:78–83

Giovannoni G, Ebers G (2007) Multiple sclerosis: the environment and causation. Curr Opin Neurol 20:261–268

Hafler DA, Compston A, Sawcer S, Lander ES, Daly MJ, De Jager PL, de Bakker PI et al; International Multiple Sclerosis Genetics Consortium (2007) Risk alleles for multiple sclerosis identified by a genome wide study. N Engl J Med 357:851–862

Haider L, Fischer MT, Frischer JM, Bauer J, Höftberger R, Botond G, Esterbauer H, Binder CJ, Witztum JL, Lassmann H (2011) Oxidative damage in multiple sclerosis lesions. Brain 134:1914–1924

He F, Sun YE (2007) Glial cells more than support cells? Int J Biochem Cell Biol 39:661–666

Hemmer B, Archelos JJ, Hartung HP (2002) New concepts in the immunopathogenesis of multiple sclerosis. Nat Rev Neurosci 3:291–301

Henderson AP, Barnett MH, Parratt JD, Prineas JW (2009) Multiple sclerosis: distribution of inflammatory cells in newly forming lesions. Ann Neurol 66:739–753

Hisahara S, Araki T, Sugiyama F, Yagami K, Suzuki M, Abe K, Yamamura K, Miyazaki J, Momoi T, Saruta T, Bernard CC, Okano H, Miura M (2000) Targeted expression of baculovirus p35 caspase inhibitor in oligodendrocytes protects mice against autoimmune-mediated demyelination. EMBO J 19:341–348

Hochmeister S, Grundtner R, Bauer J, Engelhardt B, Lyck R, Gordon G, Korosec T, Kutzelnigg A, Berger JJ, Bradl M, Bittner RE, Lassmann H (2006) Dysferlin is a new marker for leaky brain blood vessels in multiple sclerosis. J Neuropathol Exp Neurol 65:855–865

Holley JE, Gveric D, Newcombe J, Cuzner ML, Gutowski NJ (2003) Astrocyte characterization in the multiple sclerosis glial scar. Neuropathol Appl Neurobiol 29:434–444

Hövelmeyer N, Hao Z, Kranidioti K, Kassiotis G, Buch T, Frommer F, von Hoch L, Kramer D, Minichiello L, Kollias G, Lassmann H, Waisman A (2005) Apoptosis of oligodendrocytes via Fas and TNF-R1 is a key event in the induction of experimental autoimmune encephalomyelitis. J Immunol 175:5875–5884

Hu W, Lucchinetti CF (2009) The pathological spectrum of CNS inflammatory demyelinating diseases. Semin Immunopathol 31:439–453

Keegan BM, Noseworthy JH (2002) Multiple sclerosis. Annu Rev Med 53:285–302

Kermode AG, Thompson AJ, Tofts P, MacManus DG, Kendall BE, Kingsley DP, Moseley IF, Rudge P, McDonald WI (1990) Breakdown of the blood-brain barrier precedes symptoms and other MRI signs of new lesions in multiple sclerosis. Pathogenetic and clinical implications. Brain 113:1477–1489

Kerschensteiner M, Meinl E, Hohlfeld R (2009) Neuro-immune crosstalk in CNS diseases. Neuroscience 158:1122–1132

Koning N, Bö L, Hoek RM, Huitinga I (2007) Downregulation of macrophage inhibitory molecules in multiple sclerosis lesions. Ann Neurol 62:504–514

Krause DL, Müller N (2010) Neuroinflammation, microglia and implications for anti-inflammatory treatment in Alzheimer's disease. Int J Alzheimer Dis 2010:9. doi:10.4061/2010/732806

Kreutzberg GW (1996) Microglia: a sensor for pathological events in the CNS. Trends Neurosci 19:312–318

Kutzelnigg A, Lucchinetti CF, Stadelmann C, Brück W, Rauschka H, Bergmann M, Schmidbauer M, Parisi JE, Lassmann H (2005) Cortical demyelination and diffuse white matter injury in multiple sclerosis. Brain 128:2705–2712

Lassmann H (2007) Cortical, subcortical and spinal alterations in neuroimmunological diseases. J Neurol 254:15–17

Lassmann H (2011) The architecture of inflammatory demyelinating lesions: implications for studies on pathogenesis. Neuropathol Appl Neurobiol 37:698–710

Lassmann H, VanHorssen J, Mahad D (2012) Progressive multiple sclerosis: pathology and pathogenesis. Nat Rev Neurol 8:647–656

Lee LS, Xu L, Shin Y, Gardner L, Hartzes A, Dohan FC, Raine C, Homayouni R, Levin MC (2011) A potential link between autoimmunity and neurodegeneration in immune-mediated neurological disease. J Neuroimmunol 235:56–69

Lennon VA, Kryzer TJ, Pittock SJ, Verkman AS, Hinson SR (2005) IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. J Exp Med 202:473–477

Leray E, Yaouanq J, Le Page E, Coustans M, Laplaud D, Oger J, Edan G (2010) Evidence for a two-stage disability progression in multiple sclerosis. Brain 133:1900–1913

Lincoln MR, Monpetit A, Cader MZ, Saarela J, Dyment DA, Tiislar M, Fereti V, Tienari PJ, Sadovnick AD, Peltonen L, Ebers GC, Hudson TJ (2005) A predominant role for the HLA class II region in the association of the MHC region with multiple sclerosis. Nat Genet 37:1108–1112

Locatelli G, Wörtge S, Buch T, Ingold B, Frommer F, Sobottka B, Krüger M, Karram K, Bühlmann C, Bechmann I, Heppner FL, Waisman A, Becher B (2012) Primary oligodendrocyte death does not elicit anti-CNS immunity. Nat Neurosci 15:543–550

Lu F, Selak M, O'Connor J, Croul S, Lorenzana C, Butunoi C, Kalman B (2000) Oxidative damage to mitochondrial DNA and activity of mitochondrial enzymes in chronic active lesions of multiple sclerosis. J Neurol Sci 177:95–103

Lublin FD, Reingold SC (1996) Defining the clinical course of multiple sclerosis: results of an international survey. Neurology 46:907–911

Lucchinetti C, Brück W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H (1999) A quantitative analysis of oligodendrocytes in multiple sclerosis lesions. Brain 122:2279–2295

Lucchinetti C, Brück W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H (2000) Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. Ann Neurol 47:707–717

Lundmark F, Duvefelt K, Iacobaeus E, Kockum I, Wallstrom E, Khademi M, Oturai A, Ryder LP, Saarela J, Harbo HF, Celius EG, Salter H, Olsson T, Hillert J (2007) Variation in interleukin 7 receptor alpha chain (IL7R) influences risk of multiple sclerosis. Nat Genet 39:1108–1113

Mahad D, Ziabreva I, Lassmann H, Turnbull D (2008) Mitochondrial defects in acute multiple sclerosis lesions. Brain 131:1722–1735

Mahad D, Ziabreva I, Campbell G, Lax N, White K, Hanson PS, Lassmann H, Turnbull DM (2009) Mitochondrial changes within axons in multiple sclerosis. Brain 132:1161–1174

Marta CB, Montano MB, Taylor CM, Taylor AL, Bansal R, Pfeiffer SE (2005) Signaling cascades activated upon antibody cross-linking of myelin oligodendrocyte glycoprotein: potential implications for multiple sclerosis. J Biol Chem 280:8985–8993

Mathey EK, Derfuss T, Storch MK, Williams KR, Hales K, Woolley DR, Al- Hayani A, Davies SN, Rasband MN, Olsson T, Moldenhauer A, Velhin S, Hohlfeld R, Meinl E, Linington C (2007) Neurofascin as a novel target for autoantibody-mediated axonal injury. J Exp Med 204:2363–2372

Matute C (2011) Glutamate and ATP signalling in white matter pathology. J Anat 219:53–64

Matute C, Cavaliere F (2011) Neuroglial interactions mediated by purinergic signalling in the pathophysiology of CNS disorders. Semin Cell Dev Biol 22:252–259

Matute C, Pérez-Cerdá F (2005) Multiple sclerosis: novel perspectives on newly forming lesions. Trends Neurosci 28:173–175

Matute C, Alberdi E, Domercq M, Pérez-Cerdá F, Pérez-Samartín A, Sánchez-Gómez MV (2001) The link between excitotoxic oligodendroglial death and demyelinating diseases. Trends Neurosci 24:224–230

Matute C, Torre I, Pérez-Cerdá F, Pérez-Samartín A, Alberdi E, Etxebarria E, Arranz AM, Rodríguez-Antigüedad A, Sánchez- Gómez MV, Domercq M (2007) P2X7 receptor blockade prevents ATP excitotoxicity in oligodendrocytes and ameliorates experimental autoimmune encephalomyelitis. J Neurosci 7:9525–9533

Meda L, Cassatella MA, Szendrei GI, Otvos L Jr, Baron P, Villalba M (1995) Activation of microglial cells by β-amyloid protein and interferon-γ. Nature 373:647–650

Moll NM, Rietsch AM, Thomas S, Ransohoff AJ, Lee J-C, Fox R, Chang A, Ransohoff RM, Fisher E (2011) Multiple sclerosis normal-appearing white matter: pathology-imaging correlations. Ann Neurol 70:764–773

Muzzio L, Martino G, Furlan R (2007) Multifaceted aspects of inflammation in multiple sclerosis: the role of microglia. J Neuroimmunol 191:39–44

Nakahara J, Aiso S, Suzuki N (2010) Autoimmune versus oligodendrogliopathy: the pathogenesis of multiple sclerosis. Arch Immunol Ther Exp 58:325–333

Nikic I, Merkler D, Sorbara C, Brinkoetter M, Kreutzfeldt M, Bareyre FM, Brück W, Bishop D, Misgeld T, Kerschensteiner M (2011) A reversible form of axon damage in experimental autoimmune encephalomyelitis and multiple sclerosis. Nat Med 17:495–499

Ouardouz M, Coderre E, Basak A, Chen A, Zamponi GW, Hameed S, Rehak R, Yin X, Trapp BD, Stys PK (2009a) Glutamate receptors on myelinated spinal cord axons. I. GluR6 kainate receptors. Ann Neurol 65:151–159

Ouardouz M, Coderre E, Zamponi GW, Hameed S, Yin X, Trapp BD, Stys PK (2009b) Glutamate receptors on myelinated spinal cord axons. II. AMPA and GluR5 receptors. Ann Neurol 65:160–166

Pampliega O, Domercq M, Villoslada P, Sepulcre J, Rodriguez-Antiguedad A, Matute C (2008) Association of an EAAT2 polymorphism with higher glutamate concentration in relapsing multiple sclerosis. J Neuroimmunol 195:194–198

Patrikios P, Stadelmann C, Kutzelnigg A, Rauschka H, Schmidbauer M, Laursen H, Sorensen PS, Bruck W, Lucchinetti C, Lassmann H (2006) Remyelination is extensive in a subset of multiple sclerosis patients. Brain 129:3165–3172

Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, Fujihara K, Havrdova E, Hutchinson M, Kappos L, Lublin FD, Montalban X, O'Connor P, Sandberg-Wollheim M, Thompson AJ, Waubant E, Weinshenker B, Wolinsky JS (2011) Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald Criteria. Ann Neurol 69:292–302

Prineas JW, Parrat JDE (2012) Oligodendrocytes and the early multiple sclerosis lesion. Ann Neurol 72:18–31

Prineas JW, Kwon EE, Cho ES, Sharer LR, Barnett MH, Oleszak EL, Hoffman B, Morgan BP (2001) Immunopathology of secondary-progressive multiple sclerosis. Ann Neurol 50: 646–657

Prineas JW, McDonald WI, Franklin RJM (2002) Demyelinating diseases. In: Graham DI, Lantos PL (eds) Greenfield's neuropathology, vol 2. Arnold, London

Qian L, Flood PM, Hong JS (2010) Neuroinflammation is a key player in Parkinson's disease and a prime target for therapy. J Neural Transm 117:971–979

Raine CS (1994) The Dale E. McFarlin Memorial Lecture: the immunology of the multiple sclerosis lesion. Ann Neurol 36:561–572

Ren Z, Wang Y, Tao D, Liebenson D, Liggett T, Goswami R, Clarke R, Stefoski D, Balabanov R (2011) Overexpression of the dominant-negative form of interferon regulatory factor 1 in oligodendrocytes protects against experimental autoimmune encephalomyelitis. J Neurosci 31:8329–8341

Rocca MA, Absinta M, Filippi M (2012) The role of advanced magnetic resonance imaging techniques in primary progressive MS. J Neurol 259:611–621

Roemer SF, Parisi JE, Lennon VA, Benarroch EE, Lassmann H, Bruck W, Mandler RN, Weinshenker BG, Pittock SJ, Wingerchuk DM, Lucchinetti CF (2007) Pattern-specific loss of aquaporin-4 immunoreactivity distinguishes neuromyelitis optica from multiple sclerosis. Brain 130:1194–1205

Rovaris M, Confavreux C, Furlan R, Kappos L, Comi G, Filippi M (2006) Secondary progressive multiple sclerosis: current knowledge and future challenges. Lancet Neurol 5:343–354

Sánchez-Gómez MV, Alberdi E, Ibarretxe G, Torre I, Matute C (2003) Caspase-dependent and caspase-independent oligodendrocyte death mediated by AMPA and kainate receptors. J Neurosci 23:9519–9528

Sanders P, De Keyser J (2007) Janus faces of microglia in multiple sclerosis. Brain Res Rev 54:274–285

Sawcer S, Ban M, Maranian M, Yeo TW, Compston A, Kirby A, Daly MJ et al; International Multiple Sclerosis Genetics Consortium (2005) A high-density screen for linkage in multiple sclerosis. Am J Hum Genet 77:454–467

Scalfari A, Neuhaus A, Degenhardt A, Rice GP, Muraro PA, Daumer M, Ebers GC (2010) The natural history of multiple sclerosis: a geographically based study 10: relapses and long-term disability. Brain 133:1914–1929

Sharma R, Fischer MT, Bauer J, Felts PA, Smith KJ, Misu T, Fujihara K, Bradl M, Lassmann H (2010) Inflammation induced by innate immunity in the central nervous system leads to primary astrocyte dysfunction followed by demyelination. Acta Neuropathol 120:223–236

Sharp AJ, Polak PE, Simonini V, Lin SX, Richardson JC, Bongarzone ER, Feinstein DL (2008) P2X7 deficiency suppresses development of experimental autoimmune encephalomyelitis. J Neuroinflammation 5:33. doi:10.1186/1742-2094-5-33

Siffrin V, Vogt J, Radbruch H, Nitsch R, Zipp F (2010) Multiple sclerosis—candidate mechanisms underlying CNS atrophy. Trends Neurosci 33:202–210

Srinivasan R, Sailasuta N, Hurd R, Nelson S, Pelletier D (2005) Evidence of elevated glutamate in multiple sclerosis using magnetic resonance spectroscopy at 3 T. Brain 128:1016–1025

Stadelmann C, Albert M, Wegner C, Bruck W (2008) Cortical pathology in multiple sclerosis. Curr Opin Neurol 21:229–234

Stadelmann C, Wegner C, Brück W (2011) Inflammation, demyelination, and degeneration—recent insights from MS pathology. Biochim Biophys Acta 1812:275–282

Stirling DP, Stys PK (2010) Mechanisms of axonal injury: internodal nanocomplexes and calcium deregulation. Trends Mol Med 16:160–170

Stys PK (2004) Axonal degeneration in MS: is it time for neuroprotective strategies? Ann Neurol 55:601–603

Stys PK, Zamponi GW, van Minnen J, Geurts JJG (2012) Will the real multiple sclerosis please stand up? Nat Rev Neurosci 7:507–514

Thompson AJ, Polman CH, Miller DH, McDonald WI, Brochet B, Filipi M, Montalban X, De Sá J (1997) Primary progressive multiple sclerosis. Brain 120:1085–1096

Trapp BD, Nave KA (2008) Multiple sclerosis: an immune or neurodegenerative disorder? Annu Rev Neurosci 31:247–269

Trapp BD, Stys PK (2009) Virtual hypoxia and chronic necrosis of demyelinated axons in multiple sclerosis. Lancet Neurol 8:280–291

Trapp B, Peterson J, Ransohoff R, Rudick R, Mork S, Bo L (1998) Axonal transection in the lesions of multiple sclerosis. N Engl J Med 338:278–285

Vallejo-Illarramendi A, Domercq M, Pérez-Cerdá F, Ravid R, Matute C (2006) Increased expression and function of glutamate transporters in multiple sclerosis. Neurobiol Dis 21:154–164

van Horssen J, Drexhage JA, Flor T, Gerritsen W, van der Valk P, de Vries HE (2010) Nrf2 and DJ1 are consistently upregulated in inflammatory multiple sclerosis lesions. Free Radic Biol Med 8:1283–1289

van Horssen J, Witte ME, Schreibelt G, de Vries HE (2011) Radical changes in multiple sclerosis pathogenesis. Biochim Biophys Acta 1812:141–150

Veto S, Acs P, Bauer J, Lassmann H, Berente Z, Setalo G Jr, Borgulya G, Sumegi B, Komoly S, Gallyas F Jr, Illes Z (2010) Inhibiting poly(ADP-ribose) polymerase: a potential therapy against oligodendrocyte death. Brain 133:822–834

Vyshkina T, Kalman B (2008) Autoantibodies and neurodegeneration in multiple sclerosis. Lab Invest 88:796–807

Wenning GK, Stefanova N, Jellinger KA, Poewe W, Schlossmacher MG (2008) Multiple system atrophy: a primary oligodendrogliopathy. Ann Neurol 64:239–246

Williams A, Piaton G, Lubetzki C (2007) Astrocytes-friends or foes in multiple sclerosis? Glia 55:1300–1312

Witte ME, Geurts JJ, de Vries HE, van der Valk P, van Horssen J (2010) Mitochondrial dysfunction: a potential link between neuroinflammation and neurodegeneration. Mitochondrion 10:411–418

Young EA, Fowler CD, Kidd GJ, Chang A, Rudick R, Fisher E, Trapp BD (2008) Imaging correlates of decreased axonal Na+/K+ ATPase in chronic multiple sclerosis lesions. Ann Neurol 63:428–435

Zamvil SS, Steinman L (2003) Diverse targets for intervention during inflammatory and neurodegenerative phases of multiple sclerosis. Neuron 38:685–688

Zhang X, Haaf M, Todorich B, Grosstephan E, Schieremberg H, Surguladze N, Connor JR (2005) Cytokine toxicity to oligodendrocyte precursors is mediated by iron. Glia 52:199–208

Zhao C, Fancy SP, Kotter MR, Li WW, Franklin RJ (2005) Mechanisms of CNS remyelination—the key to therapeutic advances. J Neurol Sci 233:87–91

Ziabreva I, Campbell G, Rist J, Zambonin J, Rorbach J, Wydro MM, Lassmann H, Franklin RJ, Mahad D (2010) Injury and differentiation following inhibition of mitochondrial respiratory chain complex IV in rat oligodendrocytes. Glia 58:1827–1837

Zielasek J, Hartung HP (1996) Molecular mechanisms of microglial activation. Adv Neuroimmunol 6:91–202

Zipp F, Atkas O (2006) The brain as a target of inflammation: common pathways link inflammatory and neurodegenerative diseases. Trends Neurosci 29:518–527

Part III
Pathophysiology of White Matter Injury

Chapter 18
Calcium Dyshomeostasis in White Matter Injury

Elena Alberdi, Asier Ruiz, and Carlos Matute

18.1 Introduction

Central nervous system (CNS) white matter, the collection of axons and supporting glia of mammalian CNS comprises about 50 % of the human brain by volume (Zhang and Sejnowski 2000). Damage to white matter typically involves primary or secondary disruption of vital connections among CNS areas, leading to serious morbidity in a broad range of neurological disorders (Desmond 2002). Numerous brain and spinal cord disorders cause disability not only as a result of dysfunction of neurons and synapses, but also by damage to CNS axons, their myelin sheaths, and the supporting glia. Examples are numerous, ranging from trauma (spinal cord injury, traumatic brain injury), acute and chronic brain ischemia, inflammatory disorders such as multiple sclerosis, neurodegenerative diseases such as Alzheimer's disease and inherited metabolic disorders resulting in a variety of leukodystrophies usually presenting early, but occasionally manifesting only in adulthood (Chen et al. 1993; Mosser et al. 1993). Recent reports have suggested that some neuropsychiatric disorders such as schizophrenia, major depression, and autism exhibit white matter abnormalities in the brain, raising the possibility that even this group of diseases may at least partially be caused by pathological alterations of CNS myelinated tracts (Heng et al. 2010; McIntosh et al. 2008). Thus, a more thorough understanding of the basic cellular and molecular mechanisms of axo-glial injury may provide important insight into the pathogenesis and treatment of a large number of neurological disorders.

E. Alberdi • A. Ruiz • C. Matute (✉)
Departamento de Neurociencias, Achucarro Basque Center for Neuroscience, Universidad del País Vasco, Leioa, Spain

Centro de Investigación Biomédica en Red en Enfermedades Neurodegenerativas (CIBERNED), Leioa, Spain
e-mail: carlos.matute@ehu.es

S. Baltan et al. (eds.), *White Matter Injury in Stroke and CNS Disease*, Springer Series in Translational Stroke Research 4, DOI 10.1007/978-1-4614-9123-1_18,

White matter exclusively contains axons and their glial cell partners including astrocytes, oligodendrocytes (myelinating and nonmyelinating), and microglia. Axons, glia, and myelin express a complex array of conventional voltage ion channels, intracellular Ca^{2+} release channels, Ca^{2+} extrussion pumps, neurotransmitter uptake, and release mechanisms together with matching transmitter receptors. Dysregulation of ion homeostasis induced by injury or energy failure leads to an excessive elevation of Ca^{2+} concentration, lethal to white matter glia and directly disrupt axon function and structure. This chapter reviews current knowledge of the molecular mechanisms regulating Ca^{2+} homeostasis in glial cells and axons and discuss how aberrant Ca^{2+} signaling can lead to white matter signaling. Finally, we summarize the current methodology to measure calcium in glia and axons.

18.2 Characteristics of White Matter

White matter is composed of myelinating and non-myelinating axons, fibrous astrocytes, oligodendrocytes, microglia, and blood vessels. Myelinated axons in the CNS are designed to support rapid and efficient saltatory impulse propagation by means of an insulating myelin sheaths which covers 99 % of the axon surface and a high segregation of ion channels with a high density of nodal Na^+ channels and internodal K^+ channels which maintain electrical polarization and stability (Poliak and Peles 2003).

Astrocytes are found throughout the brain and spinal cord and, on the basis of number, surface area, and volume, are the predominant glial cell type. They are responsible for metabolic support, nutrition, control of ion and neurotransmitter environment, and regulation of brain-blood barrier (Verkhratsky and Butt 2007). Astrocytes exhibit regulated increases in intracellular Ca^{2+} concentration which are relevant in astrocyte-astrocyte as well as in astrocyte-neuron communication. Most white matter astrocytes are fibrous in appearance, as opposed to protoplasmic astrocytes which are the major type in gray matter. Their processes are long (up to 300 μm), though much less elaborated as compared to protoplasmic astroglia, and establish perivascular endfeet and extensions that contact axons at nodes of Ranvier, the sites of action potential propagation in myelinated axons (Verkhratsky and Butt 2007).

Oligodendrocytes are glia with few processes. The main function of oligodendrocytes is the production of myelin, which insulate axons in the CNS, and assist fast saltatory action potential propagation. Oligodendrocyte death results in demyelination, impaired axonal conduction, and ultimately axon death. Myelin is one of the most complex cellular structures in the brain. Myelin sheaths are separated along the length of axons by nodes of Ranvier.

Glial cells of all types express a variety of ligand-gated ionotropic and metabotropic receptors (Kettenmann and Steinhauser 2005; Matute 2006; Verkhratsky et al. 2009a) By contrast, myelin was, until very recently, considered to be a

relatively inert passive structure serving to insulate against leakage currents and to support saltatory conduction. Studies over the past decade have demonstrated that myelin sheaths express various ionotropic glutamate and purinergic receptors subunits (Li and Stys 2000; Brand-Schieber and Werner 2003; Micu et al. 2006; Matute et al. 2007b). These observations raised the possibility that mature myelin per se is the target of released neurotransmitter.

Microglial cells form the innate defensive system of the CNS, and their pathological potential has been extensively investigated (for review Kettenmann et al. 2011). All CNS diseases involve microglia, which typically convert from the resting/surveillant cell type in the normal brain to an activated form specialized to operate within the diseased environment. Microglial activation is classically characterized by two major changes. First, the cell shape transforms from a highly branched and ramified morphology to an amoeboid form, and second, these amoeboid cells become active phagocytes. As part of the activation routine, microglial cells release diverse substances such as reactive oxygen species (ROS), cytokines, or growth factors, which influence the pathological process during the acute and chronic phase as well as during subsequent regeneration in neurons axons oligodendrocytes and myelin (Raivich and Banati 2004).

18.3 Regulation of Ca^{2+} Signaling in White Matter

Ca^{2+} is the prime intracellular second messenger in most cell types, and numerous processes in the CNS are initiated or modulated by intracellular Ca^{2+} transients in neurons and glia (Verkhratsky et al. 2009a). Physiological effects of Ca^{2+} ions are produced by intracellular sensors, represented by enzymes, which upon Ca^{2+} binding, change their activity. Furthermore, the intracellular Ca^{2+} sensors are localized in different parts of the cell, and therefore local Ca^{2+} gradients may specifically regulate particular sets of Ca^{2+} dependent process. The molecular systems responsible for controlling intracellular Ca^{2+} homeostasis and producing Ca^{2+} signaling events are limited to several protein families represented by Ca^{2+} channels and Ca^{2+} transporters (Fig. 18.1).

Ca^{2+} signaling in both excitable and nonexcitable cells is based on the maintenance of a low concentration of cytosolic Ca^{2+} (<150 nM) as compared with the extracellular compartments (1–2 mM), and intracellular Ca^{2+} storage compartments (0.1–1 mM). This differential distribution of Ca^{2+} creates a gradient across the membrane of intracellular organelles and the plasma membrane. Cellular Ca^{2+} signaling, manifested as rapid, reversible, and often repeated, intracellular Ca^{2+} rises, may therefore result from several Ca^{2+} sources. Cytosolic Ca^{2+} levels can increase via Ca^{2+} influx from the extracellular space across the plasma membrane via Ca^{2+}-permeable ion channels, and by Ca^{2+} release from intracellular stores (Fig. 18.1). In this section, we will summarize recent knowledge about how calcium signaling is regulated in white matter.

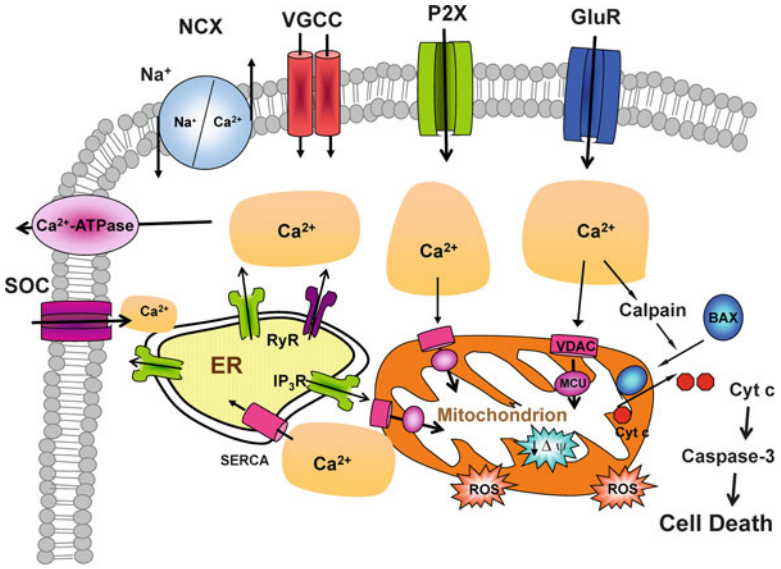


Fig. 18.1 The main "players" in Ca^{2+} signalling in white matter. Ca^{2+} flows into the cytoplasm upon glutamate and purinergic receptor stimulation, the opening of the inositol trisphosphate receptors (IP_3R), the ryanodine receptors (RyR) at the ER membranes, of the plasma membrane associated voltage (VGCC) and store (SOC) operated calcium channels and reverse operation of Na^+/Ca^{2+} exchanger (NCX). The generation of localized high Ca^{2+} concentration microdomains drives Ca^{2+} into the mitochondrial matrix via the mitochondria Ca^{2+} uniporter (MCU); this action being promoting by the voltage-dependent anion channel (VDAC), which also participates in the ER-Ca^{2+} transfer through mitochondria. Increased mitochondrial Ca^{2+} concentration stimulates TCA cycle enzymes generating NADH and increasing ATP synthesis and reactive oxygen species (ROS) production. Sustained increases in mitochondrial Ca^{2+} concentration participates in $\Delta\Psi$ attenuation and an increase in the production of ROS in mitochondria. On other hand, calpain activation by Ca^{2+} contributes to BAX translocation to mitochondria with consequent release of cytochrome c (cyt c) and induction of apoptosis. Cytosolic Ca^{2+} clearance depends on the activity of the plasma membrane Ca^{2+} ATPase (PMCA), of the plasma membrane Na^+/Ca^{2+} exchanger (NCX) and of the ER Ca^{2+} ATPase (SERCA). *ER* endoplasmic reticulum

18.3.1 Ca^{2+} Influx by Ionotropic Ca^{2+} Permeable Receptors in Glia and Axons

18.3.1.1 Ionotropic Glutamate Receptors

In white matter glial cells the ionotropic glutamate receptors involved in Ca^{2+} signaling are represented by the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate, and *N*-methyl-D-aspartate (NMDA) receptors. In particular, cells of the oligodendrocyte lineage express functional AMPA and kainate type receptors throughout a wide range of developmental stages and species, including humans (Matute et al. 2007a). In addition, immature and mature oligodendrocytes express NMDA receptors, which can be activated during injury (Káradóttir et al.

2005; Salter and Fern 2005; Micu et al. 2006; reviewed in Matute 2006). Overall, ionotropic glutamate receptors expressed in glial cells have similar properties to their neuronal counterparts. However, the fact that these receptors are edited to a lesser extent in the white matter, and that AMPA receptors in oligodendrocytes do not have GluR2 subunit suggests that they have a higher Ca^{2+} permeability than those present in gray matter (Matute et al. 2006). In turn, NMDA receptors are expressed in white matter oligodendrocytes at all developmental stages, and their activation generates a membrane depolarization and a rise in cytosolic Ca^{2+} (Bakiri et al. 2009). Interestingly, NMDA receptors are expressed in clusters on oligodendrocyte processes and myelin, whereas AMPA and kainate receptors are diffusely located on oligodendrocyte somata (Káradóttir et al. 2005; Salter and Fern 2005; Micu et al. 2006).

Mature CNS myelin expresses various ionotropic glutamate receptors subunits GluR4 (but not GluR2) AMPA subunits, KA2 kainate receptor subunit (Li and Stys 2000; Brand-Schieber and Werner 2003) and NMDA receptors in myelin sheats (Micu et al. 2006). Using transgenic mice and a technique specifically optimized for myelin Ca^{2+} imaging it was reported that a large fraction of NMDA receptor-mediated Ca^{2+} fluctuations are mediated by a "glycine-only NMDA receptor" comprised of NR1 and NR3 subunits (Piña-Crespo et al. 2010).

Ca^{2+} signaling in astrocytes is also associated with Ca^{2+} permeable ionotropic glutamate receptors. These channels were identified in freshly isolated astroglial cells, in astrocytes in culture and in brain slices (Lalo et al. 2011). AMPA receptors in astrocytes are often Ca^{2+} permeable whereas astroglial NMDA receptors have specific gating and pharmacology. They present rather weak Mg^{2+} block, are more sensitive to memantine and UBP141 and have a substantially lower Ca^{2+} permeability (Palygin et al. 2010). The biophysical and pharmacological properties indicate that astroglial NMDA receptors are assembled from NR1, NR2C/D, and NR3 subunits.

Few studies have characterized the functional expression of ionotropic glutamate receptors in microglial cells. In cultured rat microglia, AMPA-mediated currents show negligible Ca^{2+} permeability (Noda et al. 2000). The transcriptional analysis revealed the expression of GluR1 GluR2, GluR3, and GluR4 subunits for AMPA receptors and GluR5 subunit for kainate receptor in rat cultured microglia (Hagino et al. 2004). In contrast to in vitro conditions, electrophysiological recordings of microglial cells in retina or hippocampus slices failed to detect currents in response to glutamate or AMPA (Fontainhas et al. 2011; Wu and Zhuo 2008). The expression of functional NMDA receptors in microglia remains doubtful and only indirect evidence is available. Activation of microglia after transient forebrain ischemia induction leads to NMDAR1 subunit upregulation (Gottlieb and Matute 1997). The functional significance of NMDA receptor upregulation in microglia is still unknown. Moreover, NMDA injection into the somatosensory cortex of newborn rats triggered transient microglial activation (Acarin et al. 1996) whereas systemic administration of MK-801 prevented rapid microglial activation in the hippocampus secondary to ischemic insults (Streit et al. 1992) or to LPS treatment (Thomas and Kuhn 2005). Whether NMDA receptor activation controls microglial activation

directly or indirectly remains to be determined. In conclusion, further studies are necessary to characterize the existence of functional ionotropic glutamate receptors in resident and activated microglia in slices that could respond to glutamate release during synaptic activity or damage.

In axons, functional AMPA/kainate receptors are present in discrete signaling nanocomplexes that exert control over the intra-axonal Ca^{2+} stores. Axonal AMPA receptor are weakly permeable to Ca^{2+}, the entry of which in turn releases further Ca^{2+} from the axonal reticulum by opening intracellular calcium channels know as ryanodine receptors (Ouardouz et al. 2009b). In contrast, axonal kainate receptors with the GluR5 subunit are coupled to phospholipase C (PLC) activation. In addition, activation of kainate receptors with the GluR6 subunit induces a small amount of Ca^{2+} entry that stimulates nitric oxide synthase, as well as a local depolarization which activates L-type Ca^{2+} channels and subsequently ryanodine receptors in the axoplasmic reticulum (Ouardouz et al. 2009a). The functional significance of these signaling mechanisms by glutamate receptors in axons is unknown but they may serve to amplify axonal signals which seem to be weak because of the limited quantity of cation available in the narrow space.

18.3.1.2 Ionotropic Purinergic Receptors

Glial cells also express a heterogeneous repertoire of ATP receptor including an ample variety of ionotropic (P2X) and metabotropic (P2Y) purinergic receptor subtypes (Butt 2006; Verkhratsky et al. 2009b). Cells of the oligodendrocyte lineage are endowed with P2X and P2Y receptors which can act as mediators of axo-oligodendroglial communication implicated in myelination control. In particular, ATP induces a rise in cytosolic Ca^{2+} in oligodendrocytes by activating P2X7 receptors and P2Y (Kirischuk et al. 1995; Matute et al. 2007b). P2X receptors with higher affinity may be activated by ATP released during axonal electrical activity and from astrocytes (Butt 2006). In contrast, the functional significance of lower affinity P2X7 receptors in oligodendrocytes is not known, since unusual high concentrations of ATP in the extracellular space are needed to activate them. However, ATP levels may rise sufficiently upon tissue damage to stimulate P2X7 receptors and therefore they may be relevant to pathologies involving acute and chronic injury to white matter (Matute et al. 2007b; Domercq et al. 2010). Indeed, sustained activation of P2X7 receptors in oligodendrocytes in vitro and in vivo results in overload of the cytosol with Ca^{2+}, caspase-3 activation and chromatin condensation and cell death (Matute et al. 2007b). Astrocytes express various types of functional P2X purinoceptors (Franke et al. 2001; Kukley et al. 2001). Ionotropic P2XRs activation is responsible for rapid astrocytic signaling of significance for both physiological regulation and pathophysiological processes in the nervous system (Franke et al. 2006; Burnstock et al. 2011). P2X7R-mediated currents were identified in cultured rodent cortical astrocytes (Nörenberg et al. 2010) and in astrocytes patch-clamped in acute brain slices of rats and mice (Oliveira et al. 2011). P2X7Rs, through their ability to open large pores in the cell membranes, are able to damage the cytoskeleton; they appear also to trigger necrotic/

apoptotic death of glia by regulating the processing and release of interleukin-1β (IL-1β), a key mediator in neurodegeneration, chronic inflammation, and chronic pain (Skaper et al. 2010; Lenertz et al. 2011). The presence of multiple P2X and P2Y receptors at the same astrocyte may lead to an increase in $[Ca^{2+}]i$, which may trigger further second messenger pathways resulting in the neurodegenerative or proliferative reactions (Franke et al. 2004a, b).

Microglia also express a large variety of P2X and P2Y receptors which participate in microglial function. Thus, release of nucleotides/nucleosides from injured cells induces phenotypic alterations in microglia which are rapidly recruited to sites of CNS tissue damage by P2Y12. Microglia migration is mediated in part by P2X4 receptors and proliferation by P2X7 receptors whereas phagocytosis signaling is also unmasked by the upregulation of P2Y6 (Kettenmann et al. 2011).

18.3.2 Glia and Axons Express Voltage Gated Calcium Channels

Glial cells express six types of voltage gated calcium channel (VGCC) (P/Q, N, L, R, and T) on the basis of electrophysiological and pharmacological properties (MacVicar 1984; Akopian et al. 1996). Oligodendrocytes have VGCCs in distal processes which indicates that Ca^{2+} influx is restricted to sites regulating directional process growth and axon ensheathment in response to highly localized stimuli, such as axonal action potentials (Butt 2006). In turn, activation of VGCCs in astrocytes mediate glutamate release under strong depolarization and subsequent NMDA receptor-mediated neuronal excitability (Parri et al. 2001; Yaguchi and Nishizaki 2010).

VGCCs in periaxonal astrocytes participate in CNS white matter hypoxic and traumatic injury (Fern et al. 1995; Agrawal et al. 2000). These data support the hypothesis that a sustained rise in intracellular Ca^{2+} mediated through VGCCs following injury is a potential trigger for the subsequent functional and ultrastructural alterations observed in myelin.

18.3.3 The Sodium Calcium Exchanger May Be Involved in Both Ca^{2+} Extrusion and Ca^{2+} Entry

The Na^+/Ca^{2+} exchanger (NCX), a transmembrane domain protein, which couples the efflux of Ca^{2+} to the influx of Na^+ into the cell or, viceversa, the influx of Ca^{2+} to the efflux of Na^+, is involved in the regulation of diverse neuronal and glial cell functions (Annunziato et al. 2004). NCX is involved in regulating intracellular Ca^{2+} concentration under pathological conditions including ischemia-reperfusion injury (Pignataro et al. 2004), demyelinating conditions such as multiple sclerosis (MS) (Craner et al. 2004a, b), and spinal cord injury (Li et al. 2000; Tomes and Agrawal 2002).

Calcium signals mediated by NCX1, NCX2, or NCX3 play a role in oligodendrocyte maturation (Boscia et al. 2013). Notably, NCX3 knockout mice exhibit not only a reduced size of spinal cord but also a marked hypomyelination (Boscia et al. 2013). These findings indicate that calcium signaling mediated by NCX3 plays a crucial role in oligodendrocyte maturation and myelin formation.

NCXs can function in reverse mode as a consequence of transient alterations in the plasma membrane Na^+ gradient subsequent to depolarization, including that caused by glutamate receptor activation. Thus, reversal of the NCX can contribute to substantial $[Ca^{2+}]i$ increases after depolarization of astrocytes (Takuma et al. 1994), and oligodendrocyte progenitors (Liu et al. 1997). In addition, a reverse-mode NCX inhibitor reduces AMPA and kainate receptor-induced Ca^{2+} influx into the cytosol, but not the oligodendrocyte death (Alberdi et al. 2002), whereas specific inhibition of NCX1 reduces glutamate excitotoxicity in these cells (Chen et al. 2007).

In astrocytes all three types of NCXs are localized in perisynaptic processes, especially in those covering excitatory synapses (Minelli et al. 2007). Activation of astroglial ionotropic receptors (Kirischuk et al. 1997) or glutamate transporter (Rojas et al. 2007) induce substantial $[Na^+]i$ elevation which turns the exchanger into the reverse mode. Microglia also express of all three NCX isoforms (Nagano et al. 2004). Treatment with IFN-γ induces a transient increase of NCX activation which is prevented by PKC and tyrosine kinase inhibitors (Nagano et al. 2004). Ischemic insults significantly increase the expression of NCX1 in microglial cells penetrating into the infarct core (Boscia et al. 2013). The elevation of NCX1 in microglia of the postischemic brain may exert a protective role.

18.3.4 Mitochondria and Endoplasmic Reticulum Regulate Ca^{2+} Levels in Glia and Axons

Mitochondria contribute to the spatiotemporal tuning of the cytosolic Ca^{2+} concentration via Ca^{2+} uptake and release systems. The transport of Ca^{2+} by these systems controls how much Ca^{2+} enters the cell, the Ca^{2+} concentration in cytoplasmic microdomains, the frequency of oscillatory cytosolic Ca^{2+} signals and the rate of propagation of a Ca^{2+} signal. In turn, mitochondria use their Ca^{2+} transporting activity to modulate the rate of ATP synthesis in a number of ways, i.e., by activating Krebs cycle (TCA) dehydrogenases, by promoting the supply of oxidizable substrates and by regulating the activity of the ATP synthase (Fig. 18.1; Calì et al. 2012). Moderate increases in mitochondrial Ca^{2+} concentration are necessary and sufficient to adjust ATP production to cell demand, but mitochondrial Ca^{2+} overload unequivocally leads to disruption of mitochondrial membrane integrity, permeability transition, irreversible oxidative damage, and loss of ATP production, which ends up in cell demise. Endoplasmic reticulum (ER) also serves as a rapidly exchanging Ca^{2+} store and contributes to the cytosolic calcium signaling cascade by

releasing Ca^{2+} mainly through ryanodine (RyR) and IP3 (IP3R) receptors (Verkhratsky and Petersen 2002). ER and mitochondria are physically and functionally coupled by microdomains which involve IP3Rs and Ca^{2+} signaling between these organelles can induce an apoptosis crosstalk followed by the mitochondria-specific toxicity events mentioned before (for review Bononi et al. 2012). Oligodendrocyte excitotoxic insults cause Ca^{2+} influx accumulation within mitochondria, which leads to depolarization of this organelle, increased production of radical oxygen species and release of proapoptotic factors which activate caspases (Sánchez-Gómez et al. 2003). These characteristic events of mitochondrial damage are attenuated by inhibition of RyR-Ca^{2+} release in AMPA-stimulated oligodendrocytes (Ruiz et al. 2010). Moreover, Bax and calpain are essential intermediaries of the mitochondria-dependent death pathway, triggered by AMPA and kainate receptor activation in oligodendrocytes (Sánchez-Gómez et al. 2011).

In white matter tracts, Ca^{2+}-dependent injury mechanisms by ischemia involve influx of extracellular Ca^{2+} across the axolemma, and/or intra-axonal Ca^{2+} overload through ryanodine receptor-mediated release from the ER (Ouardouz et al. 2003), Ca^{2+} release via IP3R receptors in response to IP3 generation by PLC (Nikolaeva et al. 2005; Ouardouz et al. 2006) and Ca^{2+} release from mitochondria (Nikolaeva et al. 2005).

In astrocytes, cytosolic Ca^{2+} levels are also modulated by mitochondria. In stimulated astrocytes, mitochondria rapidly sequester high cytosolic Ca^{2+} via the Ca^{2+} uniporter (Reyes and Parpura 2008). As free cytosolic Ca^{2+} is removed by pumps, such as the SERCA and/or plasma membrane Ca^{2+}-ATPase, mitochondria slowly release Ca^{2+} into cytosol via the mitochondrial NCX exchanger, as well as by the formation of the mitochondrial permeability transition pore (Reyes and Parpura 2008). Mitochondrial shaping of astrocytic Ca^{2+} signals is directly involved in the regulation of exocytotic glutamate release (Reyes and Parpura 2008). However, the predominant source of Ca^{2+} for Ca^{2+}-dependent exocytosis is the ER. Since RyRs and IP3Rs show sensitivity to increased cytosolic Ca^{2+}, the initial release of Ca^{2+} is subsequently amplified in a process referred to as Ca^{2+} induced Ca^{2+} release (Verkhratsky 2005). This signaling pathway is very important for gliotransmission since thapsigargin, a specific SERCA blocker greatly reduces Ca^{2+}-dependent glutamate release from astrocytes (Hua et al. 2004).

18.4 Perturbation of Ca^{2+} Homeostasis in Glial Cells and Axons Under Pathological Conditions

Glial cells and axons, like neurons, are vulnerable to Ca^{2+} overload resulting from deregulation of channels and/or pumps. We summarize below current evidence about the mechanisms linking alterations in Ca^{2+} homeostasis to glial cell death and axonal damage, and its relevance to white matter pathology in neurological diseases.

18.4.1 Hypoxia–Ischemia-Related Diseases

Injury of central white matter is a major cause of functional disability in cerebrovascular disease (Goldberg and Ransom 2003). Damage to white matter as a consequence of hypoxic–ischemic injury occurs in periventricular leukomalacia in the neonatal period, stroke and cardiac arrest in adults, as well as in vascular dementia in the aging brain. Energy supply failure during ischemia results in ion gradient break down, membrane depolarization, and ultimately leads to Ca^{2+} overload of the cytosol which activates Ca^{2+}-dependent enzymes and intracellular Ca^{2+} release from stores, resulting in irreversible damage of white matter glia and axons (Stys 2004).

Immature and differentiated oligodendrocytes in vitro are the most sensitive cells of the white matter to transient oxygen and glucose deprivation (Matute et al. 2006; Fern and Möller 2000). In both instances, cell death is prevented in the absence of Ca^{2+} or by AMPA/kainate receptor antagonists but not by the blockade of other potential sources of Ca^{2+} influx, which suggests that Ca^{2+} entry through the receptor channels is sufficient to initiate cell demise. These findings provided a direct link between ischemic damage to oligodendrocytes and excitotoxicity. Notably, simulated ischemia induces an inward current in oligodendrocytes in situ which is partly mediated by NMDA and AMPA/kainate receptors (Káradóttir et al. 2005). In addition, Ca^{2+} levels also increase in myelin itself during ischemia, an effect which is abolished by broad-spectrum NMDA receptor antagonists, causing ultrastructural damage to both axon cylinders and myelin (Micu et al. 2006).

Excessive activation of P2X7 in oligodendrocytes is also toxic to these cells (Matute et al. 2007b). These receptors are activated during ischemia in oligodendrocytes which in turn release ATP through the opening of pannexin hemichannels and thus make an autocrine vicious loop leading to oligodendrocyte demise (Domercq et al. 2010). These data indicate that ATP released during ischemia and the subsequent activation of P2X7 receptor is critical to white matter damage during stroke.

CNS white matter becomes intrinsically more vulnerable to ischemia with aging (Baltan et al. 2008). White matter function in older animals (12 months) was not protected from ischemic injury by removal of extracellular Ca^{2+} or by blockade of reversal functioning of NCX, as is the case with young adults. Ischemic white matter injury in older mice is predominately mediated by glutamate release and activation of AMPA/kainate-type glutamate receptors. Glutamate release, attributable to reverse glutamate transport, occurs earlier and is more robust in older mice that show greater expression of the glutamate transporter of the glutamate transporter GLT-1 in astrocytes (Baltan et al. 2008). Intriguingly, blockade of NMDA receptors aggravates the outcome of ischemia in older animals (Baltan et al. 2008).

A relatively prevalent and dramatic case of hypoxia ischemia-related disease is periventricular leucomalacia (PVL), a condition that causes diffuse cerebral white matter injury as the predominant lesion. Injury to oligodendrocyte progenitors, caused in part by glutamate and the subsequent derailment of Ca^{2+} homeostasis, contributes to the pathogenesis of myelination disturbances in this illness. In the immature human brain, the susceptibility of developing oligodendrocytes to hypoxia–ischemia correlates with their expression of glutamate receptors of the

AMPA receptor subtypes on those cells in the immature human brain (Talos et al. 2006), and systemic administration of AMPA receptor antagonists attenuates injury in a rat model of PVL (Follett et al. 2004).

In addition, it has been shown that developing oligodendrocytes also express NMDA receptors and that their blockade with memantine attenuates oligodendrocytes loss and prevents the long-term reduction in cerebral mantle thickness in experimental PVL (Manning et al. 2008). Finally, perinatal ischemia triggers in addition to glutamate excitotoxicity, lethal activation of P2X7 receptors in oligodendrocyte precursors (Wang et al. 2009).

On the other hand, some evidences have shown that ischemic injury to axons is also a feature of PVL and that it occurs early in local and diffuse damage associated with this pathology (Haynes et al. 2008). Interestingly, simulated ischemic conditions in immature axons produced action potential failure and focal breakdown of the axolemma of small premyelinated axons at sites of contact with oligodendrocytes processes, which were also disrupted (Alix and Fern 2009). Axon damage is Ca^{2+}-dependent and it is prevented by NMDA and AMPA/kainate receptor blockers, and suggests that glutamate receptor-mediated injury to oligodendrocyte processes in contact with premyelinated axons precedes disruption of the underlying axon (Alix and Fern 2009).

18.4.2 Demyelinating Diseases

The major demyelinating disease of the CNS is multiple sclerosis (MS) which is the foremost disabling pathology among young adults. MS is a chronic, degenerative disease of the CNS, which is characterized by focal lesions with inflammation, demyelination, infiltration of immune cells, oligodendroglial death, and axonal degeneration (Prineas et al. 2002). These cellular alterations are accompanied by neurological deficits such as sensory disturbances, lack of motor coordination, and visual impairment. It is widely accepted that the etiology of this illness has autoimmune and inflammatory grounds and that a derailment of the immune system leads to cell and antibody-mediated attacks on myelin. Notably, treatment of oligodendrocytes with antibodies to myelin-oligodendrocyte glycoprotein (MOG) leads to an increase in Ca^{2+} influx and activation of the MAPK/Akt pathways, a signaling cascade relevant to the initial steps of MOG-mediated demyelination (Marta et al. 2005). In turn, breakdown of the blood–brain barrier caused by inflammation allows the entry into the brain parenchyma of blood constituents, which may be deleterious to neurons and glia. Thus, elevated levels of albumin induced a rise in intracellular Ca^{2+} in microglia, but not in astrocytes or macrophages, which is mediated via Src tyrosine kinase and PLC. This Ca^{2+} response is coupled to microglial proliferation suggesting that this signaling mechanism may play a role in microglial activation in pathological situations involving blood–brain barrier impairment as occurs in multiple sclerosis and in other neurodegenerative diseases (Hooper et al. 2005).

Both genetic and environmental factors contribute to MS susceptibility (Zamvil and Steinman 2003). Among them, primary and/or secondary alterations in glutamate signaling cause excitotoxicity that contribute to MS pathology. Thus, numerous studies carried out in cellular and animal models of MS as well as in postmortem brain and in patients indicate that excitotoxicity mediated by Ca^{2+}-permeable glutamate receptors contributes to oligodendrocyte death, demyelination and tissue damage in MS (Matute et al. 2001; Srinivasan et al. 2005; Vallejo-Illarramendi et al. 2006). In particular, experimental autoimmune encephalomyelitis (EAE), an animal model which exhibits the clinical and pathological features of MS, is alleviated by AMPA and kainate receptor antagonists (Pitt et al. 2000; Smith et al. 2000). Remarkably, blockade of these receptors in combination with anti-inflammatory agents is effective even at an advanced stage of unremitting EAE, as assessed by increased oligodendrocyte survival and remyelination, and corresponding decreased paralysis, inflammation, CNS apoptosis, and axonal damage (Kanwar et al. 2004). In contrast, blockade of NMDA receptors with MK-801 and genetic disruption of these receptors do not attenuate EAE symptoms (Matute 2010; Guo et al. 2012).

Glutamate levels are increased in the human brain (Srinivasan et al. 2005) as a consequence of altered glutamate homeostasis (Vallejo-Illarramendi et al. 2006) and thus, trigger excitotoxic destruction of oligodendrocytes and myelin as well as of axons (Domercq et al. 2005). Glutamate dyshomeostasis results from primary and/or secondary inflammation as a consequence of the autoimmune attack to the CNS and/or resulting from ongoing cell damage within the brain and spinal cord. Thus, activated microglia release cytokines and free radicals that diminish glutamate uptake. This in turn elevates the extracellular levels of this transmitter, resulting in overactivation of Ca^{2+}-permeable glutamate receptors, which leads to oligodendrocyte excitotoxicity (Domercq et al. 2005). Moreover, activated microglia increase their expression of the glutamate-cystine exchanger which contributes further to raising the levels of glutamate and its toxicity (Domercq et al. 2007). Other mechanisms accounting for glutamate dyshomeostasis include genetic variability in the promoter of the major glutamate transporter, EAAT2, which results in lower transporter expression (Pampliega et al. 2008). Finally, an additional component of the genetic background linking MS and deregulation of glutamate signaling and Ca^{2+}-dyshomeostasis may lie in a polymorphism in the Ca^{2+}-permeable AMPA receptor subunit GluR3, an abundantly expressed subunit in oligodendrocytes, which is associated with a subgroup of patients responding to interferon beta therapy in MS (Comabella et al. 2009).

Glutamate at nontoxic concentrations (within the micromolar range) can also contribute to demyelinating pathology by inducing Ca^{2+}-dyshomeostasis and oligodendrocyte death via sensitization of these cells to complement attack (Alberdi et al. 2006). Intriguingly, complement toxicity is induced by activation of kainate, but not of AMPA, NMDA, or metabotropic glutamate receptors. Oligodendrocyte death by complement requires the formation of the membrane attack complex, which in turn increased membrane conductance, induced Ca^{2+} overload and mitochondrial depolarization as well as a rise in the level of ROS (Alberdi et al. 2006).

Sensitization to complement attack may initiate MS lesions and thus be a pathophysiological feature of this disease or any of its neuropathological subtypes.

In addition to glutamate, ATP sustained activation of P2X7 receptors in vivo causes lesions which are reminiscent of the major features of MS lesions. Moreover, treatment of acute and chronic EAE with P2X7 antagonists reduces demyelination and ameliorates the associated clinical symptoms. Importantly, P2X7 expression is elevated in oligodendrocytes of normal-appearing axon tracts in MS patients, suggesting that signaling through this receptor is enhanced in this disease (Matute et al. 2007b). Indeed, a case–control association study revealed a polymorphism of P2X7 receptor gene more frequent in MS than in controls and displaying a gain-of-function consisting in higher Ca^{2+} permeability and larger electrophysiological responses (Oyanguren-Desez et al. 2011). The increased expression of P2X7 receptors in axon tracts before lesions are formed indicates that this feature may constitute a risk factor associated with newly forming lesions in MS; this receptor subunit may thus prove to be a diagnostic and/or prognostic clinical biomarker for MS-type leukoencephalopathy. Finally, blockade of ATP P2X7 receptors protects oligodendrocyte from dying; this property has enormous therapeutic potential for halting the progression of tissue damage in MS.

18.4.3 Psychiatric Disorders

White matter alterations have recently been detected in studies of postmortem brain from psychiatric patients including schizophrenia, bipolar disorder, and major depression. Although these diseases are distinct in nature, they share some white matter distortions including reduced oligodendrocyte number and expression of myelin constituents, which may contribute to their pathophysiology (Davis et al. 2003; Uranova et al. 2004; McIntosh et al. 2005). Neuroimaging and neuropathological studies revealed myelin defects and oligodendrocyte alterations in brain tissue from schizophrenia patients, correlating with the decreased expression of myelin-related genes (McIntosh et al. 2005). For instance, histological studies have shown an abnormal distribution and decreased density of oligodendrocytes in the frontal regions of the cerebral cortex in schizophrenia, as well as reduced cell numbers in certain cortical layers (Uranova et al. 2004). The nature of the mechanisms leading to hypomyelination and reduction of the oligodendrocyte population is not known but it has been proposed that it may be caused by alterations in Ca^{2+} homeostasis due to aberrant glutamate signaling in these insidious diseases (Davis et al. 2003). Interestingly, lithium, which is widely used for the treatment of bipolar disorder, regulates a number of components of signal transduction machinery, including Ca^{2+} homeostasis and has neuroprotective properties in experimental paradigms of excitotoxicity (Bauer et al. 2003). In addition to oligodendrocyte loss in severe psychosis and major depression, a reduction in GFAP has also been found in subjects with major depression (Fatemi et al. 2004).

18.4.4 Alzheimer's Disease

It is well known that white matter is altered in Alzheimer's disease (AD). Thus, damage in specific locations appears to impair those cognitive functions which rely on networks involving those regions, and the extent of this damage is associated with dementia severity in AD (Bronge 2002). A high percentage of AD patients exhibits evidence of white matter degeneration with severe loss of oligodendrocytes by apoptosis (Brown et al. 2000). Although the mechanisms underlying this damage are not well understood, there is evidence that they involved amyloidosis and Ca^{2+} dyshomeostasis (Mattson and Chan 2003). Amyloid beta peptides inhibit myelin formation and can damage oligodendrocytes in vitro (Horiuchi et al. 2012; Xu et al. 2001) and increase their vulnerability to being killed by glutamate (Pak et al. 2003). In addition to in vitro findings, dynamic changes in myelin aberrations and oligodendrocyte generation were described in chronic amyloidosis in mice and men (Behrendt et al. 2013). These effects have been also described in vivo by experimental injection of amyloid beta 1-42 into white matter causes axon disruption and myelin damage, as well as oligodendrocyte loss and profound gliosis (Jantaratnotai et al. 2003). On the other hand, oligodendrocytes from presenilin-1 mutant knock-in mice are more susceptible to glutamate excitotoxicity and exhibit an abnormality in Ca^{2+} regulation which is responsible for their demise (Pak et al. 2003). Moreover, when exposed to the demyelinating agent cuprizone, presenilin-1 mutant mice exhibit enhanced white matter damage and a learning and memory deficits which are not seen in wild-type mice exposed to cuprizone (Pak et al. 2003). These findings reveal that a specific presenilin-1 mutation in oligodendrocytes can have detrimental effects leading to disease, and indicate that white matter damage may well contribute to cognitive dysfunction in AD. A model of AD consists of a triple-transgenic mouse which harbors the human Swedish mutant transgene of the amyloid precursor protein, a presenilin knock-in mutation, and a tau P301L mutant transgene. Interestingly, this mouse exhibits significant region-specific alterations in myelination patterns and in oligodendrocyte marker expression profiles at time points preceding the appearance of amyloid and tau pathology (Desai et al. 2009). This finding indicates that myelin and oligodendrocyte defects in AD precede the onset of symptoms and may be key players in the development of this disease.

18.4.5 Traumatic Injury

Traumatic injury to the CNS inevitably involves damage to white matter and causes primary mechanical destruction of glia and axons. In addition, secondary impairment of tissue occurs as a consequence of a prolonged pathological response involving chronic inflammation, microglial activation, and astroglial scar formation which can ultimately result in the development of a large cavity at the site of the lesion and persistent functional deficits (Dumont et al. 2001). Ca^{2+} dyshomeostasis occurs in various ways after traumatic brain injury. Thus, tissue destruction leads to the

release of high levels of glutamate which cause Ca^{2+}-dependent excitotoxic damage to white matter astrocytes, oligodendrocytes, and myelin, but not to axons (Li and Stys 2000). ATP which is also present at high levels in the extracellular space after cell death may cause Ca^{2+}-dependent gliotoxicity either directly by activating P2X receptors, or after degradation to adenosine and subsequent activation of P1 purinergic receptors (Verkhratsky et al. 2009b), but the later putative deleterious effects on white matter have not yet been demonstrated to occur after traumatic injury. In turn, L- and N-type Ca^{2+} channels also mediate partial Ca^{2+} overload of the cytosol in white matter macroglia and the subsequent demise of these cells (Agrawal et al. 2000), whereas L-type Ca^{2+} channels also play a critical role in membrane resealing of axons (Nehrt et al. 2007). Moreover, Ca^{2+} mobilization from intracellular stores after trauma also contributes to loss of function in white matter tracts since the blockade of inositol triphosphate and ryanodine receptors can partially restore compound axon potentials (Thorell et al. 2002). Notably, glial cells distal to local mechanical trauma undergo adaptive changes including enhanced Ca^{2+} regulation which plays a protective role in the acute postinjury period (Mills et al. 2004).

Finally, it has also been shown that spinal cord injury is associated with prolonged P2X7 receptor activation and ensuing neuronal excitotoxicity (Verkhratsky et al. 2009b). Strikingly, systemic administration of a P2X7 antagonist permeable to the blood–brain barrier ameliorates the motor behavior of animals who had been subjected to spinal cord contusion indicating that neuroprotection after injury can preserve function (Peng et al. 2009). In addition, oligodendrocyte preservation by P2X7 receptor blockade may also be critical to attenuate white matter destruction and the ensuing motor and sensory deficits.

18.5 How to Measure Ca^{2+} in White Matter

Measuring the concentration of intracellular Ca^{2+} as well as analyzing its spatial and temporal dynamics is crucial for the understanding of white matter pathophysiology. This goal is now at hand with recent microscopy techniques and the development of fluorescent Ca^{2+} indicators, carried out in large part by Dr. Tsien and coworkers, who were awarded the 2008 Nobel Prize of Chemistry for the discovery and development of the green fluorescent protein (GFP). Ca^{2+} indicators are divided in two major groups: the chemically designed fluorophores and genetically encoded fluorescence proteins.

18.5.1 Chemical Fluorescent Ca^{2+} Indicators

Chemical fluorescent probes have been so far the most widely used Ca^{2+} indicators and also a straightforward method to measure intracellular free Ca^{2+} in neurons and glial cells. Many of them are derivatives of Ca^{2+} chelators like EGTA and BAPTA and produce strong light emission upon Ca^{2+} binding, which can be easily detected

Table 18.1 Widely used chemical intracellular Ca^{2+} indicators and their properties

Indicator	Affinity for Ca^{2+} (K_d)	Excitation/emission (nm)	Notes
Calcium green-1	190 nM	490/531	Single wavelength
Fluo-4	345 nM	494/516	Single wavelength
Fura-2	145 nM	363/335 ex 512 em	Ratiometric
Fura-4F	0.77 nM	336/366 ex 511 em	Ratiometric
Indo-1	230 nM	488 ex 405/488 em	Ratiometric
Oregon Green 488 BAPTA-1	170 nM	488/520	Single wavelength
X-rhod-1	0.7 nM	580/602	Single wavelength
Fluo-5N	90 μM	491/516	Single wavelength low affinity
Fura-FF	5.5 μM	335/364 ex 510 em	Ratiometric, low affinity
Rhod-2	1 μM	556/576	Single wavelength; accumulates to mitochondria
Mag-Fura-2	25 μM	329/369 ex	Ratiometric; suitable for ER Ca^{2+} measurements

Based on data from Paredes et al. (2008) and Takahashi et al. (1999)

by any fluorescence-based detection system, such as sophisticated laser scanning microscope for single cell imaging or the microplate reader for whole cell population analysis. There is a large variety of these Ca^{2+} indicators, with different loading, Ca^{2+} affinity and excitation, and emission properties (Table 18.1). For an efficient and noninvasive intracellular loading, a popular method consists on using the commercially available acetoxymethyl ester (AM ester) form of fluorescent Ca^{2+} probes. The AM esters are hydrophobic and permeable across the plasma membrane and insensitive to ions, and once inside the cell, ubiquitous intracellular esterases cleave the AM group and probes get trapped inside like ion-sensitive polyanionic indicators. Dissociation constant (K_d) for Ca^{2+} is specific and different for each indicator so that the intracellular concentration range from <50 nM to >50 μM Ca^{2+} can be properly covered. It is advisable to use the fluorescence probe to measure Ca^{2+} concentrations between 0.1 and 10 times its K_d, and to note that the K_d of an indicator in vitro could not have the same value as the K_d in vivo, since it is dependent on the pH, temperature, ionic strength, and viscosity, among others. With regard to the absorption and emission espectra, Ca^{2+} dyes are divided into the ultra-violet (UV) and light-visible (blue, green, and red) absorption spectra groups. Although the selection is usually made based on the excitation and detection systems available in the laboratory, both have their specific advantages and disadvantages depending on the properties of the sample and the Ca^{2+} signal that will be studied. For instance, UV excitation is known to be more cytotoxic than visible-wavelength irradiation, induces autofluorescence from cells, and requires specialized optical components in confocal microscopes. However, the ratiometric indicators like Indo-1 and Fura-2, which are particularly recommended for quantitative Ca^{2+} signal analysis, belong to the UV absorption category. These Ca^{2+} dyes work with either dual- excitation or dual-emission ratiometry and thus permit the correction for imaging artifacts, irregular loading, dye leakage, photobleaching,

and changes in cell volume. The ratiometric dye Fura-2 is considered one of the most successful indicators for an accurate quantification of cytosolic Ca^{2+} concentration. Its peak absorbance shifts from 380 to 340 nm when bound to Ca^{2+} and both show the same peak emission at 510 nm. Usually the membrane-permeable form of the dye and fluorescence widefield microscopy are used to quantify cytosolic Ca^{2+} changes inside the components of white matter in vitro, such as optic-nerve oligodendrocytes (Sánchez-Gómez et al. 2011), astrocytes (Li et al. 2011), axons (Jablonka et al. 2007), and microglia (Murugan et al. 2011). Nevertheless, in addition to cultured cells it is possible to carry out Fura-2 microfluorimetry on CNS slices. Heinke et al. (2004) stained lamina I neurons of rat spinal cord slices with Fura-2 via the patch-clamp pipette and measured Ca^{2+} currents through VGCCs combined with electrophysiological recordings in that area.

On the other hand, single wavelength indicators belong to the light-visible absorption spectra group. These dyes show a large increase of the fluorescence intensity upon Ca^{2+} binding without shifting the absorption/emission wavelengths or increasing the spectral bandwidth. Since spectral overlap is minimized and there is no need for dual excitation, single wavelength indicators are suitable for working simultaneously with multiple fluorophores in a laser scanning confocal microscopy. This strategy was applied to study the Ca^{2+} signaling of NG2-glia in the white matter of the mouse optic-nerve (Hamilton et al. 2010). Intact optic nerves were isolated from transgenic animals expressing DsRed fluorescent protein under the control of NG2 promoter, and incubated with Fluo-4 AM. NG2/DsRed positive and Fluo-4-labeled cell bodies were selected for cell selective imaging, which showed that axonal action potentials activate NG2 glia Ca^{2+} signaling in situ.

Chemical indicators have been shown to be useful for technically difficult white matter Ca^{2+} imaging of in vivo and ex vivo models. In order to record axonal Ca^{2+} signals from optic nerves, Verbny et al. (2002) selectively stained pure population of axons by pipetting dextran-conjugated Oregon Green BAPTA-1 dye via diffusion from the cut ends of the nerve. The membrane-impermeable indicator showed excellent dye retention and signal-to-noise ratio and Ca^{2+} fluctuations inside the axons were measured by confocal fluorescence imaging. On the other hand, different approaches have been made to measure Ca^{2+} inside myelin sheaths. Using a method developed in Peter K. Stys' lab, Micu et al. (2007) found that, compared to other Ca^{2+} dyes like BAPTA-1-AM, Fluo-3-AM, or rhod-2-AM, which remained mainly localized to the surfaces of myelinated axons, the cationic dye X-rhod-1-AM penetrated well the myelin sheath of optic nerve and dorsal column axons and emitted specific Ca^{2+} signals from cytoplasmic compartments of oligodendrocytes. To distinguish clearly somatic Ca^{2+} signals from those inside the myelin sheath, they counterstained this compartment with $DiOC_6(3)$, a strongly fluorescence cationic lipophilic membrane marker. With this technique, they measured Ca^{2+} changes in living myelin by two-photon microscopy, and found that during ischemia NMDA receptors contribute to the Ca^{2+} overload in cytoplasmic compartments of not only oligodendrocyte bodies but also of the myelin sheaths around the axons.

Measuring Ca^{2+} changes inside intracellular compartments might be one of the most important limitations of chemical fluorescent dyes. Although positive charged Rhod-2 AM accumulates mainly in the mitochondria and is the most widely used Ca^{2+}

indicator for this organelle, its localization is not fully specific and several controls and calibration procedures are recommended when using this dye. For measurements inside the endoplasmic reticulum, the main intracellular Ca^{2+} store, low-affinity indicators such as Mag-Fura-2 are commonly used. However, it also remains in the cytosol and further manipulation of the sample is required to remove nonspecific signals.

18.5.2 Genetically Encoded Ca^{2+} Indicators

Chemical Ca^{2+} indicators have proved for decades their usefulness and efficiency in the study of cytoplasmic Ca^{2+} dynamics of the CNS, especially in in vitro neuronal and glial models. However, even if they were shown to work well in in vivo models, their use as Ca^{2+} sensors in whole animals remain challenging. Another drawback of the most widely used Ca^{2+} dyes is nonspecific targeting. When loading the dye into the cytosol, it may localize also inside other cytoplasmic compartments like the endoplasmic reticulum (ER), making it difficult to get a specific signal from a single intracellular domain, including Ca^{2+} measurements inside organelles. To solve these problems, the genetically encoded Ca^{2+} indicators (GECIs) were developed in the 1990s and they have evolved since then to a large variety of probes with different intracellular localization properties, Ca^{2+} affinities, and light emission mechanisms. The genetic probes provide marked advantages over chemical dyes. First, if implemented with appropriate promoter or targeting sequences, genetically encoded probes can label specific cell types and cellular compartments and organelles. Second, once probe-encoding DNA is transfected and integrated into the genome, the protein is stably expressed and long term recordings can be achieved. Third, stable transgenic animal lines expressing functional Ca^{2+} indicators can be produced for in vivo studies. Concerning the mechanism by which they emit light upon Ca^{2+} binding, genetically encoded Ca^{2+} probes are divided into two major families: the Förster resonance energy transfer (FRET)-based cameleon type and the single GFP type such as GCaMPs and pericams (Table 18.2). Cameleons consist of two spectral variants of GFP with overlapping excitation/emission espectra, such as cyan (CFP) and yellow (YFP), linked by a Ca^{2+}-sensitive calmodulin (CaM) bound to a M13 peptide from myosine light chain kinase. When the probe binds Ca^{2+}, CaM wraps around the M13 peptide and brings the two fluorophores together (Fig. 18.2). In these conditions, the emission of the donor (CFP) excites the acceptor (YFP) and FRET signal increases, which is expressed as changes in the ratio between YFP/CFP fluorescence. As we mentioned above, one of the most remarkable advantages of the GECIs is that they allow accurate Ca^{2+} imaging in intracellular compartments, and to that aim several probes targeting mitochondria, endoplasmic reticulum, Golgi, nucleus, and plasma membrane have been designed.

We have studied mitochondrial Ca^{2+} dynamics in optic nerve-derived oligodendrocytes using the mitochondria-targeted 2mtD4cpv cameleon probe. Dissociated cells were transfected with the cameleon DNA before plating, and single cell imaging was carried out on myelin producing mature oligodendrocytes. The ratiometric probe showed a highly specific mitochondrial localization and was used to measure the mitochondrial Ca^{2+} increase during excitotoxicity in AMPA-stimulated oligodendrocytes in vitro (Fig. 18.3, unpublished data). ER Ca^{2+} dynamics have been

Table 18.2 Widely used fluorescent genetically encoded Ca^{2+} indicators and their properties

	Indicator	Fluorescent protein	Affinity for Ca^{2+} (K_d) (μM)
Single GFP	Flash pericam	YFP	0.7
	Inverse pericam	YFP	0.2
	Ratiometric pericam	YFP	1.7
	GCaMP1.6	EGFP	0.15
	GCaMP2	EGFP	0.15
	R-CaMP1.07	mApple	0.15
FRET-based	YC2.1	CFP, YFP	4.3
	YC4.6	CFP, YFP	14.4
	D1	CFP, Venus	60
	D3cpv	CFP, Venus	0.6
	D4cpv	CFP, Venus	64

Based on data from Koldenkova and Nagai (2013), Mank and Griesbeck (2008) and Miyawaki (2005)

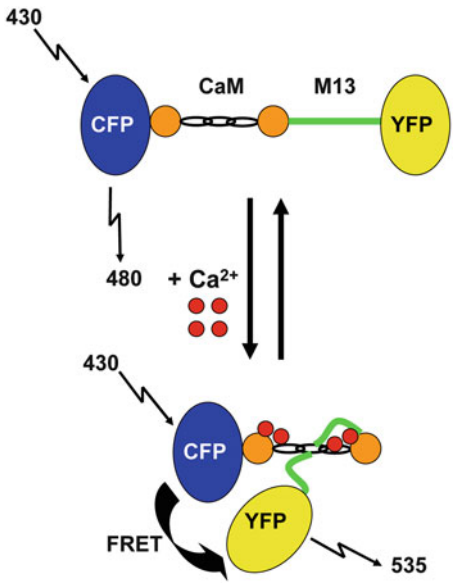


Fig. 18.2 FRET reaction between CFP and YFP proteins in a yellow cameleon after Ca^{2+} binding (adapted from http://www.embl.de/eamnet/html/calcium/proteins/cameleons.htm)

successfully studied by the D1ER cameleon, which contains an ER targeting sequence (KDEL) from calreticulin. The K_d of 60 μM of this cameleon is the appropriate for high ER Ca^{2+} levels and compared to prior cameleons, it detects small physiological [Ca^{2+}] changes in vitro (Palmer et al. 2004). On the other hand, GECIs permit cell-type selective Ca^{2+} imaging, as previously shown in rat cortical primary cultures (Tsuchiya et al. 2002). Using neuron- and astrocyte-specific promoters, enolase, and glial fibrillar acidic protein promoters respectively, cultures were transfected with a cytosolic yellow cameleon and Ca^{2+} responses were recorded separately in both cell types. Linking a cameleon DNA to a specific promoter is also a valuable tool to generate Ca^{2+}- probe-expressing transgenic mice, and thus to

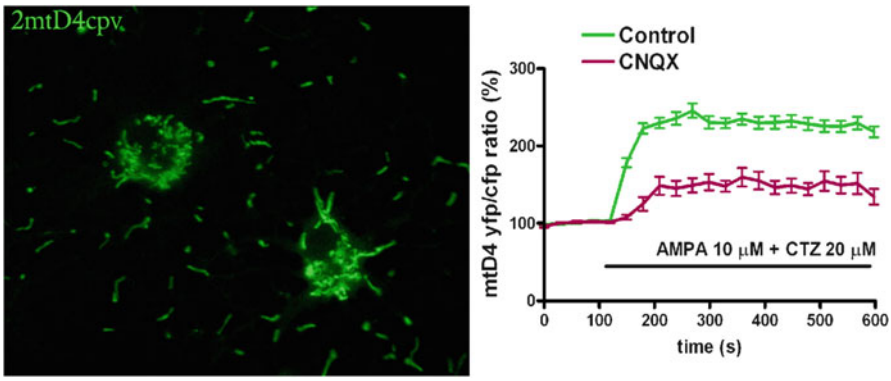


Fig. 18.3 Measuring of mitochondrial Ca^{2+} responses in oligodendrocytes by mitochondrial-targeted cameleon. Optic nerve-derived oligodendrocytes were transfected with 2mtD4cpv and exposed to AMPA (2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid) in the presence of CTZ (AMPA receptor desensitization inhibitor). Excitotoxic mitochondrial Ca^{2+} increases, which are inhibited by AMPA/kainate receptor antagonist CNQX, are expressed as FRET ratio of YFP/CFP

perform in vivo Ca^{2+} imaging experiments. Atkin and coworkers developed a transgenic mouse line which expressed YC 3.6 cameleon directed by astrocyte-specific S100β promoter and recorded Ca^{2+} signals in response to neuronal stimulation in situ by two-photon microscopy (Atkin et al. 2009).

Pericams, GCaMPs, and the latest GECOs are based as well on Ca^{2+}-sensitive calmodulin and the M13 peptide combined with a circularly permutated fluorescent protein. In particular, modifications of initial pericams resulted in a family composed by three members. Flash type pericam increases up to eightfold 520 nm fluorescence when it binds Ca^{2+} and therefore acts like Fluo-3 and Fluo-4 single wavelength dyes, while inverse pericam reduces 515 nm fluorescence upon Ca^{2+} binding. Last, the ratiometric pericam, functionally analogous to Fura-2, changes its 520 nm emission when excited at 494 and 415 nm depending on whether it is bound to Ca^{2+} or not. These indicators have been successfully used for intracellular Ca^{2+} measurements in several cell types of the CNS in vitro, as well as for intra-organelle studies with the mitochondria-targeted pericam. Moreover, like FRET-based GECIs, pericams have been shown to suit to more integral experimental models. Jasoni et al. (2007) generated transgenic mice in which a ratiometric pericam was expressed selectively in gonadotropin-releasing hormone neurons and described the properties of the spontaneous Ca^{2+} transients in these cells in situ. On the other hand, R-CaMP1.07, an improved variant of the red fluorescent R-GECO1, has been recently shown to be applicable for the monitoring of Ca^{2+} transients triggered by single action potentials in hippocampal slices (Ohkura et al. 2012).

In summary, chemical fluorescent dyes are the most straightforward and successful tool for the study of Ca^{2+} dynamics in white matter components. The large variety of dyes available, with different affinities and loading properties, allows to work with most in vitro models and detection systems, and to perform complex in

vivo experiments. However, during the last decade a lot of progress has been made in the development of the GECIs, which provide the possibility to measure Ca^{2+} accurately inside organelles and to generate probe-expressing transgenic mice for whole animal studies. To our best knowledge these indicators have not been used for in vivo white matter studies yet, but as long as new genetic approaches are made they will become an essential alternative for challenging Ca^{2+} imaging experiments in this field.

18.6 Conclusions

An excess accumulation of Ca^{2+} in axons, glial cells, and myelin itself underlies acute and chronic white matter demise in neurological and psychiatric disorders and contributes to the severity of symptoms. Ca^{2+} influx through glutamate and ATP ionotropic receptors are critical to initiate Ca^{2+} homeostasis disruption in pathology, and drugs attenuating their excitotoxic potential offer great therapeutic promise. However, the precise mechanisms leading to aberrant signaling by these two excitatory neurotransmitters and the ensuing Ca^{2+} dyshomeostasis is not fully understood. It will also be important to further clarify the contribution of intracellular sources (endoplasmic reticulum and mitochondria) to Ca^{2+} dyshomeostasis and the molecular consequences which precede axonal damage and glial cell death. Finally, since human white matter accounts for about half of the CNS and it is crucial for brain information processing, novel, effective neuroprotective strategies to treat neurological and psychiatric diseases should always include white matter targeting with specific drugs aiming at restoring Ca^{2+} homeostasis.

Acknowledgments Work in our laboratory is supported by CIBERNED and by grants from the Ministerio de Ciencia e Innovación, Gobierno Vasco, Ikerbasque, and the Universidad del País Vasco.

References

Acarin L, González B, Castellano B, Castro AJ (1996) Microglial response to N- methyl-D-aspartate-mediated excitotoxicity in the immature rat brain. J Comp Neurol 367:361–374

Agrawal SK, Nashmi R, Fehlings MG (2000) Role of L- and N-type calcium channels in the pathophysiology of traumatic spinal cord white matter injury. Neuroscience 99:179–188

Akopian G, Kressin K, Derouiche A, Steinhauser C (1996) Identified glial cells in the early postnatal mouse hippocampus display different types of Ca2+ currents. Glia 17:181–194

Alberdi E, Sánchez-Gómez MV, Marino A, Matute C (2002) Ca(2+) influx through AMPA or kainate receptors alone is sufficient to initiate excitotoxicity in cultured oligodendrocytes. Neurobiol Dis 9:234–243

Alberdi E, Sánchez-Gómez MV, Torre I, Domercq M, Pérez-Samartín A, Pérez-Cerdá F, Matute C (2006) Activation of kainate receptors sensitizes oligodendrocytes to complement attack. J Neurosci 26:3220–3228

Alix JJ, Fern R (2009) Glutamate receptor-mediated ischemic injury of premyelinated central axons. Ann Neurol 66:682–693

Annunziato L, Pignataro G, Di Renzo GF (2004) Pharmacology of brain Na+/Ca2+ exchanger: from molecular biology to therapeutic perspectives. Pharmacol Rev 56:633–654

Atkin SD, Patel S, Kocharyan A, Holtzclaw LA, Weerth SH, Schram V, Pickel J, Russell JT (2009) Transgenic mice expressing a cameleon fluorescent Ca2+ indicator in astrocytes and Schwann cells allow study of glial cell Ca2+ signals in situ and in vivo. J Neurosci Methods 181:212–226

Bakiri Y, Burzomato V, Frugier G, Hamilton NB, Káradóttir R, Attwell D (2009) Glutamatergic signaling in the brain's white matter. Neuroscience 158:266–274

Baltan S, Besancon EF, Mbow B, Ye Z, Hammer MA, Ransom BR (2008) White matter vulnerability to ischemic injury increases with age because of enhanced excitotoxicity. J Neurosci 28:1479–1489

Bauer M, Alda M, Priller J, Young LT; International Group for the Study of Lithium Treated Patients (IGSLI) (2003) Implications of the neuroprotective effects of lithium for the treatment of bipolar and neurodegenerative disorders. Pharmacopsychiatry 36:S250–S254

Behrendt G, Baer K, Buffo A, Curtis MA, Faull RL, Rees MI, Götz M, Dimou L (2013) Dynamic changes in myelin aberrations and oligodendrocyte generation in chronic amyloidosis in mice and men. Glia 61:273–286

Bononi A, Missiroli S, Poletti F, Suski JM, Agnoletto C, Bonora M, De Marchi E, Giorgi C, Marchi S, Patergnani S, Rimessi A, Wieckowski MR, Pinton P (2012) Mitochondria-associated membranes (MAMs) as hotspot Ca(2+) signaling units. Adv Exp Med Biol 740:411–437

Boscia F, D'Avanzo C, Pannaccione A, Secondo A, Casamassa A, Formisano L, Guida N, Scorziello A, Di Renzo G, Annunziato L (2013) New roles of NCX in glial cells: activation of microglia in ischemia and differentiation of oligodendrocytes. Adv Exp Med Biol 961: 307–316

Brand-Schieber E, Werner P (2003) (+/-)-Alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid and kainate receptor subunit expression in mouse versus rat spinal cord white matter: similarities in astrocytes but differences in oligodendrocytes. Neurosci Lett 345:126–130

Bronge L (2002) Magnetic resonance imaging in dementia. A study of brain white matter changes. Acta Radiol Suppl 428:1–32

Brown WR, Moody DM, Thore CR, Challa VR (2000) Cerebrovascular pathology in Alzheimer's disease and leukoaraiosis. Ann N Y Acad Sci 903:39–45

Burnstock G, Fredholm BB, Verkhratsky A (2011) Adenosine and ATP receptors in the brain. Curr Top Med Chem 11:973–1011

Butt AM (2006) Neurotransmitter-mediated calcium signalling in oligodendrocyte physiology and pathology. Glia 54:666–675

Calì T, Ottolini D, Brini M (2012) Mitochondrial Ca2+ as a key regulator of mitochondrial activities. Adv Exp Med Biol 942:53–73

Chen YQ, Rafi MA, de Gala G, Wenger DA (1993) Cloning and expression of cDNA encoding human galactocerebrosidase, the enzyme deficient in globoid cell leukodystrophy. Hum Mol Genet 2:1841–1845

Chen H, Kintner DB, Jones M, Matsuda T, Baba A, Kiedrowski L, Sun D (2007) AMPA-mediated excitotoxicity in oligodendrocytes: role for Na(+)-K(+)-Cl(−) co-transport and reversal of Na(+)/Ca(2+) exchanger. J Neurochem 102:1783–1795

Comabella M, Craig DW, Morcillo-Suárez C, Río J, Navarro A, Fernández F, Martín R, Montalban X (2009) Genome-wide scan of 500,000 single-nucleotide polymorphisms among responders and nonresponders to interferon beta therapy in multiple sclerosis. Arch Neurol 66:972–978

Craner MJ, Hains BC, Lo AC, Black JA, Waxman SG (2004a) Co-localization of sodium channel Nav1.6 and the sodium–calcium exchanger at sites of axonal injury in the spinal cord in EAE. Brain 127:294–303

Craner MJ, Newcombe J, Black JA, Hartle C, Cuzner ML, Waxman SG (2004b) Molecular changes in neurons in multiple sclerosis: altered axonal expression of Nav1.2 and Nav1.6 sodium channels and Na+/Ca2+ exchanger. Proc Natl Acad Sci U S A 101:8168–8173

Davis KL et al (2003) White matter changes in schizophrenia: evidence for myelin related dysfunction. Arch Gen Psychiatry 60:443–456
Desai MK, Sudol KL, Janelsins MC, Mastrangelo MA, Frazer ME, Bowers WJ (2009) Triple-transgenic Alzheimer's disease mice exhibit region-specific abnormalities in brain myelination patterns prior to appearance of amyloid and tau pathology. Glia 57:54–65
Desmond DW (2002) Cognition and white matter lesions. Cerebrovasc Dis 13:53–57
Domercq M, Etxebarria E, Pérez-Samartín A, Matute C (2005) Excitotoxic oligodendrocyte death and axonal damage induced by glutamate transporter inhibition. Glia 52:36–46
Domercq M, Sánchez-Gómez MV, Sherwin C, Etxebarria E, Fern R, Matute C (2007) System xc- and glutamate transporter inhibition mediates microglial toxicity to oligodendrocytes. J Immunol 178:6549–6556
Domercq M, Perez-Samartin A, Aparicio D, Alberdi E, Pampliega O, Matute C (2010) P2X7 receptors mediate ischemic damage to oligodendrocytes. Glia 58:730–740
Dumont RJ, Okonkwo DO, Verma S et al (2001) Acute spinal cord injury. Part I. Pathophysiological mechanisms. Clin Neuropharmacol 24:254–264
Fatemi SH et al (2004) Glial fibrillary acidic protein is reduced in cerebellum of subjects with major depression, but not schizophrenia. Schizophr Res 69:317–323
Fern R, Möller MT (2000) Rapid ischemic cell death in immature oligodendrocytes: a fatal glutamate release feedback loop. J Neurosci 20:34–42
Fern R, Ransom BR, Waxman SG (1995) Voltage-gated calcium channels in CNS white matter: role in anoxic injury. J Neurophysiol 74:369–377
Follett PL, Deng W, Dai W, Talos DM, Massillon LJ, Rosenberg PA, Volpe JJ, Jensen FE (2004) Glutamate receptor-mediated oligodendrocyte toxicity in periventricular leukomalacia: a protective role for topiramate. J Neurosci 24:4412–4420
Fontainhas AM, Wang M, Liang KJ, Chen S, Mettu P, Damani M, Fariss RN, Li W, Wong WT (2011) Microglial morphology and dynamic behavior is regulated by ionotropic glutamatergic and GABAergic neurotransmission. PLoS One 6:e15973
Franke H, Grosche J, Schädlich H, Krügel U, Allgaier C, Illes P (2001) P2X receptor expression on astrocytes in the nucleus accumbens of rats. Neuroscience 108:421–429
Franke H, Gunther A, Grosche J, Schmidt R, Rossner S, Reinhardt R, Faber-Zuschratter H, Schneider D, Illes P (2004a) P2X7 receptor expression after ischemia in the cerebral cortex of rats. J Neuropathol Exp Neurol 63:686–699
Franke H, Krügel U, Grosche J, Heine C, Härtig W, Allgaier C, Illes P (2004b) P2Y receptor expression on astrocytes in the nucleus accumbens of rats. Neuroscience 127:431–441
Franke H, Krügel U, Illes P (2006) P2 receptors and neuronal injury. Pflugers Arch 452:622–644
Goldberg MP, Ransom BR (2003) New light on white matter. Stroke 34:330–332
Gottlieb M, Matute C (1997) Expression of ionotropic glutamate receptor subunits in glial cells of the hippocampal CA1 area following transient forebrain ischemia. J Cereb Blood Flow Metab 17:290–300
Guo F, Maeda Y, Ko EM, Delgado M, Horiuchi M, Soulika A, Miers L, Burns T, Itoh T, Shen H, Lee E, Sohn J, Pleasure D (2012) Disruption of NMDA receptors in oligodendroglial lineage cells does not alter their susceptibility to experimental autoimmune encephalomyelitis or their normal development. J Neurosci 32:639–645
Hagino Y, Kariura Y, Manago Y, Amano T, Wang B, Sekiguchi M, Nishikawa K, Aoki S, Wada K, Noda M (2004) Heterogeneity and potentiation of AMPA type of glutamate receptors in rat cultured microglia. Glia 47:68–77
Hamilton N, Vayro S, Wigley R, Butt AM (2010) Axons and astrocytes release ATP and glutamate to evoke calcium signals in NG2-glia. Glia 58:66–79
Haynes RL, Billiards SS, Borenstein NS, Volpe JJ, Kinney HC (2008) Diffuse axonal injury in periventricular leukomalacia as determined by apoptotic marker fractin. Pediatr Res 63: 656–661
Heinke B, Balzer E, Sandkühler J (2004) Pre- and postsynaptic contributions of voltage- dependent Ca2+ channels to nociceptive transmission in rat spinal lamina I neurons. Eur J Neurosci 19:103–111

Heng S, Song AW, Sim K (2010) White matter abnormalities in bipolar disorder: insights from diffusion tensor imaging studies. J Neural Transm 117:639–654
Hooper C, Taylor DL, Pocock JM (2005) Pure albumin is a potent trigger of calcium signalling and proliferation in microglia but not macrophages or astrocytes. J Neurochem 92:1363–1376
Horiuchi M, Maezawa I, Itoh A, Wakayama K, Jin LW, Itoh T, Decarli C (2012) Amyloid β1-42 oligomer inhibits myelin sheet formation in vitro. Neurobiol Aging 33:499–509
Hua X, Malarkey EB, Sunjara V, Rosenwald SE, Li WH, Parpura V (2004) Ca2+-dependent glutamate release involves two classes of endoplasmic reticulum Ca2+ stores in astrocytes. J Neurosci Res 76:86–97
Jablonka S, Beck M, Lechner BD, Mayer C, Sendtner M (2007) Defective Ca2+ channel clustering in axon terminals disturbs excitability in motoneurons in spinal muscular atrophy. J Cell Biol 179:139–149
Jantaratnotai N, Ryu JK, Kim SU, McLarnon JG (2003) Amyloid beta peptide induced corpus callosum damage and glial activation in vivo. Neuroreport 14:1429–1433
Jasoni CL, Todman MG, Strumia MM, Herbison AE (2007) Cell type-specific expression of a genetically encoded calcium indicator reveals intrinsic calcium oscillations in adult gonadotropin-releasing hormone neurons. J Neurosci 27:860–867
Kanwar JR, Kanwar RK, Krissansen GW (2004) Simultaneous neuroprotection and blockade of inflammation reverses autoimmune encephalomyelitis. Brain 127:1313–1331
Káradóttir R, Cavelier P, Bergersen LH, Attwell D (2005) NMDA receptors are expressed in oligodendrocytes and activated in ischaemia. Nature 438:1162–1166
Kettenmann H, Steinhauser C (2005) Receptors for neurotransmitters and hormones. In: Kettenmann H, Ransom BR (eds) Neuroglia, 2nd edn. Oxford University Press, Oxford, pp 131–145
Kettenmann H, Hanisch UK, Noda M, Verkhratsky A (2011) Physiology of microglia. Physiol Rev 91:461–553
Kirischuk S, Scherer J, Kettenmann H, Verkhratsky A (1995) Activation of P2-purinoreceptors triggered Ca2+ release from InsP3-sensitive internal stores in mammalian oligodendrocytes. J Physiol 483:41–57
Kirischuk S, Kettenmann H, Verkhratsky A (1997) Na+/Ca2+ exchanger modulates kainate-triggered Ca2+ signaling in Bergmann glial cells in situ. FASEB J 11:566–572
Koldenkova VP, Nagai T (2013) Genetically encoded Ca(2+) indicators: properties and evaluation. Biochim Biophys Acta 1833(7):1787–1797. doi:10.1016/j.bbamcr.2013.01.011
Kukley M, Barden JA, Steinhäuser C, Jabs R (2001) Distribution of P2X receptors on astrocytes in juvenile rat hippocampus. Glia 36:11–21
Lalo U, Pankratov Y, Parpura V, Verkhratsky A (2011) Ionotropic receptors in neuronal-astroglial signalling: what is the role of “excitable” molecules in nonexcitable cells. Biochim Biophys Acta 1813:992–1002
Lenertz LY, Gavala ML, Zhu Y, Bertics PJ (2011) Transcriptional control mechanisms associated with the nucleotide receptor P2X7, a critical regulator of immunologic, osteogenic, and neurologic functions. Immunol Res 50:22–38
Li S, Stys PK (2000) Mechanisms of ionotropic glutamate receptor-mediated excitotoxicity in isolated spinal cord white matter. J Neurosci 20:1190–1198
Li S, Jiang Q, Stys PK (2000) Important role of reverse Na+-Ca2+ exchange in spinal cord white matter injury at physiological temperature. J Neurophysiol 84:1116–1119
Li B, Dong L, Fu H, Wang B, Hertz L, Peng L (2011) Effects of chronic treatment with fluoxetine on receptor-stimulated increase of [Ca2+]i in astrocytes mimic those of acute inhibition of TRPC1 channel activity. Cell Calcium 50:42–53
Liu H, Molina-Holgado E, Almazan G (1997) Glutamate stimulated production of inositol phosphates is mediated by Ca2+ influx in oligodendrocyte progenitors. Eur J Pharmacol 338:277–287
MacVicar BA (1984) Voltage-dependent calcium channels in glial cells. Science 226:1345–1347
Mank M, Griesbeck O (2008) Genetically encoded calcium indicators. Chem Rev 108: 1550–1564

Manning SM, Talos DM, Zhou C, Selip DB, Park HK, Park CJ, Volpe JJ, Jensen FE (2008) NMDA receptor blockade with memantine attenuates white matter injury in a rat model of periventricular leukomalacia. J Neurosci 28:6670–6678

Marta CB, Montano MB, Taylor CM, Taylor AL, Bansal R, Pfeiffer SE (2005) Signaling cascades activated upon antibody cross-linking of myelin oligodendrocyte glycoprotein: potential implications for multiple sclerosis. J Biol Chem 280:8985–8993

Mattson MP, Chan SL (2003) Neuronal and glial calcium signaling in Alzheimer's disease. Cell Calcium 34:385–397

Matute C (2006) Oligodendrocyte NMDA receptors: a novel therapeutic target. Trends Mol Med 12:289–292

Matute C (2010) Calcium dyshomeostasis in white matter pathology. Cell Calcium 47:150–157

Matute C, Alberdi E, Domercq M, Pérez-Cerdá F, Pérez-Samartín A, Sánchez-Gómez MV (2001) The link between excitotoxic oligodendroglial death and demyelinating diseases. Trends Neurosci 24:224–230

Matute C, Domercq M, Sánchez-Gómez MV (2006) Glutamate-mediated glial injury: mechanisms and clinical importance. Glia 53:212–224

Matute C, Alberdi E, Domercq M, Sánchez-Gómez MV, Pérez-Samartín A, Rodriguez-Antigüedad A, Perez-Cerdá F (2007a) Excitotoxic damage to white matter. J Anat 210:693–702

Matute C, Torre I, Perez-Cerdá F, Pérez-Samartin A, Alberdi E, Etxebarria E, Arranz AM, Ravid R, Rodriguez-Antiguedad A, Sánchez-Gómez MV, Domercq M (2007b) P2X(7) receptor blockade prevents ATP excitotoxicity in oligodendrocytes and ameliorates experimental autoimmune encephalomyelitis. J Neurosci 27:9525–9533

McIntosh AM, Job DE, Moorhead TW, Harrison LK, Lawrie SM, Johnstone EC (2005) White matter density in patients with schizophrenia, bipolar disorder and their unaffected relatives. Biol Psychiatry 58:254–257

McIntosh AM, Munoz Maniega S, Lymer GK, McKirdy J, Hall J, Sussmann JE, Bastin ME, Clayden JD, Johnstone EC, Lawrie SM (2008) White matter tractography in bipolar disorder and schizophrenia. Biol Psychiatry 64:1088–1092

Micu I, Jiang Q, Coderre E, Ridsdale A, Zhang L, Woulfe J, Yin X, Trapp BD, McRory JE, Rehak R, Zamponi GW, Wang W, Stys PK (2006) NMDA receptors mediate calcium accumulation in myelin during chemical ischaemia. Nature 439:988–992

Micu I, Ridsdale A, Zhang L, Woulfe J, McClintock J, Brantner CA, Andrews SB, Stys PK (2007) Real-time measurements of free Ca2+ changes in CNS myelin by two- photon microscopy. Nat Med 13:874–879

Mills LR, Velumian AA, Agrawal SK, Theriault E, Fehlings MG (2004) Confocal imaging of changes in glial calcium dynamics and homeostasis after mechanical injury in rat spinal cord white matter. Neuroimage 21:1069–1082

Minelli A, Castaldo P, Gobbi P, Salucci S, Magi S, Amoroso S (2007) Cellular and subcellular localization of Na+-Ca2+ exchanger protein isoforms, NCX1, NCX2, and NCX3 in cerebral cortex and hippocampus of adult rat. Cell Calcium 41:221–234

Miyawaki A (2005) Innovations in the imaging of brain functions using fluorescent proteins. Neuron 48:189–199

Mosser J, Douar AM, Sarde CO, Kioschis P, Feil R, Moser H, Poustka AM, Mandel JL, Aubourg P (1993) Putative X-linked adrenoleukodystrophy gene shares unexpected homology with ABC transporters. Nature 361:726–730

Murugan M, Sivakumar V, Lu J, Ling EA, Kaur C (2011) Expression of N-methyl D- aspartate receptor subunits in amoeboid microglia mediates production of nitric oxide via NF-κB signaling pathway and oligodendrocyte cell death in hypoxic postnatal rats. Glia 59:521–539

Nagano T, Kawasaki Y, Baba A, Takemura M, Matsuda T (2004) Up-regulation of Na+ -Ca2+ exchange activity by interferon-gamma in cultured rat microglia. J Neurochem 90:784–791

Nehrt A, Rodgers R, Shapiro S, Borgens R, Shi R (2007) The critical role of voltage dependent calcium channel in axonal repair following mechanical trauma. Neuroscience 146:1504–1512

Nikolaeva MA et al (2005) Na+-dependent sources of intra-axonal Ca2+ release in rat optic nerve during in vitro chemical ischemia. J Neurosci 25:9960–9967

Noda M, Nakanishi H, Nabekura J, Akaike N (2000) AMPA-kainate subtypes of glutamate receptor in rat cerebral microglia. J Neurosci 20:251–258

Nörenberg W, Schunk J, Fischer W, Sobottka H, Riedel T, Oliveira JF, Franke H, Illes P (2010) Electrophysiological classification of P2X7 receptors in rat cultured neocortical astroglia. Br J Pharmacol 160:1941–1952

Ohkura M, Sasaki T, Kobayashi C, Ikegaya Y, Nakai J (2012) An improved genetically encoded red fluorescent Ca2+ indicators for detecting optically evoked action potentials. PLoS One 7:e39933

Oliveira JF, Riedel T, Leichsenring A, Heine C, Franke H, Krügel U, Nörenberg W, Illes P (2011) Rodent cortical astroglia express in situ functional P2X7 receptors sensing pathologically high ATP concentrations. Cereb Cortex 21:806–820

Ouardouz M et al (2003) Depolarization-induced Ca2+ release in ischemic spinal cord white matter involves L-type Ca2+ channel activation of ryanodine receptors. Neuron 40:53–63

Ouardouz M et al (2006) Complex interplay between glutamate receptors and intracellular Ca2+ stores during ischaemia in rat spinal cord white matter. J Physiol 577:191–204

Ouardouz M, Coderre E, Basak A, Chen A, Zamponi GW, Hameed S, Rehak R, Yin X, Trapp BD, Stys PK (2009a) Glutamate receptors on myelinated spinal cord axons. I. GluR6 kainate receptors. Ann Neurol 65:151–159

Ouardouz M, Coderre E, Zamponi GW, Hameed S, Yin X, Trapp BD, Stys PK (2009b) Glutamate receptors on myelinated spinal cord axons. II. AMPA and GluR5 receptors. Ann Neurol 65:160–166

Oyanguren-Desez O, Rodríguez-Antigüedad A, Villoslada P, Domercq M, Alberdi E, Matute C (2011) Gain-of-function of P2X7 receptor gene variants in multiple sclerosis. Cell Calcium 50:468–472

Pak KJ, Chan SL, Mattson MP (2003) Presenilin-1 mutation sensitizes oligodendrocytes to glutamate and amyloid toxicities and exacerbates which matter damage and memory impairment in mice. Neuromolecular Med 3:53–64

Palmer AE, Jin C, Reed JC, Tsien RY (2004) Bcl-2-mediated alterations in endoplasmic reticulum Ca2+ analyzed with an improved genetically encoded fluorescent sensor. Proc Natl Acad Sci U S A 101:17404–17409

Palygin O, Lalo U, Verkhratsky A, Pankratov Y (2010) Ionotropic NMDA and P2X1/5 receptors mediate synaptically induced Ca2+ signalling in cortical astrocytes. Cell Calcium 48: 225–231

Pampliega O, Domercq M, Villoslada P, Sepulcre J, Rodriguez-Antiguedad A, Matute C (2008) Association of an EAAT2 polymorphism with higher glutamate concentration in relapsing multiple sclerosis. J Neuroimmunol 195:194–198

Paredes RM, Etzler JC, Watts LT, Zheng W, Lechleiter JD (2008) Chemical calcium indicators. Methods 46:143–151

Parri HR, Gould TM, Crunelli V (2001) Spontaneous astrocytic Ca2+ oscillations in situ drive NMDAR-mediated neuronal excitation. Nat Neurosci 4:803–812

Peng W, Cotrina ML, Han X et al (2009) Systemic administration of an antagonist of the ATP-sensitive receptor P2X7 improves recovery after spinal cord injury. Proc Natl Acad Sci U S A 106:12489–12493

Pignataro G, Gala R, Cuomo O, Tortiglione A, Giaccio L, Castaldo P, Sirabella R, Matrone C, Canitano A, Amoroso S, Di Renzo GF, Annunziato L (2004) Two sodium/calcium exchanger gene products, NCX1 and NCX3, play a major role in the development of permanent focal cerebral ischemia. Stroke 35:2566–2570

Piña-Crespo JC, Talantova M, Micu I, States B, Chen HS, Tu S, Nakanishi N, Tong G, Zhang D, Heinemann SF, Zamponi GW, Stys PK, Lipton SA (2010) Excitatory glycine responses of CNS myelin mediated by NR1/NR3 “NMDA” receptor subunits. J Neurosci 30:11501–11505

Pitt D, Werner P, Raine CS (2000) Glutamate excitotoxicity in a model of multiple sclerosis. Nat Med 6:67–70

Poliak S, Peles P (2003) The local differentiation of myelinated axons at nodes of Ranvier. Nat Rev Neurosci 4:968–980

Prineas JW, McDonald WI, Franklin RJM (2002) Demyelinating diseases. In: Graham DI, Lantos PL (eds) Greenfield's neuropathology, vol 2. Arnold, London, pp 471–550
Raivich G, Banati R (2004) Brain microglia and blood-derived macrophages: molecular profiles and functional roles in multiple sclerosis and animal models of autoimmune demyelinating disease. Brain Res Rev 46:261–281
Reyes RC, Parpura V (2008) Mitochondria modulate Ca2+-dependent glutamate release from rat cortical astrocytes. J Neurosci 28:9682–9691
Rojas H, Colina C, Ramos M, Benaim G, Jaffe EH, Caputo C, DiPolo R (2007) Na+ entry via glutamate transporter activates the reverse Na+/Ca2+ exchange and triggers Ca2+-induced Ca2+ release in rat cerebellar type-1 astrocytes. J Neurochem 100:1188–1202
Ruiz A, Matute C, Alberdi E (2010) Intracellular Ca2+ release through ryanodine receptors contributes to AMPA receptor-mediated mitochondrial dysfunction and ER stress in oligodendrocytes. Cell Death Dis 1:e54
Salter MG, Fern R (2005) NMDA receptors are expressed in developing oligodendrocyte processes and mediate injury. Nature 438:1167–1171
Sánchez-Gómez MV, Alberdi E, Ibarretxe G, Torre I, Matute C (2003) Caspase-dependent and caspase-independent oligodendrocyte death mediated by AMPA and kainate receptors. J Neurosci 23:9519–9528
Sánchez-Gómez MV, Alberdi E, Pérez-Navarro E, Alberch J, Matute C (2011) Bax and calpain mediate excitotoxic oligodendrocyte death induced by activation of both AMPA and kainate receptors. J Neurosci 31:2996–3006
Skaper SD, Debetto P, Giusti P (2010) The P2X7 purinergic receptor: from physiology to neurological disorders. FASEB J 24:337–345
Smith T, Groom A, Zhu B, Turski L (2000) Autoimmune encephalomyelitis ameliorated by AMPA antagonists. Nat Med 6:62–66
Srinivasan R, Sailasuta N, Hurd R, Nelson S, Pelletier D (2005) Evidence of elevated glutamate in multiple sclerosis using magnetic resonance spectroscopy at 3 T. Brain 128:1016–1025
Streit WJ, Morioka T, Kalehua AN (1992) MK-801 prevents microglial reaction in rat hippocampus after forebrain ischemia. Neuroreport 3:146–148
Stys PK (2004) White matter injury mechanisms. Curr Mol Med 4:113–130
Takahashi A, Camacho P, Lechleiter JD, Herman B (1999) Measurements of intracellular calcium. Physiol Rev 79:1089–1125
Takuma K, Matsuda T, Hashimoto H, Asano S, Baba A (1994) Cultured rat astrocytes possess Na+-Ca2+ exchanger. Glia 12:336–342
Talos DM, Follett PL, Folkerth RD, Fishman RE, Trachtenberg FL, Volpe JJ, Jensen FE (2006) Developmental regulation of alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor subunit expression in forebrain and relationship to regional susceptibility to hypoxic/ischemic injury. II. Human cerebral white matter and cortex. J Comp Neurol 497:61–77
Thomas DM, Kuhn DM (2005) MK-801 and dextromethorphan block microglial activation and protect against methamphetamine-induced neurotoxicity. Brain Res 1050:190–198
Thorell WE, Leibrock LG, Agrawal SK (2002) Role of RyRs and IP3 receptors after traumatic injury to spinal cord white matter. J Neurotrauma 19:335–342
Tomes DJ, Agrawal SK (2002) Role of Na+-Ca2+ exchanger after traumatic or hypoxic/ischemic injury to spinal cord white matter. Spine J 2:35–40
Tsuchiya R, Yoshiki F, Kudo Y, Morita M (2002) Cell type-selective expression of green fluorescent protein and the calcium indicating protein, yellow cameleon, in rat cortical primary cultures. Brain Res 956:221–229
Uranova NA, Vostrikov VM, Orlovskaya DD, Rachmanova VI (2004) Oligodendroglial density in the prefrontal cortex in schizophrenia and mood disorders: a study from the Stanley Neuropathology Consortium. Schizophr Res 67:269–275
Vallejo-Illarramendi A, Domercq M, Pérez-Cerdá F, Ravid R, Matute C (2006) Increased expression and function of glutamate transporters in multiple sclerosis. Neurobiol Dis 21:154–164
Verbny Y, Zhang CL, Chiu SY (2002) Coupling of calcium homeostasis to axonal sodium in axons of mouse optic nerve. J Neurophysiol 88:802–816

Verkhratsky A (2005) Physiology and pathophysiology of the calcium store in the endoplasmic reticulum of neurons. Physiol Rev 85:201–279
Verkhratsky A, Butt A (2007) Glial neurobiology. A textbook. Wiley, Chichester
Verkhratsky A, Petersen OH (2002) The endoplasmic reticulum as an integrating signalling organelle: from neuronal signalling to neuronal death. Eur J Pharmacol 447:141–154
Verkhratsky A, Anderova M, Chvatal A (2009a) Differential calcium signalling in neuronal-glial networks. Front Biosci 14:2004–2016
Verkhratsky A, Krishtal OA, Burnstock G (2009b) Purinoceptors on neuroglia. Mol Neurobiol 39:190–208
Wang L-Y, Cai W-Q, Chen P-H, Deng Q-Y, Zhao C-M (2009) Downregulation of P2X7 receptor expression in rat oligodendrocyte precursor cells after hypoxia ischemia. Glia 57:307–319
Wu LJ, Zhuo M (2008) Resting microglial motility is independent of synaptic plasticity in mammalian brain. J Neurophysiol 99:2026–2032
Xu J, Chen S, Ahmed SH, Chen H, Ku G, Goldberg MP, Hsu CY (2001) Amyloid-beta peptides are cytotoxic to oligodendrocytes. J Neurosci 21(1):118
Yaguchi T, Nishizaki T (2010) Extracellular high K+ stimulates vesicular glutamate release from astrocytes by activating voltage-dependent calcium channels. J Cell Physiol 225:512–518
Zamvil SS, Steinman L (2003) Diverse targets for intervention during inflammatory and neurodegenerative phases of multiple sclerosis. Neuron 38:685–688
Zhang K, Sejnowski TJ (2000) A universal scaling law between gray matter and white matter of cerebral cortex. Proc Natl Acad Sci U S A 97:5621–5626

Chapter 19
Inflammation and White Matter Injury in Animal Models of Ischemic Stroke

Lyanne C. Schlichter, Sarah Hutchings, and Starlee Lively

Abbreviations

AchAo	Anterior choroidal artery occlusion
AMPA	2-Amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid
APC	Adenomatous polyposis coli
APP	Amyloid precursor protein
ATP	Adenosine triphosphate
BBB	Blood–brain barrier
BCCAo	Bilateral common carotid artery occlusion
CBF	Cerebral blood flow
CNS	Central nervous system
COX-2	Cyclooxygenase-2
CR3A	Complement component receptor 3 alpha
dMBP	Degraded myelin basic protein
eNOS	Endothelial nitric oxide synthase
ET-1	Endothelin-1
FACS	Fluorescence-activated cell sorting
GABA	Gamma-aminobutyric acid
GFP	Green fluorescent protein

The last two authors contributed equally.

L.C. Schlichter (✉) • S. Hutchings
Genes & Development, Toronto Western Research Institute,
University Health Network, Toronto, ON, Canada

Department of Physiology, University of Toronto, Toronto, ON, Canada
e-mail: schlicht@uhnres.utoronto.ca; sarah.hutchings@utoronto.ca

S. Lively
Genes & Development,Toronto Western Research Institute,
University Health Network,Toronto, ON, Canada
e-mail: slively@uhnresearch.ca

S. Baltan et al. (eds.), *White Matter Injury in Stroke and CNS Disease*, Springer Series in Translational Stroke Research 4, DOI 10.1007/978-1-4614-9123-1_19,

H&E	Hematoxylin and eosin
HI	Hypoxia–ischemia
IB_4	Isolectin B4
Iba1	Ionized calcium binding adapter molecule 1
ICA	Internal carotid artery
ICH	Intracerebral hemorrhage
IL	Interleukin
ITGAM	Integrin alpha M
LCA	Leukocyte common antigen
LFB	Luxol fast blue
Mac-1	Macrophage-1 alpha antigen
MBP	Myelin basic protein
MCAo	Middle cerebral artery occlusion
MHC	Major histocompatibility complex
MMP	Matrix metallopeptidase
MPO	Myeloperoxidase
MRI	Magnetic resonance imaging
NKT	Natural killer T cells
NO	Nitric oxide
NOS	Nitric oxide synthase
OGD	Oxygen-glucose deprivation
RAG1	Recombination activating gene 1
TGFβ	Transforming growth factor beta
TIMP	Tissue inhibitor of metallopeptidase
TNF-α	Tumor necrosis factor alpha
tPA	Tissue plasminogen activator

19.1 Introduction

19.1.1 Statement of the Problem

This entire book focuses on white matter; ranging from structure and distribution in the central nervous system (CNS), to methods for monitoring injury and repair, and mechanisms underlying white matter injury. Many types of CNS insult in patients and in animal models are considered, and several chapters deal specifically with stroke in patients, and with ischemia in animal models. Ischemic stroke is much more prevalent than hemorrhagic stroke (Adams et al. 1993) and is the leading cause of long-term disability in the USA and the second leading cause of death worldwide (Kelly-Hayes et al. 1998; Lopez and Mathers 2006). Primary ischemic stroke can be global (usually the result of cardiac arrest or an aortic occlusion) (Traystman 2003) or focal, if caused by a transient or permanent occlusion of a major cerebral artery or one or more deep blood vessels (so-called lacunar stroke) (Durukan and Tatlisumak 2007). Intracerebral hemorrhage (ICH) results from rupture of small brain arteries or arterioles, and accounts for the remaining 10–15 % of strokes in

Western populations (Anderson et al. 1994). Up to 30–40 % of ischemic strokes spontaneously undergo hemorrhagic transformation (Wang and Lo 2003), and ICH patients are at a high risk of developing global ischemia (Qureshi et al. 2009). In addition, ischemic stroke is a risk factor when treating hemorrhage with the blood-clot promoter; factor VIIa, the only drug used for treating ICH. While factor VIIa can decrease hematoma expansion (Diringer et al. 2008; Mayer et al. 2005, 2008), the outcome is often unimproved (Mayer et al. 2008; Lin et al. 2012).

The only effective drug treatment for ischemic stroke patients is the thrombolytic, tissue plasminogen activator (tPA). While tPA can, in limited cases, produce a remarkable reperfusion of damaged tissue, its use is limited by the need to inject it within the first 3–4.5 h of stroke onset (Wang et al. 2004). Because most patients are not in hospital and assessed in that time window, only about 5 % of patients can currently benefit from tPA therapy (del Zoppo 1998). This has spawned a huge and ongoing effort to identify new therapeutic targets and drugs. Hundreds of chemicals that target early neurotoxicity have been identified, and dozens have proven promising in preclinical studies in rodents but have been ineffective in improving survival or functional outcomes in clinical trials (Brott and Bogousslavsky 2000; Ginsberg 2009; Lakhan et al. 2009). This failure has led to deeper consideration of potential shortcomings of experimental approaches and models used in preclinical stroke research (Braeuninger and Kleinschnitz 2009; Dirnagl 2006; O'Collins et al. 2006). Several reports from European and North American Stroke Consortia (Stroke Therapy Academic Industry Roundtable) (Endres et al. 2008; Meairs et al. 2006; Fisher 1999) have emphasized that most experimental studies target events that are too early to be "drug-able," are restricted to healthy, young male rodents, lack relevant comorbidities (e.g., hypertension, diabetes), monitor infarct size as their primary outcome measure, and have been highly neurocentric. Most stroke research has explored neural cell death and neural cell repair (Petty and Wettstein 1999). Many studies have relied on showing treatment-related changes in neuron apoptosis (e.g., TUNEL, caspase 3 activation), damaged (e.g., Fluorojade) or remaining neurons (e.g., NeuN staining). Recommendations from stroke consortia for improving preclinical studies include: (1) focus more on white matter injury; (2) identify strategies to protect vascular and glial cells, not just neurons; (3) focus more on aged animals; (4) develop small animal models of lacunar stroke and compare mechanisms of damage with large vessel occlusion; and (5) identify and understand neuroimmune interactions and develop therapeutic strategies to target inflammation. This chapter will address several of these recommendations for preclinical studies.

19.1.2 Why Study Inflammation and White Matter Injury After Ischemic Stroke?

Historically, white matter was thought to be less susceptible to stroke than gray matter (Marcoux et al. 1982). More recent advances in imaging technology have led to a paradigm shift. Development of neuroimaging techniques, such as magnetic resonance imaging (MRI) and Diffuse Tensor Imaging, has provided the means

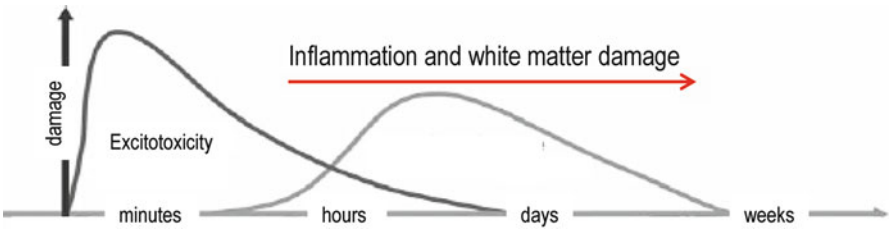


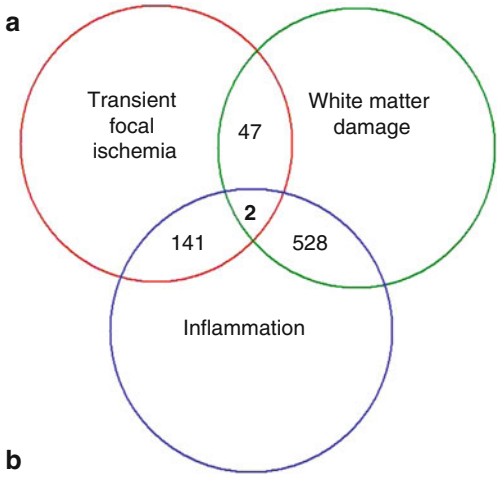
Fig. 19.1 The generally accepted time course of damaging events after stroke in humans (Sect. 19.1.2). Until recently, most experimental studies in animals focused on neurotoxicity, which is initiated very rapidly after stroke. This chapter addresses the inflammation phase which is delayed and prolonged

to map networks of white matter in the human brain at the macroscopic level. Resulting clinical observations have shown that ischemic stroke is rarely confined to gray matter (Goldberg and Ransom 2003), and primary white matter injury occurs in about 25 % of human strokes (Matute et al. 2013). At the microscopic level, more recent research has demonstrated the sensitivity of the major cellular components of white matter to ischemia. Several animal models have shown the susceptibility to ischemic damage of oligodendrocytes, the myelin forming cells of the CNS (reviewed by (Arai and Lo 2009)), myelin and axons (Petty and Wettstein 1999; Hughes et al. 2003; Irving et al. 2001; Lively and Schlichter 2012; Medana and Esiri 2003; Moxon-Emre and Schlichter 2010, 2011; Pantoni et al. 1996). Thus, white matter is now considered an important contributor to stroke outcome, and a tractable therapeutic target.

A second paradigm shift has been the change in focus of preclinical investigations searching for new post-stroke therapies. The emphasis is moving away from the rapidly occurring primary neurotoxicity to the secondary injury phase, which occurs in a time window that is amenable to treatment in hospital. This secondary phase is characterized by a prominent inflammatory response within and surrounding the core infarct that can last for many hours to days (Emsley and Tyrrell 2002; Jin et al. 2010; Zhang and Stanimirovic 2002). However, inflammation is complex. Understanding its temporal-spatial development and which components are harmful versus beneficial will be essential for developing better therapeutic strategies. Figure 19.1 illustrates the commonly proposed time course of events after stroke in humans.

19.1.3 Scope of This Chapter

Much of this book is devoted to white matter damage after acute injury, including ischemic stroke and intracerebral and subarachnoid hemorrhage. Thus, we will not review findings that focus solely on white matter damage. Instead, the chapter will focus on the intersection of inflammation and white matter injury in the context of experimental models of ischemia. In focusing on ischemic stroke (the vast majority), the chapter begins by describing the main models used to monitor white matter injury



b

Stroke-related	White matter-related	Inflammation-related
Transient focal ischemia	White matter	Inflammation
Hypoxia ischemia	Axon damage	Microglia
	Myelin	Macrophage
	Corpus callosum	Neutrophil
	Oligodendrocytes	Lymphocyte
	Internal capsule	Dendritic cell

Fig. 19.2 As discussed in Sect. 19.1.3, very few studies consider all three elements. (**a**) Venn diagram showing the approximate number of articles found when each pair of terms or the combined term "transient focal ischemia AND white matter AND inflammation" was entered into PubMed. (Human studies were excluded.) (**b**) To broaden the scope of the search, additional search terms and combinations were included. There was a slight variation in the number of articles found, depending on which search terms were combined. However, in every case, the intersection of elements from all three columns retrieved very few papers

in experimental stroke studies (mainly rodents). We describe methods used to monitor white matter damage in these models, and provide some figures to illustrate why we prefer some approaches over others. Because this is the only chapter in this book that explicitly deals with inflammation, we then provide a brief primer on inflammation. This section describes the main immune cells involved in animal models of ischemia, how to monitor them, and key findings. Then, we summarize the limited literature concerning the intersection of all three elements (ischemic stroke, white matter injury, inflammation) in both adult and neonatal rodent ischemia models. The wrap-up section comments on needs for further research.

In selecting literature related to this chapter, we searched the PubMed database up to March 2013. As shown in Fig. 19.2, the search began with original and review articles that contained all three topics: transient focal ischemia + white matter + inflammation. Remarkably, only two publications were found. We then broadened the search using combinations of multiple search terms, and included studies that examined inflammation and white matter damage in neonatal hypoxia–ischemia and adult chronic ischemia models.

19.2 Animal Models Used to Study White Matter Injury After Ischemia

19.2.1 White Matter Locations in the Brain

It is often noted that the ratio of white matter to gray matter in the human brain is 60:40, but much lower in the rat (14:86) and mouse (10:90) (Krafft et al. 2012). While this might be considered an impediment to studying white matter injury, rodents have several brain regions with high densities of white matter (Johnson et al. 2012). Several of these regions are commonly used to study white matter damage in rodents. The corpus callosum is comprised of white matter tracts (nerve fibers) that connect the two cerebral hemispheres, transferring and integrating information from the left and right hemispheres (Bruni and Montemurro 2009). The internal capsule is a compact band of fibers that lies deep in the brain, separating the caudate nucleus from the putamen. It consists of projection fibers that relay information from the cortex and spinal cord, brain stem, and subcortical structures (Bruni and Montemurro 2009). The optic nerve, formed by axons of retinal ganglion cells, carries information from the retina to the thalamus and other subcortical nuclei (Perry and Cowey 1984; Perry et al. 1984). Other white matter-dense brain regions include, but are not limited to, the anterior commissure, cingulum, extreme capsule, and external capsule (Bruni and Montemurro 2009). Some brain regions such as the striatum provide an excellent template for studying white and gray matter injury side-by-side. As Fig. 19.3 illustrates, the striatum contains a large number of myelinated axon bundles that are surrounded by nerve cell bodies and dendrites, and astroglial and microglial cells. We have exploited this structure in rats to simultaneously analyze inflammation, neuronal death, and damage to myelin and axons after both ischemic and hemorrhagic stroke (Lively and Schlichter 2012; Moxon-Emre and Schlichter 2010, 2011; Lively et al. 2011; Wasserman and Schlichter 2007, 2008; Wasserman et al. 2008).

19.2.2 Ischemia Models Used to Study White Matter Damage

It is clear that no animal model can fully recapitulate all components of human stroke (Durukan and Tatlisumak 2007; Howells et al. 2010). Most preclinical studies are conducted in small animals, especially rodents (Durukan and Tatlisumak 2007), and almost all results relevant to this chapter come from rats and mice. While mouse models facilitate using transgenic animals, which are of limited availability in rats (Durukan and Tatlisumak 2007), we mainly exploit rats for several reasons. The cerebrovascular anatomy and physiology is reasonably similar between rats and humans (Macrae 1992). In the most commonly used mouse model of ischemia (middle cerebral artery occlusion (MCAo), discussed below), the infarct size

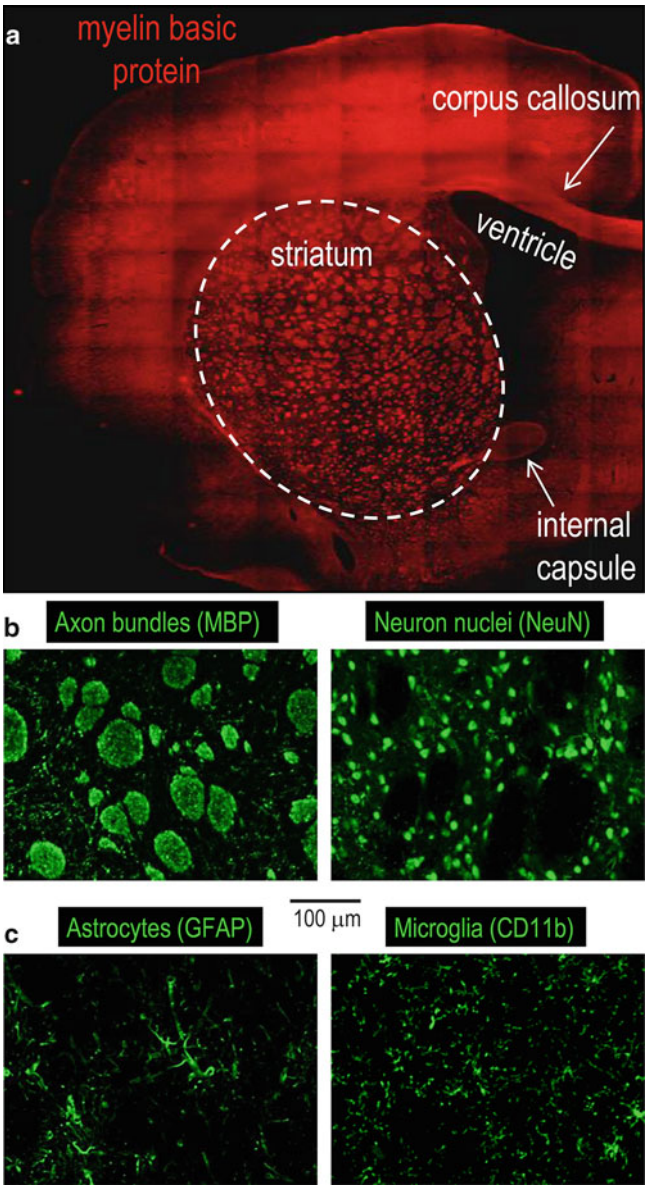


Fig. 19.3 As discussed in Sect. 19.2.1, the architecture of the rat striatum provides advantages for studying white matter and gray matter together with glial responses and inflammation. (**a**) Coronal section of healthy adult rat brain labeled with an antibody against myelin basic protein (MBP). Note the structure of the striatum (*circled*), which contains numerous white matter bundles (axon tracts) cut in cross section. Also shown are two regions of relatively pure white matter: the corpus callosum and internal capsule. Low magnification images were digitally stitched to show an entire hemisphere. (Modified from Moxon-Emre I and Schlichter LC. 2010. Evolution of inflammation and white matter injury in a model of transient focal ischemic stroke. J. Neuropathol. Expt'l Neurol. 69:1–15.) (**b**) Higher magnification images show bundles of myelinated axons (*left*: MBP labeled), surrounded by gray matter and neuropil (*right*) in which neuronal nuclei are labeled with NeuN antibody. (**c**) Outside the axon bundles, there are astrocytes (immunolabeled for glial fibrillary acidic protein, GFAP) and ramified, resting microglia (labeled with OX-42 antibody, which recognizes CD11b)

is highly variable and shows strain-dependence that is not seen in rats (Carmichael 2005). The positive correlation between brain size and amount of white matter (Zhang and Sejnowski 2000) and larger brain size of rats yields more material for analysis. The larger size is especially useful when one seeks to analyze several parameters or stains, and to quantify differences across brain regions.

19.2.2.1 Vessel Occlusion

Middle cerebral artery occlusion. Most human ischemic strokes occur in the area surrounding the middle cerebral artery (MCA). A rodent model of MCAo was developed early and has since been refined (Koizumi et al. 1986; Robinson et al. 1975). Robinson et al. (1975) used craniotomy to expose the MCA and distally ligated the artery, which produced an ischemic lesion that extended into the cortex. Now, a more commonly used method for occluding blood flow is to advance an intraluminal suture through the internal carotid artery (ICA) until it reaches the origin of the MCA (del Zoppo et al. 1992). With this method, transient ischemia is produced by removing the suture to allow reperfusion. The duration of MCAo needed to produce a significant nonlethal lesion is variable. The common duration for rat is 60, 90, or 120 min (Carmichael 2005), and usually results in neuron death throughout the striatum and into the dorsolateral cortex, but not in contralateral brain regions (Garcia et al. 1995). Based on the distribution of injury, the MCAo model is useful for simultaneously analyzing damage that is mainly isolated to gray matter (in the cortex), white matter (in the corpus callosum), or occurs in both (in the striatum). Despite its popularity, an important limitation of the MCAo model is the variable size and location of the infarct (Liu and McCullough 2011). Some of the variability seen in the literature can be attributed to differences in size and quality of the suture/filament used (Kuge et al. 1995); silicone-coated thread induces a larger lesion than uncoated thread (Laing et al. 1993). When using mice, some strains demonstrate substantially larger infarcts after MCAo than others (Connolly et al. 1996a). Other limitations of the MCAo model are the risk of subarachnoid hemorrhage (Carmichael 2005), and the added difficulty in aged animals, which have less flexible blood vessels and higher mortality (Liu and McCullough 2011).

Fig. 19.4 (continued) putamen+caudate of the striatum. Staining with 2 % TTC (2,3,5,-triphenyl-2H-tetrazolium chloride) appears pale in the metabolically compromised infarcted regions. (**c**) Example of sampling sites for quantification in a TTC-stained section at Day 7 after ET-1 injection: infarct core (*red*), edge (*white*), surrounding striatum (*blue*), uninjured contralateral striatum (*green*). We sample multiple regions to quantify parameters that include staining area, staining intensity, density of specific cell types. For statistical comparisons, the average of four "boxes" from each of several sections is then averaged for multiple animals. Comparisons include: naïve animals, saline-injected control animals, and the contralateral hemisphere of ET-1 injected animals. (Modified from Moxon-Emre I and Schlichter LC. 2010. Evolution of inflammation and white matter injury in a model of transient focal ischemic stroke. J. Neuropathol. Expt'l Neurol. 69: 1–15)

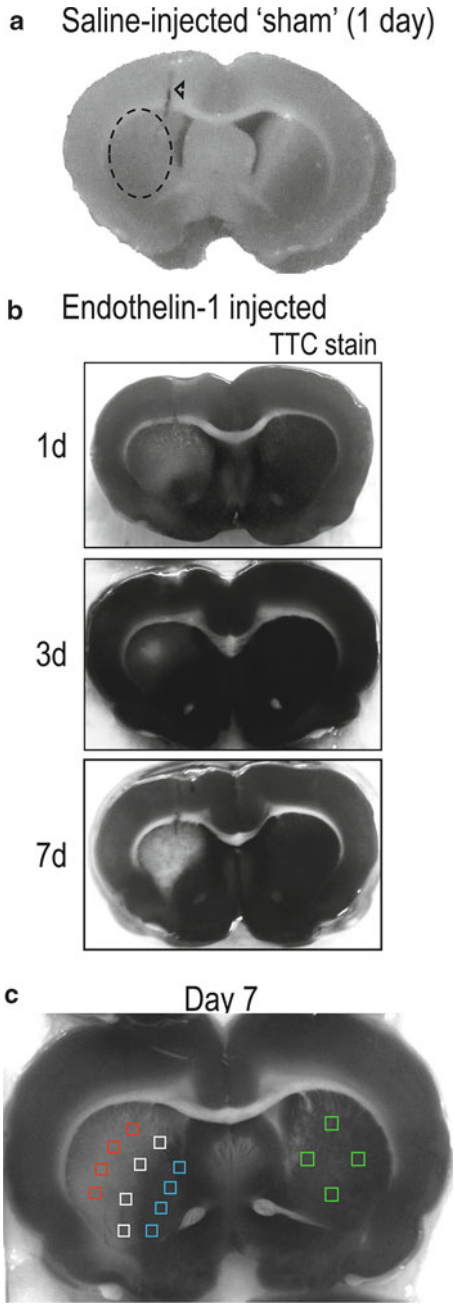


Fig. 19.4 Focal ischemia induced by endothelin-1 (ET-1) injection in the rat (Sect. 19.2.2.2). (**a**) There was no infarct in saline-injected control rats. The location of the needle penetration track is indicated by a small amount of bleeding seen 1 day after saline injection (*arrowhead*). (**b**) Stereotaxic injection of ET-1 into the anterior striatum produced a lesion that was restricted to the

Anterior choroidal artery occlusion. Occlusion of the anterior choroidal artery (AchAo) can be obtained by advancing an intraluminal suture through the ICA to a region *proximal* to the MCA (He et al. 1999). As illustrated in detail in their follow-up paper, the infarct location is similar in MCAo and AchAo (He et al. 2000). The main differences are that after AchAo, the infarct is generally smaller but nearly always encompasses the internal capsule. Thus, AchAo reliably produces ischemia in a white matter-dense region, and facilitates studies of deep lacunar strokes with a less-distributed lesion than MCAo.

19.2.2.2 Vasoconstriction

An increasingly popular model uses injection of the potent vasoconstrictor, endothelin-1 (ET-1). ET-1 is a naturally occurring, 21 amino acid peptide produced by endothelial cells, which binds to the endothelial receptors, ET_A and ET_B (Verhaar et al. 1998). There are several advantages of this model over vessel occlusion models. Provided the potency of each ET-1 batch is tested, the injected dose can be titrated to obtain different degrees of transient ischemia, and lesion sizes that are relatively reproducible. For instance, with moderate amounts of ET-1 injected directly into the brain parenchyma, a reduction of cerebral blood flow (CBF) of ~60 % for up to 3 h has been reported (Hughes et al. 2003).

ET-1 is sometimes applied to the MCA to cause constriction, instead of ligating the artery in rats (Gresle et al. 2006; Robinson et al. 1990). Importantly, stereotaxic ET-1 injection can be used to produce a focal infarct in brain regions that are selected to focus on white matter (e.g., corpus callosum, internal capsule), gray matter (e.g., cortex), or both (e.g., striatum) (Sozmen et al. 2012). When injected into white matter-rich regions, ET-1 produces hallmarks of white matter injury seen in humans, including axonal damage, demyelination, inflammation, and glial scar formation (Hughes et al. 2003; Lively and Schlichter 2012; Moxon-Emre and Schlichter 2010). Figure 19.4 shows the development of a typical infarct after ET-1 injection into the anterior striatum (putamen + caudate) of the rat, and illustrates sample sites for quantitative spatial analysis.

There are few mouse studies using ET-1 injection to evoke transient ischemia, and the results have been inconsistent. Injecting ET-1 into the striatum failed to induce ischemia in multiple strains of mice, and increasing the dose increased mortality without inducing a lesion (Horie et al. 2008). Another study showed a dose-dependent increase in infarct size in mouse brain subjected to multiple focal injections of ET-1 (Sozmen et al. 2009). Differences in expression of endothelin receptor subtypes might be a confounding factor. Mouse brain expresses a lower proportion of ET_A receptors, which evoke the vasoconstriction needed to produce ischemia, than ET_B receptors, which generally cause vasodilation (but see below) (Sozmen et al. 2012; Wiley and Davenport 2004). The possibility was raised that the larger proportion of ET_B receptors interferes with induction of ischemia in the mouse brain (Sozmen et al. 2012). That differences in ET-1 affinities are responsible

is unlikely: the K_d for ET-1 binding is 0.12 nM for ET_B and 0.6 nM for ET_A receptors (Haynes et al. 1995). A further complication is that in humans, ET_B receptors can mediate both vasodilation and vasoconstriction (Haynes et al. 1995; Clozel et al. 1992; Seo et al. 1994). This might explain the finding that although the rat striatum contains mainly ET_B receptors (Tayag et al. 1996), a focal ET-1 injection reliably produces an ischemic infarct (described above). Further research is needed to clarify the regional and cell-specific expression, and roles of the ET-1 receptor subtypes in rodents. Nevertheless, focal ET-1 injection into the rat CNS is a widely accepted model of transient ischemia that can create reproducible lesions restricted to a desired region of the brain (Sozmen et al. 2012).

19.2.2.3 Neonatal Hypoxia–Ischemia Models

The most commonly used model of ischemic injury in immature rodents is the hypoxia–ischemia (HI) model (Vannucci and Hagberg 2004). To induce ischemia, the common carotid artery is permanently ligated, and the whole body is temporarily exposed to hypoxia (often 8 % oxygen for up to 3 h). This procedure reduces CBF to the ipsilateral brain region by 40–60 %, and then CBF returns to normal immediately after re-exposure to normoxic conditions (Vannucci et al. 1988). Unlike adult rodents, ligation or hypoxia alone does not induce ischemia in neonates: both are required (Vannucci and Hagberg 2004). The resulting infarct is large but restricted to the ipsilateral hemisphere; damage to the contralateral hemisphere is rare (Towfighi et al. 1995; Vannucci and Vannucci 1997). On the ipsilateral side, the infarct extends through the cerebral cortex, striatum, thalamus, hippocampus, and most importantly for the topic of this chapter, the subcortical white matter. HI is exploited as an animal model of cerebral palsy (Johnston et al. 2005), a disorder seen in about 1/300 8-year-old children in the USA (Kirby et al. 2011). Several modifications of the HI model have also been used, including bilateral carotid ligation or variations in the duration of hypoxia. In this chapter, we will restrict discussion to rodent models using unilateral ligation followed by 6–8 % oxygen.

19.2.2.4 Optic Nerve Ischemia

The myelinated axon tracts that make up the optic nerve have proven extremely useful for studying mechanisms underlying white matter injury. Much of the stroke-related mechanistic work has used isolated optic nerves subjected to varying periods of oxygen-glucose deprivation (OGD) to simulate ischemia (Arai and Lo 2009). However, the optic nerve is one of the most vulnerable white matter regions following experimental chronic ischemia (Wakita et al. 1994). ET-1 has been injected into the microvasculature of the optic nerve in vivo (Cioffi et al. 1995), and some consider the resulting ischemia and white matter injury to be a good model of human glaucoma (Cioffi 2005).

19.2.3 Methods Used to Monitor White Matter Damage After Ischemia

As mentioned above, neuronal axons and the myelin sheath are both susceptible to ischemic injury. Despite their intimate connections, the timing and mechanisms underlying damage to these structures differs. The vulnerability of oligodendrocytes to ischemic injury can lead to myelin loss and prevent its repair. Therefore, to comprehensively examine white matter injury after ischemia, it is important to distinguish myelin from axons (both healthy and damaged) and to monitor oligodendrocytes.

19.2.3.1 Staining Healthy and Damaged Myelin

Histological stains for myelin have been in use for many years, with loss of staining indicating white matter pathology. Healthy myelin can be labeled with colorimetric stains, such as the lipid soluble dyes, Luxol Fast Blue (LFB) (Kluver and Barrera 1953; Salthouse 1962) and Sudan Black B (Stilwell 1957) but these dyes provide low contrast and resolution (Schmued et al. 2008). There are several recent improvements in myelin labeling that are less expensive than antibody-based methods. Gold-chloride and, especially, the improved gold-phosphate complex ("Black-Gold") provide excellent contrast under bright-field and dark-field illumination (Wasserman and Schlichter 2008; Schmued et al. 2008). If fluorescence is required, FluoroMyelin™ stain is attractive because it can be combined with immunohistochemistry in fixed tissue. For live imaging, FluoroMyelin™ Red is a vital dye that labels myelinated axons (Monsma and Brown 2012).

The use of antibody-based immunohistochemistry for detecting myelin damage has become popular because it can provide excellent contrast and allows use of multiple stains. The most commonly used protein target is myelin basic protein (MBP), which constitutes up to 30 % of myelin in the CNS (Sternberger et al. 1978). A reduction in antibody staining for MBP has been used to monitor myelin damage or loss in animal models of transient ischemia (Irving et al. 2001; Lively and Schlichter 2012; Moxon-Emre and Schlichter 2010; Souza-Rodrigues et al. 2008) and HI (Carty et al. 2008; Villapol et al. 2011). A cautionary note in relying solely on MBP labeling is that the staining can appear faint if healthy myelinated axon bundles are very tightly packed (Sternberger et al. 1978). If so, then the swelling of damaged axon bundles might actually increase the MBP labeling. An important advance that overcomes this limitation is the immunological detection of degraded MBP (dMBP) (Matsuo et al. 1997). We prefer this approach and, as shown in Fig. 19.5, have found that the increase in dMBP labeling coincides temporally and spatially with loss of MBP after transient ischemia evoked by injecting the vasoconstrictor, ET-1, into the rat striatum.

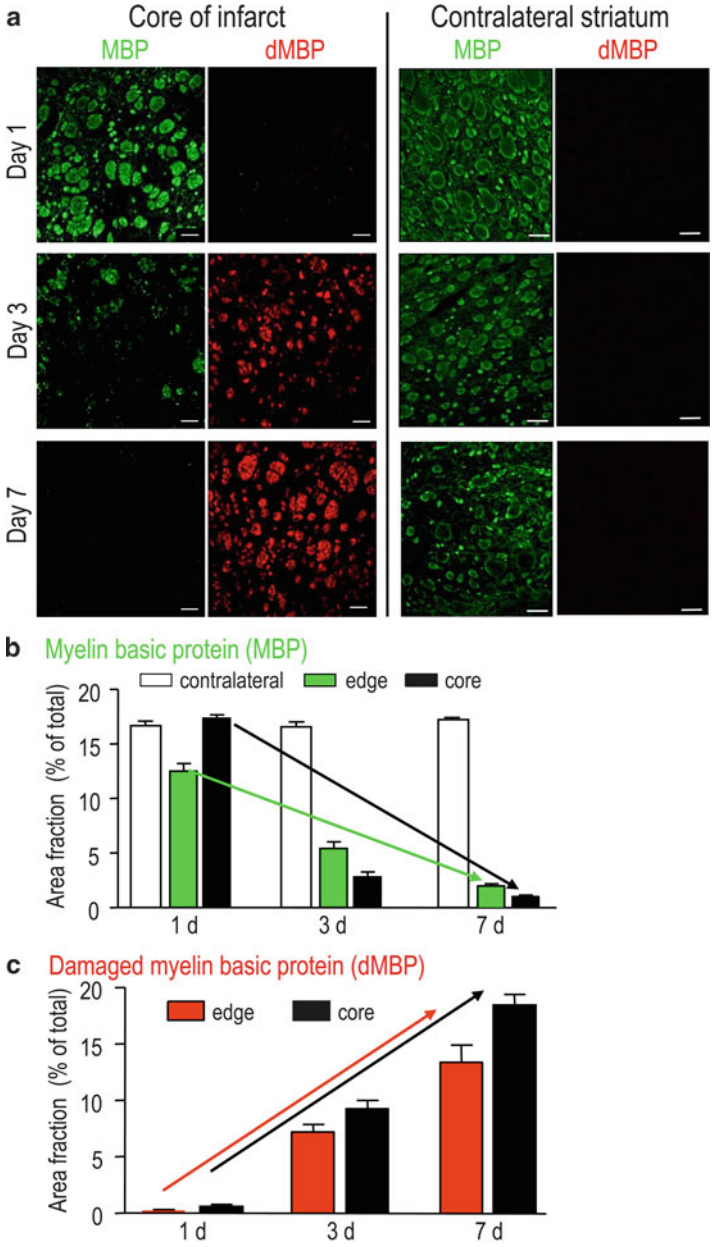


Fig. 19.5 Temporal evolution of myelin damage in the infarct core is shown during the first week after ET-1 injection into the rat striatum (Sects. 19.2.3.1 and 19.4.1). (**a**) The *left-hand panels* show sections immunolabeled for normal myelin (*green*; mouse monoclonal anti-MBP antibody) and damaged myelin (*red*; rabbit polyclonal anti-dMBP antibody). The *right-hand panels* show the normal myelin staining in uninjured contralateral striatum. (**b**) Quantification shows the loss of normal myelin basic protein staining in the infarct core and at the infarct edge. In the contralateral striatum (*white bars*), there was no loss of staining. The area of staining (expressed as percent of total area examined) was determined with ImageJ software, averaged for four rats at each time point, and shown as mean ± SEM. (**c**) A time-dependent increase in staining for damaged MBP is seen, using the same quantification procedure as in (**b**). Only the core and edge were analyzed because there was no dMBP staining on the undamaged contralateral side (see (**a**), *right*). (Modified from Moxon-Emre I and Schlichter LC. 2010. Evolution of inflammation and white matter injury in a model of transient focal ischemic stroke. J. Neuropathol. Expt'l Neurol. 69: 1–15)

19.2.3.2 Identifying Damaged Axons

For histological assessment of axon pathology, the Bielschowski silver impregnation method was developed many years ago (Nauta 1952). This stain labels degenerating axon terminals, and although it is still in use, it is capricious and cannot be used for co-labeling (Castellani et al. 2007). With increased understanding of axon pathology, new markers of damaged axons continue to be identified. For instance, when fast axonal transport is disrupted in injured axons, there is swelling and accumulation of proteins and organelles. Accumulation of amyloid precursor protein (APP) can be readily detected by immunohistochemistry (Fig. 19.6). APP is considered a more reliable indicator of early axon injury than traditional silver stains (Ferguson et al. 1997; Gentleman et al. 1995; Smith et al. 2003) but accumulation does not indicate whether axons are terminally damaged or can recover. As shown in Fig. 19.6, we recently found that the matricellular molecule, SC1/hevin, identifies early axon damage after transient ischemia (and other forms of acute damage) much like APP (Lively and Schlichter 2012). Another indication of damage is loss of integrity of the axon cytoskeleton, which can be monitored using antibodies raised against neurofilaments (e.g., NF200) (Lively and Schlichter 2012; Moxon-Emre and Schlichter 2011; McCracken et al. 2002). In the brain, the heavy neurofilament chain is considered the most highly phosphorylated protein, and changes in its phosphorylation state have been used to detect axon damage (reviewed in (Petzold 2005)). Nonphosphorylated heavy neurofilaments can be detected using the SMI-32 antibody (Sternberger and Sternberger 1983), which labels damaged axons in humans following cerebral ischemia (Leifer and Kowall 1993). The interpretation of changes in SMI-32 staining intensity might be complicated. After transient forebrain ischemia in rats, an increase in SMI-32 intensity was seen in swollen, degenerating axons in the core of the infarct, and the staining gradually decreased as white

Fig. 19.6 (continued) axon bundles (not showing MBP). Scale bar: 100 μm. Panel C shows time-dependent increases in the fractional area of SC1 and APP staining (percent of total area examined). Values are the mean ± SEM of three animals, with each measurement being the mean of four sampling regions per striatum. (**d**) APP and SC1/hevin distribution in damaged white matter tracts; examples are 1 day after a hemorrhagic stroke in the striatum of adult rats. The labels are: NF200 for axon neurofilaments (*green*), DAPI (*blue*) for cell nuclei, and SC1 or APP (*red*). *Left*: The image from a normal white matter tract in the contralateral striatum shows NF200 distribution, and lack of APP. In the surrounding striatum, further from the lesion, an apparently normal white matter tract is labeled for NF200, but not SC1. *Right*: At the edge of the lesion, adjacent serial sections were labeled for NF200 to align the images, and either APP or SC1. The distributions of APP and SC1 were similar. (**e**) High-magnification, deconvolved images of cross-sectioned axons (color-separated and merged) near the inner edge of the lesion. The immunostains are for: SC1 + NF200, SC1 + pan-axonal neurofilament, SC1 + MBP. Scale bar = 5 μm. The axons in the *top* and *bottom* images are cut in cross-section, while axons in the center image were selected to be at a more oblique angle. In damaged white matter tracts, SC1 staining was inside swollen axons (compare with NF200). ((**b**–**e**) are modified from Lively S and Schlichter LC. 2012. SC1/hevin identifies early white matter injury after ischemia and intracerebral hemorrhage in young and aged rats. J. Neuropathol. Expt'l Neurol. 71:480–493)

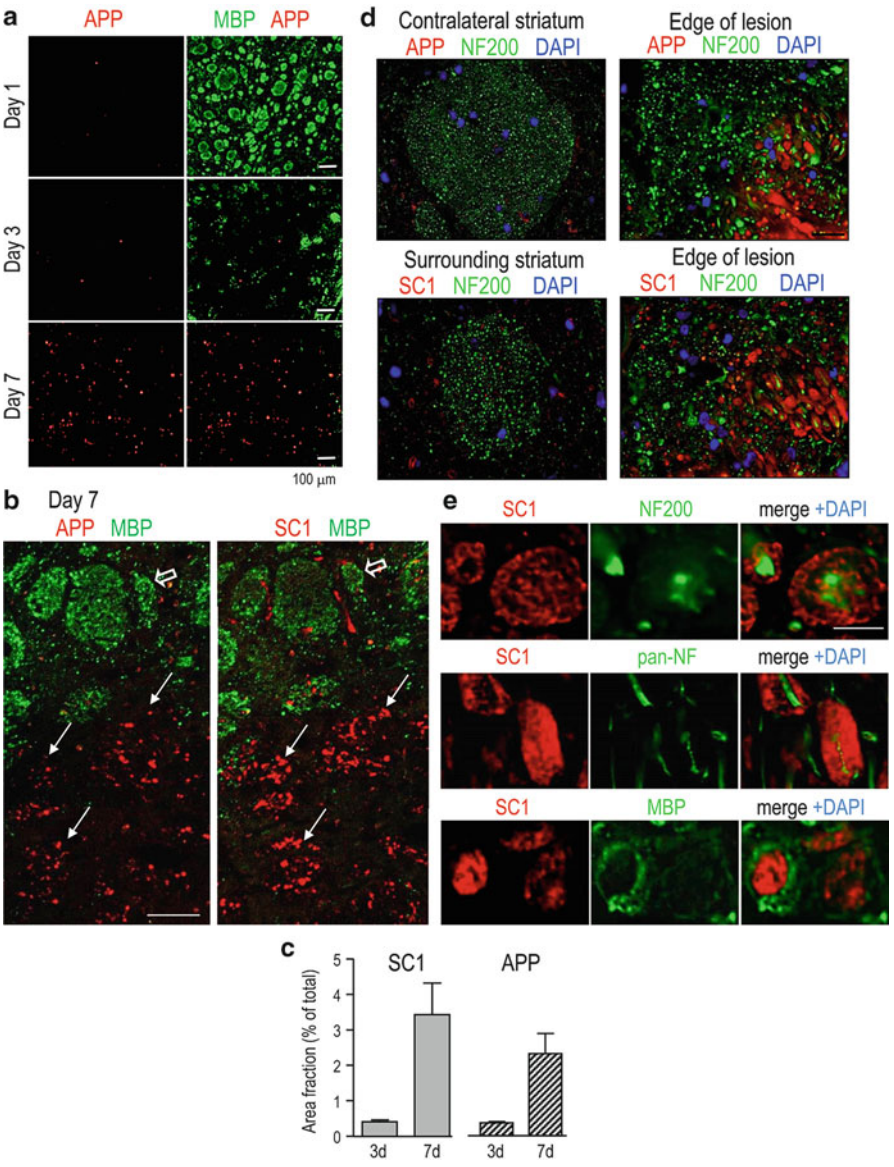


Fig. 19.6 To monitor axon damage after ET-1 induced ischemia, we quantified accumulation of amyloid precursor protein (APP) and the matricellular molecule, SC1 (also known as hevin) (Sects. 19.2.3.2 and 19.4.1). (**a**) In the infarct core, APP (rabbit polyclonal anti-APP antibody, *red*) accumulated in injured axons as staining for normal myelin decreased (mouse monoclonal anti-MBP antibody, *green*). (Modified from Moxon-Emre I and Schlichter LC. 2010. Evolution of inflammation and white matter injury in a model of transient focal ischemic stroke. J. Neuropathol. Expt'l Neurol. 69:1–15.) (**b**, **c**) Confocal images taken at the edge of the infarct 7 days after ischemia (**b**). Adjacent serial sections were immunolabeled for MBP, and individual axon bundles were identified for alignment purposes (*open arrows*). SC1 and APP were prominent in damaged

matter damage progressed (Gresle et al. 2006). However, at the inside edge of the lesion (delineated by ballistic light analysis), increased staining was also seen in morphologically normal-appearing axons. The authors suggest that SMI-32 immunohistochemistry might distinguish the edge of the peri-infarct region.

19.2.3.3 Monitoring Oligodendrocyte Damage

The microtubule-associated protein, Tau-1, might be a good early indicator of oligodendrocyte pathology. After transient (Valeriani et al. 2000) and permanent ischemia (Valeriani et al. 2000; Irving et al. 1997), Tau-1 immunoreactivity increases rapidly (<1 h) and transiently (1–3 days) in oligodendrocytes within the infarct. However, the fate of the Tau-1-labeled cells is unclear and they are not necessarily lost (Gresle et al. 2006). Tau-1 is normally present in healthy axons so it is necessary to either show lack of co-labeling with a neuron marker, or the presence of co-labeling with an oligodendrocyte marker. Loss of oligodendrocytes can be assessed using markers of mature oligodendrocytes, including antibodies directed against 2′, 3′cyclic nucleotide 3′ phosphodiesterase (CNPase) (Sprinkle et al. 1983) and adenomatous polyposis coli (APC) protein (Bhat et al. 1996). For a comprehensive review of oligodendrocyte markers used during development, please see (Baumann and Pham-Dinh 2001).

19.3 A Primer on CNS Inflammation After Ischemia

19.3.1 Overview

The brain is highly vascularized, and when healthy, is protected by the blood–brain barrier (BBB) from cells and molecules of the circulatory system (reviewed in (Engelhardt and Sorokin 2009)). This isolation from blood and lymphatic systems contributed to the long-standing notion that the CNS is immune-privileged (Streilein 1993). However, tissues that are at high risk of infection generally possess resident macrophages (e.g., Kupffer cells in the liver, “dust cells” in the lung) that are positioned to rapidly respond, and to help recruit blood-borne immune cells into the injured tissue. The CNS is no exception: it possesses microglia, resident macrophage-like cells that rapidly respond to a wide variety of insults, and with a myriad of outcomes (discussed below). After a stroke, the BBB integrity is compromised and CNS inflammation then involves “activation” and extravasation of blood-borne innate immune cells (neutrophils, monocytes). The innate immune system can respond rapidly because, unlike the adaptive immune system mediated by T and B lymphocytes, it does not require antigen recognition, processing, cell priming, and proliferation. Thus, it is expected that within minutes of arriving at the damage site, resident microglia and infiltrating innate immune cells (neutrophils, macrophages) can begin to

secrete inflammatory mediators. From in vitro work, we (Kaushal et al. 2007; Kaushal and Schlichter 2008; Sivagnanam et al. 2010) and many others (Colton and Gilbert 1987; Mao et al. 2007; Piani et al. 1991; Smith et al. 1998) have shown that activated microglia and macrophages can produce pro-inflammatory molecules, excitatory amino acids, lipases, proteases, and reactive oxygen and nitrogen species.

When considering acute inflammation after stroke, the damage-induced ("danger") response is generally more relevant than a pathogen-induced ("stranger") response. However, stroke often occurs in an inflammatory environment (Jin et al. 2010; Chapman et al. 2009; Denes et al. 2010; Drake et al. 2011; McColl et al. 2009; Whiteley et al. 2009), with comorbidity arising from bacterial infection, atherosclerosis, hypertension, diabetes, or obesity (Drake et al. 2011; Fischer et al. 2006). Inflammation in peripheral tissue or within the brain is thought to be an important determinant of stroke outcome (Kleinig and Vink 2009). In rodent models of transient ischemia, a wave of inflammation occurs within the brain during the first week: microglial activation is seen early, followed by infiltration of blood-borne immune cells (macrophages, neutrophils) (Moxon-Emre and Schlichter 2010; Stevens et al. 2002). Infiltration of blood-borne immune cells is mediated by their interactions with endothelial cells through receptors that include the lymphocyte adhesion receptor α4 integrin; and significantly, blocking this interaction reduced the infarct volume and improved the neurological outcome after transient MCAo (Becker et al. 2001; Relton et al. 2001).

Animal models are widely used to study inflammation after transient ischemia; for detailed reviews, see (Jin et al. 2010; Ceulemans et al. 2010; Jordan et al. 2008; Wang et al. 2007). Often called a "double-edged sword," there is strong evidence that inflammation can both contribute to secondary damage and to post-stroke recovery. A simplistic view has been that early inflammatory responses are directed toward destroying damaged cells, and later responses help resolve the pro-inflammatory state, promoting tissue recovery and repair. There are many recent reviews on microglia (Kettenmann et al. 2011; Luo and Chen 2010; Saijo and Glass 2011) and macrophages (Van Dyken and Locksley 2013; Wynn et al. 2013) summarizing that these cells can function as antigen-presenting cells, phagocytose damaged cells and debris, and produce a plethora of cytokines, chemokines, proteases, and growth factors that affect themselves and other cells. Here, we will not catalogue individual inflammatory molecules and cell functions. Instead, we will briefly present methods for monitoring the most relevant inflammatory cells, and then present an overview of findings on inflammation in rodent models of transient focal ischemia.

19.3.2 Methods to Assess Inflammation in Ischemia Models

19.3.2.1 Monitoring Neutrophils

After transient ischemia, neutrophils have been identified using routine hematoxylin and eosin (H&E) staining, by recognizing cells with a unique multi-lobed

nucleus (Phillips et al. 2000; Williams et al. 2003; Zhao et al. 2009). Limitations are that the staining resolution is relatively low, especially in densely packed tissues, and it is not conducive to double- and triple labeling. The extent of neutrophil infiltration after ischemia (and other types of injury) is most commonly monitored by biochemical detection of myeloperoxidase (MPO) activity, which is high in activated neutrophils (Barone et al. 1991; Batteur-Parmentier et al. 2000; Beray-Berthat et al. 2003; Bradley et al. 1982; Lerouet et al. 2002). A major limitation of this indirect detection is its inability to reveal spatial and cell-specific MPO expression. This problem can be overcome by exploiting anti-MPO antibodies, as has been done by us and numerous others in animal models of transient ischemia (Moxon-Emre and Schlichter 2010; Justicia et al. 2003; Matsuo et al. 1994). Immunodetection is more sensitive than the biochemical assay (Zhang and Chopp 1997) and can be paired with other cell-specific antibodies to identify the cellular source (Weston et al. 2007). This is important because MPO can also be expressed by activated macrophages and microglia, as shown after transient MCAo (Zhang and Chopp 1997). MPO levels increase when microglia and macrophages become phagocytic, and they can also phagocytose neutrophils that contain MPO (Weston et al. 2007). In mice, neutrophils have also been labeled in situ by an antibody against the Ly6G antigen, which is commonly used in fluorescence-activated cell sorting (FACS) analysis (Gelderblom et al. 2009). Two papers used antibodies against unknown neutrophil antigens: HB199 (Hughes et al. 2003) and MBS-1, with antigen retrieval (Souza-Rodrigues et al. 2008).

19.3.2.2 Monitoring Microglia and Macrophages

In the healthy adult brain, resting microglia can be readily distinguished from other cell types by their tiny cell bodies and highly ramified processes, and by immunohistochemical staining (reviewed in (Kettenmann et al. 2011)). There are several markers that are widely used to detect microglia: the "integrin alpha M" subunit (ITGAM), which is also known as CD11b, complement component receptor 3 alpha (CR3A), and macrophage-1 alpha antigen (Mac-1A) (Akiyama

Fig. 19.7 (continued) indistinguishable from macrophages. After transient ischemia induced by ET-1 injection, ramified microglial cells are still present in the striatum surrounding the infarct, and are found throughout the uninjured contralateral striatum. Cells in the core of the infarct rapidly adopt a macrophage-like appearance. (**c**) By 3 days after ischemia, the infarct is defined by large numbers of activated microglia/macrophages that can be labeled with Iba1 or the OX-42 antibody, which labels CD11b (or several other markers, see Sect. 19.3.2.2). (**d**) Spatial correlation of activated microglia/macrophages with damaged axons. *Left*: Activated microglia/macrophages were immunolabeled with Iba1 (*red*) and ED1 (*green*). *Middle*: the adjacent serial section was immunolabeled with APP to indicate damaged axons, and ED1 to label activated microglia/macrophages. *Right*: Higher magnification images of the boxed regions show activated microglia inside damaged axon bundles closer to the infarct. Scale bars: 100 μm (*left*, *middle*), 10 μm (*right*). ((**d**) is from Moxon-Emre I and Schlichter LC. 2011. Neutrophil depletion reduces BBB breakdown, axon injury inflammation after intracerebral hemorrhage. J. Neuropathol. Expt'l Neurol. 70:218–235)

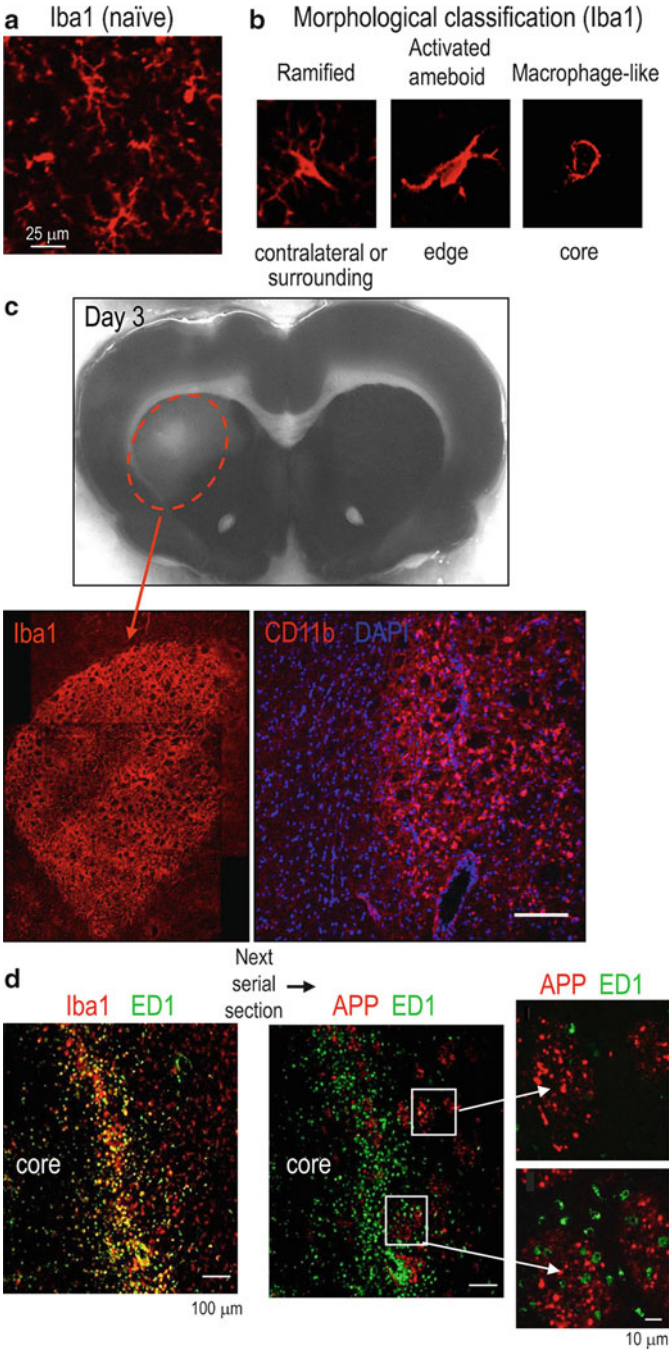


Fig. 19.7 Immunolabeling for microglia and macrophages (Sects. 19.3.2.2 and 19.4.1). (**a**) Iba1 labels ramified, resting microglia in the normal striatum of a healthy young adult rat. (**b**) Iba1 labeling shows that as microglia activate, their processes are retracted until they are morphologically

and McGeer 1990; Robinson et al. 1986); "ionized calcium binding adapter molecule 1" (Iba1) (Imai et al. 1996; Ito et al. 1998); and the surface glycoprotein, F4/80 (Perry et al. 1985). ITGAM and F4/80 also label dendritic cells (Whiteland et al. 1995), endogenous macrophage-like cells that are mainly in myelinated fiber tracts (e.g., corpus callosum, striatal axon bundles, fimbria) and at sites of interstitial fluid drainage (i.e., perivascular space, interface between choroid plexus and cerebral spinal fluid) (reviewed in (Colton 2013)). Microglia (and infiltrating macrophages) can also be identified by lectin staining, notably *Griffonia simplicifolia* B_4 isolectin (IB_4) and tomato lectin; however, these stains are less specific and also label blood vessels (Acarin et al. 1994; Streit and Kreutzberg 1987). Figure 19.7 illustrates some markers we exploit for labeling resting microglia in the healthy rat brain, and both activated microglia and macrophages after transient ischemia.

After acute brain damage, some of the markers are up-regulated (Isaksson et al. 1999; Ito et al. 2001) but the continuing challenge is to discriminate microglia from blood-derived macrophages in damaged tissue. Guillemin and Brew (Guillemin and Brew 2004) have reviewed biochemical and morphological similarities and differences between activated microglia and macrophages. The key issues are that microglia respond to injury by retracting their processes and can adopt a rounded morphology that closely resembles perivascular macrophages and infiltrating macrophages, and all three cell types share many markers. Because of the difficulty in distinguishing between these cells in the damaged brain, we prefer using the combined term, "activated microglia/macrophages." Matters are further complicated because the commonly used marker, CD11b, is also present on neutrophils. After infiltrating the brain following transient ischemia, neutrophils can also label with IB_4 (Matsumoto et al. 2007), although staining might also reflect phagocytosis by neutrophils of microglia and/or macrophages. CD11b is part of an integrin complex on leukocytes, whose interaction with adhesion molecules on endothelial cells allows cell extravasation. Consequently, CD11b inhibition has been tested as a means of reducing stroke damage. Intravenous injection of a function-blocking anti-CD11b antibody at the time of reperfusion after MCAo, reduced neutrophil infiltration, infarct volume, and neurological deficits (Chen et al. 1994). This was presumed to result from reduced neutrophil infiltration but the possibility that macrophage infiltration and microglial activation were affected was not addressed. Another antibody often used as a pan-macrophage marker is CD68 (also known as ED1), which was initially thought to label an intracellular antigen (Dijkstra et al. 1985). Later, ED1 was shown to be a lysosomal marker whose increased expression correlates with the amount of phagocytic activity (Damoiseaux et al. 1994). In the acutely damaged rat brain, ED1 labels phagocytic microglia and macrophages (Moxon-Emre and Schlichter 2010, 2011; Wasserman et al. 2008; Hansen et al. 2001) (and see Fig. 19.7). In an attempt to differentiate between microglia and macrophages, an alternative approach has been to use FACS analysis. It is most common to co-label with CD11b and the antigen, CD45 (also known as leukocyte common antigen [LCA]), which is a protein tyrosine phosphatase. Resting microglia are $CD11b^{+}CD45^{low}$; whereas, infiltrating macrophages are $CD11b^{+}CD45^{high}$

(Ford et al. 1995). One limitation is that CD45 can be up-regulated in activated microglial cells, which then closely resemble invading macrophages (Kettenmann et al. 2011; Sedgwick et al. 1991). Moreover, because all information about spatial localization of the cells is lost, FACS analysis is not useful for addressing the relationship between specific immune cells and white matter damage.

19.3.2.3 Monitoring Lymphocytes and Other Immune Cells

When examining lymphocytes, it can be useful to first monitor the presence of both activated T and B cells by staining with an antibody directed against CD25 (the IL-2 receptor alpha chain) (Whiteland et al. 1995). More specific markers can be used to distinguish between T and B cells. B cells can be labeled for CD45R/B220 (Whiteland et al. 1995), Ki-B1R antigen (Koch et al. 2008), CD19 or Pax5 (Ward et al. 2006); and the pan-T cell marker, CD3, is very useful for showing their presence in rodent tissues (Ward et al. 2006). All of these markers can be used both for immunohistochemistry on brain sections and for FACS analysis. Immunohistochemistry is of limited use for identifying T cell subtypes and double labeling is usually required. For example, CD4 labels helper T cells, and CD8 labels cytotoxic T cells and NK cells (Whiteland et al. 1995), but in rats, both have been reported to label macrophages and dendritic cells (Gibbings and Befus 2009). To further discriminate T cell subtypes, it is better to use FACS analysis (Liesz et al. 2011).

19.3.3 Findings on Brain Inflammation in Rodent Models of Ischemia

Here, we will address the intersection of inflammation and ischemia, with emphasis on the major immune cells involved, and animal models using vessel occlusion or vasoconstriction, which are more closely aligned with human ischemic stroke seen in the clinical setting. Microglia, macrophages, and neutrophils are the most abundant types of immune cells found in the ischemic infarct (Gelderblom et al. 2009), and consequently, have been the focus of most experimental stroke studies.

19.3.3.1 Results on Neutrophils

Circulating blood leukocytes are thought to have a more prominent and damaging role in transient ischemia, which is followed by reperfusion, than in permanent ischemia, in which blood flow is arrested. Reperfusion after transient ischemia increases endothelial cell expression of adhesion molecules that attract circulating neutrophils, and this recruitment can further clog blood vessels (Connolly et al. 1996b; del Zoppo et al. 1991; Hartl et al. 1996; Prestigiacomo et al. 1999). The prevailing view

is that neutrophils are the first hematogenous cells to infiltrate the ischemic infarct. For instance, using the ET-1 model of transient ischemia, we (Moxon-Emre and Schlichter 2010) and others (Souza-Rodrigues et al. 2008) observed peak neutrophil levels at 1 day, and they were undetectable by 7 days. However, another study reported a complete absence of neutrophils (Hughes et al. 2003). The reasons for this discrepancy are not clear but might reflect the labeling methods; i.e., different antibodies, occasional use of antigen retrieval (Sect. 19.3.2.1). The idea that neutrophils are harmful after ischemia is long-standing. The extent of ischemic injury correlates with the increase in neutrophil numbers (Weston et al. 2007). Neutrophils express several pro-inflammatory mediators, and although activated neutrophils are short-lived (1–3 days) (Moxon-Emre and Schlichter 2010; Lerouet et al. 2002; Matsuo et al. 1994; Weston et al. 2007), they can contribute to the early rise in cytokines, reactive oxygen species, and matrix metallopeptidases (MMPs) that can exacerbate BBB breakdown and tissue damage (Justicia et al. 2003; Hartl et al. 1996; Nguyen et al. 2007).

The most compelling evidence comes from studies that have depleted the pool of circulating neutrophils or inhibited their extravasation (reviewed in (Hartl et al. 1996)). Antibody-mediated neutrophil depletion has been exploited in several studies of cerebral ischemia (Matsuo et al. 1994; Gautier et al. 2009; Harris et al. 2005; Petrault et al. 2005). We used this method in a study of ICH in the rat striatum, and showed how blood neutrophil depletion affects their density in the lesion (Moxon-Emre and Schlichter 2011). Several studies implicate neutrophils in BBB breakdown, edema, and infarct volume (Matsuo et al. 1994; Gautier et al. 2009) but others reported that neutrophil depletion did not affect the infarct size (Beray-Berthat et al. 2003; Yilmaz et al. 2006). One possible explanation is that the contribution of neutrophils to ischemic damage might be region dependent. After transient MCAo, MPO activity was elevated for up to 3 days in the cortex and striatum of adult rats (Weston et al. 2007). Depleting neutrophils with vinblastine reduced MPO activity in the cortex and striatum, but oxidative stress and infarct volume decreased only in the cortex (Beray-Berthat et al. 2003). This is consistent with neutrophils being more abundant and long-lasting (up to 15 days post-ischemia) in the cortex than in the striatum (Weston et al. 2007). However, while increased MPO activity reflected accumulation of neutrophils, the sustained elevation at later times reflected neutrophil phagocytosis by microglia and macrophages (Weston et al. 2007).

In considering neutrophils as a potential target for reducing stroke damage, it is important to note their interactions with capillary endothelial cells, which regulate the BBB and control neutrophil infiltration (reviewed in (Tonnesen 1989)). Endothelial cells are more resistant to ischemic insults than neurons but are still vulnerable, and their damage can contribute to BBB breakdown and secondary injury (Won et al. 2011). A salient in vitro study that exposed bovine endothelial cells to OGD showed that they express inducible nitric oxide synthase (NOS-2) and produce nitric oxide (NO), which contributes to their OGD-induced apoptosis (Xu et al. 2000). However, not all sources of NO are detrimental. There is evidence that toxicity depends on the cellular source, type of NO synthase used (e.g., inducible versus constitutive), and timing. Some studies suggest that NO

production very early after transient ischemia is beneficial. That is, infarct volume and neutrophil infiltration increased if the NOS inhibitor, L-NAME (N^G-nitro-L-arginine methyl ester), was injected intraperitoneally immediately (but not 1 h) after the onset of ischemia (Batteur-Parmentier et al. 2000). That study suggested that high NO might limit neutrophil entry. Transgenic mice lacking constitutive endothelial NOS had larger infarcts after focal ischemia (Huang et al. 1996). A recent study has challenged the accepted view that neutrophils infiltrate the brain parenchyma after transient MCAo. Neutrophils did accumulate but remained in the capillary lumen or perivascular space in mice and in samples from human stroke patients (Enzmann et al. 2013). These studies have contributed to an ongoing debate concerning whether the contribution of neutrophils to stroke pathology has been overestimated (Kalimo et al. 2013).

19.3.3.2 Results on Microglia and Macrophages

A burgeoning area of experimental stroke research (reviewed in (Weinstein et al. 2010)) is the roles of macrophage-related innate immune cells: microglia, macrophages, and dendritic cells. Their roles are widely debated. A serious confounding factor is that most studies have failed to discriminate between activated microglia and macrophages (Sect. 19.3.2.2). Both cell types have the capacity to produce molecules and perform functions that can potentially either exacerbate damage or aid in repair. In vitro studies show that purified populations of microglia can produce pro-inflammatory cytokines (e.g., tumor necrosis factor (TNF)-α, interleukin (IL)-1β), and reactive oxygen and nitrogen species that can be cytotoxic (Wang et al. 2007; Hanisch and Kettenmann 2007). Conversely, microglia can produce anti-inflammatory cytokines (e.g., IL-10, TGFβ) that dampen the inflammatory response, and growth factors and neuroprotective molecules that can promote neuron survival and repair (Turrin and Rivest 2006; Wang and Dore 2007). In vivo studies are just beginning to discriminate their roles using selective monocyte depletion, genetic labels, and chimeric or parabiotic animals.

As CNS-resident cells, microglia have the potential to respond rapidly to ischemic events, including the initial insult to neurons, and the timing of events has sometimes been used to implicate microglia. After transient MCAo, one study concluded that microglia progress to the phagocytic state before macrophages enter (Schilling et al. 2005). As noted (Sect. 19.3.2.2), ED1 is up-regulated in phagocytic microglia and macrophages. Using the ET-1 injection model in rats, one study saw accumulation of ED1-labeled cells at 1 day, with maximal accumulation at 3 and 7 days (Souza-Rodrigues et al. 2008), while we (Moxon-Emre and Schlichter 2010) and others (Hughes et al. 2003) did not see them at 1 day. The timing of macrophage entry after stroke is uncertain, possibly reflecting different stroke models, but certainly because of identification issues. After transient MCAo in rats, round, $Iba1^+$ cells were present in the infarct at 12–24 h and interpreted as "may be blood-borne macrophages" (Ito et al. 2001). However, there was no proof of their identity. A similar study claiming monocyte infiltration as early as 1 day used H&E staining

and showed cells accumulating in the lumen of blood vessels (Clark et al. 1994). The timing of extravasation was not determined and the authors stated that macrophages were abundant in the infarct by 3 days. A better approach is to systemically inject super-paramagnetic iron oxide nanoparticles, which are endocytosed by blood-borne monocytes and can be detected by MRI in the brain. Using this method after cortical photo-thrombotic stroke in rats, blood-derived macrophages were detected in the ischemic infarct only after 5–8 days (Kleinschnitz et al. 2003). Based on their late entry, the authors postulate that after stroke, macrophages are involved in remodeling functions rather than acute responses.

Another major issue in considering potential roles of activated microglia and macrophages is defining what is meant by "activation." For microglia it is essential to move beyond conventional descriptions of morphological changes, and instead, assess their biological functions. Recent attempts to better define microglial activation have used macrophage activation as a starting point (Luo and Chen 2010; Boche et al. 2013; Colton 2009; Noda and Suzumura 2012). Over the past decade, up to five modes of macrophage activation have been proposed to describe their responses, with each mode depending on the stimulus and affecting different inflammatory mediators and cellular functions (reviewed in (Van Dyken and Locksley 2013; Gordon 2003; Varin and Gordon 2009)). In the absence of microbe infiltration, classical and alternative activation are especially relevant to stroke. While classical activation is evoked by bacterial lipopolysaccharide, it is postulated to occur after exposure to "danger" signals during early stroke events. Classical activation results in secretion of reactive oxygen and nitrogen species and pro-inflammatory mediators. Alternative activation induces anti-inflammatory molecules that antagonize the actions of the pro-inflammatory mediators produced during classical activation. Thus, it is thought to be involved in tissue remodeling and repair. Experimentally, alternative activation can be evoked by IL-4 and IL-13. The analogy between activation of macrophages and microglia is probably too simplistic. Monocytes are blood-borne cells that mature into macrophages in multiple tissues, while microglial cells normally reside behind the BBB. These very different chemical and structural environments will undoubtedly influence the specific responses and contributions of these two cell types to brain pathology.

Because blood-derived macrophages are absent from the healthy brain, it is much easier to analyze functions of "resting" microglia. Several studies have addressed motility, and we now know that the ramified processes of resting microglia are actually very active in sensing the environment. The processes make contact with various neural tissue elements, including neurons, astrocytes, and blood vessels, and engulf tissue particles that are then retrograde-transported to their somata (Nimmerjahn et al. 2005). The ramified processes of microglia monitor synaptic conditions, and their motility is modulated by neuron activity. Live imaging using two-photon microscopy shows that cortical microglia make brief (~5 min) contacts with synapses as often as once an hour, and the frequency decreases during reduced neuron activity (Wake et al. 2009). There is evidence that multiple neurotransmitters affect microglial motility. In an ex vivo retinal explant model, glutamatergic neurotransmission through AMPA and kainate receptors maintained microglial branching complexity and increased process motility indirectly through purinergic signaling

(Fontainhas et al. 2011). Ionotropic GABAergic neurotransmission had the opposite effects; it decreased branching complexity and reduced process motility. Changes in motility of microglial processes occur after ischemia. One study addressed this in the peri-infarct region after photo-thrombotic focal ischemia in the mouse somatosensory cortex (Wake et al. 2009). As early as 30 min after ischemia, the duration of contact between microglia and synapses increased, and was often followed by loss of the presynaptic terminal. A recent study found that after 60 min of focal ischemia, there was an increase in microglial branching complexity (hypertrophy) in the ischemic striatum but a decrease in number of contacts per microglia in the cortex (Morrison and Filosa 2013). However, following reperfusion for 8 or 24 h, progressive retraction of microglial processes (de-ramification) occurred in both regions.

Phagocytosis is an important function of microglia and macrophages after stroke; indeed, microglia were once considered the brain's "garbage men." The bulk of the evidence is that microglial cells can rapidly transform into active phagocytes, in some cases before macrophages have even begun to enter the brain. One such study depleted peripheral monocytes using clodronate-loaded liposomes, and then subjected the rats to photo-thrombotic focal ischemia (Schroeter et al. 1997). Accumulation of phagocytic cells in the peri-infarct area was mainly microglia at 3 days but both microglia and macrophages at 6 days. A study of transient MCAo in mice used a transgenic, bone-marrow chimera in which blood-borne monocytes were labeled with green fluorescent protein (GFP) (Schilling et al. 2005). Microglial activation, as judged by morphological changes of unlabeled cells, was seen as early as 1 day, was coincident with phagocytosis of neuronal debris, and preceded macrophage infiltration, which began on day 2. By 7 days, massive accumulation of these cells was observed in the infarct, but macrophages accounted for only 20 % of the cells. Using the ET-1 injection model in rats, our laboratory also observed huge numbers of activated microglia/macrophages in the infarct by 7 days, but we could not distinguish between the two cell types (Moxon-Emre and Schlichter 2010). It has been postulated that a protective role of microglia is to phagocytose infiltrating neutrophils, thereby reducing toxic molecules in the extracellular space (Denes et al. 2007; Neumann et al. 2008). However, it is also possible that by releasing inflammatory molecules during phagocytosis, microglia might damage bystander neurons. Given that most acute neuron death (gray matter injury) has already occurred by the time the majority of macrophages infiltrate, the question is: What are macrophages doing? Do they perpetrate delayed white matter damage? Are they mainly involved in repair mechanisms? These questions and others highlight the need to determine, in time and space, the distribution of the two cell types, what molecules they produce, and what specific cellular functions they perform.

19.3.3.3 Results on Lymphocytes and Other Immune Cells

The peripheral immune system is suppressed after stroke, perhaps as an attempt to offset the inflammatory reactions occurring in the brain, and T lymphocytes are thought to be involved in this process (Planas and Chamorro 2009). However, there is a continuing debate concerning the degree to which lymphocytes enter the

brain after stroke and whether they contribute to brain pathology. An early study reported T cell infiltration in the rat cortex as early as 1 day following photothrombotic stroke (Jander et al. 1995). FACS analysis and immunohistochemistry have identified both T and B cells in the ischemic hemispheres of mice 3 days after transient MCAo (Stevens et al. 2002; Gelderblom et al. 2009). Some findings suggest that T cells play a more prominent role in the evolving stroke damage, and they have been receiving more attention in experimental stroke research (reviewed in (Brait et al. 2012)). Transgenic mice in which "recombination activating gene 1" (RAG1) is deleted are deficient in both T and B cells, and RAG1-null mice had 70 % smaller ischemic infarcts at 24 h after reperfusion than their wild-type counterparts. If RAG1-null mice were reconstituted with $CD3^+$ T cells, the ischemic infarcts were larger and comparable to wild-type mice; whereas, reconstitution with B cells did not affect the infarct size (Kleinschnitz et al. 2010).

Differences in stroke models, subclasses of T cells examined and gender contribute to controversies about the role of T cells. A transient increase in natural killer T cells (NKT) cells (but not NK cells) has been identified by FACS analysis in the ipsilateral mouse hemisphere 3 days after transient MCAo (Gelderblom et al. 2009) but their role is unknown. As expected, T cells infiltrate better after reperfusion in a transient ischemia model, but surprisingly, the brains of male mice contained more T cells (Brait et al. 2010). On the one hand, there is evidence for a beneficial role of regulatory T cells (T_{reg}) in a permanent MCAo model in mice. Depletion of T_{reg} cells using a CD25 function-blocking antibody increased the infarct size, microglial "activation," neutrophil invasion, levels of pro-inflammatory cytokines in the brain and blood, and neurological deficits (Liesz et al. 2009). Conversely, a detrimental role was seen in mice after transient MCAo. Selective genetic depletion of T_{reg} cells in DEpletion of REGulatory T cells (DEREG) mice reduced the infarct size and improved neurological outcome scores (Kleinschnitz et al. 2012). Intriguingly, that study found that T_{reg} cells can exacerbate ischemic brain damage by causing dysfunction of the microvasculature, rather than through their typical immunosuppressive functions. Roles of other T cell subsets are beginning to be investigated. After transient MCAo, mice lacking $CD4^+$ or $CD8^+$ T cells had smaller infarct volumes, which correlated with less leukocyte adhesion and recruitment as early as 4 h after reperfusion (Yilmaz et al. 2006). Given that an adaptive immune response generally takes days to develop, this rapid effect is surprising and the mechanism is unknown. Related studies of T cell roles after ischemia in other organs, such as liver (Zwacka et al. 1997) and kidney (Yokota et al. 2002) further demonstrate their complexity, and highlight the need to clarify their roles in cerebral ischemia. We lack studies of the contributions of T cells to white matter injury after stroke; however, future investigations can be informed by studies that associate specific T cell subsets with white matter damage in multiple sclerosis (Scheikl et al. 2010; Walker et al. 2011).

19.4 Intersection of Inflammation and White Matter Damage After Transient Ischemia

19.4.1 Adult Rodents

The preceding section addressed the intersection of inflammation and transient ischemia (see Venn diagram in Fig. 19.2), focusing on the immune cells involved. Here, we add the third component: white matter damage. Much of the limited information about effects of inflammation on white matter injury after ischemia is based on the correlation of microglia and macrophage accumulation in regions of white matter damage.

As one of surprisingly few groups that address this three-way intersection, we have exploited the transient focal ischemia model induced by a single 400 pmol ET-1 injection into the anterior striatum (putamen + caudate) of adult Sprague–Dawley rats. In our first study, having determined that saline injection alone caused minimal damage at each time point, we quantitatively compared changes in the core and edge of the infarct with the undamaged contralateral striatum (Moxon-Emre and Schlichter 2010). We showed that neutrophils (strongly immunoreactive for MPO) were present in the ischemic infarct at 1 day following the insult, and were no longer detected by 7 days. When present, neutrophils were inside and around MBP-labeled myelinated white matter tracts. As previously discussed (Sect. 19.3.2.2), after microglia had rounded-up we could not readily distinguish them from macrophages, and therefore used the collective term: activated microglia/macrophages. [See Fig. 19.7 for examples of staining for CD11b, Iba1, ED1.] As shown in Fig. 19.8, by 3 days after ischemia, Iba1-labeled activated microglia/macrophages began to infiltrate MBP-labeled white matter tracts in the infarct. By 7 days, there was a massive infiltration of Iba1-labeled cells into white matter tracts that were damaged, as judged by loss of MBP signal. By co-immunolabeling adjacent serial sections for MBP and dMBP, we were further able to show that the infiltrated bundles were damaged. A similar study of transient ischemia injected a much smaller dose of ET-1 (10 pmol) into the striatum of adult Wistar rats (Souza-Rodrigues et al. 2008), and did not include quantitative analysis. Qualitative similarities include progressive loss of MBP staining in striatal axon bundles that was most prominent by 7 days; infiltration by 1 day of neutrophils (labeled with MBS-1); and later accumulation, at 3 and 7 days, of ED1-positive cells in the ischemic infarct. However, we must be cautious in comparing the results because that study did not show the infarct, report its volume, or show the position of the sample site. It also did not compare the locations of neutrophils or microglia/macrophages in the infarct, or their spatial relation to the damaged white matter.

We know very little about the specific activation state of the microglia/macrophages or their contribution to the evolving white matter injury. However, in the two studies just described, their increased expression of the lysosomal marker, ED1 (see Fig. 19.7), suggests that they are actively phagocytic; perhaps removing myelin

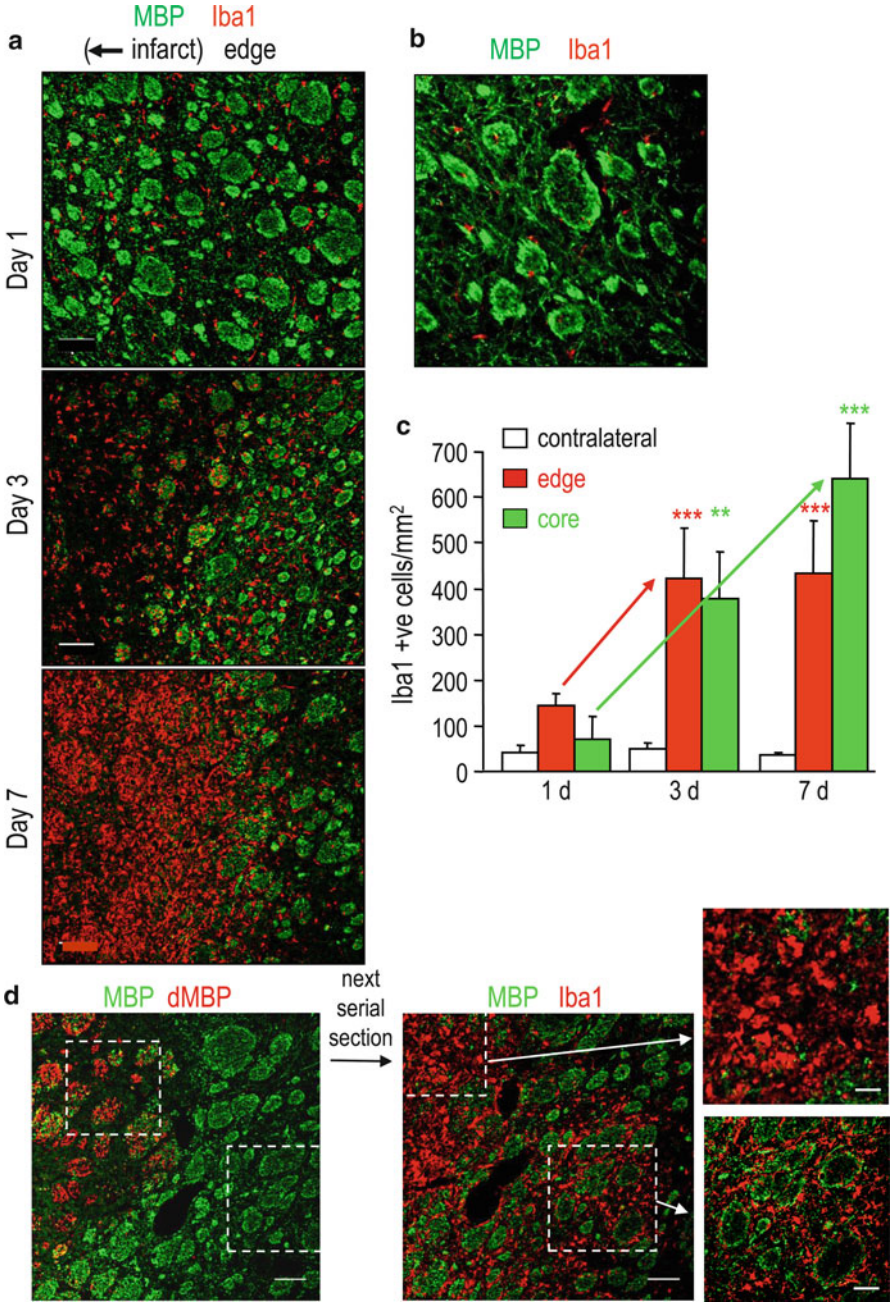


Fig. 19.8 Relationship between activated microglia/macrophages and myelin damage after ET-1-induced ischemia (Sect. 19.4.1). (**a**, **b**) At the edge of the infarct (**a**), there is a spatial-temporal correlation of the accumulation of activated microglia/macrophages (immunolabeled with rabbit polyclonal anti-Iba1 antibody, *red*) with loss of normal MBP-labeled myelin (labeled

debris. Both microglia and macrophages can phagocytose myelin in vitro (Smith 1993; Trotter et al. 1986). Myelin degradation products have been found in vivo within lesion-associated cells in the corpus callosum of cuprizone-fed mice (Olah et al. 2012). The authors concluded that these were microglia because this model of demyelination and re-myelination apparently has minimal involvement of peripheral immune cells.

More information comes from models of neonatal hypoxia–ischemia (Sect. 19.2.2.3) and adult chronic ischemia. In humans, chronic hypo-perfusion resulting from blocked vessels or impaired vascular function is thought to underlie vascular dementia (Roman et al. 2002), and is associated with neurodegeneration, impaired cognitive processes and psychiatric disorders (reviewed in (Farkas et al. 2007)). The main animal model of chronic hypo-perfusion uses bilateral common carotid artery occlusion (BCCAo) in adult rats, which results in memory impairment (Ohta et al. 1997). Two earlier studies used this model to correlate inflammation with white matter damage, as judged by LFB (Wakita et al. 1994) or by ultra-structural changes, including large vacuoles in swollen axons, and myelin sheaths that were irregular and loosely wrapped (Farkas et al. 2004). In the latter study, there was a prolonged (13 weeks) accumulation of $CD11b^+$ cells in the corpus callosum, internal capsule and, most notably, in the optic tract (Farkas et al. 2004). The white matter damage correlated with accumulation of $CD11b^+$ microglia (macrophages?), often in close proximity to damaged fibers (Farkas et al. 2004), and with infiltration of $CD4^+$ and $CD8^+$ lymphocytes (Wakita et al. 1994).

Accumulation of major histocompatibility complex (MHC) class I-labeled cells began as early as 1 day, was much more prominent in white matter than gray matter, and preceded the appearance of white matter lesions (Wakita et al. 1994). These cells were called "microglia" at the earlier time points (1–3 days). At 1 day, the white matter infiltrating cells had shortened processes, hypertrophic somata, and increased staining for MHC class I (Wakita et al. 1994). By 3 days, the bundle-infiltrating cells began to express MHC class II (Ia) antigen and LCA (CD45). Their numbers increased with time to a peak at 7–14 days, at which time they were round, looked like foamy macrophages, and from this time on, the authors called them "microglia/macrophages." In a recent study that did not directly correlate

Fig. 19.8 (continued) with mouse monoclonal anti-MBP, *green*). Scale bars=100 μm. For comparison, staining in the uninjured contralateral striatum is shown ((**b**), same scale). (**c**) There was a time-dependent increase in density of Iba1-labeled activated microglia/macrophages in the core and at the edge of the infarct. Values are shown as mean ± SD for four animals at each time point, and differences from day 1 are indicated (**$p<0.01$, ***$p<0.001$). (**d**) Activated microglia/macrophages infiltrate axon bundles displaying damaged myelin. *Left* and *middle*: Sections were double-stained with antibodies that recognize normal myelin (MBP) and damaged myelin (dMBP). The *boxes* outline the same white matter bundles in the adjacent serial sections. Scale bars = 100 μm. *Right*: Higher magnification images of the boxed regions from the *middle panels*. Scale bars = 50 μm. (Modified from Moxon-Emre I and Schlichter LC. 2010. Evolution of inflammation and white matter injury in a model of transient focal ischemic stroke. J. Neuropathol. Expt'l Neural. 69: 1–15)

inflammation with white matter damage, increased numbers of MHC class II-labeled microglia (macrophages?) were detected at 13 weeks after BCCAo in the hippocampal fimbria of rats exhibiting spatial memory impairments (Choi et al. 2011).

Several studies have used drugs to target inflammation following BCCAo. Rats that received intraperitoneal injections of the immunosuppressant, cyclosporin A, had less white matter damage, as judged by LFB and Bielschowsky silver stain (Wakita et al. 1995). The white matter tracts also contained fewer "microglia/macrophages" that were immunostained for LCA, MHC class I or II antigens. A very similar study by the same group used the immunosuppressant, FK506, and found similar results; i.e., less white matter damage and fewer bundle-infiltrating microglia/macrophages (Wakita et al. 1998). A third BCCAo study tested a 2 week treatment with the anti-inflammatory compound, minocycline (Cho et al. 2006). They found reduced white matter injury (LFB, Bielschowsky silver, MBP staining) in the corpus callosum and optic tract, and reduced microglia (and macrophage?) activation, as measured by the $CD11b^+$ area in the corpus callosum. Similarly, after MCAo in adult mice, axon damage and oligodendrocyte death were reduced by melatonin, which is considered to act an anti-inflammatory agent (Lee et al. 2005).

19.4.2 Neonatal Rodents

Although there are few investigations of the specific intersection between ischemia, inflammation, and white matter damage in neonatal animals, there are more publications than for adult animals. Neonatal stroke studies almost entirely exploit the HI model in rodents, which causes damage to both white and gray matter (Calvert and Zhang 2005). White matter damage is often assessed as the degree of myelin loss, axonal damage, and oligodendrocyte death (Carty et al. 2008; Villapol et al. 2011; Biran et al. 2006; Deng et al. 2008; Uehara et al. 1999; Wang et al. 2013). Subcortical white matter damage correlates with decreased numbers of immature (Carty et al. 2008; Wang et al. 2013) and mature oligodendrocytes (Carty et al. 2008; Villapol et al. 2011). Several HI studies have monitored inflammation concomitantly with white matter injury and have shown an increase in numbers of microglia/macrophages in white matter tracts. For instance, after HI in neonatal rats, white matter injury was accompanied by a 2–3 fold increase in the number of microglia (macrophages?) (IB_4 or CD11b labeled), which were assumed to be "activated" (Biran et al. 2006). However, their specific activation states have not been defined and, for the reasons addressed in Sect. 19.3.3.2, it is not known whether these two immune cells play similar or different roles.

It is well known from in vitro studies from our lab (Kaushal et al. 2007; Kaushal and Schlichter 2008; Sivagnanam et al. 2010; Fordyce et al. 2005; Khanna et al. 2001; Schlichter et al. 2010) and others (Colton and Gilbert 1987; Mao et al. 2007; Piani et al. 1991; Smith et al. 1998; Lai and Todd 2008; Rock et al. 2004) that activated neonatal microglia can produce neurotoxic molecules. Less is known about their products and roles in white matter injury in vivo. Several studies of neonatal HI have directly addressed correlations between inflammatory cell numbers and white matter

damage. Evidence that these cells directly contribute to white matter damage is the phagocytosis of immature oligodendrocytes in damaged white matter, as judged by colocalization of the microglia/macrophage marker, Iba1, and the immature oligodendrocyte marker, O1 (Biran et al. 2006). This is expected to hamper recovery and remyelination. In the corpus callosum of rat pups, a decrease in the density and activation of microglia (macrophages?) (Iba1 staining) accompanied a decrease in myelin loss (MBP staining intensity) and increase in premyelinating (O4+ cells) and immature oligodendrocytes (O1+ cells) (Carty et al. 2008). More recently, when the density of microglia (macrophages?) (tomato lectin staining) was reduced by melatonin administration, there was a decrease in HI-evoked white matter damage (MBP loss) and an increase in mature oligodendrocytes (APC-labeled cells) (Villapol et al. 2011).

Several studies support the view that inflammation, microglia/macrophage activation, and specific pro-inflammatory molecules contribute to white matter damage after neonatal HI. Some studies have begun to target functions of specific inflammatory mediators. Increased expression of the potentially toxic cytokines, IL-1β, and TNF-α, was seen in the rat corpus callosum after HI (Deng et al. 2008; Wang et al. 2013; Brochu et al. 2011; Carty et al. 2011). Administering the IL-1 receptor antagonist reduced white matter injury in the internal capsule (Girard et al. 2012). IL-1β can be processed to its active form by MMP9 (Schonbeck et al. 1998) and, after HI in MMP9-null mice, there were fewer activated microglia (macrophages?) (IB_4 staining) in the damaged white matter, which was judged by loss of MBP (Svedin et al. 2007). MMPs are regulated by several "tissue inhibitors of metallopeptidases" (TIMPs), and there is evidence from knockout mice that TIMP-3 contributes to TNF-α-dependent death of immature oligodendrocytes after MCAo in the adult (Yang et al. 2011). Cyclooxygenase-2 (COX-2) is an interesting target because it is inhibited by nonsteroidal anti-inflammatory drugs (such as aspirin and ibuprofen), and it increases after ischemic stroke in humans, rodents, and nonhuman primates (reviewed in (Candelario-Jalil and Fiebich 2008)). In rats, COX-2 was found in activated microglia (macrophages?) (Bauer et al. 1997), and the nonsteroidal anti-inflammatory drug, ibuprofen, inhibited COX-2 induction after HI (Carty et al. 2011). In mice, the pro-inflammatory cytokine, IL-18, was markedly increased in microglia and astrocytes after HI, and IL-18-null mice had less subcortical white matter damage (Hedtjarn et al. 2002, 2005). Several anti-inflammatory drugs reduced white matter damage in rodent HI models. Ibuprofen reduced IL-1β and TNF-α, and increased the amount of intact myelin (MBP stained) and immature (O1+) and mature oligodendrocytes (O4+) (Carty et al. 2011). White matter damage (loss of MBP) was also reduced by minocycline (Carty et al. 2008) and melatonin (Villapol et al. 2011).

19.5 Future Studies Needed

In 2010, we published our first paper on inflammation and white matter damage after focal ischemia in rat (Moxon-Emre and Schlichter 2010). At that time we stated that: "Despite substantial progress in understanding the pathogenesis of neuronal injury after stroke, most preclinical studies have failed to consider damage to

the white matter. Only recently have stroke studies begun to focus on the secondary injury phase and prominent inflammatory response, which is delayed, prolonged, and more amenable to treatment than acute neurotoxicity." White matter is increasingly recognized as a vulnerable region following ischemia (reviewed in (Petty and Wettstein 1999)); thus, we were surprised how little was known about the spatial and temporal progression of white matter damage. As described in Sect. 19.4.1, only two studies had addressed inflammation and white matter damage after transient ischemia in adult rodents (Hughes et al. 2003; Souza-Rodrigues et al. 2008), and they came to different conclusions about the infiltration of neutrophils and microglia/macrophages into the ischemic infarct. Thus, we set out to quantitatively analyze the temporal and spatial coevolution of white matter damage and inflammation using ET-1 injection to produce a transient, focal ischemic stroke in the rat striatum (Moxon-Emre and Schlichter 2010). ED1-labeled microglia/macrophages selectively infiltrated damaged white matter tracts in the rat striatum as early as 3 days after focal ischemia, but remained outside undamaged white matter tracts. As the Venn diagram in Fig. 19.2 illustrates, we found no additional publications addressing the intersection of inflammation and white matter damage after transient ischemia in adult rodents. This dearth of experimental animal research means there are many important aspects to address in future. Here, we will briefly discuss a few that are especially relevant to the material in this chapter.

1. While the ET-1 model of ischemia has shown that microglia/macrophages infiltrate damaged white matter tracts, we do not know their specific activation state or what functions they are carrying out (Sect. 19.3.2.2). We do not know if, or how, this infiltration affects neurotransmission, neuron survival, repair, or ultimate behavioral outcomes. We know little, if anything, about whether other inflammatory cells (e.g., lymphocytes, dendritic cells) contribute to white matter damage after transient ischemia (Sect. 19.3.2.3). We know almost nothing about how the inflammatory response affects white matter damage in aged or hypertensive animals, which are better models of most human strokes than using young, healthy animals.
2. Very few studies have *quantified* specific responses in transient ischemic stroke models. We need more detailed information on position- and time-dependent changes in the staining intensity or area of damaged white matter, whether it is myelin or axon damage, the density or numbers of specific immune cells, inflammatory molecules they are producing, and how these responses are changed by treatments.
3. As addressed throughout this chapter (e.g., Sect. 19.3.2), a major challenge is distinguishing activated microglia from infiltrating macrophages. Over the years, several experimental manipulations have been used in an attempt to distinguish their contributions. Several studies have ablated circulating monocytes using clodronate-loaded liposomes (Schroeter et al. 1997; Kanematsu et al. 2011; Mawhinney et al. 2012) or by bone marrow irradiation (Schilling et al. 2005). The recent development of knock-in fluorescent protein reporter mice for the fractalkine receptor (CX_3CR_1+/GFP) and chemokine receptor 2 (CCR2+/RFP) promises to help differentiate endogenous microglia from invading macrophages (Mizutani et al. 2012).

4. Recent insights into the ontogeny of microglia show that parenchymal CNS microglial cells develop earlier than previously thought, and from a surprising location: the embryonic yolk sac (Ginhoux et al. 2010). This site of origin differs from bone-marrow derived macrophages. A better understanding of their development and possible differential gene expression might provide insight into how to distinguish between activated microglia and infiltrating macrophages. There is some controversy about the potential of peripheral macrophages to replenish microglia after CNS injury. In transgenic mice engineered to allow ganciclovir-mediated depletion of proliferating microglia (CD11b-HSVTK transgenic mice), circulating Iba1$^+$ macrophages entered the brain, responded to ATP gradients and migrated towards sites of kainate-induced neuron death (Varvel et al. 2012). Unlike native resting microglia, which are highly ramified and have small somata, the infiltrating macrophages had large somata and short, irregular processes. This suggests that infiltrating macrophages remain as a distinct population. In future, identifying molecular expression profiles underlying the unique morphological and developmental differences might be useful in distinguishing microglia from macrophages.
5. It has been postulated that white matter is particularly vulnerable to inflammatory mediators (Coleman and Perry 2002). This means that inflammation after stroke might damage white matter at times and in locations where frank loss of neurons is not seen. Our discovery (Sect. 19.2.3.2) that SC1/hevin is a new early marker of axon damage might prove very useful. Its spatiotemporal expression correlated with microglia/macrophage infiltration of white matter bundles, and it showed aging-related differences in the progression of white matter damage (Lively and Schlichter 2012).
6. In neonatal HI models, attempts have been made to more closely evaluate the intersection of ischemia, white matter damage, and inflammation (Sect. 19.4.2). Most studies have been correlational, showing that white matter damage in a lesioned area was accompanied by an increase in microglia/macrophage numbers. While several studies used broad-spectrum anti-inflammatory drugs (minocycline, ibuprofen, melatonin), further research is needed to address the mechanisms. As for adult animals, an over-arching need is to parse out specific aspects of the inflammatory response, and determine whether they exacerbate the damage, aid in repair, or both.

References

Stroke therapy Academic Industry Roundtable, Fisher MC (1999) Recommendations for standards regarding preclinical neuroprotective and restorative drug development. Stroke 30:2752–2758

Acarin L, Vela JM, Gonzalez B et al (1994) Demonstration of poly-N-acetyl lactosamine residues in ameboid and ramified microglial cells in rat brain by tomato lectin binding. J Histochem Cytochem 42:1033–1041

Adams HP Jr, Bendixen BH, Kappelle LJ et al (1993) Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. Stroke 24:35–41

Akiyama H, McGeer PL (1990) Brain microglia constitutively express beta-2 integrins. J Neuroimmunol 30:81–93

Anderson CS, Chakera TM, Stewart-Wynne EG et al (1994) Spectrum of primary intracerebral haemorrhage in Perth, Western Australia, 1989–90: incidence and outcome. J Neurol Neurosurg Psychiatry 57:936–940

Arai K, Lo EH (2009) Experimental models for analysis of oligodendrocyte pathophysiology in stroke. Exp Transl Stroke Med 1:6

Barone FC, Hillegass LM, Price WJ et al (1991) Polymorphonuclear leukocyte infiltration into cerebral focal ischemic tissue: myeloperoxidase activity assay and histologic verification. J Neurosci Res 29:336–345

Batteur-Parmentier S, Margaill I, Plotkine M (2000) Modulation by nitric oxide of cerebral neutrophil accumulation after transient focal ischemia in rats. J Cereb Blood Flow Metab 20:812–819

Bauer MK, Lieb K, Schulze-Osthoff K et al (1997) Expression and regulation of cyclooxygenase-2 in rat microglia. Eur J Biochem 243:726–731

Baumann N, Pham-Dinh D (2001) Biology of oligodendrocyte and myelin in the mammalian central nervous system. Physiol Rev 81:871–927

Becker K, Kindrick D, Relton J et al (2001) Antibody to the alpha4 integrin decreases infarct size in transient focal cerebral ischemia in rats. Stroke 32:206–211

Beray-Berthat V, Croci N, Plotkine M et al (2003) Polymorphonuclear neutrophils contribute to infarction and oxidative stress in the cortex but not in the striatum after ischemia-reperfusion in rats. Brain Res 987:32–38

Bhat RV, Axt KJ, Fosnaugh JS et al (1996) Expression of the APC tumor suppressor protein in oligodendroglia. Glia 17:169–174

Biran V, Joly LM, Heron A et al (2006) Glial activation in white matter following ischemia in the neonatal P7 rat brain. Exp Neurol 199:103–112

Boche D, Perry VH, Nicoll JA (2013) Review: activation patterns of microglia and their identification in the human brain. Neuropathol Appl Neurobiol 39:3–18

Bradley PP, Priebat DA, Christensen RD et al (1982) Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. J Invest Dermatol 78:206–209

Braeuninger S, Kleinschnitz C (2009) Rodent models of focal cerebral ischemia: procedural pitfalls and translational problems. Exp Transl Stroke Med 1:8

Brait VH, Jackman KA, Walduck AK et al (2010) Mechanisms contributing to cerebral infarct size after stroke: gender, reperfusion, T lymphocytes, and Nox2-derived superoxide. J Cereb Blood Flow Metab 30:1306–1317

Brait VH, Arumugam TV, Drummond GR et al (2012) Importance of T lymphocytes in brain injury, immunodeficiency, and recovery after cerebral ischemia. J Cereb Blood Flow Metab 32:598–611

Brochu ME, Girard S, Lavoie K et al (2011) Developmental regulation of the neuroinflammatory responses to LPS and/or hypoxia-ischemia between preterm and term neonates: an experimental study. J Neuroinflammation 8:55

Brott T, Bogousslavsky J (2000) Treatment of acute ischemic stroke. N Engl J Med 343:710–722

Bruni EJ, Montemurro DG (2009) Human neuroanatomy: a text, brain atlas, and laboratory dissection guide, 3rd edn. Oxford University Press, New York

Calvert JW, Zhang JH (2005) Pathophysiology of an hypoxic-ischemic insult during the perinatal period. Neurol Res 27:246–260

Candelario-Jalil E, Fiebich BL (2008) Cyclooxygenase inhibition in ischemic brain injury. Curr Pharm Des 14:1401–1418

Carmichael ST (2005) Rodent models of focal stroke: size, mechanism, and purpose. NeuroRx 2:396–409

Carty ML, Wixey JA, Colditz PB et al (2008) Post-insult minocycline treatment attenuates hypoxia-ischemia-induced neuroinflammation and white matter injury in the neonatal rat: a comparison of two different dose regimens. Int J Dev Neurosci 26:477–485

Carty ML, Wixey JA, Reinebrant HE et al (2011) Ibuprofen inhibits neuroinflammation and attenuates white matter damage following hypoxia-ischemia in the immature rodent brain. Brain Res 1402:9–19

Castellani RJ, Alexiev BA, Phillips D et al (2007) Microscopic investigations in neurodegenerative diseases. In: Mendez-Vilas A, Diaz J (eds) Modern research and educational topics in microscopy, vol 1. Formatex, Badajoz, Spain, pp 171–182

Ceulemans AG, Zgavc T, Kooijman R et al (2010) The dual role of the neuroinflammatory response after ischemic stroke: modulatory effects of hypothermia. J Neuroinflammation 7:74

Chapman KZ, Dale VQ, Denes A et al (2009) A rapid and transient peripheral inflammatory response precedes brain inflammation after experimental stroke. J Cereb Blood Flow Metab 29:1764–1768

Chen H, Chopp M, Zhang RL et al (1994) Anti-CD11b monoclonal antibody reduces ischemic cell damage after transient focal cerebral ischemia in rat. Ann Neurol 35:458–463

Cho KO, La HO, Cho YJ et al (2006) Minocycline attenuates white matter damage in a rat model of chronic cerebral hypoperfusion. J Neurosci Res 83:285–291

Choi BR, Kwon KJ, Park SH et al (2011) Alternations of septal-hippocampal system in the adult wistar rat with spatial memory impairments induced by chronic cerebral hypoperfusion. Exp Neurobiol 20:92–99

Cioffi GA (2005) Ischemic model of optic nerve injury. Trans Am Ophthalmol Soc 103:592–613

Cioffi GA, Orgul S, Onda E et al (1995) An in vivo model of chronic optic nerve ischemia: the dose-dependent effects of endothelin-1 on the optic nerve microvasculature. Curr Eye Res 14:1147–1153

Clark RK, Lee EV, White RF et al (1994) Reperfusion following focal stroke hastens inflammation and resolution of ischemic injured tissue. Brain Res Bull 35:387–392

Clozel M, Gray GA, Breu V et al (1992) The endothelin ETB receptor mediates both vasodilation and vasoconstriction in vivo. Biochem Biophys Res Commun 186:867–873

Coleman MP, Perry VH (2002) Axon pathology in neurological disease: a neglected therapeutic target. Trends Neurosci 25:532–537

Colton CA (2009) Heterogeneity of microglial activation in the innate immune response in the brain. J Neuroimmune Pharmacol 4:399–418

Colton CA (2013) Immune heterogeneity in neuroinflammation: dendritic cells in the brain. J Neuroimmune Pharmacol 8:145–162

Colton CA, Gilbert DL (1987) Production of superoxide anions by a CNS macrophage, the microglia. FEBS Lett 223:284–288

Connolly ES Jr, Winfree CJ, Stern DM et al (1996a) Procedural and strain-related variables significantly affect outcome in a murine model of focal cerebral ischemia. Neurosurgery 38:523–531, discussion 32

Connolly ES Jr, Winfree CJ, Springer TA et al (1996b) Cerebral protection in homozygous null ICAM-1 mice after middle cerebral artery occlusion. Role of neutrophil adhesion in the pathogenesis of stroke. J Clin Invest 97:209–216

Damoiseaux JG, Dopp EA, Calame W et al (1994) Rat macrophage lysosomal membrane antigen recognized by monoclonal antibody ED1. Immunology 83:140–147

del Zoppo GJ (1998) Clinical trials in acute stroke: why have they not been successful? Neurology 51:S59–S61

del Zoppo GJ, Schmid-Schonbein GW, Mori E et al (1991) Polymorphonuclear leukocytes occlude capillaries following middle cerebral artery occlusion and reperfusion in baboons. Stroke 22:1276–1283

del Zoppo GJ, Poeck K, Pessin MS et al (1992) Recombinant tissue plasminogen activator in acute thrombotic and embolic stroke. Ann Neurol 32:78–86

Denes A, Vidyasagar R, Feng J et al (2007) Proliferating resident microglia after focal cerebral ischaemia in mice. J Cereb Blood Flow Metab 27:1941–1953

Denes A, Thornton P, Rothwell NJ et al (2010) Inflammation and brain injury: acute cerebral ischaemia, peripheral and central inflammation. Brain Behav Immun 24:708–723

Deng Y, Lu J, Sivakumar V et al (2008) Amoeboid microglia in the periventricular white matter induce oligodendrocyte damage through expression of proinflammatory cytokines via MAP kinase signaling pathway in hypoxic neonatal rats. Brain Pathol 18:387–400

Dijkstra CD, Dopp EA, Joling P et al (1985) The heterogeneity of mononuclear phagocytes in lymphoid organs: distinct macrophage subpopulations in the rat recognized by monoclonal antibodies ED1, ED2 and ED3. Immunology 54:589–599
Diringer MN, Skolnick BE, Mayer SA et al (2008) Risk of thromboembolic events in controlled trials of rFVIIa in spontaneous intracerebral hemorrhage. Stroke 39:850–856
Dirnagl U (2006) Bench to bedside: the quest for quality in experimental stroke research. J Cereb Blood Flow Metab 26:1465–1478
Drake C, Boutin H, Jones MS et al (2011) Brain inflammation is induced by co-morbidities and risk factors for stroke. Brain Behav Immun 25:1113–1122
Durukan A, Tatlisumak T (2007) Acute ischemic stroke: overview of major experimental rodent models, pathophysiology, and therapy of focal cerebral ischemia. Pharmacol Biochem Behav 87:179–197
Emsley HC, Tyrrell PJ (2002) Inflammation and infection in clinical stroke. J Cereb Blood Flow Metab 22:1399–1419
Endres M, Engelhardt B, Koistinaho J et al (2008) Improving outcome after stroke: overcoming the translational roadblock. Cerebrovasc Dis 25:268–278
Engelhardt B, Sorokin L (2009) The blood–brain and the blood-cerebrospinal fluid barriers: function and dysfunction. Semin Immunopathol 31:497–511
Enzmann G, Mysiorek C, Gorina R et al (2013) The neurovascular unit as a selective barrier to polymorphonuclear granulocyte (PMN) infiltration into the brain after ischemic injury. Acta Neuropathol 125:395–412
Farkas E, Donka G, de Vos RA et al (2004) Experimental cerebral hypoperfusion induces white matter injury and microglial activation in the rat brain. Acta Neuropathol 108:57–64
Farkas E, Luiten PG, Bari F (2007) Permanent, bilateral common carotid artery occlusion in the rat: a model for chronic cerebral hypoperfusion-related neurodegenerative diseases. Brain Res Rev 54:162–180
Ferguson B, Matyszak MK, Esiri MM et al (1997) Axonal damage in acute multiple sclerosis lesions. Brain 120(pt 3):393–399
Fischer U, Arnold M, Nedeltchev K et al (2006) Impact of comorbidity on ischemic stroke outcome. Acta Neurol Scand 113:108–113
Fontainhas AM, Wang M, Liang KJ et al (2011) Microglial morphology and dynamic behavior is regulated by ionotropic glutamatergic and GABAergic neurotransmission. PLoS One 6:e15973
Ford AL, Goodsall AL, Hickey WF et al (1995) Normal adult ramified microglia separated from other central nervous system macrophages by flow cytometric sorting. Phenotypic differences defined and direct ex vivo antigen presentation to myelin basic protein-reactive CD4+ T cells compared. J Immunol 154:4309–4321
Fordyce CB, Jagasia R, Zhu X et al (2005) Microglia Kv1.3 channels contribute to their ability to kill neurons. J Neurosci 25:7139–7149
Garcia JH, Liu KF, Ho KL (1995) Neuronal necrosis after middle cerebral artery occlusion in Wistar rats progresses at different time intervals in the caudoputamen and the cortex. Stroke 26:636–642, discussion 43
Gautier S, Ouk T, Petrault O et al (2009) Neutrophils contribute to intracerebral haemorrhages after treatment with recombinant tissue plasminogen activator following cerebral ischaemia. Br J Pharmacol 156:673–679
Gelderblom M, Leypoldt F, Steinbach K et al (2009) Temporal and spatial dynamics of cerebral immune cell accumulation in stroke. Stroke 40:1849–1857
Gentleman SM, Roberts GW, Gennarelli TA et al (1995) Axonal injury: a universal consequence of fatal closed head injury? Acta Neuropathol 89:537–543
Gibbings D, Befus AD (2009) CD4 and CD8: an inside-out coreceptor model for innate immune cells. J Leukoc Biol 86:251–259
Ginhoux F, Greter M, Leboeuf M et al (2010) Fate mapping analysis reveals that adult microglia derive from primitive macrophages. Science 330:841–845

Ginsberg MD (2009) Current status of neuroprotection for cerebral ischemia: synoptic overview. Stroke 40:S111–S114
Girard S, Sebire H, Brochu ME et al (2012) Postnatal administration of IL-1Ra exerts neuroprotective effects following perinatal inflammation and/or hypoxic-ischemic injuries. Brain Behav Immun 26:1331–1339
Goldberg MP, Ransom BR (2003) New light on white matter. Stroke 34:330–332
Gordon S (2003) Alternative activation of macrophages. Nat Rev Immunol 3:23–35
Gresle MM, Jarrott B, Jones NM et al (2006) Injury to axons and oligodendrocytes following endothelin-1-induced middle cerebral artery occlusion in conscious rats. Brain Res 1110:13–22
Guillemin GJ, Brew BJ (2004) Microglia, macrophages, perivascular macrophages, and pericytes: a review of function and identification. J Leukoc Biol 75:388–397
Hanisch UK, Kettenmann H (2007) Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nat Neurosci 10:1387–1394
Hansen R, Sauder C, Czub S et al (2001) Activation of microglia cells is dispensable for the induction of rat retroviral spongiform encephalopathy. J Neurovirol 7:501–510
Harris AK, Ergul A, Kozak A et al (2005) Effect of neutrophil depletion on gelatinase expression, edema formation and hemorrhagic transformation after focal ischemic stroke. BMC Neurosci 6:49
Hartl R, Schurer L, Schmid-Schonbein GW et al (1996) Experimental antileukocyte interventions in cerebral ischemia. J Cereb Blood Flow Metab 16:1108–1119
Haynes WG, Strachan FE, Webb DJ (1995) Endothelin ETA and ETB receptors cause vasoconstriction of human resistance and capacitance vessels in vivo. Circulation 92:357–363
He Z, Yamawaki T, Yang S et al (1999) Experimental model of small deep infarcts involving the hypothalamus in rats: changes in body temperature and postural reflex. Stroke 30:2743–2751, discussion 51
He Z, Yang SH, Naritomi H et al (2000) Definition of the anterior choroidal artery territory in rats using intraluminal occluding technique. J Neurol Sci 182:16–28
Hedtjarn M, Leverin AL, Eriksson K et al (2002) Interleukin-18 involvement in hypoxic-ischemic brain injury. J Neurosci 22:5910–5919
Hedtjarn M, Mallard C, Arvidsson P et al (2005) White matter injury in the immature brain: role of interleukin-18. Neurosci Lett 373:16–20
Horie N, Maag AL, Hamilton SA et al (2008) Mouse model of focal cerebral ischemia using endothelin-1. J Neurosci Methods 173:286–290
Howells DW, Porritt MJ, Rewell SS et al (2010) Different strokes for different folks: the rich diversity of animal models of focal cerebral ischemia. J Cereb Blood Flow Metab 30:1412–1431
Huang Z, Huang PL, Ma J et al (1996) Enlarged infarcts in endothelial nitric oxide synthase knockout mice are attenuated by nitro-L-arginine. J Cereb Blood Flow Metab 16:981–987
Hughes PM, Anthony DC, Ruddin M et al (2003) Focal lesions in the rat central nervous system induced by endothelin-1. J Neuropathol Exp Neurol 62:1276–1286
Imai Y, Ibata I, Ito D et al (1996) A novel gene iba1 in the major histocompatibility complex class III region encoding an EF hand protein expressed in a monocytic lineage. Biochem Biophys Res Commun 224:855–862
Irving EA, Yatsushiro K, McCulloch J et al (1997) Rapid alteration of tau in oligodendrocytes after focal ischemic injury in the rat: involvement of free radicals. J Cereb Blood Flow Metab 17:612–622
Irving EA, Bentley DL, Parsons AA (2001) Assessment of white matter injury following prolonged focal cerebral ischaemia in the rat. Acta Neuropathol 102:627–635
Isaksson J, Farooque M, Holtz A et al (1999) Expression of ICAM-1 and CD11b after experimental spinal cord injury in rats. J Neurotrauma 16:165–173
Ito D, Imai Y, Ohsawa K et al (1998) Microglia-specific localisation of a novel calcium binding protein, Iba1. Brain Res Mol Brain Res 57:1–9

Ito D, Tanaka K, Suzuki S et al (2001) Enhanced expression of Iba1, ionized calcium-binding adapter molecule 1, after transient focal cerebral ischemia in rat brain. Stroke 32:1208–1215
Jander S, Kraemer M, Schroeter M et al (1995) Lymphocytic infiltration and expression of intercellular adhesion molecule-1 in photochemically induced ischemia of the rat cortex. J Cereb Blood Flow Metab 15:42–51
Jin R, Yang G, Li G (2010) Inflammatory mechanisms in ischemic stroke: role of inflammatory cells. J Leukoc Biol 87:779–789
Johnson GA, Calabrese E, Badea A et al (2012) A multidimensional magnetic resonance histology atlas of the Wistar rat brain. Neuroimage 62:1848–1856
Johnston MV, Ferriero DM, Vannucci SJ et al (2005) Models of cerebral palsy: which ones are best? J Child Neurol 20:984–987
Jordan J, Segura T, Brea D et al (2008) Inflammation as therapeutic objective in stroke. Curr Pharm Des 14:3549–3564
Justicia C, Panes J, Sole S et al (2003) Neutrophil infiltration increases matrix metalloproteinase-9 in the ischemic brain after occlusion/reperfusion of the middle cerebral artery in rats. J Cereb Blood Flow Metab 23:1430–1440
Kalimo H, del Zoppo GJ, Paetau A et al (2013) Polymorphonuclear neutrophil infiltration into ischemic infarctions: myth or truth? Acta Neuropathol 125:313–316
Kanematsu Y, Kanematsu M, Kurihara C et al (2011) Critical roles of macrophages in the formation of intracranial aneurysm. Stroke 42:173–178
Kaushal V, Schlichter LC (2008) Mechanisms of microglia-mediated neurotoxicity in a new model of the stroke penumbra. J Neurosci 28:2221–2230
Kaushal V, Koeberle PD, Wang Y et al (2007) The Ca2+-activated K+ channel KCNN4/KCa3.1 contributes to microglia activation and nitric oxide-dependent neurodegeneration. J Neurosci 27:234–244
Kelly-Hayes M, Robertson JT, Broderick JP et al (1998) The American Heart Association stroke outcome classification. Stroke 29:1274–1280
Kettenmann H, Hanisch UK, Noda M et al (2011) Physiology of microglia. Physiol Rev 91:461–553
Khanna R, Roy L, Zhu X et al (2001) K+ channels and the microglial respiratory burst. Am J Physiol Cell Physiol 280:C796–C806
Kirby RS, Wingate MS, Van Naarden BK et al (2011) Prevalence and functioning of children with cerebral palsy in four areas of the United States in 2006: a report from the Autism and Developmental Disabilities Monitoring Network. Res Dev Disabil 32:462–469
Kleinig TJ, Vink R (2009) Suppression of inflammation in ischemic and hemorrhagic stroke: therapeutic options. Curr Opin Neurol 22:294–301
Kleinschnitz C, Bendszus M, Frank M et al (2003) In vivo monitoring of macrophage infiltration in experimental ischemic brain lesions by magnetic resonance imaging. J Cereb Blood Flow Metab 23:1356–1361
Kleinschnitz C, Schwab N, Kraft P et al (2010) Early detrimental T-cell effects in experimental cerebral ischemia are neither related to adaptive immunity nor thrombus formation. Blood 115:3835–3842
Kleinschnitz C, Kraft P, Dreykluft A et al (2012) Regulatory T cells are strong promoters of acute ischemic stroke in mice by inducing dysfunction of the cerebral microvasculature. Blood 121:679–691
Kluver H, Barrera E (1953) A method for the combined staining of cells and fibers in the nervous system. J Neuropathol Exp Neurol 12:400–403
Koch M, Broecker V, Heratizadeh A et al (2008) Induction of chronic renal allograft injury by injection of a monoclonal antibody against a donor MHC Ib molecule in a nude rat model. Transpl Immunol 19:187–191
Koizumi J, Yoshida Y, Nakazawa T et al (1986) Experimental studies of ischemic brain edema, I: a new experimental model of cerebral embolism in rats in which recirculation can be introduced in the ischemic area. Jpn J Stroke 8:1–8

Krafft PR, Bailey EL, Lekic T et al (2012) Etiology of stroke and choice of models. Int J Stroke 7:398–406
Kuge Y, Minematsu K, Yamaguchi T et al (1995) Nylon monofilament for intraluminal middle cerebral artery occlusion in rats. Stroke 26:1655–1657, discussion 8
Lai AY, Todd KG (2008) Differential regulation of trophic and proinflammatory microglial effectors is dependent on severity of neuronal injury. Glia 56:259–270
Laing RJ, Jakubowski J, Laing RW (1993) Middle cerebral artery occlusion without craniectomy in rats. Which method works best? Stroke 24:294–297
Lakhan SE, Kirchgessner A, Hofer M (2009) Inflammatory mechanisms in ischemic stroke: therapeutic approaches. J Transl Med 7:97
Lee EJ, Lee MY, Chen HY et al (2005) Melatonin attenuates gray and white matter damage in a mouse model of transient focal cerebral ischemia. J Pineal Res 38:42–52
Leifer D, Kowall NW (1993) Immunohistochemical patterns of selective cellular vulnerability in human cerebral ischemia. J Neurol Sci 119:217–228
Lerouet D, Beray-Berthat V, Palmier B et al (2002) Changes in oxidative stress, iNOS activity and neutrophil infiltration in severe transient focal cerebral ischemia in rats. Brain Res 958:166–175
Liesz A, Suri-Payer E, Veltkamp C et al (2009) Regulatory T cells are key cerebroprotective immunomodulators in acute experimental stroke. Nat Med 15:192–199
Liesz A, Zhou W, Mracsko E et al (2011) Inhibition of lymphocyte trafficking shields the brain against deleterious neuroinflammation after stroke. Brain 134:704–720
Lin Y, Stanworth S, Birchall J et al (2012) Recombinant factor VIIa for the prevention and treatment of bleeding in patients without haemophilia. Cochrane Database Syst Rev (3):CD005011
Liu F, McCullough LD (2011) Middle cerebral artery occlusion model in rodents: methods and potential pitfalls. J Biomed Biotechnol 2011:464701
Lively S, Schlichter LC (2012) SC1/hevin identifies early white matter injury after ischemia and intracerebral hemorrhage in young and aged rats. J Neuropathol Exp Neurol 71:480–493
Lively S, Moxon-Emre I, Schlichter LC (2011) SC1/hevin and reactive gliosis after transient ischemic stroke in young and aged rats. J Neuropathol Exp Neurol 70:913–929
Lopez AD, Mathers CD (2006) Measuring the global burden of disease and epidemiological transitions: 2002–2030. Ann Trop Med Parasitol 100:481–499
Luo XG, Chen SD (2010) The changing phenotype of microglia from homeostasis to disease. Transl Neurodegener 1:9
Macrae IM (1992) New models of focal cerebral ischaemia. Br J clin Pharmacol 34:302–308
Mao H, Fang X, Floyd KM et al (2007) Induction of microglial reactive oxygen species production by the organochlorinated pesticide dieldrin. Brain Res 1186:267–274
Marcoux FW, Morawetz RB, Crowell RM et al (1982) Differential regional vulnerability in transient focal cerebral ischemia. Stroke 13:339–346
Matsumoto H, Kumon Y, Watanabe H et al (2007) Antibodies to CD11b, CD68, and lectin label neutrophils rather than microglia in traumatic and ischemic brain lesions. J Neurosci Res 85:994–1009
Matsuo Y, Onodera H, Shiga Y et al (1994) Correlation between myeloperoxidase-quantified neutrophil accumulation and ischemic brain injury in the rat. Effects of neutrophil depletion. Stroke 25:1469–1475
Matsuo A, Lee GC, Terai K et al (1997) Unmasking of an unusual myelin basic protein epitope during the process of myelin degeneration in humans: a potential mechanism for the generation of autoantigens. Am J Pathol 150:1253–1266
Matute C, Domercq M, Perez-Samartin A et al (2013) Protecting white matter from stroke injury. Stroke 44:1204–1211
Mawhinney LA, Thawer SG, Lu WY et al (2012) Differential detection and distribution of microglial and hematogenous macrophage populations in the injured spinal cord of lys-EGFP-ki transgenic mice. J Neuropathol Exp Neurol 71:180–197
Mayer SA, Brun NC, Broderick J et al (2005) Safety and feasibility of recombinant factor VIIa for acute intracerebral hemorrhage. Stroke 36:74–79

Mayer SA, Brun NC, Begtrup K et al (2008) Efficacy and safety of recombinant activated factor VII for acute intracerebral hemorrhage. N Engl J Med 358:2127–2137
McColl BW, Allan SM, Rothwell NJ (2009) Systemic infection, inflammation and acute ischemic stroke. Neuroscience 158:1049–1061
McCracken E, Fowler JH, Dewar D et al (2002) Grey matter and white matter ischemic damage is reduced by the competitive AMPA receptor antagonist, SPD 502. J Cereb Blood Flow Metab 22:1090–1097
Meairs S, Wahlgren N, Dirnagl U et al (2006) Stroke research priorities for the next decade–a representative view of the European scientific community. Cerebrovasc Dis 22:75–82
Medana IM, Esiri MM (2003) Axonal damage: a key predictor of outcome in human CNS diseases. Brain 126:515–530
Mizutani M, Pino PA, Saederup N et al (2012) The fractalkine receptor but not CCR2 is present on microglia from embryonic development throughout adulthood. J Immunol 188:29–36
Monsma PC, Brown A (2012) FluoroMyelin Red is a bright, photostable and non-toxic fluorescent stain for live imaging of myelin. J Neurosci Methods 209:344–350
Morrison HW, Filosa JA (2013) A quantitative spatiotemporal analysis of microglia morphology during ischemic stroke and reperfusion. J Neuroinflammation 10:4
Moxon-Emre I, Schlichter LC (2010) Evolution of inflammation and white matter injury in a model of transient focal ischemia. J Neuropathol Exp Neurol 69:1–15
Moxon-Emre I, Schlichter LC (2011) Neutrophil depletion reduces blood–brain barrier breakdown, axon injury, and inflammation after intracerebral hemorrhage. J Neuropathol Exp Neurol 70:218–235
Nauta WJ (1952) Selective silver impregnation of degenerating axons in the central nervous system. Stain Technol 27:175–179
Neumann J, Sauerzweig S, Ronicke R et al (2008) Microglia cells protect neurons by direct engulfment of invading neutrophil granulocytes: a new mechanism of CNS immune privilege. J Neurosci 28:5965–5975
Nguyen HX, O'Barr TJ, Anderson AJ (2007) Polymorphonuclear leukocytes promote neurotoxicity through release of matrix metalloproteinases, reactive oxygen species, and TNF-alpha. J Neurochem 102:900–912
Nimmerjahn A, Kirchhoff F, Helmchen F (2005) Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 308:1314–1318
Noda M, Suzumura A (2012) Sweepers in the CNS: microglial migration and phagocytosis in the Alzheimer disease pathogenesis. Int J Alzheimers Dis 2012:891087
O'Collins VE, Macleod MR, Donnan GA et al (2006) 1,026 experimental treatments in acute stroke. Ann Neurol 59:467–477
Ohta H, Nishikawa H, Kimura H et al (1997) Chronic cerebral hypoperfusion by permanent internal carotid ligation produces learning impairment without brain damage in rats. Neuroscience 79:1039–1050
Olah M, Amor S, Brouwer N et al (2012) Identification of a microglia phenotype supportive of remyelination. Glia 60:306–321
Pantoni L, Garcia JH, Gutierrez JA (1996) Cerebral white matter is highly vulnerable to ischemia. Stroke 27:1641–1646, discussion 7
Perry VH, Cowey A (1984) Retinal ganglion cells that project to the superior colliculus and pretectum in the macaque monkey. Neuroscience 12:1125–1137
Perry VH, Oehler R, Cowey A (1984) Retinal ganglion cells that project to the dorsal lateral geniculate nucleus in the macaque monkey. Neuroscience 12:1101–1123
Perry VH, Hume DA, Gordon S (1985) Immunohistochemical localization of macrophages and microglia in the adult and developing mouse brain. Neuroscience 15:313–326
Petrault O, Ouk T, Gautier S et al (2005) Pharmacological neutropenia prevents endothelial dysfunction but not smooth muscle functions impairment induced by middle cerebral artery occlusion. Br J Pharmacol 144:1051–1058
Petty MA, Wettstein JG (1999) White matter ischaemia. Brain Res Brain Res Rev 31:58–64

Petzold A (2005) Neurofilament phosphoforms: surrogate markers for axonal injury, degeneration and loss. J Neurol Sci 233:183–198
Phillips JB, Williams AJ, Adams J et al (2000) Proteasome inhibitor PS519 reduces infarction and attenuates leukocyte infiltration in a rat model of focal cerebral ischemia. Stroke 31:1686–1693
Piani D, Frei K, Do KQ et al (1991) Murine brain macrophages induced NMDA receptor mediated neurotoxicity in vitro by secreting glutamate. Neurosci Lett 133:159–162
Planas AM, Chamorro A (2009) Regulatory T cells protect the brain after stroke. Nat Med 15:138–139
Prestigiacomo CJ, Kim SC, Connolly ES Jr et al (1999) CD18-mediated neutrophil recruitment contributes to the pathogenesis of reperfused but not nonreperfused stroke. Stroke 30:1110–1117
Qureshi AI, Mendelow AD, Hanley DF (2009) Intracerebral haemorrhage. Lancet 373: 1632–1644
Relton JK, Sloan KE, Frew EM et al (2001) Inhibition of alpha4 integrin protects against transient focal cerebral ischemia in normotensive and hypertensive rats. Stroke 32:199–205
Robinson RG, Shoemaker WJ, Schlumpf M et al (1975) Effect of experimental cerebral infarction in rat brain on catecholamines and behaviour. Nature 255:332–334
Robinson AP, White TM, Mason DW (1986) Macrophage heterogeneity in the rat as delineated by two monoclonal antibodies MRC OX-41 and MRC OX-42, the latter recognizing complement receptor type 3. Immunology 57:239–247
Robinson MJ, Macrae IM, Todd M et al (1990) Reduction of local cerebral blood flow to pathological levels by endothelin-1 applied to the middle cerebral artery in the rat. Neurosci Lett 118:269–272
Rock RB, Gekker G, Hu S et al (2004) Role of microglia in central nervous system infections. Clin Microbiol Rev 17:942–964, table of contents
Roman GC, Erkinjuntti T, Wallin A et al (2002) Subcortical ischaemic vascular dementia. Lancet Neurol 1:426–436
Saijo K, Glass CK (2011) Microglial cell origin and phenotypes in health and disease. Nat Rev Immunol 11:775–787
Salthouse TN (1962) Luxol fast blue ARN: a new solvent azo dye with improved staining qualities for myelin and phospholipids. Stain Technol 37:313–316
Scheikl T, Pignolet B, Mars LT et al (2010) Transgenic mouse models of multiple sclerosis. Cell Mol Life Sci 67:4011–4034
Schilling M, Besselmann M, Muller M et al (2005) Predominant phagocytic activity of resident microglia over hematogenous macrophages following transient focal cerebral ischemia: an investigation using green fluorescent protein transgenic bone marrow chimeric mice. Exp Neurol 196:290–297
Schlichter LC, Kaushal V, Moxon-Emre I et al (2010) The Ca2+ activated SK3 channel is expressed in microglia in the rat striatum and contributes to microglia-mediated neurotoxicity in vitro. J Neuroinflammation 7:4
Schmued L, Bowyer J, Cozart M et al (2008) Introducing Black-Gold II, a highly soluble gold phosphate complex with several unique advantages for the histochemical localization of myelin. Brain Res 1229:210–217
Schonbeck U, Mach F, Libby P (1998) Generation of biologically active IL-1 beta by matrix metalloproteinases: a novel caspase-1-independent pathway of IL-1 beta processing. J Immunol 161:3340–3346
Schroeter M, Jander S, Huitinga I et al (1997) Phagocytic response in photochemically induced infarction of rat cerebral cortex. The role of resident microglia. Stroke 28:382–386
Sedgwick JD, Schwender S, Imrich H et al (1991) Isolation and direct characterization of resident microglial cells from the normal and inflamed central nervous system. Proc Natl Acad Sci U S A 88:7438–7442
Seo B, Oemar BS, Siebenmann R et al (1994) Both ETA and ETB receptors mediate contraction to endothelin-1 in human blood vessels. Circulation 89:1203–1208

Sivagnanam V, Zhu X, Schlichter LC (2010) Dominance of E. coli phagocytosis over LPS in the inflammatory response of microglia. J Neuroimmunol 227:111–119
Smith ME (1993) Phagocytosis of myelin by microglia in vitro. J Neurosci Res 35:480–487
Smith ME, van der Maesen K, Somera FP (1998) Macrophage and microglial responses to cytokines in vitro: phagocytic activity, proteolytic enzyme release, and free radical production. J Neurosci Res 54:68–78
Smith DH, Meaney DF, Shull WH (2003) Diffuse axonal injury in head trauma. J Head Trauma Rehabil 18:307–316
Souza-Rodrigues RD, Costa AM, Lima RR et al (2008) Inflammatory response and white matter damage after microinjections of endothelin-1 into the rat striatum. Brain Res 1200:78–88
Sozmen EG, Kolekar A, Havton LA et al (2009) A white matter stroke model in the mouse: axonal damage, progenitor responses and MRI correlates. J Neurosci Methods 180:261–272
Sozmen EG, Hinman JD, Carmichael ST (2012) Models that matter: white matter stroke models. Neurotherapeutics 9:349–358
Sprinkle TJ, Sheedlo HJ, Buxton TB et al (1983) Immunochemical identification of 2′, 3′-cyclic nucleotide 3′-phosphodiesterase in central and peripheral nervous system myelin, the Wolfgram protein fraction, and bovine oligodendrocytes. J Neurochem 41:1664–1671
Sternberger LA, Sternberger NH (1983) Monoclonal antibodies distinguish phosphorylated and nonphosphorylated forms of neurofilaments in situ. Proc Natl Acad Sci U S A 80:6126–6130
Sternberger NH, Itoyama Y, Kies MW et al (1978) Myelin basic protein demonstrated immunocytochemically in oligodendroglia prior to myelin sheath formation. Proc Natl Acad Sci U S A 75:2521–2524
Stevens SL, Bao J, Hollis J et al (2002) The use of flow cytometry to evaluate temporal changes in inflammatory cells following focal cerebral ischemia in mice. Brain Res 932:110–119
Stilwell DL (1957) A sudan black B myelin stain for peripheral nerves. Stain Technol 32:19–23
Streilein JW (1993) Immune privilege as the result of local tissue barriers and immunosuppressive microenvironments. Curr Opin Immunol 5:428–432
Streit WJ, Kreutzberg GW (1987) Lectin binding by resting and reactive microglia. J Neurocytol 16:249–260
Svedin P, Hagberg H, Savman K et al (2007) Matrix metalloproteinase-9 gene knock-out protects the immature brain after cerebral hypoxia-ischemia. J Neurosci 27:1511–1518
Tayag EC, Jeng AY, Savage P et al (1996) Rat striatum contains pure population of ETB receptors. Eur J Pharmacol 300:261–265
Tonnesen MG (1989) Neutrophil-endothelial cell interactions: mechanisms of neutrophil adherence to vascular endothelium. J Invest Dermatol 93:53S–58S
Towfighi J, Zec N, Yager J et al (1995) Temporal evolution of neuropathologic changes in an immature rat model of cerebral hypoxia: a light microscopic study. Acta Neuropathol 90:375–386
Traystman RJ (2003) Animal models of focal and global cerebral ischemia. ILAR J 44:85–95
Trotter J, DeJong LJ, Smith ME (1986) Opsonization with antimyelin antibody increases the uptake and intracellular metabolism of myelin in inflammatory macrophages. J Neurochem 47:779–789
Turrin NP, Rivest S (2006) Molecular and cellular immune mediators of neuroprotection. Mol Neurobiol 34:221–242
Uehara H, Yoshioka H, Kawase S et al (1999) A new model of white matter injury in neonatal rats with bilateral carotid artery occlusion. Brain Res 837:213–220
Valeriani V, Dewar D, McCulloch J (2000) Quantitative assessment of ischemic pathology in axons, oligodendrocytes, and neurons: attenuation of damage after transient ischemia. J Cereb Blood Flow Metab 20:765–771
Van Dyken SJ, Locksley RM (2013) Interleukin-4- and interleukin-13-mediated alternatively activated macrophages: roles in homeostasis and disease. Annu Rev Immunol 31:317–343
Vannucci SJ, Hagberg H (2004) Hypoxia-ischemia in the immature brain. J Exp Biol 207:3149–3154

Vannucci RC, Vannucci SJ (1997) A model of perinatal hypoxic-ischemic brain damage. Ann N Y Acad Sci 835:234–249
Vannucci RC, Lyons DT, Vasta F (1988) Regional cerebral blood flow during hypoxia-ischemia in immature rats. Stroke 19:245–250
Varin A, Gordon S (2009) Alternative activation of macrophages: immune function and cellular biology. Immunobiology 214:630–641
Varvel NH, Grathwohl SA, Baumann F et al (2012) Microglial repopulation model reveals a robust homeostatic process for replacing CNS myeloid cells. Proc Natl Acad Sci U S A 109:18150–18155
Verhaar MC, Strachan FE, Newby DE et al (1998) Endothelin-A receptor antagonist-mediated vasodilatation is attenuated by inhibition of nitric oxide synthesis and by endothelin-B receptor blockade. Circulation 97:752–756
Villapol S, Fau S, Renolleau S et al (2011) Melatonin promotes myelination by decreasing white matter inflammation after neonatal stroke. Pediatr Res 69:51–55
Wake H, Moorhouse AJ, Jinno S et al (2009) Resting microglia directly monitor the functional state of synapses in vivo and determine the fate of ischemic terminals. J Neurosci 29:3974–3980
Wakita H, Tomimoto H, Akiguchi I et al (1994) Glial activation and white matter changes in the rat brain induced by chronic cerebral hypoperfusion: an immunohistochemical study. Acta Neuropathol 87:484–492
Wakita H, Tomimoto H, Akiguchi I et al (1995) Protective effect of cyclosporin A on white matter changes in the rat brain after chronic cerebral hypoperfusion. Stroke 26:1415–1422
Wakita H, Tomimoto H, Akiguchi I et al (1998) Dose-dependent, protective effect of FK506 against white matter changes in the rat brain after chronic cerebral ischemia. Brain Res 792:105–113
Walker CA, Huttner AJ, O'Connor KC (2011) Cortical injury in multiple sclerosis; the role of the immune system. BMC Neurol 11:152
Wang J, Dore S (2007) Inflammation after intracerebral hemorrhage. J Cereb Blood Flow Metab 27:894–908
Wang X, Lo EH (2003) Triggers and mediators of hemorrhagic transformation in cerebral ischemia. Mol Neurobiol 28:229–244
Wang X, Tsuji K, Lee SR et al (2004) Mechanisms of hemorrhagic transformation after tissue plasminogen activator reperfusion therapy for ischemic stroke. Stroke 35:2726–2730
Wang Q, Tang XN, Yenari MA (2007) The inflammatory response in stroke. J Neuroimmunol 184:53–68
Wang Y, Li B, Li Z et al (2013) Improvement of hypoxia-ischemia-induced white matter injury in immature rat brain by ethyl pyruvate. Neurochem Res 38:742–752
Ward JM, Erexson CR, Faucette LJ et al (2006) Immunohistochemical markers for the rodent immune system. Toxicol Pathol 34:616–630
Wasserman JK, Schlichter LC (2007) Neuron death and inflammation in a rat model of intracerebral hemorrhage: effects of delayed minocycline treatment. Brain Res 1136:208–218
Wasserman JK, Schlichter LC (2008) White matter injury in young and aged rats after intracerebral hemorrhage. Exp Neurol 214:266–275
Wasserman JK, Yang H, Schlichter LC (2008) Glial responses, neuron death and lesion resolution after intracerebral hemorrhage in young vs. aged rats. Eur J Neurosci 28:1316–1328
Weinstein JR, Koerner IP, Moller T (2010) Microglia in ischemic brain injury. Future Neurol 5:227–246
Weston RM, Jones NM, Jarrott B et al (2007) Inflammatory cell infiltration after endothelin-1-induced cerebral ischemia: histochemical and myeloperoxidase correlation with temporal changes in brain injury. J Cereb Blood Flow Metab 27:100–114
Whiteland JL, Nicholls SM, Shimeld C et al (1995) Immunohistochemical detection of T-cell subsets and other leukocytes in paraffin-embedded rat and mouse tissues with monoclonal antibodies. J Histochem Cytochem 43:313–320

Whiteley W, Jackson C, Lewis S et al (2009) Inflammatory markers and poor outcome after stroke: a prospective cohort study and systematic review of interleukin-6. PLoS Med 6:e1000145
Wiley KE, Davenport AP (2004) Endothelin receptor pharmacology and function in the mouse: comparison with rat and man. J Cardiovasc Pharmacol 44(suppl 1):S4–S6
Williams AJ, Hale SL, Moffett JR et al (2003) Delayed treatment with MLN519 reduces infarction and associated neurologic deficit caused by focal ischemic brain injury in rats via antiinflammatory mechanisms involving nuclear factor-kappaB activation, gliosis, and leukocyte infiltration. J Cereb Blood Flow Metab 23:75–87
Won SM, Lee JH, Park UJ et al (2011) Iron mediates endothelial cell damage and blood–brain barrier opening in the hippocampus after transient forebrain ischemia in rats. Exp Mol Med 43:121–128
Wynn TA, Chawla A, Pollard JW (2013) Macrophage biology in development, homeostasis and disease. Nature 496:445–455
Xu J, He L, Ahmed SH et al (2000) Oxygen-glucose deprivation induces inducible nitric oxide synthase and nitrotyrosine expression in cerebral endothelial cells. Stroke 31:1744–1751
Yang Y, Jalal FY, Thompson JF et al (2011) Tissue inhibitor of metalloproteinases-3 mediates the death of immature oligodendrocytes via TNF-alpha/TACE in focal cerebral ischemia in mice. J Neuroinflammation 8:108
Yilmaz G, Arumugam TV, Stokes KY et al (2006) Role of T lymphocytes and interferon-gamma in ischemic stroke. Circulation 113:2105–2112
Yokota N, Daniels F, Crosson J et al (2002) Protective effect of T cell depletion in murine renal ischemia-reperfusion injury. Transplantation 74:759–763
Zhang ZG, Chopp M (1997) Measurement of myeloperoxidase immunoreactive cells in ischemic brain after transient middle cerebral artery occlusion in the rat. Neurosci Res Commun 20:85–91
Zhang K, Sejnowski TJ (2000) A universal scaling law between gray matter and white matter of cerebral cortex. Proc Natl Acad Sci U S A 97:5621–5626
Zhang W, Stanimirovic D (2002) Current and future therapeutic strategies to target inflammation in stroke. Curr Drug Targets Inflamm Allergy 1:151–166
Zhao BQ, Chauhan AK, Canault M et al (2009) von Willebrand factor-cleaving protease ADAMTS13 reduces ischemic brain injury in experimental stroke. Blood 114:3329–3334
Zwacka RM, Zhang Y, Halldorson J et al (1997) CD4(+) T-lymphocytes mediate ischemia/reperfusion-induced inflammatory responses in mouse liver. J Clin Invest 100:279–289

Chapter 20
Oxidative Stress in White Matter Injury

Hideyuki Yoshioka, Takuma Wakai, Hiroyuki Kinouchi, and Pak H. Chan

Abbreviations

4-HNE	4-Hydroxynonenal
APP	Amyloid precursor protein
eNOS	Endothelium nitric oxide synthase
H_2O_2	Hydrogen peroxide
iNOS	Inducible nitric oxide synthase
MBP	Myelin basic protein
MCA	Middle cerebral artery
MDA	Malondialdehyde
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide

H. Yoshioka, M.D., Ph.D. • T. Wakai, M.D.
Department of Neurosurgery, Stanford University School of Medicine, Stanford, CA, USA

Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, CA, USA

Program in Neurosciences, Stanford University School of Medicine, Stanford, CA, USA

Department of Neurosurgery, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Yamanashi, Japan

H. Kinouchi, M.D., Ph.D.
Department of Neurosurgery, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Yamanashi, Japan

P.H. Chan, Ph.D. (✉)
Department of Neurosurgery, Stanford University School of Medicine, Stanford, CA, USA

Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, CA, USA

Program in Neurosciences, Stanford University School of Medicine, Stanford, CA, USA
e-mail: phchan@stanford.edu

S. Baltan et al. (eds.), *White Matter Injury in Stroke and CNS Disease*, Springer Series in Translational Stroke Research 4, DOI 10.1007/978-1-4614-9123-1_20,

NOS	Nitric oxide synthase
NOX	Nicotinamide adenine dinucleotide phosphate oxidase
$O_2^{\bullet-}$	Superoxide anions
$^{\bullet}OH$	Hydroxyl radicals
$ONOO^-$	Peroxynitrite
PWMI	Periventricular white matter injury
RIP	Receptor-interacting protein
ROS	Reactive oxygen species
SMI-32	An antibody against a non-phosphorylated neurofilament epitope
SOD	Superoxide dismutase
SOD1	Copper/zinc superoxide dismutase
SOD2	Manganese superoxide dismutase

20.1 Introduction

White matter is the region of the brain underlying gray matter and is primarily composed of axonal bundles ensheathed with myelin. The cells forming these sheaths are oligodendrocytes, which tend to be arranged in rows parallel to axonal tracts. White matter comprises over half the human brain, a far greater proportion than in other animals (Fields 2008). Because white matter is at risk for ischemic injury throughout life, from periventricular white matter injury (PWMI) in neonates to stroke and vascular dementia in later life, this injury is of great clinical interest. The failure to ameliorate ischemic white matter injury is a major factor in the failure to translate preclinical studies into therapy (Dewar et al. 1999). Accordingly, ischemic white matter injury has been the focus of much attention in recent years.

White matter is extremely vulnerable to ischemic stress. First, blood flow in white matter is lower than in gray matter and there is little collateral blood supply in deep white matter (Xing et al. 2012). Second, once injured by ischemic stress, the structural integrity of axons and the myelin sheath is weakened and demyelinated axons become more susceptible to ischemic injury due to their heightened metabolic requirements caused by loss of energy-efficient saltatory conduction and leaky sodium channels (Lo et al. 2003; Trapp and Stys 2009). Third, axons, oligodendrocytes, and their progenitors are highly sensitive to oxidative stress (Dewar et al. 2003).

Oxidative stress is a condition under which reactive oxygen species (ROS) generation is enhanced and ROS metabolism is impaired. Axons are exceedingly vulnerable to oxidative stress, since axons contain abundant mitochondria, organelles that are a main source of ROS (Xing et al. 2012). In addition, the myelin sheath contains numerous lipids, which can be peroxidized after oxidative stress (Ueno et al. 2009). Oligodendrocytes are also vulnerable to oxidative stress. One of the reasons for this vulnerability is that they generate ROS for production and maintenance of myelin. Oligodendrocytes have been estimated to have the highest metabolic rate of any cell in the brain for production and maintenance of the myelin sheath (Connor and Menzies 1996). Because myelin production is energy dependent,

large amounts of adenosine triphosphate are consumed in the process and toxic oxidants are produced as byproducts of adenosine triphosphate synthesis (McTigue and Tripathi 2008). Another reason for the vulnerability is that many metabolic and myelin synthetic enzymes require iron as a cofactor (Connor and Menzies 1996). Accordingly, oligodendrocytes and oligodendrocyte progenitors have the largest intracellular stores of iron in the brain (20-fold greater than astrocytes under baseline culture conditions) (Thorburne and Juurlink 1996; Cheepsunthorn et al. 1998). Iron is necessary for myelin production, while it is also highly reactive and can evoke free radical formation and lipid peroxidation. In addition, low concentrations of glutathione (a robust antioxidative enzyme) in oligodendrocytes contribute to the vulnerability to oxidative stress (Thorburne and Juurlink 1996). Oxidative stress in white matter injury is deeply involved in a number of pathological conditions, including trauma, stroke, and multiple sclerosis.

20.2 Generation of Oxygen Free Radicals

Free radicals are molecular species that contain one or more unpaired valence electrons not contributing to intramolecular bonding. Free radicals are highly interactive with other molecules, such as DNA and lipids, pairing with their single electrons and causing oxidation of those molecules (Dröge 2002). Several oxygen free radicals (oxidants) and their derivatives are generated after ischemia, including superoxide anions ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($^{\bullet}OH$). $O_2^{\bullet -}$ are formed when oxygen acquires an additional electron, leaving the molecule with only one unpaired electron. A major source of $O_2^{\bullet -}$ is mitochondria where approximately 2–5 % of the molecular oxygen consumed during normal physiological respiration is converted into $O_2^{\bullet -}$ and H_2O_2 (Boveris and Chance 1973). Pro-oxidant enzymes, such as cyclooxygenase, xanthine dehydrogenase, xanthine oxidase, myeloperoxidase, monoamine oxidase, and nicotinamide adenine dinucleotide phosphate oxidase (NOX), also catalyze the generation of $O_2^{\bullet -}$. Among them, much attention has recently been focused on the role of NOX in cerebral ischemia. NOX is a multi-subunit enzyme that transfers an electron from nicotinamide adenine dinucleotide phosphate to molecular oxygen to generate $O_2^{\bullet -}$. Originally discovered in phagocytic cells, expression and distribution of NOX have subsequently been found in many other cell types, such as neurons, astrocytes, and microglia, in the cortex, hippocampus, and cerebellum (Kim et al. 2005; Tejada-Simon et al. 2005; Infanger et al. 2006; Bedard and Krause 2007). It is becoming clear that overactivation of NOX plays a role in many neurodegenerative diseases, such as stroke and Alzheimer's disease (Walder et al. 1997; Bedard and Krause 2007; Chen et al. 2009; Kim et al. 2009; Yoshioka et al. 2011a). NOX may also play an important role in the pathogenesis of white matter injury.

Peroxynitrite ($ONOO^-$) generated by inducible nitric oxide synthase (iNOS) and NOX in activated microglia injures oligodendrocytes (Li et al. 2005). $O_2^{\bullet -}$ can react with nitric oxide (NO) to produce $ONOO^-$ ($NO + O_2^{\bullet -} \rightarrow ONOO^-$), which is a strong oxidative radical that causes protein nitration and dysfunction (Beckman et al. 1990).

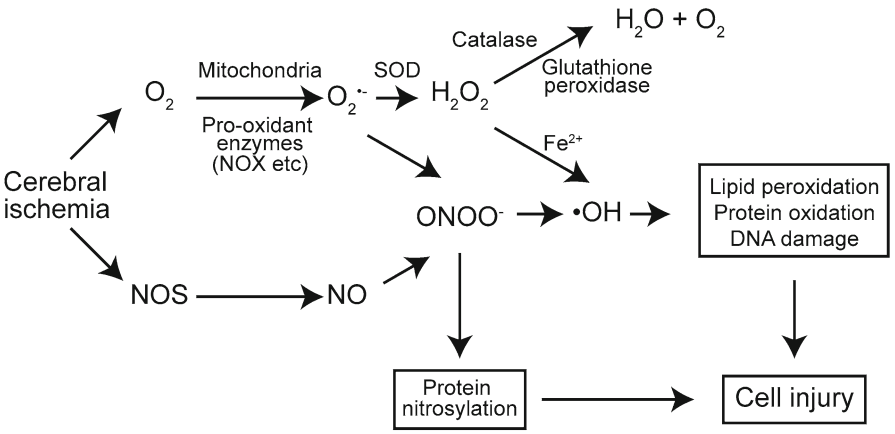


Fig. 20.1 Oxidants and NO generated after ischemia induce lipid peroxidation, protein oxidation, DNA damage, and protein nitrosylation, all of which cause cell injury

NO is produced by nitric oxide synthase (NOS) using arginine and O_2 as substrates. Three isoforms of NOS exist in central nervous system parenchyma: neuronal nitric oxide synthase (nNOS), a constitutive isoform that is localized in neurons; iNOS, an isoform that is induced in microglia/macrophages and astrocytes and endothelial cells; and eNOS, a constitutive form that is localized in the endothelium. nNOS and eNOS activity is Ca^{2+}-dependent, whereas iNOS is Ca^{2+}-independent. NO produced by nNOS and iNOS has been implicated in both in vitro cell culture injury and in vivo ischemic brain damage. NO produced by eNOS is known to be neuroprotective because of its vasodilative effects (Chan 2001).

Superoxide dismutases (SODs) are specific enzymes and have three isoforms: copper/zinc SOD (SOD1), manganese SOD (SOD2), and extracellular SOD. All three SOD isoforms detoxify $O_2^{\bullet-}$ to H_2O_2 ($O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2$), which is further converted to H_2O by catalase or glutathione peroxidase ($2H_2O_2 \rightarrow 2H_2O + O_2$) (Chan 2001). $^{\bullet}OH$ are extremely reactive oxidants produced by H_2O_2 through the Fenton reaction ($H_2O_2 + Fe^{2+} \rightarrow OH^- + Fe^{3+} + {}^{\bullet}OH$) and the Haber-Weiss reaction ($O_2^{\bullet-} + H_2O_2 \rightarrow {}^{\bullet}OH + HO^- + O_2$) or by $ONOO^-$ (Beckman et al. 1990; Chan 1996). Experimental studies using transgenic or knockout animals have shown that both SOD1 and SOD2 are neuroprotective against ischemic injury (Kinouchi et al. 1991; Chan 2001; Niizuma et al. 2010; Chen et al. 2011). In addition to antioxidant enzymes, other antioxidants, including glutathione, ascorbic acid, and vitamin E, are also involved in the detoxification of oxidants (Chan 1996, 2001). A constitutively low concentration of oxidants is necessary; they act as signaling molecules for various functions, such as regulation of vascular tone, monitoring of oxygen tension, and erythropoietin production (Dröge 2002). However, excessive oxidants may irreversibly oxidize macromolecules such as DNA, lipids, and protein, and cause severe cell injury. Figure 20.1 summarizes the generation of oxidants in vivo that leads to brain cell damage under ischemic conditions.

20.3 Oxidative Stress in Ischemic White Matter Injury

Three pathologic conditions are deeply connected to ischemic oxidative injury of white matter, namely, PWMI, acute cerebral ischemia, and chronic cerebral hypoperfusion.

20.3.1 Oxidative Stress in Ischemic Injury of Oligodendrocyte Progenitors

Oligodendroglial lineages are vulnerable to ischemic conditions. Among them, oligodendrocyte progenitors are much more vulnerable to ischemic insults than mature oligodendrocytes (Husain and Juurlink 1995; Fern and Möller 2000). PWMI is related to the ischemic vulnerability of oligodendrocyte progenitors and is the major pathological finding underlying cerebral palsy, subsequently observed in survivors of premature birth. Cerebral palsy is a motor disorder affecting 10 % of very low-birth-weight (<1,500 g) premature infants who survive the intensive care nursery. The pathogenesis of PWMI is multifactorial and likely involves damage related to ischemic injury in the critically ill premature infant with impaired regulation of cerebral blood flow, as well as inflammation-induced brain injury associated with maternal and/or fetal infection (Haynes et al. 2005).

PWMI includes a spectrum of cerebral injuries that ranges from focal cystic necrotic lesions (periventricular leukomalacia) to extensive white matter lesions (diffuse PWMI). Periventricular leukomalacia commonly occurs in the subventricular zone adjacent to the lateral ventricle and involves injury to all cellular elements. In contrast, diffuse PWMI is characterized by deep cerebral white matter that contains extensive regions of numerous reactive astrocytes (diffuse gliosis) and diffuse myelination disturbances. Although diffuse gliosis is believed to arise in response to extensive white matter damage, the types of injured cells that provoke this gliosis remain unknown, but are hypothesized to be oligodendrocyte progenitors because such lesions occur in a similar distribution in regions with myelination disturbance (Back and Rivkees 2004).

Oligodendrocytes develop a well-established lineage, precisely defined by antibodies that are stage-specific for sequentially expressed oligodendrocyte cell-surface and myelin-specific epitope (Back and Rivkees 2004). Since the major period of vulnerability for PWMI (23–32 weeks gestation) occurs before the onset of myelination, Back and Volpe (1997) first proposed that the myelination disturbances in PWMI might arise from targeted death of oligodendrocyte progenitors. Several lines of evidence support a role for targeted death of oligodendrocyte progenitors in the pathogenesis of PWMI, derived from in vitro and in vivo experimental models (Back and Rivkees 2004).

Ischemic oxidative stress is thought to be closely connected to the targeted death of oligodendrocyte progenitors. In human brain samples, immunohistochemical studies showed evidence of oxidative and nitrative stress in PWMI. Expression of the markers of lipid peroxidation (4-hydroxynonenal [4-HNE] and malondialdehyde

[MDA]) and nitrative stress (nitrotyrosine) increases in premyelinating oligodendrocytes within PWMI. These cells are also positive for staining with terminal deoxynucleotidyl transferase-mediated uridine 5′-triphosphate-biotin nick end labeling, which indicates the vulnerability of premyelinating oligodendrocytes. Oxidative and nitrative stress markers are also expressed in reactive astrocytes within the lesion. However, astrocytes are expected to protect surrounding oligodendrocytes because they do not undergo cell death (Haynes et al. 2003).

The release of ROS is related to ischemia that leads to a decrease in the cellular antioxidant glutathione, failure of membrane channels that regulate ionic and osmotic homeostasis of the cells, and excessive release of glutamate from injured axons, which results in a subsequent release of ROS. Ischemia also induces the upregulation of iNOS in reactive astrocytes (Haynes et al. 2003). In addition to ischemia, inflammatory response associated with infection is another source of ROS. Upregulation of cytokines, including tumor necrosis factor-α, interleukin-2, interleukin-6, and interferon-γ (Deguchi et al. 1996; Yoon et al. 1997; Kadhim et al. 2001, 2002; Folkerth et al. 2004b), activates astrocytes and microglia in the surrounding lesions, which releases ROS and reactive nitrogen species (Haynes et al. 2003). Recent evidence has revealed that the vulnerability of oligodendrocyte progenitors to oxidative stress results, in part, from developmental mismatch in antioxidant capacity in white matter of the human fetus. During the period of greatest risk for PWMI, expression of the peroxide-generating enzymes SOD1 and SOD2 significantly lags behind that of peroxide-degrading catalase and glutathione peroxidase, which alters a crucial balance in oxidant metabolism (Folkerth et al. 2004a).

Several experimental models have been developed to study the mechanism of PWMI in rodents, rabbits, sheep, and nonhuman primates. These models include ischemia, injection of endotoxin, administration of excitotoxic agents, and chronic sublethal hypoxia. Although rodent acute hypoxia–ischemia models (usually achieved through bilateral or unilateral carotid artery occlusion followed by exposure to hypoxia) generate extensive cortical and subcortical neuronal death and do not reproduce the many distinct physiological features unique to the premature human infant, these models have been widely used to study the cellular and molecular mechanisms of the injury. At postnatal day 2 (P2), white matter of rodents contains predominantly oligodendrocyte progenitors as in premature infants (Craig et al. 2003). The vulnerable period for white matter injury in rodents is around P2, then declines thereafter, coincident with the onset of oligodendrocyte differentiation and myelination between P7 and P14. In rodents, oligodendrocyte progenitors are also more vulnerable to ischemic stress than mature oligodendrocytes (Back et al. 2002), and developmental mismatch in the antioxidant capacity in rat oligodendrocyte progenitors is also reported in human fetuses. Expression and activity of the antioxidant SOD2 in oligodendrocyte progenitors are lower than in mature oligodendrocytes (Baud et al. 2004). Poor capability of oligodendrocyte progenitors to treat free iron is another mechanism of this vulnerability. Normal development of the brain requires iron as a cofactor for many enzymes; however, free iron can enhance oxidative damage by the Fenton reaction (Halliwell 1989). Because oligodendroglial lineage, especially oligodendroglia progenitors, has high iron stores but

low glutathione, peroxide remains at dangerously high levels if iron is released from iron stores (Thorburne and Juurlink 1996; Juurlink et al. 1998). In addition, the delayed appearance of ferritin in oligodendrocytes contributes to the vulnerability of oligodendrocyte progenitors. Ferritin not only makes iron available within cells, but also provides protection from iron-induced oxidative damage (Harrison and Arosio 1996), and there is a shift in ferritin-containing cell types during development from predominantly microglia at P5 to predominantly oligodendrocytes by P30 (Cheepsunthorn et al. 1998). This delayed appearance of ferritin also makes oligodendrocyte progenitors susceptible to ischemic oxidative stress (Cheepsunthorn et al. 2001).

These studies indicate that oxidative stress is implicated in white matter injury in the developing brain, and some studies using neonatal rodent models have revealed that free radical scavengers are protective against this injury. The concentrations of 4-HNE and MDA in the brain increased 1–24 h after hypoxia–ischemia, and 4-HNE was expressed in pyknotic O_4-positive oligodendrocyte progenitors. α-Phenyl-*n-tert*-butyl-nitrone, a spin-trapping agent, attenuated this oxidative stress and injury in axons and oligodendrocyte progenitors after hypoxia–ischemia (Lin et al. 2004). Edaravone, a free radical scavenger clinically used in Japan, also protected white mater after mouse neonatal hypoxia–ischemia (Shen et al. 2012). In addition to free radical scavengers, some reagents were reported to reduce oxidative stress and alleviate ischemic white matter injury. Minocycline is a bacteriostatic agent with broad-spectrum antimicrobial activity shown to provide neuroprotection against ischemic brain injury in adult and neonatal rodents (Yrjänheikki et al. 1998; Arvin et al. 2002; Xu et al. 2004). Cai et al. (2006) reported that minocycline alleviated hypoxia–ischemia injury to developing oligodendrocytes due to reduction in oxidative damage. They showed that minocycline reduced hypoxia–ischemia-induced oxidative and nitrosative stress, as indicated by 4-HNE- and nitrotyrosine-positive oligodendrocytes. They also showed a decrease in 8-isoprostane, an oxidative stress marker, in minocycline-treated rats compared with vehicle-treated rats. Administration of 17β-estradiol, which plays an important function in the developing brain, protected oligodendrocyte progenitors against oxidative stress induced by cysteine depletion and alleviated white matter ischemic injury in a neonatal rat unilateral carotid ligation model (Gerstner et al. 2009). Although no clinically effective therapy for PWMI has been established, reduction in oxidative stress is a strategy for treatment.

20.3.2 Oxidative Stress in Ischemic Injury of Mature Oligodendrocytes

Susceptibility to oxidative stress is not restricted to oligodendrocyte progenitors. Mature oligodendrocytes in culture are also susceptible to oxidative stress. H_2O_2 induced cell death in bovine oligodendrocytes in culture (Kim and Kim 1991). Adult rat oligodendrocytes were damaged by H_2O_2 formed by redox cycling of catecholamines (Noble et al. 1994). NO also damaged oligodendrocytes in vitro

(Mitrovic et al. 1995, 1996). Compared with other cell types tested under similar hypoxic conditions, oligodendrocytes are less vulnerable than neurons, but are much more quickly injured than astrocytes, microglia, or endothelial cells (Lyons and Kettenmann 1998; Xu et al. 2000; Dewar et al. 2003).

Several factors related to the susceptibility of oligodendrocytes have been reported. Juurlink et al. (1998) found that not only oligodendrocyte progenitors but also mature oligodendrocytes had low levels of reduced glutathione and high levels of iron content. Hence, mature oligodendrocytes have a poor ability to scavenge peroxides in a way similar to oligodendrocyte progenitors. Bernardo et al. (2003) reported that this susceptibility was related to the low expression of SOD2 and catalase in mature oligodendrocytes. In the next sections, acute cerebral ischemia and chronic hypoperfusion, in which ischemic injury of mature oligodendrocytes participates, will be discussed.

20.3.2.1 Acute Ischemic Injury

Much attention has been focused on gray matter in ischemic stroke, while white matter has been considered less vulnerable than gray matter to ischemic injury. However, almost all cases of ischemic stroke involve white matter, and ischemia sometimes primarily involves white matter (Bogousslavsky and Regli 1992). Therefore, white matter is now being recognized as highly vulnerable to the effects of ischemia (Pantoni et al. 1996).

White matter injury after acute ischemia has been studied in both focal and global ischemia rodent models. In a rat permanent middle cerebral artery (MCA) occlusion model, morphologic changes in oligodendrocytes and myelinated axons in white matter occurred as rapidly as in neuronal perikarya. After 3 h of ischemia most oligodendrocytes were lethally injured and preceded the appearance of necrotic neurons in the cortex and basal ganglia by several hours. Vacuolation and pallor of white matter were very marked after 24 h and reflected the segmental swelling of myelinated axons, the formation of spaces between myelin sheaths, and axolemma (Pantoni et al. 1996). Rapid injury of white matter oligodendrocytes was confirmed by immunostaining with tau, a microtubule-associated protein (Dewar and Dawson 1995; Irving et al. 1997). For detection of axonal injury after ischemia, immunostaining with amyloid precursor protein (APP) has been widely used. APP is conveyed by fast anterograde axonal transport, and the presence of APP within axons at the site of injury is thought to be due to its accumulation after inhibition of axoplasmic flow (Shigematsu and McGeer 1992). Increased APP immunoreactivity has been demonstrated in white matter of rats after transient and permanent MCA occlusion and thromboembolic stroke (Stephenson et al. 1992; Yam et al. 1997; Dietrich et al. 1998; Imai et al. 2001; Gresle et al. 2006). One of the problems in detecting axonal injury by APP is that the immunostaining is restricted mainly to the margins of the ischemic lesion, and hence is largely absent from the ischemic core (Yam et al. 1997; Gresle et al. 2006). Another immunostaining marker used to detect axonal injury is SMI-32. The SMI-32 antibody reacts with dephosphorylated

neurofilament H within the neuronal and axonal cytoskeleton. Neurofilament proteins are highly phosphorylated under physiological conditions and axonal injury causes a decrease in phosphorylated neurofilament and an increase in dephosphorylated neurofilament (Trapp et al. 1998). Therefore, the SMI-32 antibody can be used to detect axonal injury under many conditions (Gresle et al. 2006; Yoshioka et al. 2011b). In contrast to APP immunostaining, SMI-32 immunostaining can visualize axons within the ischemic core as changes in the state of phosphorylation. Myelin impairment has been evaluated using immunostaining with myelin basic protein (MBP). In histologically normal tissue, MBP immunoreactivity was detected in myelinated fiber tracts. Changes in MBP staining after cerebral ischemia differ among ischemia models and conditions. Marked reduction in MBP levels within ischemic tissue was detected 1–2 weeks after MCA occlusion (Irving et al. 2001), while in endothelin-1-injection ischemia models MBP staining was reduced 1–7 days after injection (Souza-Rodrigues et al. 2008; Sozmen et al. 2009; Moxon-Emre and Schlichter 2010).

White matter injury has also been reported in transient global cerebral ischemia models. Some authors reported axonal injury after global ischemia in the stratum radiatum of the CA1 subregion, the cerebral subcortical region, and the corpus callosum in rodent models (Pluta et al. 2006; Kubo et al. 2009; Walker and Rosenberg 2010). Oligodendrocytes are also vulnerable to global ischemia. The number of CC-1-positive mature oligodendrocytes in the corpus callosum significantly decreased 3 days after transient global ischemia (Walker and Rosenberg 2010). Petito et al. (1998) reported that oligodendrocytes were more vulnerable than neurons in the cerebral cortex and thalamus in a rat transient global cerebral ischemia model. We reported severe injury in oligodendrocytes and axons in the striatum after prolonged transient global cerebral ischemia (Yoshioka et al. 2011b). The cell processes of oligodendrocytes depicted with receptor-interacting protein (RIP) immunostaining in the striatum began to change 1–3 days after transient global ischemia, and disappeared 7 days after ischemia. Intense expression of SMI-32 was observed in the fiber fascicles of the internal capsule of the striatum 3 days after ischemia. At the same time point, MBP staining of the fiber fascicles became coarse, and vacuolation of the fascicles was observed (Fig. 20.2) (Yoshioka et al. 2011b).

Participation of oxidative stress in white matter injury after acute ischemia and the effectiveness of antioxidants have been investigated using these rodent models and immunostaining markers. 4-HNE staining was observed in axons within core and peri-lesion areas after transient MCA occlusion in rats (Imai et al. 2001). Pretreatment with α-phenyl-*n*-*tert*-butyl-nitrone reduced by 55 % the number of tau-positive injured oligodendrocytes in the subcortical white matter of the ischemic hemisphere compared with untreated animals after permanent MCA occlusion (Irving et al. 1997). Ebselen, which has potent antioxidant effects by acting as glutathione peroxidase and phospholipid glutathione peroxidase mimics, reduced oxidative stress in white matter and consequently lessened axon and oligodendrocyte injury in a rat MCA occlusion model (Imai et al. 2001). Administration of Edaravone 60 min after transient global cerebral ischemia reduced axonal damage in

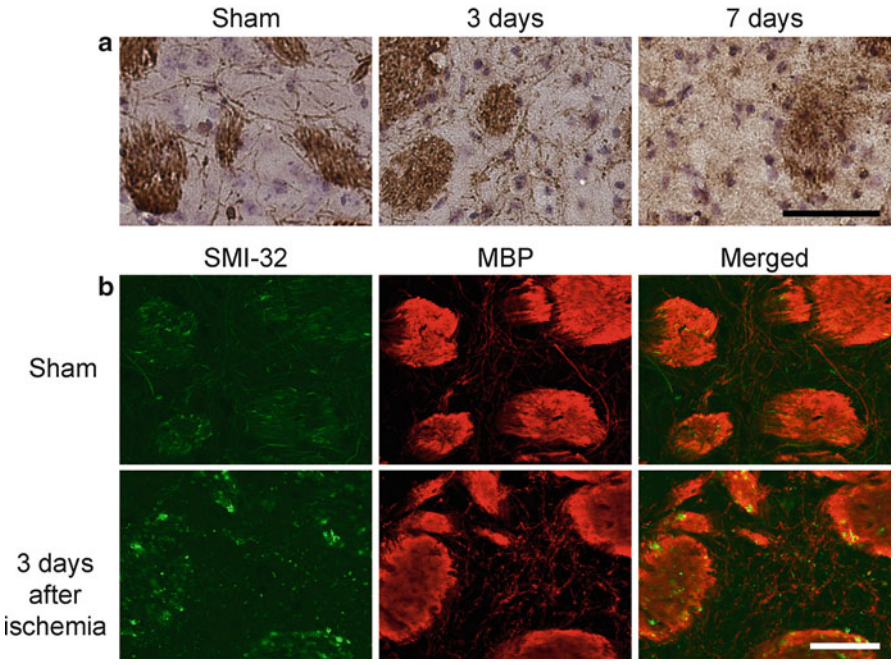


Fig. 20.2 White matter injury in the striatum after transient global cerebral ischemia. (**a**) Representative photomicrographs of RIP staining. RIP-positive processes were thin and smooth in the sham animals. Three days after bilateral common carotid artery occlusion, they became tangled and intermittent. Seven days after bilateral common carotid artery occlusion, RIP-positive processes disappeared, though staining of cell bodies was observed. Nuclei were counterstained with hematoxylin. Scale bar: 50 μm. (**b**) Representative photomicrographs of SMI-32 (*green*) and MBP (*red*) staining of the striatum. Three days after ischemia, intense expression of SMI-32 was observed in the fiber fascicles of the internal capsule of the striatum. With MBP staining, coarse change and vacuolation of the fiber fascicles were also observed. Scale bar: 50 μm

the hippocampus CA1 subregion and the corpus callosum in rats (Kubo et al. 2009). These studies support a pivotal role for oxidative stress in acute ischemic white matter injury.

20.3.2.2 Chronic Hypoperfusion

White matter lesions are often observed in patients with ischemic cerebrovascular diseases (such as Binswanger disease). They are most likely caused by chronic cerebral ischemia and are believed to be responsible for cognitive impairment (Pantoni and Garcia 1997). Neuropathological changes in these lesions are characterized by diffuse demyelination, the loss of axons, and gliosis (Shibata et al. 2004). Many processes, including inflammation and apoptosis of oligodendrocytes, contribute to white matter lesions, and oxidative stress is an important factor among them.

Nonhuman primates, dogs, cats, and rodents have been used to investigate pathological processes of white matter lesions. Nonhuman primates probably represent the best model because their vascular architecture and gyrencephalic brain with extensive white matter more closely resemble those of humans. Nevertheless, most experiments are performed in rodents because of lower cost and higher acceptability from ethical committees (Ginsberg and Busto 1989). Permanent occlusion of both common carotid arteries has been frequently used in rats (Wakita et al. 1994; Ihara et al. 2001; Ueno et al. 2002), while permanent narrowing of both common carotid arteries has been used in gerbils and mice, in which posterior communicating arteries are undeveloped (Hattori et al. 1992; Kudo et al. 1993; Kurumatani et al. 1998; Shibata et al. 2004). Several studies have identified the optic tract and the corpus callosum as predominant locations of vulnerable white matter lesions in the rat brain, while the internal capsule and the caudoputamen seem to be preserved (Wakita et al. 1994; Takizawa et al. 2003; Farkas et al. 2004). Hattori et al. (1992) reported that after more than 8-weeks' duration of brain hypoperfusion in gerbils, there were two types of white matter lesions: one similar to that found in gray matter, and the other observed only in white matter, including in the internal capsule, corpus callosum, ventral hippocampal commissure, and the fiber bundle of the caudoputamen. In mice, white matter lesions were most intense in the median of the corpus callosum, moderate in the paramedian of the corpus callosum, caudoputamen, and internal capsule, and slight in the anterior commissure and the optic tract (Shibata et al. 2004).

The temporal profile of white matter lesions is also different among species. In rats, white matter lesions in the optic nerve and the optic tract were observed 3–7 days after surgery, and those in the corpus callosum, anterior commissure, internal capsule, and the fiber bundle of the caudoputamen were not detected until 14 days (Wakita et al. 1994). In gerbil brains, the lesions specific to white matter were not detected before 8 weeks, and significantly increased in number and size by 12 weeks after surgery (Hattori et al. 1992), while white matter lesions in a mouse model were not detected until 14 days after surgery (Shibata et al. 2004). The different time courses of white matter lesions among these species may be attributed to the degree of change in cerebral blood flow and the vulnerability of white matter.

Antioxidants and free radical scavengers are effective against white matter injury after chronic hypoperfusion in rodent models. Edaravone suppressed accumulation of 4-HNE and 8-hydroxy-deoxyguanosine (an oxidative stress marker) and loss of oligodendrocytes in the corpus callosum after ligation of the bilateral common carotid artery (Ueno et al. 2009). Quercetin, a flavonoid known to scavenge free radicals, reduced vacuolar changes in the optic tract after ligation of the bilateral common carotid artery (Takizawa et al. 2003). Angiotensin-converting enzyme inhibitor and angiotensin II type 1 receptor blocker suppress superoxide production in experimental acute cerebral ischemia models (Ravati et al. 1999; Iwai et al. 2004; Sugawara et al. 2005; Wakai et al. 2011). These reagents are also reported to have protective effects against white matter injury after chronic hypoperfusion due to scavenging free radicals. Angiotensin-converting enzyme inhibitor treatment significantly suppressed the level of MDA and the oxidized glutathione–total

glutathione ratio and reduced white matter lesions in the optic tract, anterior commissure, corpus callosum, internal capsule, and caudoputamen after chronic hypoperfusion (Kim et al. 2008). Telmisartan, an angiotensin II type 1 receptor blocker with peroxisome proliferator-activated receptor-γ-modulating activity, reduced the degree of oxidative stress in vascular endothelial cells in the corpus callosum and attenuated oligodendrocyte loss and demyelinating change in the corpus callosum (Washida et al. 2010). Direct renin inhibition via aliskiren ameliorated NOX activity in the brain and a hypoperfusion-induced increase in cerebral nitrotyrosine levels, and protected the corpus callosum against chronic hypoperfusion (Dong et al. 2011). In addition to these reagents related to the renin–angiotensin system, cilostazol, a potent inhibitor of type III phosphodiesterase, markedly suppressed accumulation of 4-HNE and loss of oligodendrocytes (Watanabe et al. 2006). This evidence supports the participation of oxidative stress in the development of white matter lesions after chronic hypoperfusion and suggests that oxidative stress could be a target of treatment.

Acknowledgments We thank Liza Reola and Bernard Calagui for technical assistance.

This work was supported by grants PO1 NS014543, RO1 NS025372, and RO1 NS038653, from the National Institutes of Health, and by the James R. Doty Endowment.

References

Arvin KL, Han BH, Du Y, S-Z L, Paul SM, Holtzman DM (2002) Minocycline markedly protects the neonatal brain against hypoxic-ischemic injury. Ann Neurol 52:54–61

Back SA, Rivkees SA (2004) Emerging concepts in periventricular white matter injury. Semin Perinatol 28:405–414

Back SA, Volpe JJ (1997) Cellular and molecular pathogenesis of periventricular white matter injury. Ment Retard Dev Disabil Res Rev 3:96–107

Back SA, Han BH, Luo NL, Chricton CA, Xanthoudakis S, Tam J, Arvin KL, Holtzman DM (2002) Selective vulnerability of late oligodendrocyte progenitors to hypoxia–ischemia. J Neurosci 22:455–463

Baud O, Haynes RF, Wang H, Folkerth RD, Li J, Volpe JJ, Rosenberg PA (2004) Developmental up-regulation of MnSOD in rat oligodendrocytes confers protection against oxidative injury. Eur J Neurosci 20:29–40

Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA (1990) Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci U S A 87:1620–1624

Bedard K, Krause K-H (2007) The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. Physiol Rev 87:245–313

Bernardo A, Greco A, Levi G, Minghetti L (2003) Differential lipid peroxidation, Mn superoxide, and bcl-2 expression contribute to the maturation-dependent vulnerability of oligodendrocytes to oxidative stress. J Neuropathol Exp Neurol 62:509–519

Bogousslavsky J, Regli F (1992) Centrum ovale infarcts: subcortical infarction in the superficial territory of the middle cerebral artery. Neurology 42:1992–1998

Boveris A, Chance B (1973) The mitochondrial generation of hydrogen peroxide. general properties and effect of hyperbaric oxygen. Biochem J 134:707–716

Cai Z, Lin S, Fan L-W, Pang Y, Rhodes PG (2006) Minocycline alleviates hypoxic–ischemic injury to developing oligodendrocytes in the neonatal rat brain. Neuroscience 137:425–435

Chan PH (1996) Role of oxidants in ischemic brain damage. Stroke 27:1124–1129
Chan PH (2001) Reactive oxygen radicals in signaling and damage in the ischemic brain. J Cereb Blood Flow Metab 21:2–14
Cheepsunthorn P, Palmer C, Connor JR (1998) Cellular distribution of ferritin subunits in postnatal rat brain. J Comp Neurol 400:73–86
Cheepsunthorn P, Palmer C, Menzies S, Roberts RL, Connor JR (2001) Hypoxic/ischemic insult alters ferritin expression and myelination in neonatal rat brains. J Comp Neurol 431:382–396
Chen H, Song YS, Chan PH (2009) Inhibition of NADPH oxidase is neuroprotective after ischemia–reperfusion. J Cereb Blood Flow Metab 29:1262–1272
Chen H, Yoshioka H, Kim GS, Jung JE, Okami N, Sakata H, Maier CM, Narasimhan P, Goeders CE, Chan PH (2011) Oxidative stress in ischemic brain damage: mechanisms of cell death and potential molecular targets for neuroprotection. Antioxid Redox Signal 14:1505–1517
Connor JR, Menzies SL (1996) Relationship of iron to oligodendrocytes and myelination. Glia 17:83–93
Craig A, Luo NL, Beardsley DJ, Wingate-Pearse N, Walker DW, Hohimer AR, Back SA (2003) Quantitative analysis of perinatal rodent oligodendrocyte lineage progression and its correlation with human. Exp Neurol 181:231–240
Deguchi K, Mizuguchi M, Takashima S (1996) Immunohistochemical expression of tumor necrosis factor α in neonatal leukomalacia. Pediatr Neurol 14:13–16
Dewar D, Dawson D (1995) Tau protein is altered by focal cerebral ischaemia in the rat: an immunohistochemical and immunoblotting study. Brain Res 684:70–78
Dewar D, Yam P, McCulloch J (1999) Drug development for stroke: importance of protecting cerebral white matter. Eur J Pharmacol 375:41–50
Dewar D, Underhill SM, Goldberg MP (2003) Oligodendrocytes and ischemic brain injury. J Cereb Blood Flow Metab 23:263–274
Dietrich WD, Kraydieh S, Prado R, Stagliano NE (1998) White matter alterations following thromboembolic stroke: a β-amyloid precursor protein immunocytochemical study in rats. Acta Neuropathol 95:524–531
Dong Y-F, Kataoka K, Toyama K, Sueta D, Koibuchi N, Yamamoto E, Yata K, Tomimoto H, Ogawa H, Kim-Mitsuyama S (2011) Attenuation of brain damage and cognitive impairment by direct renin inhibition in mice with chronic cerebral hypoperfusion. Hypertension 58: 635–642
Dröge W (2002) Free radicals in the physiological control of cell function. Physiol Rev 82:47–95
Farkas E, Donka G, de Vos RAI, Mihály A, Bari F, Luiten PGM (2004) Experimental cerebral hypoperfusion induces white matter injury and microglial activation in the rat brain. Acta Neuropathol 108:57–64
Fern R, Möller T (2000) Rapid ischemic cell death in immature oligodendrocytes: a fatal glutamate release feedback loop. J Neurosci 20:34–42
Fields RD (2008) White matter in learning, cognition and psychiatric disorders. Trends Neurosci 31:361–370
Folkerth RD, Haynes RL, Borenstein NS, Belliveau RA, Trachtenberg F, Rosenberg PA, Volpe JJ, Kinney HC (2004a) Developmental lag in superoxide dismutases relative to other antioxidant enzymes in premyelinated human telencephalic white matter. J Neuropathol Exp Neurol 63:990–999
Folkerth RD, Keefe RJ, Haynes RL, Trachtenberg FL, Volpe JJ, Kinney HC (2004b) Interferon-γ expression in periventricular leukomalacia in the human brain. Brain Pathol 14:265–274
Gerstner B, Lee J, DeSilva TM, Jensen FE, Volpe JJ, Rosenberg PA (2009) 17β-Estradiol protects against hypoxic/ischemic white matter damage in the neonatal rat brain. J Neurosci Res 87: 2078–2086
Ginsberg MD, Busto R (1989) Rodent models of cerebral ischemia. Stroke 20:1627–1642
Gresle MM, Jarrott B, Jones NM, Callaway JK (2006) Injury to axons and oligodendrocytes following endothelin-1-induced middle cerebral artery occlusion in conscious rats. Brain Res 1110:13–22

Halliwell B (1989) Oxidants and the central nervous system: some fundamental questions. Is oxidant damage relevant to Parkinson's disease, Alzheimer's disease, traumatic injury or stroke? Acta Neurol Scand Suppl 126:23–33

Harrison PM, Arosio P (1996) The ferritins: molecular properties, iron storage function and cellular regulation. Biochim Biophys Acta 1275:161–203

Hattori H, Takeda M, Kudo T, Nishimura T, Hashimoto S (1992) Cumulative white matter changes in the gerbil brain under chronic cerebral hypoperfusion. Acta Neuropathol 84:437–442

Haynes RL, Folkerth RD, Keefe RJ, Sung I, Swzeda LI, Rosenberg PA, Volpe JJ, Kinney HC (2003) Nitrosative and oxidative injury to premyelinating oligodendrocytes in periventricular leukomalacia. J Neuropathol Exp Neurol 62:441–450

Haynes RL, Baud O, Li J, Kinney HC, Volpe JJ, Folkerth RD (2005) Oxidative and nitrative injury in periventricular leukomalacia: a review. Brain Pathol 15:225–233

Husain J, Juurlink BHJ (1995) Oligodendroglial precursor cell susceptibility to hypoxia is related to poor ability to cope with reactive oxygen species. Brain Res 698:86–94

Ihara M, Tomimoto H, Kinoshita M, Oh J, Noda M, Wakita H, Akiguchi I, Shibasaki H (2001) Chronic cerebral hypoperfusion induces MMP-2 but not MMP-9 expression in the microglia and vascular endothelium of white matter. J Cereb Blood Flow Metab 21:828–834

Imai H, Masayasu H, Dewar D, Graham DI, Macrae IM (2001) Ebselen protects both gray and white matter in a rodent model of focal cerebral ischemia. Stroke 32:2149–2154

Infanger DW, Sharma RV, Davisson RL (2006) NADPH oxidases of the brain: distribution, regulation, and function. Antioxid Redox Signal 8:1583–1596

Irving EA, Yatsushiro K, McCulloch J, Dewar D (1997) Rapid alteration of tau in oligodendrocytes after focal ischemic injury in the rat: involvement of free radicals. J Cereb Blood Flow Metab 17:612–622

Irving EA, Bentley DL, Parsons AA (2001) Assessment of white matter injury following prolonged focal cerebral ischaemia in the rat. Acta Neuropathol 102:627–635

Iwai M, Liu H-W, Chen R, Ide A, Okamoto S, Hata R, Sakanaka M, Shiuchi T, Horiuchi M (2004) Possible inhibition of focal cerebral ischemia by angiotensin II type 2 receptor stimulation. Circulation 110:843–848

Juurlink BHJ, Thorburne SK, Hertz L (1998) Peroxide-scavenging deficit underlies oligodendrocyte susceptibility to oxidative stress. Glia 22:371–378

Kadhim H, Tabarki B, Verellen G, De Prez C, Rona A-M, Sébire G (2001) Inflammatory cytokines in the pathogenesis of periventricular leukomalacia. Neurology 56:1278–1284

Kadhim H, Tabarki B, De Prez C, Rona A-M, Sébire G (2002) Interleukin-2 in the pathogenesis of perinatal white matter damage. Neurology 58:1125–1128

Kim YS, Kim SU (1991) Oligodendroglial cell death induced by oxygen radicals and its protection by catalase. J Neurosci Res 29:100–106

Kim MJ, Shin K-S, Chung Y-B, Jung KW, Cha CI, Shin DH (2005) Immunohistochemical study of $p47^{Phox}$ and $gp91^{Phox}$ distributions in rat brain. Brain Res 1040:178–186

Kim J-S, Yun I, Choi YB, Lee K-S, Kim Y-I (2008) Ramipril protects from free radical induced white matter damage in chronic hypoperfusion in the rat. J Clin Neurosci 15:174–178

Kim GS, Jung JE, Niizuma K, Chan PH (2009) CK2 is a novel negative regulator of NADPH oxidase and a neuroprotectant in mice after cerebral ischemia. J Neurosci 29:14779–14789

Kinouchi H, Epstein CJ, Mizui T, Carlson E, Chen SF, Chan PH (1991) Attenuation of focal cerebral ischemic injury in transgenic mice overexpressing CuZn superoxide dismutase. Proc Natl Acad Sci U S A 88:11158–11162

Kubo K, Nakao S, Jomura S, Sakamoto S, Miyamoto E, Xu Y, Tomimoto H, Inada T, Shingu K (2009) Edaravone, a free radical scavenger, mitigates both gray and white matter damages after global cerebral ischemia in rats. Brain Res 1279:139–146

Kudo T, Takeda M, Tanimukai S, Nishimura T (1993) Neuropathologic changes in the gerbil brain after chronic hypoperfusion. Stroke 24:259–264

Kurumatani T, Kudo T, Ikura Y, Takeda M (1998) White matter changes in the gerbil brain under chronic cerebral hypoperfusion. Stroke 29:1058–1062

Li J, Baud O, Vartanian T, Volpe JJ, Rosenberg PA (2005) Peroxynitrite generated by inducible nitric oxide synthase and NADPH oxidase mediates microglial toxicity to oligodendrocytes. Proc Natl Acad Sci U S A 102:9936–9941

Lin S, Rhodes PG, Lei M, Zhang F, Cai Z (2004) α-Phenyl-*n-tert*-butyl-nitrone attenuates hypoxic–ischemic white matter injury in the neonatal rat brain. Brain Res 1007:132–141

Lo EH, Dalkara T, Moskowitz MA (2003) Mechanisms, challenges and opportunities in stroke. Nat Rev Neurosci 4:399–415

Lyons SA, Kettenmann H (1998) Oligodendrocytes and microglia are selectively vulnerable to combined hypoxia and hypoglycemia injury *in vitro*. J Cereb Blood Flow Metab 18:521–530

McTigue DM, Tripathi RB (2008) The life, death, and replacement of oligodendrocytes in the adult CNS. J Neurochem 107:1–19

Mitrovic B, Ignarro LJ, Vinters HV, Akers M-A, Schmid I, Uittenbogaart C, Merrill JE (1995) Nitric oxide induces necrotic but not apoptotic cell death in oligodendrocytes. Neuroscience 65:531–539

Mitrovic B, Parkinson J, Merrill JE (1996) An *in vitro* model of oligodendrocyte destruction by nitric oxide and its relevance to multiple sclerosis. Methods 10:501–513

Moxon-Emre I, Schlichter LC (2010) Evolution of inflammation and white matter injury in a model of transient focal ischemia. J Neuropathol Exp Neurol 69:1–15

Niizuma K, Yoshioka H, Chen H, Kim GS, Jung JE, Katsu M, Okami N, Chan PH (2010) Mitochondrial and apoptotic neuronal death signaling pathways in cerebral ischemia. Biochim Biophys Acta 1802:92–99

Noble PG, Antel JP, Yong VW (1994) Astrocytes and catalase prevent the toxicity of catecholamines to oligodendrocytes. Brain Res 633:83–90

Pantoni L, Garcia JH (1997) Pathogenesis of leukoaraiosis. Stroke 28:652–659

Pantoni L, Garcia JH, Gutierrez JA (1996) Cerebral white matter is highly vulnerable to ischemia. Stroke 27:1641–1646

Petito CK, Olarte J-P, Roberts B, Nowak TS Jr, Pulsinelli WA (1998) Selective glial vulnerability following transient global ischemia in rat brain. J Neuropathol Exp Neurol 57:231–238

Pluta R, Ułamek M, Januszewski S (2006) Micro-blood-brain barrier openings and cytotoxic fragments of amyloid precursor protein accumulation in white matter after ischemic brain injury in long-lived rats. Acta Neurochir Suppl 96:267–271

Ravati A, Junker V, Kouklei M, Ahlemeyer B, Culmsee C, Krieglstein J (1999) Enalapril and moexipril protect from free radical-induced neuronal damage in vitro and reduce ischemic brain injury in mice and rats. Eur J Pharmacol 373:21–33

Shen Y, Liu X-B, Pleasure DE, Deng W (2012) Axon–glia synapses are highly vulnerable to white matter injury in the developing brain. J Neurosci Res 90:105–121

Shibata M, Ohtani R, Ihara M, Tomimoto H (2004) White matter lesions and glial activation in a novel mouse model of chronic cerebral hypoperfusion. Stroke 35:2598–2603

Shigematsu K, McGeer PL (1992) Accumulation of amyloid precursor protein in neurons after intraventricular injection of colchicine. Am J Pathol 140:787–794

Souza-Rodrigues RD, Costa AMR, Lima RR, Dos Santos CD, Picanço-Diniz CW, Gomes-Leal W (2008) Inflammatory response and white matter damage after microinjections of endothelin-1 into the rat striatum. Brain Res 1200:78–88

Sozmen EG, Kolekar A, Havton LA, Carmichael ST (2009) A white matter stroke model in the mouse: axonal damage, progenitor responses and MRI correlates. J Neurosci Methods 180: 261–272

Stephenson DT, Rash K, Clemens JA (1992) Amyloid precursor protein accumulates in regions of neurodegeneration following focal cerebral ischemia in the rat. Brain Res 593:128–135

Sugawara T, Kinouchi H, Oda M, Shoji H, Omae T, Mizoi K (2005) Candesartan reduces superoxide production after global cerebral ischemia. Neuroreport 16:325–328

Takizawa S, Fukuyama N, Hirabayashi H, Kohara S, Kazahari S, Shinohara Y, Nakazawa H (2003) Quercetin, a natural flavonoid, attenuates vacuolar formation in the optic tract in rat chronic cerebral hypoperfusion model. Brain Res 980:156–160

Tejada-Simon MV, Serrano F, Villasana LE, Kanterewicz BI, Wu G-Y, Quinn MT, Klann E (2005) Synaptic localization of a functional NADPH oxidase in the mouse hippocampus. Mol Cell Neurosci 29:97–106

Thorburne SK, Juurlink BHJ (1996) Low glutathione and high iron govern the susceptibility of oligodendroglial precursors to oxidative stress. J Neurochem 67:1014–1022

Trapp BD, Stys PK (2009) Virtual hypoxia and chronic necrosis of demyelinated axons in multiple sclerosis. Lancet Neurol 8:280–291

Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mörk S, Bö L (1998) Axonal transection in the lesions of multiple sclerosis. N Engl J Med 338:278–285

Ueno M, Tomimoto H, Akiguchi I, Wakita H, Sakamoto H (2002) Blood–brain barrier disruption in white matter lesions in a rat model of chronic cerebral hypoperfusion. J Cereb Blood Flow Metab 22:97–104

Ueno Y, Zhang N, Miyamoto N, Tanaka R, Hattori N, Urabe T (2009) Edaravone attenuates white matter lesions through endothelial protection in a rat chronic hypoperfusion model. Neuroscience 162:317–327

Wakai T, Yoshioka H, Yagi T, Kato T, Kinouchi H (2011) Effects of valsartan on neuroprotection and neurogenesis after ischemia. Neuroreport 22:385–390

Wakita H, Tomimoto H, Akiguchi I, Kimura J (1994) Glial activation and white matter changes in the rat brain induced by chronic cerebral hypoperfusion: an immunohistochemical study. Acta Neuropathol 87:484–492

Walder CE, Green SP, Darbonne WC, Mathias J, Rae J, Dinauer MC, Curnutte JT, Thomas GR (1997) Ischemic stroke injury is reduced in mice lacking a functional NADPH oxidase. Stroke 28:2252–2258

Walker EJ, Rosenberg GA (2010) Divergent role for MMP-2 in myelin breakdown and oligodendrocyte death following transient global ischemia. J Neurosci Res 88:764–773

Washida K, Ihara M, Nishio K, Fujita Y, Maki T, Yamada M, Takahashi J, Wu X, Kihara T, Ito H, Tomimoto H, Takahashi R (2010) Nonhypotensive dose of telmisartan attenuates cognitive impairment partially due to peroxisome proliferator-activated receptor-γ activation in mice with chronic cerebral hypoperfusion. Stroke 41:1798–1806

Watanabe T, Zhang N, Liu M, Tanaka R, Mizuno Y, Urabe T (2006) Cilostazol protects against brain white matter damage and cognitive impairment in a rat model of chronic cerebral hypoperfusion. Stroke 37:1539–1545

Xing C, Arai K, Lo EH, Hommel M (2012) Pathophysiologic cascades in ischemic stroke. Int J Stroke 7:378–385

Xu J, He L, Ahmed SH, Chen S-W, Goldberg MP, Beckman JS, Hsu CY (2000) Oxygen-glucose deprivation induces inducible nitric oxide synthase and nitrotyrosine expression in cerebral endothelial cells. Stroke 31:1744–1751

Xu L, Fagan SC, Waller JL, Edwards D, Borlongan CV, Zheng J, Hill WD, Feuerstein G, Hess DC (2004) Low dose intravenous minocycline is neuroprotective after middle cerebral artery occlusion-reperfusion in rats. BMC Neurol 4:7

Yam PS, Takasago T, Dewar D, Graham DI, McCulloch J (1997) Amyloid precursor protein accumulates in white matter at the margin of a focal ischaemic lesion. Brain Res 760:150–157

Yoon BH, Romero R, Kim CJ, Koo JN, Choe G, Syn HC, Chi JG (1997) High expression of tumor necrosis factor-α and interleukin-6 in periventricular leukomalacia. Am J Obstet Gynecol 177:406–411

Yoshioka H, Niizuma K, Katsu M, Okami N, Sakata H, Kim GS, Narasimhan P, Chan PH (2011a) NADPH oxidase mediates striatal neuronal injury after transient global cerebral ischemia. J Cereb Blood Flow Metab 31:868–880

Yoshioka H, Niizuma K, Katsu M, Sakata H, Okami N, Chan PH (2011b) Consistent injury to medium spiny neurons and white matter in the mouse striatum after prolonged transient global cerebral ischemia. J Neurotrauma 28:649–660

Yrjänheikki J, Keinänen R, Pellikka M, Hökfelt T, Koistinaho J (1998) Tetracyclines inhibit microglial activation and are neuroprotective in global brain ischemia. Proc Natl Acad Sci U S A 95:15769–15774

Chapter 21
Acute Axonal Injury in White Matter Stroke

Jason D. Hinman and S. Thomas Carmichael

21.1 Introduction

Stroke is a leading cause of death and disability. Thrombolysis and revascularization have greatly enhanced our ability to treat acute strokes secondary to large vessel occlusions. Despite these advances, stroke due to small vessel disease remains an important clinical problem accounting for up to 25 % of the 795,000 new strokes occurring annually in the USA (Roger et al. 2012). The vast majority of these small vessel infarcts affect brain white matter, resulting in focal myelin loss and axonal degeneration that produces physical and mental disability. The percentage of new small vessel strokes is likely to grow as the rates of type II diabetes and metabolic syndrome, both associated with small vessel disease (Bokura et al. 2008; Gouw et al. 2008a), are predicted to increase over the next decade. There is also increasing evidence that repeated small vessel infarcts within the white matter increase the risk of large vessel cortical strokes (Kobayashi et al. 1997; Rost et al. 2010) and may place patients at risk for the development of vascular dementia (Dufouil et al. 2009). Revascularization therapies are rarely available for the acute treatment of white matter stroke and current therapies for this type of cerebrovascular disease focus primarily on secondary prevention (Schwartz et al. 2005). These treatments are modest in their effect and do not address the cascade of molecular events that occurs both locally and distantly in the brain after stroke. In this chapter, we set out to define a new therapeutic target for the treatment of white matter stroke; one focused on an enhanced understanding of the neurobiology of brain white matter (the axoglial unit) and the interaction between white matter injury and the response of cortical neurons whose axons are distantly affected by injury.

J.D. Hinman • S.T. Carmichael, M.D. (✉)
Department of Neurology, University of California, Los Angeles, Los Angeles, CA, USA
e-mail: scarmichael@mednet.ucla.edu

S. Baltan et al. (eds.), *White Matter Injury in Stroke and CNS Disease*, Springer Series in Translational Stroke Research 4, DOI 10.1007/978-1-4614-9123-1_21,

21.2 The Progressive Nature of White Matter Stroke

The clinical care of patients presenting with small vessel infarcts can be frustrating. In addition to poorly controlled vascular risk factors, these patients rarely present acutely. When they do, these patients often have an isolated clinical deficit, thereby limiting the availability of revascularization therapies, either due to time or outsized risk–benefit ratios. A subset of these patients show clinical worsening during the first 24–48 h, often while under care in the hospital (Fig. 21.1). Since this type of stroke is essentially untreated in the acute and subacute phases, clinical recognition of stroke expansion during these phases and achieving an enhanced understanding of the molecular pathways that underlie stroke expansion provide an opportunity for new therapeutic intervention in this cohort of patients. Such clinical recognition will undoubtedly rely on magnetic resonance imaging (MRI) as a diagnostic modality. There are several imaging studies that support the idea that white matter strokes expand in patients. White matter hyperintensities on MRI in patients with vascular comorbidities are thought to be consistent with ischemic lesions. In a cohort of patients with white matter hyperintensities on MRI, follow-up imaging demonstrated that over time, new strokes develop within or adjacent to prior lesions (Gouw et al. 2008b). Imaging studies also show that two-thirds of acute subcortical strokes progress to cavitary lesions over several months (Koch et al. 2011) providing the clearest evidence that white matter stroke is a progressive illness. In the tissue surrounding white matter stroke, fractional anisotropy decreases (Maillard et al. 2011), a phenomenon that these authors termed a penumbra of white matter stroke. Fractional anisotropy is a measure of the directional diffusivity of water in MRI diffusion tensor imaging. A decrease after stroke has been linked to glial reaction or tissue destruction from the ischemic damage (Chin et al. 2010). These studies suggest that the abrupt onset of ischemia within the white matter leads to complete degeneration of axons, myelin, and oligodendrocytes within the central region of ischemia. The degree to which partial ischemia or a ischemia-induced molecular

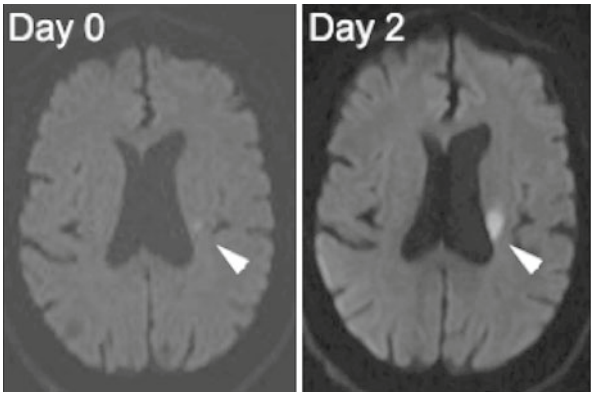


Fig. 21.1 MRI images obtained from patient at UCLA Stroke Center who presented initially with mild right-sided weakness and subtle evidence of white matter stroke in the left hemisphere and progressed in hospital to near complete right-sided paralysis associated with stroke expansion on MRI (*arrowheads*)

pathway producing damage to surviving axons and oligodendrocytes in the white matter surrounding the ischemic core is unknown.

A second cohort of patients with small vessel disease and white matter stroke never present clinically with focal deficits but rather have a delayed clinical presentation with moderate to abundant disease producing gait abnormalities, vascular dementia, or functional decline (Inzitari et al. 2009; Rosenberg 2009; Xiong and Mok 2011; Sahathevan et al. 2012). While undiagnosed and under-treated chronic cerebrovascular atherosclerotic disease may account for a fair proportion of these patients (Liao et al. 1996), there is likely a progressive component to the brain lesions that is unique to white matter stroke. White matter stroke produces both local effects on the axons and oligodendrocytes present at the lesion site and a retrograde effect on the neurons whose axons have been affected. In other diseases of brain white matter, such as multiple sclerosis, this delayed neuronal response to white matter lesions is postulated to account for cortical thinning, cognitive dysfunction and progressive disability (Sailer et al. 2003; Pomeroy et al. 2008; Calabrese et al. 2010). Because leukoaraiosis caused by small vessel disease results in similar axonal loss and injury, it likely produces a similar delayed neuronal response (Seo et al. 2012a, b). Thus, in this second cohort of patients, the local expansion of white matter strokes is perhaps less important than the progressive damage to neurons resulting from distant ischemic axonal injury.

The clinical evidence for both local and distant disease progression after white matter stroke exists. Yet our understanding of the molecular pathways that contribute to small vessel disease, local disruption of the axoglial unit, and the delayed response of the neuron to ischemic axonal injury remains poor and no treatments exist. In part, this lack of understanding is due to the limited availability of experimental models that replicate the human condition. The relatively little data available from animal models supports the concept of white matter stroke as a progressive disease.

21.3 Experimental Evidence of Progressive Lesion Development

Models of white matter ischemia span a wide gamut of preparations, from in vitro slices to animal models including rodents, gerbils, and primates (Sozmen et al. 2012). None of these models truly represent the clinical presentation of most cases of white matter stroke: the aged patient with chronic microvascular risk factors such as hyperglycemia, hypertension, and dyslipidemia with one or more focal ischemic lesions. Rodent models are most amenable to rapid laboratory analysis while providing the deepest access to modern molecular biology tools. In these models, focal white matter ischemia is produced using local vasoconstrictive compounds. While not perfect, such rodent models are reproducible and show many of the features seen in human white matter stroke (Hughes et al. 2003; Sozmen et al. 2009). In the rat, white matter stroke produced by focal injection of endothelin-1 results in myelin

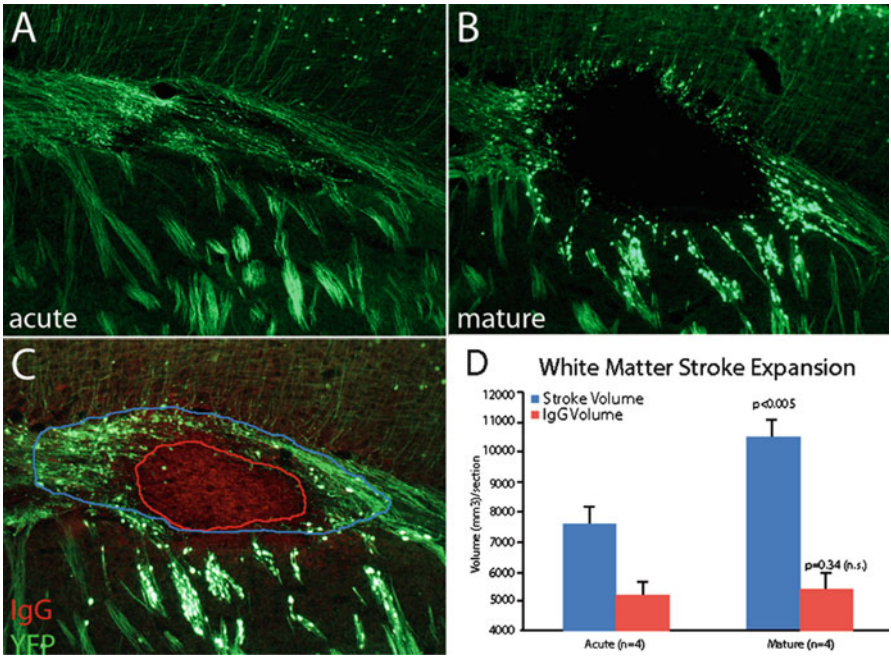


Fig. 21.2 Acute (3 h, (**a**)) and mature (7 days, (**b**)) strokes in YFP-H mouse line shows evolution of white matter stroke with progressive axonal damage. Scattered YFP+ axonal swellings are seen acutely, while by 7 days YFP+ axonal retraction bulbs are prominent. Stereologic volume measurements of acute vs. mature strokes demonstrates an increase in stroke volume by 2.9 mm^3 (38 %) (*graph*). A volumetric ratio of endogenous IgG leakage to YFP+ axonal swellings (**c**), shows that stroke progression is dependent on triggered axonal degeneration rather than focal ischemia (*Asterisk* denotes $p < 0.005$)

loss, astrocytosis, and axonal loss that expands from 24 h to 7 days to include surrounding tissue (Hughes et al. 2003). Over a period of 7–14 days, white matter stroke in the mouse leads to focal loss of myelin, apoptosis of oligodendrocytes, and axonal loss (Sozmen et al. 2009). In this model, as in the rat, the axonal injury expands over time to affect a greater area than affected by focal ischemia. In a pilot experiment using the mouse model of white matter stroke, local blood–brain barrier breakdown (evidenced by IgG leakage into the white matter) was measured 3 h after stroke compared to 7 days after stroke. The area of IgG leakage does not increase significantly, while the area of axonal injury (evidenced by axonal swelling) increases by 30–40 % (Fig. 21.2). These studies provide experimental evidence supporting the clinical observations about white matter stroke; there is a progressive element of axonal loss that follows white matter stroke. With the half-life of the most commonly used vasoconstrictive compounds being extremely short (hours), stroke expansion is far more likely to be a result of progressive molecular pathways triggered by ischemia than ongoing ischemia in the surrounding tissue. Thus, interventions designed to target these molecular pathways provide a new target for the treatment of brain injury related to white matter stroke.

The pathways triggered by white matter ischemia are partially characterized and include activation of *bcl-2* (Zhuo et al. 2007), calcium-dependent proteolysis via the calpain system (Vosler et al. 2008) as well as excitotoxicity via excessive activation of NMDA and purine receptors found on oligodendrocytes (Matute 2006; Matute et al. 2007a, b). The role of many of these acute changes in ionic flux and neurotransmitter signaling in axonal death (degeneration) is discussed elsewhere in this text. Due to the technical limitations of studying ionic changes and neurotransmitter signaling in vivo, few of these studies have examined these changes in a true disease model like the focal white matter stroke models previously mentioned. Instead, they employ ex vivo slice preparations or teased axonal preparations that limit the extrapolation of findings to the clinical condition of white matter stroke. Fewer still examine the white matter surrounding the focal injury or the neuronal response to axonal ischemia. Yet these are the sites of injury that are likely therapeutic targets for white matter stroke. Thus, the focus of understanding white matter ischemia must change from axonal molecular cascades that produce irreversible changes and Wallerian degeneration in in vitro model systems to identifying new molecular pathways that incorporate all the cellular elements of white matter and the cortex: the axoglial unit.

21.4 The Axoglial Unit

Stroke is considered a classical neuroanatomic illness with the deficit corresponding directly to the area of infarcted cortex or involved axonal pathways. This remains largely true for the acute presentation of large vessel cortical strokes producing hemiparesis, hemianopsia, aphasia, etc. However, there is increasing evidence suggesting stroke serves as a stimulus for profound changes in the electrophysiological properties (Carmichael and Chesselet 2002; Clarkson et al. 2010), neurotransmitter signaling (Brailowsky et al. 1986; Kumami et al. 1988; De Ryck et al. 1990), and growth-promoting molecular pathways (Kee et al. 2001; Carmichael et al. 2005; Carmichael 2006; Li and Carmichael 2006; Papadopoulos et al. 2009; Kilic et al. 2010) in neurons as far away as the contralateral cortex. In this context, stroke affects the entire brain with regional inhibitory and growth-promoting signals that initiate a partial but incomplete recovery. Comparatively, white matter stroke is more enigmatic in its clinical presentation with lesions frequently appearing silently but likely producing an equally broad effect on the entire brain. One key reason for this is that while the lesion of white matter stroke is focal, it has both local effects on the involved and neighboring tissue (axons, myelin, oligodendrocytes) but also more distant effects on the associated neurons. This view of brain white matter, not as a glial-predominant anatomic structure distinct from that of the neuron-abundant cortex, but rather as an integrative part of the cortex without which the cortex cannot thrive, is a minority view but one gaining increasing support (Silbert et al. 2012; Zhuang et al. 2012). In this context, healthy brain white matter is better viewed as a multicellular system termed the axoglial unit (Fig. 21.3). The axoglial unit includes

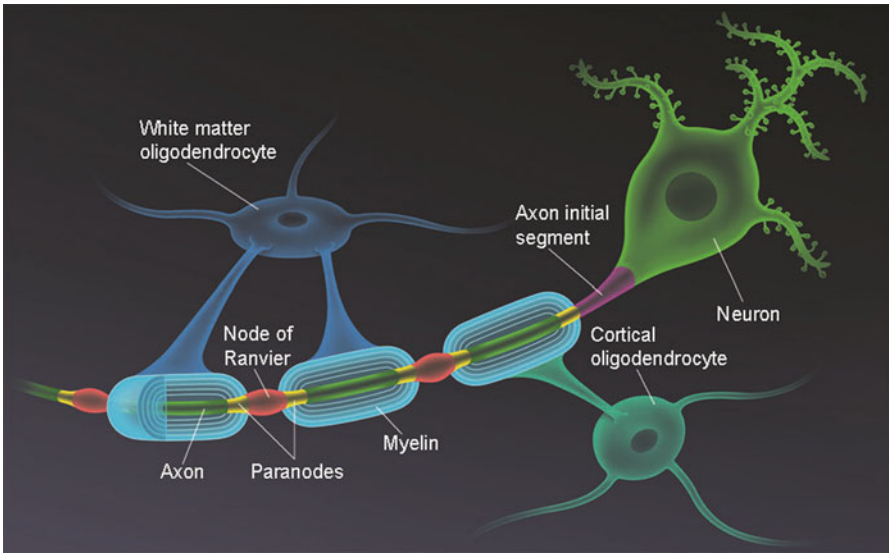


Fig. 21.3 The axoglial unit includes all elements of the CNS that could be affected by white matter injury, including stroke. Axoglial contact occurs between white matter oligodendrocytes (*blue*) and the axon (*green*) at paranodes (*yellow*). Nodes of Ranvier are discretely located along the axon and essential for axonal function. Disruption of axoglial contact and these axonal microdomains occurs after white matter stroke. This disruption is part of the initial response to ischemia and also occurs in the tissue surrounding the stroke. Additional elements of the axoglial unit that can be injured or participate in recovery include the neuronal cell body (*green*) and its vital axonal microdomain, the axon initial segment (*purple*) responsible for triggering action potentials that are conducted down the axon. Cortical oligodendrocytes are also part of the axoglial unit and may contribute to proximal and neuronal survival after injury through energy transfer or other neurotrophic mechanisms. Focal white matter stroke can produce changes in all these elements of the axoglial unit

the neuron, its axon and associated axonal microdomains, as well as myelin and oligodendrocytes, both in the white matter and in the lower layers of cortex. A variety of scientific evidence supports the concept of a tight symbiotic relationship between the neurons, axons, and oligodendrocytes comprising the axoglial unit that is dependent on the following: (1) neuronal activity, (2) initial cell–cell adhesion, (3) maintenance of cell–cell adhesion, and (4) energy transfer.

Initially during development, neurons drive the formation of the axoglial unit with electrical activity of neurons necessary for oligodendrocyte survival (Gary et al. 2012) and sufficient to stimulate oligodendrocytes to initiate myelination (Wake et al. 2011; Gary et al. 2012). Developing, immature axons express a variety of known and unknown molecular cues that drive the initiation of myelination by oligodendrocytes (Taveggia et al. 2010). Most of the known molecules are cell-surface molecules expressed on the axonal membrane that drive myelination (Taveggia et al. 2010). Once myelin is elaborated, the axon responds by organizing into specific microdomains (node, paranode, and juxtaparanode; Fig. 21.3), each

with specific functions in axonal health. The maintenance of unique axonal microdomains is driven by the molecular interaction between myelinating oligodendrocytes and the axonal membrane at paranodes. This distinct microdomain keeps voltage-gated sodium channels (Na_V) segregated appropriately to the node of Ranvier, thereby facilitating normal high-speed neuronal communication in the axon and limiting energy-dependent ionic flux to discrete regions of the axon. The presence of intact paranodes not only dictates clustering of Na_V at the node during development (Rasband et al. 1999), but also drives the specific isotype of Na_V localized to the nodal region (Rasband et al. 2003), indicating a paranodal-derived feedback mechanism between myelination and the neuronal transcriptome. Genetic removal of any of the key axoglial cell–cell adhesion molecules present at the paranode results in long-term axonal degeneration (Bhat et al. 2001; Boyle et al. 2001; Pillai et al. 2009) indicating this cell–cell adhesion complex is essential for axonal maintenance. Finally, axons appear to be energetically dependent on oligodendrocytes, requiring oligodendrocyte-to-axon transfer of lactate for the survival of at least some motor neurons (Lee et al. 2012). Thus, white matter shares a highly symbiotic relationship with subcortically projecting neurons within the cortex and white matter injury should be viewed in the context of how injury affects all elements of the axoglial unit, not just those present in the white matter.

In demyelinating injury, disruption of myelin affects the tripartite molecular signaling complex at the paranode (NF-155, contactin, and caspr). Such disruption can be measured by assessing paranodal length and morphology as has been shown in multiple sclerosis (Howell et al. 2006) and multiple models of both genetic and acquired dysmyelination (Wiley-Livingston and Ellisman 1981; Bray et al. 1983; Bartsch et al. 1995; Teigler et al. 2009). Paranodal disruption leads to a redistribution of voltage-gated sodium channels (Na_V) along the length of the axon (Dupree et al. 1999). Over time, this redistribution of Na_V outside of the normal nodal region causes axonal damage both by altering the ionic flux along the length of axonal segments as well as by changing the energy-dependency of axons now requiring an increased amount of ATP-dependent ionic transport (Wingerchuk et al. 2001). Besides these alterations in ion channel localization, disrupted axoglial contact after injury also interrupts incompletely characterized molecular signals and trophic support mechanisms that mediate healthy axons.

In white matter stroke, the paradigm is slightly different than in demyelinating injury. The ischemic core experiences ionic flux and energy deprivation that facilitate each other and can lead to rapid axonal degeneration (Mattson et al. 2008; Matute 2010), while oligodendrocytes appear to have a unique predisposition to ischemic injury (McTigue and Tripathi 2008; McIver et al. 2010). This typically leads to a focal area of oligodendrocyte cell death. The loss of ATP within the ischemic core triggers irreversible calcium signaling that can trigger downstream enzymatic activation. In the tissue adjacent to the infarct, more mild effects of focal ischemia impact surviving axoglial units surrounding the ischemic core in ways that may lead to progressive damage. Several examples exist to suggest that mature oligodendrocytes respond to ischemia by process retraction and dysregulation at the paranode. In a spinal cord preparation, application of glutamate agonists to fresh

tissue resulted in splitting and retraction of myelin at paranodes (Fu et al. 2009). This appeared to be dependent on glutamate signaling through NMDA and kainate receptors, both of which triggered calcium activation. Calcium ionophores and calpain inhibition prevented paranodal myelin splitting. In a rodent MCA stroke model, a population of oligodendrocytes within the white matter of an MCA distribution stroke demonstrates process retraction and myelin fragmentation with the first 24–48 h (McIver et al. 2010). While this study did not specifically look at axonal microdomain organization in the affected white matter, oligodendrocyte process retraction likely indicates that axoglial contact is lost in the white matter following MCA stroke. In a study of mild cerebral hypoperfusion in the mouse, axoglial contact at paranodes in rapidly disrupted within 3 days of mild hypoperfusion and the effects worsen over time (Reimer et al. 2011). In our studies of focal white matter stroke in the mouse, there is an even more rapid elongation of paranodes that occurs within 3 h of focal ischemia before there is significant axonal damage (unpublished data). This suggests that lost axoglial contact is an early feature of white matter stroke. Extra myelin loops at the paranodal and disrupted molecular organization of axons are also found in normally aged rodents and primates (Hinman et al. 2006; Shepherd et al. 2012), indicating that the molecular interactions at the paranode are crucial for integrity of the axoglial unit.

These studies indicate that white matter ischemia activates a number of pathways that lead to axonal degeneration and ultimately to lacunar infarction. In addition to this complete destruction of axons in the infarct core, disruption of axoglial contact, particularly disruption of the cell–cell adhesion between oligodendrocytes and axons present at paranodes, remains an important feature of white matter stroke. Few studies have examined the effect stroke has on the molecular systems that maintain the axoglial unit. An improved understanding of white matter injury will include studies of the molecular interactions between axons, oligodendrocytes, and the neuron, all working together as part of the axoglial unit.

21.5 Delayed Neuronal Effects of White Matter Stroke

As was just discussed, local disruption of the axoglial unit within the white matter produces dysfunctional axons adjacent to the site of injury. In white matter stroke, this local injury occurs in several stages. First, during the acute stage within the ischemic core, there is altered ionic flux (particularly sodium and calcium) that initiates a variety of rapidly deleterious signaling and proteolytic mechanisms. In addition, within the ischemic core, there is also absolute or significant energy deprivation that impairs mitochondrial function and leads to dysfunctional and degenerative axons. In a second subacute stage, there is lost axoglial contact and progressive axonal degeneration surrounding the initial ischemic core. In a delayed third stage, these local injury pathways exert effects on the more distant element of the axoglial unit: the neuron. This third stage presents a unique therapeutic opportunity for neuroprotection and neural repair and remains vastly understudied.

Neurons respond to direct ischemia with rapid upregulation of immediate early genes including *c-fos*, *c-jun*, and *krox-20* (Gusev and Skvortsova 2003) as well as neurotrophic factors, erythropoietin, *bcl-2*, and heat shock proteins which appear to drive cell survival and stimulate axonal sprouting to replace the injured tract (Blanco et al. 2006). Focal cerebral ischemia induces growth cone-promoting genes and axonal sprouting in ipsilateral and contralateral hemispheres in patterns that change with age (Li and Carmichael 2006; Carmichael 2008). In contrast to direct cerebral ischemia, in white matter stroke the parent cell body is unharmed by local changes in its environment. Rather, damage to the axon results in Wallerian degeneration in the portion of the axon distal to the site of injury. The molecular mechanisms that underlie Wallerian degeneration are increasingly understood and can be partially delayed by increased availability of nicotinamide (NAD+) (Mack et al. 2001; Wang et al. 2005; Wang and He 2009). Considerably less is known about axonal dieback, which occurs in the proximal portion of the axon that remains connected to the soma (Richardson et al. 2009). This process is not dependent on NAD+ (Cheng and Burke 2010). Most of the data regarding neuronal responses to axotomy come from peripheral nerve injury. Nerve injury in dorsal root ganglion cells results in an increase in the mRNAs in the cell body (Mesnard et al. 2010). This increase is dependent on retrograde axonal transport (Murphy et al. 1999) indicating that there must be retrograde signals conducted back to the soma by axonal injury. In the peripheral nervous system, studies suggest that these signals include a mix of negative signals, leading to cell death and a local inflammatory response (Ha et al. 2008; Hydman et al. 2005) as well as positive signals that trigger axonal regeneration by increases in GAP-43 (Richardson et al. 2009) and nuclear translocation of STAT3 (Lee et al. 2004; Ben-Yaakov et al. 2012) and upregulation of brain-derived neurotrophic factor (BDNF) (Tonra et al. 1998). GAP-43 and BDNF are both upregulated in peri-infarct cortex (Comelli et al. 1992; Carmichael et al. 2005) and clearly contribute to axonal sprouting and cortical stroke recovery. The extent to which these molecular pathways are triggered by white matter stroke is unknown.

The peripheral cell body response to axonal injury also includes activation of the gp130 cytokine family, including IL-6, leukemia inhibitory factor, and ciliary neurotrophic factor (Richardson et al. 2009; Zigmond 2011). These molecules appear to participate in a local axonal injury signaling cascade that includes the retrograde transport of transcription factors important in the cellular response to axonal injury. It remains unclear whether these cytokines are produced or released from injured axons or are part of a multicellular response to peripheral nerve injury. In several studies, LIF and CNF were shown to be released from Schwann cells rather than synthesized from neurons (Kurek et al. 1996; Xu et al. 2009). Regardless, in the PNS, there is clearly a retrograde protein complex that translocates from the axon to the neuronal nucleus to trigger axonal regeneration. Importin beta-1 is a part of cytoplasmic protein complex that binds molecules with nuclear localization signal (NLS) and brings them to the nuclear envelope. Importin beta-1 is upregulated in axons after local peripheral nerve injury and coordinates the retrograde delivery of a protein complex to the neuronal nucleus, serving to signal distal axonal injury. Deletion of the 3′ UTR of the importin beta-1 subunit prevents axonal localization of this

molecule and impairs recovery (Ben-Tov Perry et al. 2012). In related work, peripheral nerve injury results in increased axonal translation of STAT3, which is then retrogradely transported to the nucleus and impacts neuronal survival after injury (Ben-Yaakov et al. 2012). c-Jun is also upregulated after axonal injury, initially by rapid changes in phosphorylation mediated by axonal Jun kinase activation (Yoshimura et al. 2011; Lindwall et al. 2005) and later by increased transcription and translation of c-Jun (Kenney et al. 1998). This activation of c-Jun appears to promote axonal regeneration (Lindwall et al. 2004). Recently, the dual leucine zipper kinase (DLK) has been shown to be crucial for the phosphorylation of STAT3 and c-Jun by providing an essential role in the retrograde transport of these transcription factors back to the nucleus (Shin et al. 2012). The contribution of these molecules to a CNS response to axonal injury has not been well-studied. This is partly secondary to the complexity of inducing axonal injury alone in the CNS. Focal white matter stroke is one condition that may be amenable to the identification of retrograde axonal signals resulting from CNS axonal injury. While the initial injury is focal, its effect on all elements of the axoglial unit provide an excellent disease model to better understand the biologic interplay between cortex and white matter.

21.6 Targets for Prevention and Repair

The design of treatments that can protect brain white matter and minimize patient disability hinges on an improved knowledge of the molecular events that put individuals at risk for and follow white matter stroke, both in the immediate phase and the delayed recovery phase. With both local damage to axoglial units within the white matter and distant damage to the associated neurons, white matter stroke provides a number of molecular targets that are ripe for the development of new therapeutics. Though not the focus of this discussion, an improved understanding of the microvascular pathology that places patients at risk for white matter stroke could open up a new category of preventive treatments for microvascular arteriosclerosis. This field remains largely unstudied and lacking in appropriate animal and in vitro models.

Acute and subacute protection of axoglial units surrounding the infarct could ameliorate the initial worsening that many patients experience. Molecular targets that might be amenable to early intervention after stroke include preventing calcium-dependent proteolytic activation with a goal of protecting the axonal cytoskeleton and maintaining axoglial contact at paranodes. In addition, blockade of a yet-unknown axonal injury molecule or molecular pathway might prevent or lessen the local inflammatory response, thus protecting axoglial units in and around the stroke site. Another local acute protective strategy would be molecular augmentation of axoglial signaling so that the white matter surrounding the initial stroke is immune to the progressive destruction of axoglial units that follows white matter stroke. All of these strategies require identification of new molecules that are undoubtedly part

of the disease process but difficult to discover because of the multicellular nature of the axoglial unit limiting in vitro modeling.

Local repair of white matter after injury has proven difficult. Molecules that promote remyelination after a demyelinating injury have only recently been developed into therapeutics and only one of them is currently in clinical trial development (BIIB033 which blocks LINGO-1). Repair of brain white matter after stroke will likely necessitate a multifaceted molecular approach requiring activation and maturation of oligodendrocyte precursors, blockade of glial scarring, and triggering of axonal regrowth. Though ambitious, cocktail molecular therapy to promote remyelination after white matter stroke would offer treatment to many patients who have suffered prior white matter stroke.

Therapies to prevent delayed neuronal injury after stroke should be viewed in a different light than prior attempts at neuroprotective agents for stroke. The barriers to neuroprotection in acute stroke have been well documented (Cook and Tymianski 2011). Many of these would not be relevant to neuroprotection for white matter stroke. Because the neurons affected are often not close to the site of injury, poor blood flow would not prevent delivery of drug to the target. Secondly, because the injury is delayed, requiring retrograde axon to neuron signaling, there is likely an extended therapeutic window that would allow many patients to qualify for treatment. Thirdly, these targets could range from anti-apoptotic therapies to simply prevent neuronal death to neural repair therapies that shift an injured neuron back into a growth pattern, thus promoting axonal sprouting and ultimately white matter repair and recovery.

White matter stroke is an important clinical problem, affecting a multitude of patients and representing 25 % of the annual stroke burden in the USA alone (Roger et al. 2012). This unique subtype of stroke has many unanswered fundamental questions including the pathophysiology of the microvasculature that places patients at risk for the disease, the acute molecular events that lead to stroke expansion, and the molecular pathways that prevent white matter repair. An enhanced understanding of both the local response of the axoglial unit and the delayed effects of white matter stroke on the neuronal cell body can lead to the design of new therapeutic strategies for this common condition.

References

Bartsch U, Montag D et al (1995) Multiply myelinated axons in the optic nerve of mice deficient for the myelin-associated glycoprotein. Glia 14(2):115–122

Ben-Tov Perry R, Doron-Mandel E et al (2012) Subcellular knockout of importin beta1 perturbs axonal retrograde signaling. Neuron 75(2):294–305

Ben-Yaakov K, Dagan SY et al (2012) Axonal transcription factors signal retrogradely in lesioned peripheral nerve. EMBO J **31**(6):1350–1363

Bhat MA, Rios JC et al (2001) Axon-glia interactions and the domain organization of myelinated axons requires neurexin IV/Caspr/Paranodin. Neuron 30(2):369–383

Blanco M, Lizasoain I et al (2006) Ischemic preconditioning: a novel target for neuroprotective therapy. Cerebrovasc Dis 21(suppl 2):38–47
Bokura H, Yamaguchi S et al (2008) Metabolic syndrome is associated with silent ischemic brain lesions. Stroke 39(5):1607–1609
Boyle ME, Berglund EO et al (2001) Contactin orchestrates assembly of the septate-like junctions at the paranode in myelinated peripheral nerve. Neuron 30(2):385–397
Brailowsky S, Knight RT et al (1986) Gamma-aminobutyric acid-induced potentiation of cortical hemiplegia. Brain Res 362(2):322–330
Bray GM, Duncan ID et al (1983) 'Shaking pups': a disorder of central myelination in the spaniel dog. IV. Freeze-fracture electron microscopic studies of axons, oligodendrocytes and astrocytes in the spinal cord white matter. Neuropathol Appl Neurobiol 9(5):369–378
Calabrese M, Rinaldi F et al (2010) Widespread cortical thinning characterizes patients with MS with mild cognitive impairment. Neurology 74(4):321–328
Carmichael ST (2006) Cellular and molecular mechanisms of neural repair after stroke: making waves. Ann Neurol 59(5):735–742
Carmichael ST (2008) Themes and strategies for studying the biology of stroke recovery in the poststroke epoch. Stroke 39(4):1380–1388
Carmichael ST, Chesselet MF (2002) Synchronous neuronal activity is a signal for axonal sprouting after cortical lesions in the adult. J Neurosci 22(14):6062–6070
Carmichael ST, Archibeque I et al (2005) Growth-associated gene expression after stroke: evidence for a growth-promoting region in peri-infarct cortex. Exp Neurol 193(2):291–311
Cheng HC, Burke RE (2010) The Wld(S) mutation delays anterograde, but not retrograde, axonal degeneration of the dopaminergic nigro-striatal pathway in vivo. J Neurochem 113(3):683–691
Chin Y, Sato Y et al (2010) Transient decrease in cerebral motor pathway fractional anisotropy after focal ischemic stroke in monkey. Neurosci Res 66:406–411
Clarkson AN, Huang BS et al (2010) Reducing excessive GABA-mediated tonic inhibition promotes functional recovery after stroke. Nature 468(7321):305–309
Comelli MC, Seren MS et al (1992) Photochemical stroke and brain-derived neurotrophic factor (BDNF) mRNA expression. Neuroreport 3(6):473–476
Cook DJ, Tymianski M (2011) Translating promising preclinical neuroprotective therapies to human stroke trials. Expert Rev Cardiovasc Ther 9(4):433–449
De Ryck M, Duytschaever H et al (1990) Ionic channels, cholinergic mechanisms, and recovery of sensorimotor function after neocortical infarcts in rats. Stroke 21(11 suppl):III158–III163
Dufouil C, Godin O et al (2009) Severe cerebral white matter hyperintensities predict severe cognitive decline in patients with cerebrovascular disease history. Stroke 40(6):2219–2221
Dupree JL, Girault JA et al (1999) Axo-glial interactions regulate the localization of axonal paranodal proteins. J Cell Biol 147(6):1145–1152
Fu Y, Sun W et al (2009) Glutamate excitotoxicity inflicts paranodal myelin splitting and retraction. PLoS One 4(8):e6705
Gary DS, Malone M et al (2012) Electrical stimulation promotes the survival of oligodendrocytes in mixed cortical cultures. J Neurosci Res 90(1):72–83
Gouw AA, van der Flier WM et al (2008a) Progression of white matter hyperintensities and incidence of new lacunes over a 3-year period: the Leukoaraiosis and Disability study. Stroke 39(5):1414–1420
Gouw AA, van der Flier WM et al (2008b) On the etiology of incident brain lacunes: longitudinal observations from the LADIS study. Stroke 39(11):3083–3085
Gusev EI, Skvortsova VI (2003) Brain ischemia. Springer, Berlin
Ha GK, Parikh S et al (2008) Influence of injury severity on the rate and magnitude of the T lymphocyte and neuronal response to facial nerve axotomy. **199**(1-2):18–23
Hinman JD, Peters A et al (2006) Age-related molecular reorganization at the node of Ranvier. J Comp Neurol 495(4):351–362
Howell OW, Palser A et al (2006) Disruption of neurofascin localization reveals early changes preceding demyelination and remyelination in multiple sclerosis. Brain 129(pt 12):3173–3185

Hughes PM, Anthony DC et al (2003) Focal lesions in the rat central nervous system induced by endothelin-1. J Neuropathol Exp Neurol 62(12):1276–1286
Hydman J, Svensson M et al (2005) Neuronal survival and glial reactions after recurrent laryngeal nerve resection in the rat. Laryngoscope **115**(4):619–624
Inzitari D, Pracucci G et al (2009) Changes in white matter as determinant of global functional decline in older independent outpatients: three year follow-up of LADIS (leukoaraiosis and disability) study cohort. BMJ 339:b2477
Kee NJ, Preston E et al (2001) Enhanced neurogenesis after transient global ischemia in the dentate gyrus of the rat. Exp Brain Res 136(3):313–320
Kenney AM, and Kocsis JD (1998) Peripheral axotomy induces long-term c-jun amino-terminal kinase-1 activation and activator protein-1 binding activity by c-Jun and junD in adult rat dorsal root ganglia In vivo. **18**(4):1318–1328
Kilic E, ElAli A et al (2010) Role of Nogo-A in neuronal survival in the reperfused ischemic brain. J Cereb Blood Flow Metab 30(5):969–984
Kobayashi S, Okada K et al (1997) Subcortical silent brain infarction as a risk factor for clinical stroke. Stroke 28(10):1932–1939
Koch S, McClendon MS et al (2011) Imaging evolution of acute lacunar infarction: leukoariosis or lacune? Neurology 77(11):1091–1095
Kumami K, Mrsulja BB et al (1988) Effect of ischemia on noradrenergic and energy-related metabolites in the cerebral cortex of young and adult gerbils. Metab Brain Dis 3(4):273–277
Kurek JB, Austin L et al (1996) Up-regulation of leukaemia inhibitory factor and interleukin-6 in transected sciatic nerve and muscle following denervation. **6**(2):105–114
Lee Y, Morrison BM, Li Y, Lengacher S, Farah MH, Hoffman PN, Liu Y, Tsingalia A, Jin L, Zhang PW, Pellerin L, Magistretti PJ, Rothstein JD (2012) Oligodendroglia metabolically support axons and contribute to neurodegeneration. Nature 487(7408):443–448
Lee N, Neitzel KL, et al (2004) STAT3 phosphorylation in injured axons before sensory and motor neuron nuclei: potential role for STAT3 as a retrograde signaling transcription factor. **474**(4):535–545
Li S, Carmichael ST (2006) Growth-associated gene and protein expression in the region of axonal sprouting in the aged brain after stroke. Neurobiol Dis 23(2):362–373
Liao D, Cooper L et al (1996) Presence and severity of cerebral white matter lesions and hypertension, its treatment, and its control. The ARIC Study. Atherosclerosis Risk in Communities Study. Stroke 27(12):2262–2270
Lindwall C, Dahlin L, et al (2004) Inhibition of c-jun phosphorylation reduces axonal outgrowth of adult rat nodose ganglia and dorsal root ganglia sensory neurons. **27**(3):267–279
Lindwall C and Kanje M (2005) Retrograde axonal transport of JNK signaling molecules influence injury induced nuclear changes in p-c-jun and ATF3 in adult rat sensory neurons. **29**(2): 269–282
Mack TG, Reiner M et al (2001) Wallerian degeneration of injured axons and synapses is delayed by a Ube4b/Nmnat chimeric gene. Nat Neurosci 4(12):1199–1206
Maillard P, Fletcher E et al (2011) White matter hyperintensity penumbra. Stroke 42(7):1917–1922
Mattson MP, Gleichmann M et al (2008) Mitochondria in neuroplasticity and neurological disorders. Neuron 60(5):748–766
Matute C (2006) Oligodendrocyte NMDA receptors: a novel therapeutic target. Trends Mol Med 12(7):289–292
Matute C (2010) Calcium dyshomeostasis in white matter pathology. Cell Calcium 47(2):150–157
Matute C, Alberdi E et al (2007a) Excitotoxic damage to white matter. J Anat 210(6):693–702
Matute C, Torre I et al (2007b) P2X(7) receptor blockade prevents ATP excitotoxicity in oligodendrocytes and ameliorates experimental autoimmune encephalomyelitis. J Neurosci 27(35):9525–9533
McIver SR, Muccigrosso M et al (2010) Oligodendrocyte degeneration and recovery after focal cerebral ischemia. Neuroscience 169(3):1364–1375
McTigue DM, Tripathi RB (2008) The life, death, and replacement of oligodendrocytes in the adult CNS. J Neurochem 107(1):1–19

Mesnard NA, Alexander TD et al (2010) Use of laser microdissection in the investigation of facial motoneuron and neuropil molecular phenotypes after peripheral axotomy. **225**(1):94–103
Murphy PG, Borthwick LS et al (1999) Nature of the retrograde signal from injured nerves that induces interleukin-6 mRNA in neurons. **19**(10):3791–3800
Papadopoulos CM, Tsai SY et al (2009) Motor recovery and axonal plasticity with short-term amphetamine after stroke. Stroke 40(1):294–302
Pillai AM, Thaxton C et al (2009) Spatiotemporal ablation of myelinating glia-specific neurofascin (Nfasc NF155) in mice reveals gradual loss of paranodal axoglial junctions and concomitant disorganization of axonal domains. J Neurosci Res 87(8):1773–1793
Pomeroy IM, Jordan EK et al (2008) Diffuse cortical atrophy in a marmoset model of multiple sclerosis. Neurosci Lett 437(2):121–124
Rasband MN, Peles E et al (1999) Dependence of nodal sodium channel clustering on paranodal axoglial contact in the developing CNS. J Neurosci 19(17):7516–7528
Rasband MN, Kagawa T et al (2003) Dysregulation of axonal sodium channel isoforms after adult-onset chronic demyelination. J Neurosci Res 73(4):465–470
Reimer MM, McQueen J et al (2011) Rapid disruption of axon-glial integrity in response to mild cerebral hypoperfusion. J Neurosci 31(49):18185–18194
Richardson PM, Miao T et al (2009) Responses of the nerve cell body to a xotomy. Neurosurgery **65**(4 Suppl): A74–79
Roger VL, Go AS et al (2012) Heart disease and stroke statistics—2012 update: a report from the American Heart Association. Circulation 125(1):e2–e220
Rosenberg GA (2009) Inflammation and white matter damage in vascular cognitive impairment. Stroke 40(3 suppl):S20–S23
Rost NS, Fitzpatrick K et al (2010) White matter hyperintensity burden and susceptibility to cerebral ischemia. Stroke 41(12):2807–2811
Sahathevan R, Brodtmann A et al (2012) Dementia, stroke, and vascular risk factors: a review. Int J Stroke 7(1):61–73
Sailer M, Fischl B et al (2003) Focal thinning of the cerebral cortex in multiple sclerosis. Brain 126(pt 8):1734–1744
Schwartz GL, Fornage M et al (2005) Treatment of leukoaraiosis. Curr Treat Options Cardiovasc Med 7(3):173–177
Seo SW, Lee JM et al (2012a) Cortical thinning related to periventricular and deep white matter hyperintensities. Neurobiol Aging 33(7):1156–1167
Seo SW, Lee JM et al (2012b) Cardiovascular risk factors cause cortical thinning in cognitively impaired patients: relationships among cardiovascular risk factors, white matter hyperintensities, and cortical atrophy. Alzheimer Dis Assoc Disord 26(2):106–112
Shepherd MN, Pomicter AD et al (2012) Paranodal reorganization results in the depletion of transverse bands in the aged central nervous system. Neurobiol Aging 33(1):203.e13–24
Shin JE, Cho Y et al (2012) Dual leucine zipper kinase is required for retrograde injury signaling and axonal regeneration. Neuron 74(6):1015–1022
Silbert LC, Dodge HH et al (2012) Trajectory of white matter hyperintensity burden preceding mild cognitive impairment. Neurology 79(8):741–747
Sozmen EG, Kolekar A et al (2009) A white matter stroke model in the mouse: axonal damage, progenitor responses and MRI correlates. J Neurosci Methods 180(2):261–272
Sozmen EG, Hinman JD et al (2012) Models that matter: white matter stroke models. Neurotherapeutics 9(2):349–358
Taveggia C, Feltri ML et al (2010) Signals to promote myelin formation and repair. Nat Rev Neurol 6(5):276–287
Teigler A, Komljenovic D et al (2009) Defects in myelination, paranode organization and Purkinje cell innervation in the ether lipid-deficient mouse cerebellum. Hum Mol Genet 18(11):1897–1908
Tonra JR, Curtis R et al (1998) Axotomy upregulates the anterograde transport and expression of brain-derived neurotrophic factor by sensory neurons. **18**(11):4374–4383
Vosler PS, Brennan CS et al (2008) Calpain-mediated signaling mechanisms in neuronal injury and neurodegeneration. Mol Neurobiol 38(1):78–100

Wake H, Lee PR et al (2011) Control of local protein synthesis and initial events in myelination by action potentials. Science 333(6049):1647–1651
Wang J, He Z (2009) NAD and axon degeneration: from the Wlds gene to neurochemistry. Cell Adh Migr 3(1):77–87
Wang J, Zhai Q et al (2005) A local mechanism mediates NAD-dependent protection of axon degeneration. J Cell Biol 170(3):349–355
Wiley-Livingston CA, Ellisman MH (1981) Myelination-dependent axonal membrane specializations demonstrated in insufficiently myelinated nerves of the dystrophic mouse. Brain Res 224(1):55–67
Wingerchuk DM, Lucchinetti CF et al (2001) Multiple sclerosis: current pathophysiological concepts. Lab Invest 81(3):263–281
Xiong YY, Mok V (2011) Age-related white matter changes. J Aging Res 2011:617927
Xu JJ, Chen EY et al (2009) Recombinant ciliary neurotrophic factor promotes nerve regeneration and induces gene expression in silicon tube-bridged transected sciatic nerves in adult rats. **16**(6):812–817
Yoshimura K, Ueno M et al (2011) c-jun N-terminal kinase induces axonal egeneration and limits motor recovery after spinal cord injury in mice." **71**(3):266–277
Zhuang L, Sachdev PS et al (2012) Microstructural white matter changes in cognitively normal individuals at risk of amnestic MCI. Neurology 79(8):748–754
Zhuo PH, Xiong Y et al (2007) Expression of bcl-2 protein and apoptosis of neurocytes in neonatal rats with brain white matter damage. Zhongguo Dang Dai Er Ke Za Zhi 9(2):164–168
Zigmond RE (2011) gp130 cytokines are positive signals triggering changes in gene expression and axon outgrowth in peripheral neurons following injury. Front Mol Neurosci 4:62

Part IV
Other White Matter Injuries

Chapter 22
The Interplay Between White Matter, Mitochondria, and Neuroprotection

R. Anne Stetler, Rehana K. Leak, Zheng Jing, Xiaoming Hu, Yanqin Gao, Guodong Cao, and Jun Chen

22.1 Introduction

White matter (WM) is predominantly composed of astroglia, oligodendrocytes, and microglia in the CNS and Schwann cells in the PNS, but also includes critical elements of the blood–brain barrier, such as pericytes. The impact of these non-neuronal cells on brain function remains an open field of investigation, partly because of the greater historical emphasis on studying neurons. Although WM was long held to be a passive tissue, a recent surge of interest in white matter injury (WMI) has revealed a significant influence of WM on the major functions of the

R.A. Stetler, Ph.D. • X. Hu, Ph.D. • G. Cao, Ph.D. • J. Chen, M.D. (✉)
Department of Neurology and Center of Cerebrovascular Disease Research, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

State Key Laboratory of Medical Neurobiology and Institute of Brain Sciences, Fudan University Shanghai Medical College, Shanghai, China

Geriatric Research, Educational and Clinical Center, Veterans Affairs Pittsburgh Health Care System, Pittsburgh, PA, USA
e-mail: chenj2@upmc.edu

R.K. Leak, Ph.D.
Division of Pharmaceutical Sciences, Mylan School of Pharmacy, Duquesne University, Pittsburgh, PA, USA

Z. Jing, Ph.D.
Department of Neurology and Center of Cerebrovascular Disease Research, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

Geriatric Research, Educational and Clinical Center, Veterans Affairs Pittsburgh Health Care System, Pittsburgh, PA, USA

Y. Gao, M.D.
State Key Laboratory of Medical Neurobiology and Institute of Brain Sciences, Fudan University Shanghai Medical College, Shanghai, China

S. Baltan et al. (eds.), *White Matter Injury in Stroke and CNS Disease*, Springer Series in Translational Stroke Research 4, DOI 10.1007/978-1-4614-9123-1_22,

nervous system, including the generation of adaptive behavior. In humans, WM accounts for a remarkable ~90 % of the total cells in the brain (Baumann and Pham-Dinh 2001). In addition to its striking abundance, WM performs highly diverse and specialized functions, many of which change throughout development or with environmental circumstances. This plasticity does not support the traditional view of WM as a passive tissue. Instead, recent research has revealed that the interplay between WM, neuronal function, and mitochondria is a highly dynamic activity that is both regionally and temporally regulated. This powerful set of processes deeply impinges on basic neural functions as well as response to and recovery from injury or disease states. Furthermore, the interactions between WM, neurons, and mitochondria have novel implications for the mechanism of action of pharmacotherapies and draw into question whether neuroprotection afforded by antioxidants or mitochondrial-directed therapies directly protect neurons, or whether the protection of neurons is largely secondary to the protection of astroglia and myelin. Fortunately, answering this question forces researchers to take a more global perspective of brain function that encompasses both neural and nonneural cells. This is important because an understanding of the basic hierarchical organization of WMI and grey matter injury may revolutionize how we conceptualize therapeutic strategies in the future.

22.2 Neuronal Mitochondria

The classic descriptions of mitochondria revolve around their role as the energy powerhouses of the cell. As the brain uses more energy than any other organ, the importance of mitochondria in the central nervous system cannot be overstated. Although other metabolic pathways may bypass the need for mitochondrial energy output, neurons rely heavily on their mitochondria for high amounts of ATP during two energetically expensive processes: neurotransmission and the maintenance of ionic gradients across neuronal membranes. As such, neurons contribute considerably to the high metabolic rate of the brain (Kann and Kovacs 2007) and are exquisitely sensitive to mitochondrial dysfunction.

Mitochondria produce ATP via oxidative phosphorylation in the electron transport chain (ETC) in a process that is fed by glycolytically derived pyruvate from the cytosol. Although neurons can produce their own pyruvate in the cytosol, WM cells, including astrocytes and oligodendrocytes, may also generate glycolytically derived pyruvate and/or lactate and shuttle them to neurons via monocarboxylate transporters (MCT) (Tekkok et al. 2005; Brown et al. 2001; Funfschilling et al. 2012). Partly because of this relationship, neuronal mitochondria can be damaged either directly with neuronal insults or indirectly through the perturbation of WM.

Unlike most other cells, neurons are highly polarized and therefore have disparate energetic needs in distinct cellular regions. Not surprisingly, spatial differences in mitochondrial densities and ETC activity have been reported in growth cones and dendritic protrusions (Morris and Hollenbeck 1993; Li et al. 2004), in regions of

intense activity (Kann and Kovacs 2007), and in specific regions of myelinated axons (Campbell and Mahad 2012). In fact, the dynamic nature of mitochondria was first discovered in neurons. Neuronal mitochondria are able to rapidly translocate to distal cytosolic regions that experience higher metabolic loads (Li et al. 2004; Kann and Kovacs 2007). Mitochondria also undergo fission/fusion events distal to the soma. Because mitochondria are often not in close proximity to the synthetic machinery of the rough endoplasmic reticulum, they must rely on local protein synthesis as well as the transcription of their own mitochondrial DNA (mtDNA) (Amiri and Hollenbeck 2008). These active processes are highly sensitive to perturbations in WM such as demyelination. The relationship between WMI and neuronal mitochondria will be discussed in these contexts below.

22.3 White Matter (WM) Cells and Their Mitochondria

The roles and energetic requirements of the mitochondrial organelle within astroglia, oligodendrocytes, and microglia are still under intense scrutiny. Although mitochondria were long thought to function in roughly the same capacity in most cell types, recent studies reveal cell-type dependent variations in mitochondrial morphology, number, and function, including relatively underexplored mitochondrial signaling capacities. Thus, WMI may result in different types of mitochondrial dysfunction in distinct subtypes of WM as well as in neuronal subtypes. Naturally, this will render it more challenging to treat mitochondrial dysfunction in disease or injury states with specific pharmacological tools.

22.4 Mitochondria in Oligodendrocytes

As one of the major subtypes of WM cells, oligodendrocytes are essential for the myelination of neurons and have an especially tight association with the energy-rich axonal regions. During brain development, oligodendrocytes are the last neural cell to differentiate; in humans, the full process of oligodendrocyte maturation and neuronal myelination is not complete until the third decade of life (Bradl and Lassmann 2010). Oligodendrocytic precursor cells (OPCs) originate from several distinct populations and migrate long distances toward regions of neuronal activity. Although the details of the process are still unclear, a combination of neuronal activity and other axonal signals may stimulate the maturation of oligodendrocytes. Myelination occurs only during a finite and rapid period in the maturation process. After the axon is ensheathed in myelin and the oligodendrocyte is fully differentiated, it appears to be unable to elicit remyelination. This inability to remyelinate after oligodendrocyte differentiation is complete may have ramifications for the plastic response to WMI. However, little is known about internal oligodendrocytic energetic capacity over the maturation process until recently.

New observations indicate that the different stages in oligodendrocytic maturation are unique in their energetic requirements, and that these differences may permit the optimization of brain energetic capacity. During migration and myelination, the developing oligodendrocyte is theorized to demand an enormous energetic load to synthesize enough myelin to wrap the axon several times over (Harris and Attwell 2012). Nevertheless, this process is energetically favorable in the long term by virtue of the reduced energetic requirements of myelinated axons (Harris and Attwell 2012). In further support of differential energetic capacities during maturation, another study suggests that mature oligodendrocytes do not rely on the ETC for stability (Funfschilling et al. 2012). The latter authors made a conditional mutation in an essential element of cytochrome c oxidase (COX, or Complex IV) and found that myelinated axons in the CNS were resilient and did not undergo demyelination or axonal degeneration, contrary to what was observed in Schwann cells of the PNS or in developing oligodendrocytes (Funfschilling et al. 2012). Using a variety of other tools, the authors also found evidence for enhanced glycolysis, rather than reliance on mitochondrial ATP as the primary source of energy for mature oligodendrocytes. Thus, these findings suggest a developmental stage-specific role for oligodendrocytic mitochondria; developing oligodendrocytes may rely on aerobic respiration and the ETC, whereas fully developed oligodendrocytes may switch to glycolytic pathways for energy support. Although many questions about this interesting switch remain, the shift from ETC-derived energetic substrates during oligodendrogenesis and myelination to enhanced glycolysis in mature oligodendrocytes raises important questions on whether sensitivity to WMI varies by developmental stage or with aging.

22.5 Oligodendrocytic Injury and Neuronal Mitochondria

For many years, oligodendrocytes were thought to function solely as a means to promote rapid conduction of electrical signals by virtue of axonal myelination. However, more recent studies suggest that oligodendrocytes not only aid electrical conduction but may also contribute to the metabolic support of axonal mitochondria. As discussed above, recent evidence indicates that mature oligodendrocytes switch to enhanced glycolysis for maintenance of myelin structure and axonal integrity (Funfschilling et al. 2012). The major by-products of aerobic glycolysis are pyruvate and lactate. Because lactate and pyruvate can be used by axonal mitochondria as an alternate energy source (Tekkok et al. 2005; Brown et al. 2001), the movement of lactate and pyruvate via MCT from glial cells to neurons is hypothesized to serve as a source of neuronal energetic substrates (Pierre and Pellerin 2005). In support of the concept of intercellular shuttling of lactate, several MCTs are expressed in brain, both on neurons and glia (Pierre and Pellerin 2005). Recently, MCT1 has been found to be highly expressed in oligodendrocytes and knockdown of oligodendrocytic MCT1 led to degeneration of neuronal axons (Lee et al. 2012). Whether oligodendrocytic MCT1 is necessary for neuronal metabolism or whether it serves in an alternative, unexplored capacity is not yet known. Currently, there are

competing hypotheses against the shuttling of lactate to neurons (DiNuzzo et al. 2010; Jolivet et al. 2010). Mathematical models have been used to argue that the shuttling of pyruvate or lactate would be unfavorable under normal conditions and that lactate uptake in glial cells would instead be used by glia and thus allow neurons full access to plasma glucose. However, the model has not yet been tested under conditions of energy depletion. Nevertheless, these studies confirm the close bond between oligodendrocyte function and neuronal viability.

In addition to metabolic support of axonal mitochondria, proper formation of myelin and the nodes of Ranvier are essential for maintaining axonal mitochondrial dynamics. Over the length of the internodal regions, specific sodium channels and exchangers are positioned to facilitate smooth propagation of the action potential along the axon (Fig. 22.1). For example, the Na^+/K^+ ATPase is placed along the myelinated regions of the axon and requires a constant supply of ATP (Young et al. 2008). Axonal mitochondria are also located along the internodes (Edgar et al. 2008), co-residing with ATPase-dependent channels in myelinated axonal regions. However, in the context of WMI, damage to the myelin sheath disrupts conduction and results in a redistribution of ion exchangers in an effort to compensate for the loss of signal transduction (Edgar et al. 2008; Andrews et al. 2006; Bradl and Lassmann 2010; Campbell and Mahad 2012). This redistribution of ion channels correlates with a redistribution of and increase in axonal mitochondria and COX activity. In the absence of remyelination, it is postulated that the energy sources necessary for the ion exchangers eventually become depleted, and that the exchangers fail and potentially reverse, leading to a catastrophic build up of intracellular sodium, then calcium, and then axonal degeneration (Campbell and Mahad 2012). Thus, WMI may not only affect the availability of metabolic substrates for axons, but can also lead to rapid changes in axonal mitochondrial dynamics and stress.

Mitochondrial dysfunction within the developing brain is likely to have an even more severe impact on axons. Although post-myelination oligodendrocytes do not require functional COXIV for survival, cultured oligodendrocytes (which are maintained in an immature state) are sensitive to perturbation in COXIV function and rapidly degenerate (Funfschilling et al. 2012). Coupled with the higher risk for periventricular WMI in the immature brain prior to the onset of myelination (Back et al. 2001, 2007), alterations in energetic states and/or mitochondrial function may underlie the age-related susceptibility to WMI.

22.6 Mitochondria in Astrocytes

Manifold roles of astrocytes in the brain in normal and injured states have been described and remain under investigation. Largely viewed as support cells for neurons, astrocytes contribute to neural homeostasis by their uptake of extracellular neurotransmitters, regulation of pH and ion concentrations, and provision of trophic support (Chen and Swanson 2003). These processes either directly or indirectly require functional mitochondria, and as such, bolstering astrocytic mitochondrial

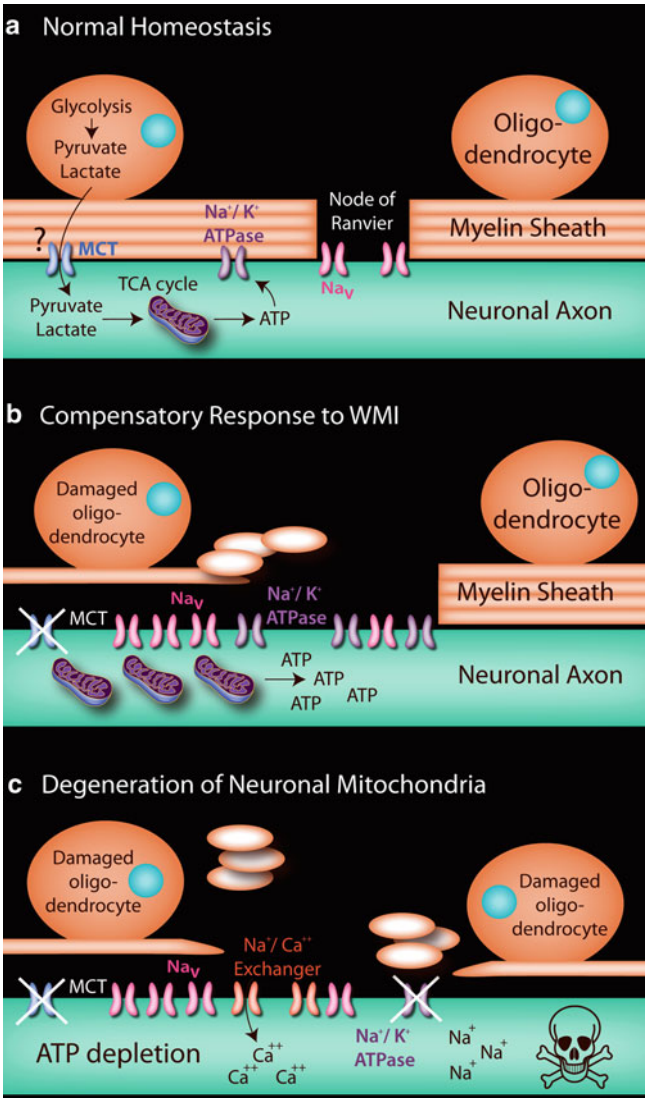


Fig. 22.1 Hypothetical interplay between oligodendrocytes and axonal mitochondria in neurons. (**a**) Under normal homeostatic conditions, mature myelin sheaths regulate the sequestration of axonal mitochondria into clusters near which ATPase ion channels are concentrated. (**b**) Following WMI, there is an increased dependence on these ion channels and the associated mitochondria. Once the myelin sheath fragments and is not adequately cleared, remyelination is impeded. The loss of myelin increases expression of the ATPase ion channels to maintain neurotransmission. As a result of the increased need for ATP, mitochondrial recruitment and activity are both raised in the demyelinated axon. (**c**) After prolonged periods of WMI and demyelination, there is catastrophic depletion of ATP and reversal of the sodium–calcium (Na^+/Ca^{2+}) exchanger that normally clears calcium during action potentials. These responses directly damage the neuron. In addition, damage to the oligodendrocyte may inhibit pyruvate or lactate shuttling into neurons, further contributing to ATP depletion and axonal injury

function has been proposed as a potential therapeutic target, whereas their dysfunction has been posited as a contributor to neural injury.

Differences between astrocytic mitochondria and mitochondria from other neural cell types may underlie their differential susceptibility to neural injury. For example, mitochondria isolated from astrocytes were responsive to cyclosporine A-mediated inhibition of calcium overload opening of the mitochondrial permeability transition pore (PTP), whereas neuronal mitochondria were insensitive to the same stimulus (Bambrick et al. 2006). In whole cells, both cyclosporine A and FK506 inhibited mitochondrial depolarization induced by a calcium ionophore in astrocytes, but not in neurons (Kahraman et al. 2011). However, this effect appeared to be the result of a dampening in the rise of cytosolic calcium in astrocytes, indicating an indirect effect on mitochondrial PTP. Nevertheless, these results illustrate that the control of events leading to downstream mitochondrial alterations may differ between neural subtypes.

The efficiency of mtDNA repair following oxidative stress also differs between astrocytes and other neural cells (Hollensworth et al. 2000; LeDoux et al. 2007), in that astrocytes are more efficient at mtDNA repair than neurons and other glial cell types. In addition, morphological differences have been noted in cristae orientation (Kristian et al. 2006), although the significance of this structural variance is not known. More recently, mitochondrial differences between astrocytic subpopulations (i.e., striatal astrocytes versus cortical astrocytes) in their Ca^{2+} buffering capacities have also been identified. Similar to striatal neurons, striatal astrocytes exhibited a decreased capacity to buffer Ca^{2+} compared to cortical astrocytes (Oliveira and Goncalves 2009). These functional differences between astrocytic populations from different brain regions may underlie regional selectivity in WMI, or neural injury in general. Likewise, specific characteristics of neuronal versus astrocytic mitochondria may also contribute to the established differences in sensitivity of neurons and astrocytes to injury (Bambrick et al. 2004). Ongoing research is still addressing these issues and attempting to develop therapeutic strategies targeted at specific mitochondrial subpopulations.

In addition to the direct response of astrocytic mitochondria to altered neural states, a direct energetic link between astrocytic function and neuronal mitochondria has also been hypothesized. Brain tissue is known to possess the ability to utilize glycolysis and synthesize lactate (Vaishnavi et al. 2010; Mangia et al. 2009a), and glia (and to a lesser extent, neurons) also have a high affinity for lactate uptake (Boumezbeur et al. 2010; Gandhi et al. 2009). Lactate and/or pyruvate are hypothesized to be shuttled to neurons via astrocytes for use as metabolic substrates (feeding into gluconeogenesis) in times of heightened energy demand (Pellerin and Magistretti 1994). These hypotheses have also been supported by recent in vivo data demonstrating that lactate levels increase with brain activation (Mangia et al. 2009a, b), and that neurons in particular may metabolize the majority of excess plasma lactate (Boumezbeur et al. 2010). Despite the indirect support for an astrocyte-neuron lactate shuttle, mathematical models suggest that astrocytic glycogen synthesis (or plasma lactate uptake from the bloodstream) does not shuttle to neurons, but rather serves as a source of energy for astrocytes (DiNuzzo et al. 2010). This mechanism

would, in effect, allow neurons full access to blood glucose. The results from the mathematical model still need to be further extrapolated to pathological events when blood glucose is critically depleted, as in stroke. Indeed, a separate mathematical model did provide support for the shuttling of lactate from astrocytes to neurons under hypoxic conditions (Genc et al. 2011). It will be interesting to see how these mathematical models translate to cellular systems.

In addition to the link between astrocytes and neuronal energetic processes via a lactate shuttle, recent findings suggest that the presence of neurons may affect astrocytic mitochondrial behavior. DJ-1 is a critical redox-sensitive chaperone involved in mitochondrial dynamics. Disruption of DJ-1 in astrocytes yielded different mitochondrial responses to the Complex I inhibitor rotenone in astrocyte-enriched cultures versus astrocyte–neuron cocultures (Larsen et al. 2011). In particular, astrocytes with diminished (knockdown) DJ-1 protein levels had reduced mitochondrial motility, with no effect on fission/fusion either in the presence or absence of rotenone. However, in the presence of both neurons and rotenone, astrocytic knockdown of DJ-1 significantly reduced fusion of mitochondria within astrocytes. The precise nature of these intercellular events involving astrocytes, neurons, and their mitochondria and their biological significance remain unknown. Nevertheless, these studies reveal a curious and as-yet unexplored intercellular interplay between neurons and astrocytes to which mitochondria are highly sensitive.

22.7 Astrocytic Mitochondria and Neural Protection

Given the critical role of astrocytes in maintaining neural homeostasis, it is not surprising that astrocytic dysfunction leads to neural injury. However, the contributions of astrocytic mitochondrial dysfunction to WMI or neural injury, and that of astrocytic injury to neuronal mitochondrial dysfunction, are still under investigation. Coculture systems and cell-specific promoters have only recently made it possible to manipulate processes within one cell type and assess their effect on another cell type. Nevertheless, strategies directly targeting astrocytic mitochondria have already shown promise. We will discuss some of the more recent findings in this field, with an emphasis on neural protection.

Astrocytes are less sensitive to ischemic neural injury compared to oligodendrocytes and neurons; this difference may relate to the ability of astrocytes to produce ATP via glycolysis of glycogen under hypoxic conditions (Dugan and Kim-Han 2004). Given these findings, one might expect astrocytes to also be less sensitive to depletion of blood glucose. Consistent with a protective role of glycogenolysis, boosting astrocytic glycogen stores improved neuronal survival following glucose deprivation in a coculture system (Swanson and Choi 1993). Although the ability to maintain ATP production in acute injury states promotes the survival of both astrocytes and their associated neurons, glycolytic utilization of glucose produces lactic acid, which can affect pH in cultures (Hochachka and Mommsen 1983; Chen and Swanson 2003). Once the pH falls below 6.6, astrocytes can no longer produce ATP

and become irreversibly injured (Swanson and Benington 1996; Swanson et al. 1997; Chen and Swanson 2003). As one might expect, this astrocytic injury can also contribute to neuronal loss.

Suboptimal astroglial mitochondrial function during ischemic injury can also lead to insufficient overall production of the ATP that is necessary for the increase in ATP-dependent signaling and ATPase ionic pumps during ischemic injury (Zheng et al. 2013). This would be particularly critical in conditions such as edema, where compromised ionic pumps could either initiate or exacerbate the injured state (Fig. 22.2). Early studies found that application of purines (e.g., ATP and adenosine) inhibited both mitochondrial dysfunction and astrocytic cell death in an in vitro model of hydrogen peroxide and glucose deprivation (Yoo et al. 2005). The purinergic receptor $P2Y_1R$ localizes to astrocytic mitochondria (Krzeminski et al. 2007), and upon agonist (e.g., purine) stimulation, increases intracellular calcium levels via IP_3 channels (Idestrup and Salter 1998) and leads to increased mitochondrial function (McCormack et al. 1990). In line with the protection of neurons by astrocytic mitochondria, several studies indicate that stimulation of the glial-specific $P2Y_1R$ decreases ischemic injury both in neuron–astroglia cocultures as well as in ischemic embolism and traumatic brain injury models in vivo (Wu et al. 2007; Zheng et al. 2010, 2013; Talley Watts et al. 2013). Furthermore, agonists of $P2Y_1R$ decrease edema in cultured astrocytes and in animals in a mitochondria-dependent manner (Wu et al. 2007; Zheng et al. 2010). These findings suggest that mitochondrial dysfunction in astrocytes significantly contributes to edema and cell vulnerability under acute injury.

Other approaches to deal with increased ROS generation and astrocytic damage have been proposed. Targeted expression of the amyotrophic lateral sclerosis (ALS)-related mutant superoxide dismutase-1 (SOD1-G93A) disrupts mitochondrial function in astrocytes (Cassina et al. 2008). Antioxidants, such as ubiquinone or carboxy-proxyl nitroxide, when coupled with a triphenylphosphonium cation, preferentially act in the mitochondrial space (Kelso et al. 2001). Addition of these mitochondria-targeted antioxidants suppressed mitochondrial dysfunction in SOD1-G93A expressing astrocyte cultures. An intercellular effect of astrocytic mitochondria protection was demonstrated in astrocytic–motor neuronal cocultures. Incubation of normal astrocytes with motor neurons sustained motor neuron survival, whereas astrocytes overexpressing SOD1-G93A were toxic in cocultures with normal motor neurons. However, when SOD1-G93A astrocytes were preincubated with the mitochondria-targeted antioxidants, motor neuron survival rates were maintained at control levels despite the astrocytic presence of SOD-G93A (Cassina et al. 2008). Similarly, astrocytic targeting of SOD2 (a mitochondrial matrix protein that also reduces superoxide levels) reduced damage to CA1 neurons in a global ischemia model (Xu et al. 2010). These studies demonstrate that targeting mitochondrial dysfunction by increasing antioxidants in astrocytes may enhance neural survival.

Other model systems also support the concept that astrocytic mitochondrial function normally helps maintain neural survival. For example, perturbation of mitochondrial function in glia increased sensitivity of cocultured neurons to

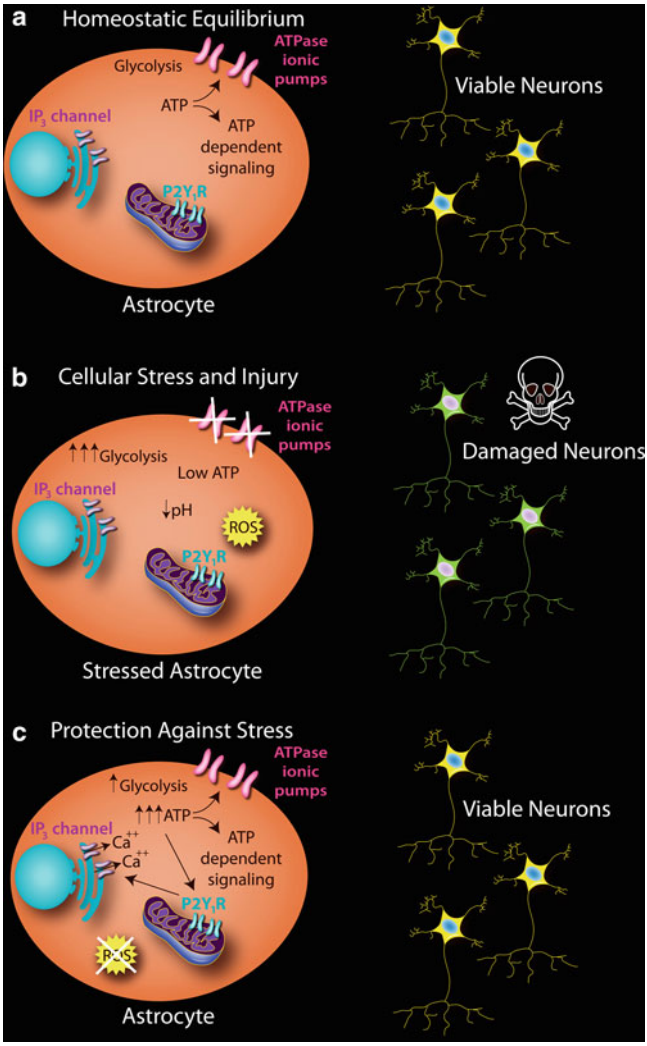


Fig. 22.2 Impact of astrocytic mitochondria on neuronal viability. (**a**) Healthy astrocytes preserve homeostatic equilibrium partly by maintaining ionic gradients with ATPase ionic pumps as well as ATP dependent signaling. (**b**) Following stress-induced mitochondrial impairment, the ATPase ionic pumps cannot effectively maintain ionic gradients, resulting in an increased demand for ATP. Glycolysis may then rapidly increase, which can both decrease pH and increase ROS generation, leading to astrocytic stress and decreased neuronal viability. (**c**) Protection against stress may be conferred by bolstering mitochondrial function via mitochondrial purinergic receptor stimulation. This increases ATP levels in astrocytes through the stimulation of IP_3 channels and release of intracellular calcium stores. Astrocytes with healthier mitochondria can more readily protect neighboring neurons

glutamate toxicity (Volobouéva et al. 2007). Astrocytes are also vulnerable to pro-apoptotic microRNAs such as miR-181a, which targets and degrades critical mitochondrial proteins such as Bcl-2 family members (Ouyang et al.). miR-181a inhibition effectively prevented loss of mitochondrial membrane potential,

mitochondrial dysfunction, and sensitivity to glucose deprivation in astrocytes (Ouyang et al. 2012). Likewise, as mentioned earlier, astrocytic knockdown of DJ-1 impaired astrocytic mitochondrial function only in the presence of both neurons and the Complex I inhibitor, rotenone (Larsen et al. 2011). Furthermore, astrocytic knockdown of DJ-1 increased neuronal sensitivity to rotenone in a coculture setting (Mullett and Hinkle 2009; Mullett et al. 2013). Conversely, overexpression of DJ-1 in astrocytes significantly increased neuronal protection against rotenone (Mullett et al. 2013). In an in vitro model related to Alzheimer's disease and other amyloid pathologies, beta amyloid caused loss of mitochondrial potential in astrocytes, but not in neurons, and increased production of ROS (Abramov et al. 2004). The differential effect of beta amyloid on neurons versus astrocytes may relate to preferential astrocytic uptake of protein aggregates, as astrocytes take up and degrade beta amyloid (Wyss-Coray et al. 2003). The effects of beta amyloid on astrocytes occurred via the activity of NADPH oxidase and phospholipase A2 (Abramov et al. 2004; Zhu et al. 2006). Taken together, these results implicate astrocytic mitochondrial dysfunction as a possible contributor to ROS generation and oxidative stress in models of Alzheimer's disease, Parkinson's disease, and other oxidative stress- or amyloid-related neuropathologies.

22.8 Clinical Overlap Between White Matter Diseases and Mitochondrial Dysfunction

Recent clinical observations suggest a link between mitochondrial dysfunction and WMI, as the symptoms of mitochondrial genetic diseases can significantly overlap with the symptoms of classic WM diseases such as multiple sclerosis (MS) (Finsterer et al. 2012; Yu-Wai-Man et al. 2011). Leber's hereditary optic neuropathy (LHON), an optic neuropathy caused by point mutations in mtDNA, and autosomal-dominant optic atrophy (DOA), an optic neuropathy commonly associated with mutations in the mitochondrial OPA1 gene, can both be clinically indistinguishable from other WM degenerative diseases such as Charcot-Marie-Tooth and MS (Yu-Wai-Man et al. 2011). In one case study, a patient was diagnosed with MS based on clinical presentation, MRI, and oligoclonal bands in the CSF (Finsterer et al. 2012). However, steroid treatment for MS exacerbated the symptoms and drugs targeting the immune system were ineffective. Sixteen years after the original misdiagnosis, the patient was reevaluated for a mitochondrial disorder based on new symptoms and a recent diagnosis of mitochondrial disorder in her mother. Tests then confirmed the presence of a mitochondrial disorder in this patient (Finsterer et al. 2012). Similarly, in separate case studies, individuals with LHON (confirmed by the presence of a specific mtDNA point mutation) presented with MS-like symptoms (Bhatti and Newman 1999; Olsen et al. 1995). This overlap in clinical presentation renders it difficult for neurological examinations and clinical testing to conclusively differentiate between MS and mitochondrial disorders. If possible, it seems best to rule out mitochondrial disorders before a final MS diagnosis is reached.

One potential explanation for the inability to distinguish between some presentations of mitochondrial disorders and neurodegenerative diseases may lie in our uncertainty of the underlying cause of diseases such as MS, or the full extent of pathology resulting from mitochondrial disorders, such as LHON or Kearns–Sayre syndrome. Recent histological studies have demonstrated extensive WM loss in Kearns–Sayre syndrome coupled with high levels of mtDNA deletions (Lax et al. 2012). Likewise, WM lesions have been identified in individual LHON patients presenting with MS-like illness (Harding et al. 1992; Lev et al. 2002; Kovacs et al. 2005). Thus, WM injury appears to correlate with at least a subset of mitochondrial dysfunctions.

Partly due to the overlap in mitochondrial diseases (e.g., LHON) and MS-like symptom presentation, MS has been postulated to arise from mitochondrial dysfunction (Harding et al. 1992; Mao and Reddy 2010). Consistent with this hypothesis, recent reports of a variety of mitochondrial dysfunctions have surfaced in clinical case studies of MS patients (reviewed in Mao and Reddy 2010). Additionally, a decrease in the components necessary for astrocytic glycogenolysis has been detected in WM astrocytes from MS patients (Cambron et al. 2012). As discussed above, decreased glycolysis in astrocytes may perturb the levels of energetic substrates available for neurons, either by necessitating increased use of glucose by glial cells or by the unavailability of pyruvate or lactate as additional energetic substrates. However, there are still no direct mitochondrial links unifying the demyelination and inflammatory processes that are so characteristic of MS. Furthermore, randomly sampled MS patients have been screened for LHON mtDNA mutations, but only very few (all women) were found to harbor these mutations (Hwang et al. 2001; Nishimura et al. 1995; Kellar-Wood et al. 1994). The etiology and cause(s) for the wide variation in the progression of MS are as yet unknown, and thus the possibility still exists that mitochondrial dysfunction may underlie some cases of MS pathology.

22.9 Conclusions

The interplay between WM, mitochondria, and neural injury is a highly complex and underexplored area of pathology. In particular, the roles of WM have only recently begun to be addressed due to the inherent limitations of previous model systems. This recent work contradicts traditional notions of WM as a passive tissue and has unveiled an integral role for WM in the determination of neuronal survival, through the control of neuronal mitochondria, as in the case of oligodendrocytic myelination, through WM mitochondria themselves, or through the supply of metabolic substrates to neuronal mitochondria. Unfortunately, the specific role of mitochondria in microglia still remains uncharted territory, despite the emerging evidence that microglia exert a complicated and potentially powerful role in WMI. Finally, we must appreciate that WM fills a much larger percentage of the human brain than in other animals. Because of the abundance of WM in humans, experimental models of WMI in rodents may underestimate how essential WM and its

mitochondria are to human grey matter and neuronal mitochondria. Although we currently model WMI in lower species for both cost and ethical reasons, we may find one day that the impact of WMI on mitochondria and neuronal survival is actually deepest in humans and other primates. With improvements in animal models, coculture systems, in vivo cell-specific targeting and imaging, and a more sophisticated understanding of mitochondrial behavior, additional research within the next few years will hopefully explore further links between WM, mitochondria, and neuroprotection and support the global view of brain cells as one well-integrated functional unit.

References

Abramov AY, Canevari L, Duchen MR (2004) Beta-amyloid peptides induce mitochondrial dysfunction and oxidative stress in astrocytes and death of neurons through activation of NADPH oxidase. J Neurosci 24(2):565–575

Amiri M, Hollenbeck PJ (2008) Mitochondrial biogenesis in the axons of vertebrate peripheral neurons. Dev Neurobiol 68(11):1348–1361

Andrews H, White K, Thomson C, Edgar J, Bates D, Griffiths I, Turnbull D, Nichols P (2006) Increased axonal mitochondrial activity as an adaptation to myelin deficiency in the Shiverer mouse. J Neurosci Res 83(8):1533–1539

Back SA, Luo NL, Borenstein NS, Levine JM, Volpe JJ, Kinney HC (2001) Late oligodendrocyte progenitors coincide with the developmental window of vulnerability for human perinatal white matter injury. J Neurosci 21(4):1302–1312

Back SA, Riddle A, McClure MM (2007) Maturation-dependent vulnerability of perinatal white matter in premature birth. Stroke 38(2 suppl):724–730

Bambrick L, Kristian T, Fiskum G (2004) Astrocyte mitochondrial mechanisms of ischemic brain injury and neuroprotection. Neurochem Res 29(3):601–608

Bambrick LL, Chandrasekaran K, Mehrabian Z, Wright C, Krueger BK, Fiskum G (2006) Cyclosporin A increases mitochondrial calcium uptake capacity in cortical astrocytes but not cerebellar granule neurons. J Bioenerg Biomembr 38(1):43–47

Baumann N, Pham-Dinh D (2001) Biology of oligodendrocyte and myelin in the mammalian central nervous system. Physiol Rev 81(2):871–927

Bhatti MT, Newman NJ (1999) A multiple sclerosis-like illness in a man harboring the mtDNA 14484 mutation. J Neuroophthalmol 19(1):28–33

Boumezbeur F, Petersen KF, Cline GW, Mason GF, Behar KL, Shulman GI, Rothman DL (2010) The contribution of blood lactate to brain energy metabolism in humans measured by dynamic 13C nuclear magnetic resonance spectroscopy. J Neurosci 30(42):13983–13991

Bradl M, Lassmann H (2010) Oligodendrocytes: biology and pathology. Acta Neuropathol 119(1):37–53

Brown AM, Wender R, Ransom BR (2001) Metabolic substrates other than glucose support axon function in central white matter. J Neurosci Res 66(5):839–843

Cambron M, D'Haeseleer M, Laureys G, Clinckers R, Debruyne J, De Keyser J (2012) White-matter astrocytes, axonal energy metabolism, and axonal degeneration in multiple sclerosis. J Cereb Blood Flow Metab 32(3):413–424

Campbell GR, Mahad DJ (2012) Mitochondrial changes associated with demyelination: consequences for axonal integrity. Mitochondrion 12(2):173–179

Cassina P, Cassina A, Pehar M, Castellanos R, Gandelman M, de Leon A, Robinson KM, Mason RP, Beckman JS, Barbeito L, Radi R (2008) Mitochondrial dysfunction in SOD1G93A-bearing astrocytes promotes motor neuron degeneration: prevention by mitochondrial-targeted antioxidants. J Neurosci 28(16):4115–4122

Chen Y, Swanson RA (2003) Astrocytes and brain injury. J Cereb Blood Flow Metab 23(2): 137–149

DiNuzzo M, Mangia S, Maraviglia B, Giove F (2010) Glycogenolysis in astrocytes supports blood-borne glucose channeling not glycogen-derived lactate shuttling to neurons: evidence from mathematical modeling. J Cereb Blood Flow Metab 30(12):1895–1904

Dugan LL, Kim-Han JS (2004) Astrocyte mitochondria in in vitro models of ischemia. J Bioenerg Biomembr 36(4):317–321

Edgar JM, McCulloch MC, Thomson CE, Griffiths IR (2008) Distribution of mitochondria along small-diameter myelinated central nervous system axons. J Neurosci Res 86(10):2250–2257

Finsterer J, Hoftberger R, Stollberger C, Rolinski B (2012) Mimicry between mitochondrial disorder and multiple sclerosis. Metab Brain Dis 27(2):217–220

Funfschilling U, Supplie LM, Mahad D, Boretius S, Saab AS, Edgar J, Brinkmann BG, Kassmann CM, Tzvetanova ID, Mobius W, Diaz F, Meijer D, Suter U, Hamprecht B, Sereda MW, Moraes CT, Frahm J, Goebbels S, Nave KA (2012) Glycolytic oligodendrocytes maintain myelin and long-term axonal integrity. Nature 485(7399):517–521

Gandhi GK, Cruz NF, Ball KK, Dienel GA (2009) Astrocytes are poised for lactate trafficking and release from activated brain and for supply of glucose to neurons. J Neurochem 111(2):522–536

Genc S, Kurnaz IA, Ozilgen M (2011) Astrocyte-neuron lactate shuttle may boost more ATP supply to the neuron under hypoxic conditions—in silico study supported by in vitro expression data. BMC Syst Biol 5:162

Harding AE, Sweeney MG, Miller DH, Mumford CJ, Kellar-Wood H, Menard D, McDonald WI, Compston DA (1992) Occurrence of a multiple sclerosis-like illness in women who have a Leber's hereditary optic neuropathy mitochondrial DNA mutation. Brain 115(pt 4):979–989

Harris JJ, Attwell D (2012) The energetics of CNS white matter. J Neurosci 32(1):356–371

Hochachka PW, Mommsen TP (1983) Protons and anaerobiosis. Science 219(4591):1391–1397

Hollensworth SB, Shen C, Sim JE, Spitz DR, Wilson GL, LeDoux SP (2000) Glial cell type-specific responses to menadione-induced oxidative stress. Free Radic Biol Med 28(8):1161–1174

Hwang JM, Chang BL, Park SS (2001) Leber's hereditary optic neuropathy mutations in Korean patients with multiple sclerosis. Ophthalmologica 215(6):398–400

Idestrup CP, Salter MW (1998) P2Y and P2U receptors differentially release intracellular Ca2+ via the phospholipase c/inositol 1,4,5-triphosphate pathway in astrocytes from the dorsal spinal cord. Neuroscience 86(3):913–923

Jolivet R, Allaman I, Pellerin L, Magistretti PJ, Weber B (2010) Comment on recent modeling studies of astrocyte-neuron metabolic interactions. J Cereb Blood Flow Metab 30(12):1982–1986

Kahraman S, Bambrick LL, Fiskum G (2011) Effects of FK506 and cyclosporin a on calcium ionophore-induced mitochondrial depolarization and cytosolic calcium in astrocytes and neurons. J Neurosci Res 89(12):1973–1978

Kann O, Kovacs R (2007) Mitochondria and neuronal activity. Am J Physiol Cell Physiol 292(2):C641–C657

Kellar-Wood H, Robertson N, Govan GG, Compston DA, Harding AE (1994) Leber's hereditary optic neuropathy mitochondrial DNA mutations in multiple sclerosis. Ann Neurol 36(1):109–112

Kelso GF, Porteous CM, Coulter CV, Hughes G, Porteous WK, Ledgerwood EC, Smith RA, Murphy MP (2001) Selective targeting of a redox-active ubiquinone to mitochondria within cells: antioxidant and antiapoptotic properties. J Biol Chem 276(7):4588–4596

Kovacs GG, Hoftberger R, Majtenyi K, Horvath R, Barsi P, Komoly S, Lassmann H, Budka H, Jakab G (2005) Neuropathology of white matter disease in Leber's hereditary optic neuropathy. Brain 128(pt 1):35–41

Kristian T, Hopkins IB, McKenna MC, Fiskum G (2006) Isolation of mitochondria with high respiratory control from primary cultures of neurons and astrocytes using nitrogen cavitation. J Neurosci Methods 152(1–2):136–143

Krzeminski P, Misiewicz I, Pomorski P, Kasprzycka-Guttman T, Baranska J (2007) Mitochondrial localization of P2Y1, P2Y2 and P2Y12 receptors in rat astrocytes and glioma C6 cells. Brain Res Bull 71(6):587–592

Larsen NJ, Ambrosi G, Mullett SJ, Berman SB, Hinkle DA (2011) DJ-1 knock-down impairs astrocyte mitochondrial function. Neuroscience 196:251–264
Lax NZ, Campbell GR, Reeve AK, Ohno N, Zambonin J, Blakely EL, Taylor RW, Bonilla E, Tanji K, DiMauro S, Jaros E, Lassmann H, Turnbull DM, Mahad DJ (2012) Loss of myelin-associated glycoprotein in kearns-sayre syndrome. Arch Neurol 69(4):490–499
LeDoux SP, Druzhyna NM, Hollensworth SB, Harrison JF, Wilson GL (2007) Mitochondrial DNA repair: a critical player in the response of cells of the CNS to genotoxic insults. Neuroscience 145(4):1249–1259
Lee Y, Morrison BM, Li Y, Lengacher S, Farah MH, Hoffman PN, Liu Y, Tsingalia A, Jin L, Zhang PW, Pellerin L, Magistretti PJ, Rothstein JD (2012) Oligodendroglia metabolically support axons and contribute to neurodegeneration. Nature 487(7408):443–448
Lev D, Yanoov-Sharav M, Watemberg N, Leshinsky-Silver E, Lerman-Sagie T (2002) White matter abnormalities in Leber's hereditary optic neuropathy due to the 3460 mitochondrial DNA mutation. Eur J Paediatr Neurol 6(2):121–123
Li Z, Okamoto K, Hayashi Y, Sheng M (2004) The importance of dendritic mitochondria in the morphogenesis and plasticity of spines and synapses. Cell 119(6):873–887
Mangia S, Giove F, Tkac I, Logothetis NK, Henry PG, Olman CA, Maraviglia B, Di Salle F, Ugurbil K (2009a) Metabolic and hemodynamic events after changes in neuronal activity: current hypotheses, theoretical predictions and in vivo NMR experimental findings. J Cereb Blood Flow Metab 29(3):441–463
Mangia S, Simpson IA, Vannucci SJ, Carruthers A (2009b) The in vivo neuron-to-astrocyte lactate shuttle in human brain: evidence from modeling of measured lactate levels during visual stimulation. J Neurochem 109(suppl 1):55–62
Mao P, Reddy PH (2010) Is multiple sclerosis a mitochondrial disease? Biochim Biophys Acta 1802(1):66–79
McCormack JG, Halestrap AP, Denton RM (1990) Role of calcium ions in regulation of mammalian intramitochondrial metabolism. Physiol Rev 70(2):391–425
Morris RL, Hollenbeck PJ (1993) The regulation of bidirectional mitochondrial transport is coordinated with axonal outgrowth. J Cell Sci 104(pt 3):917–927
Mullett SJ, Hinkle DA (2009) DJ-1 knock-down in astrocytes impairs astrocyte-mediated neuroprotection against rotenone. Neurobiol Dis 33(1):28–36
Mullett SJ, Di Maio R, Greenamyre JT, Hinkle DA (2013) DJ-1 expression modulates astrocyte-mediated protection against neuronal oxidative stress. J Mol Neurosci 49(3):507–511
Nishimura M, Obayashi H, Ohta M, Uchiyama T, Hao Q, Saida T (1995) No association of the 11778 mitochondrial DNA mutation and multiple sclerosis in Japan. Neurology 45(7):1333–1334
Oliveira JM, Goncalves J (2009) In situ mitochondrial Ca2+ buffering differences of intact neurons and astrocytes from cortex and striatum. J Biol Chem 284(8):5010–5020
Olsen NK, Hansen AW, Norby S, Edal AL, Jorgensen JR, Rosenberg T (1995) Leber's hereditary optic neuropathy associated with a disorder indistinguishable from multiple sclerosis in a male harbouring the mitochondrial DNA 11778 mutation. Acta Neurol Scand 91(5):326–329
Ouyang YB, Lu Y, Yue S, Giffard RG (2012) miR-181 targets multiple Bcl-2 family members and influences apoptosis and mitochondrial function in astrocytes. Mitochondrion 12(2):213–219
Pellerin L, Magistretti PJ (1994) Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. Proc Natl Acad Sci U S A 91(22):10625–10629
Pierre K, Pellerin L (2005) Monocarboxylate transporters in the central nervous system: distribution, regulation and function. J Neurochem 94(1):1–14
Swanson RA, Benington JH (1996) Astrocyte glucose metabolism under normal and pathological conditions in vitro. Dev Neurosci 18(5–6):515–521
Swanson RA, Choi DW (1993) Glial glycogen stores affect neuronal survival during glucose deprivation in vitro. J Cereb Blood Flow Metab 13(1):162–169
Swanson RA, Farrell K, Stein BA (1997) Astrocyte energetics, function, and death under conditions of incomplete ischemia: a mechanism of glial death in the penumbra. Glia 21(1):142–153

Talley Watts L, Sprague S, Zheng W, Garling RJ, Jimenez D, Digicaylioglu M, Lechleiter J (2013) Purinergic 2Y(1) receptor stimulation decreases cerebral edema and reactive gliosis in a traumatic brain injury model. J Neurotrauma 30(1):55–66

Tekkok SB, Brown AM, Westenbroek R, Pellerin L, Ransom BR (2005) Transfer of glycogen-derived lactate from astrocytes to axons via specific monocarboxylate transporters supports mouse optic nerve activity. J Neurosci Res 81(5):644–652

Vaishnavi SN, Vlassenko AG, Rundle MM, Snyder AZ, Mintun MA, Raichle ME (2010) Regional aerobic glycolysis in the human brain. Proc Natl Acad Sci U S A 107(41):17757–17762

Volobouеva LA, Suh SW, Swanson RA, Giffard RG (2007) Inhibition of mitochondrial function in astrocytes: implications for neuroprotection. J Neurochem 102(4):1383–1394

Wu J, Holstein JD, Upadhyay G, Lin DT, Conway S, Muller E, Lechleiter JD (2007) Purinergic receptor-stimulated IP3-mediated Ca2+ release enhances neuroprotection by increasing astrocyte mitochondrial metabolism during aging. J Neurosci 27(24):6510–6520

Wyss-Coray T, Loike JD, Brionne TC, Lu E, Anankov R, Yan F, Silverstein SC, Husemann J (2003) Adult mouse astrocytes degrade amyloid-beta in vitro and in situ. Nat Med 9(4):453–457

Xu L, Emery JF, Ouyang YB, Volobouеva LA, Giffard RG (2010) Astrocyte targeted overexpression of Hsp72 or SOD2 reduces neuronal vulnerability to forebrain ischemia. Glia 58(9): 1042–1049

Yoo BK, Choi JW, Yoon SY, Ko KH (2005) Protective effect of adenosine and purine nucleos(t) ides against the death by hydrogen peroxide and glucose deprivation in rat primary astrocytes. Neurosci Res 51(1):39–44

Young EA, Fowler CD, Kidd GJ, Chang A, Rudick R, Fisher E, Trapp BD (2008) Imaging correlates of decreased axonal Na+/K+ ATPase in chronic multiple sclerosis lesions. Ann Neurol 63(4):428–435

Yu-Wai-Man P, Griffiths PG, Chinnery PF (2011) Mitochondrial optic neuropathies—disease mechanisms and therapeutic strategies. Prog Retin Eye Res 30(2):81–114

Zheng W, Watts LT, Holstein DM, Prajapati SI, Keller C, Grass EH, Walter CA, Lechleiter JD (2010) Purinergic receptor stimulation reduces cytotoxic edema and brain infarcts in mouse induced by photothrombosis by energizing glial mitochondria. PLoS One 5(12):e14401

Zheng W, Talley Watts L, Holstein DM, Wewer J, Lechleiter JD (2013) P2Y(1)R-initiated, IP(3) R-dependent stimulation of astrocyte mitochondrial metabolism reduces and partially reverses ischemic neuronal damage in mouse. J Cereb Blood Flow Metab 33(4):600–611

Zhu D, Lai Y, Shelat PB, Hu C, Sun GY, Lee JC (2006) Phospholipases A2 mediate amyloid-beta peptide-induced mitochondrial dysfunction. J Neurosci 26(43):11111–11119

Chapter 23
Heavy Metals and White Matter Injury

Yang V. Li

23.1 Introduction

Heavy metal ion dyshomeostasis is a well-recognized cofactor in several neurodegenerative disorders (Li and Zhang 2012a, b). They are either essential nutrients in brain development and function, or a major environmental and occupational hazard. Examples of exogenous heavy metal ions include cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), selenium (Se), and zinc (Zn). These metal ions form a crucial part in normal biological functioning of cells and participate in controlling various metabolic and signaling pathways. Among them, Zn, Cu, and Fe play important role in regulating brain excitability and neuronal plasticity through their action in neurotransmission or function as second messengers (Li and Zhang 2012b). The metal ions (Zn, Fe, Cu, Co, Mn, and Se) are bound up in metal–protein complexes or metalloproteins, such as enzymes, transport and storage proteins, in which metals ion are critical in proper protein folding, structural stability, or enzymatic catalysis (Hanna and Doudna 2000; Frausto da Silva and Williams 2001). Considerable evidence has emerged regarding the role of heavy metals in neurotoxicity as well as neuroprotection. Among them, the administration of Se may benefit early recovery or reduce the risk of stroke. However, the overloads of heavy metals, as cytotoxic factors, have been associated with the brain injury including white matter injury. Therefore, these metals both represent essential components for the maintenance of normal biological functions and have a central role in many biochemical pathways in glial cells.

Several exogenous heavy metals such as cadmium (Cd), nickel (Ni), arsenic (As), mercury (Hg), and Lead (Pb) are important environmental pollutants, and also linked to the pathophysiology of neurological conditions (Li and Zhang 2012b).

Y.V. Li (✉)
Department of Biomedical Sciences, Ohio University, Athens, OH 45701, USA
e-mail: liy1@ohio.edu

S. Baltan et al. (eds.), *White Matter Injury in Stroke and CNS Disease*, Springer Series in Translational Stroke Research 4, DOI 10.1007/978-1-4614-9123-1_23,

A variety of heavy metals are toxic to many cell types, with the degree of toxicity depending on cell type, metal type, metal concentration, and duration of exposure. Exogenous metal dyshomeostasis, although generally no known useful physiological role is associated with them, has attracted the interest of researchers investigating the etiology of a variety of neurological conditions. Chronic heavy metal contaminations are becoming an emerging epidemic and pose a major worldwide health problem. Heavy metals may enter the human body through inhalation of dust, direct ingestion of soil, and consumption of food plants grown in metal-contaminated soil. Toxic effects of these metals share some chemical similarities with the above essential metals, and when present in excess can induce the production of reactive radicals as well as interact with nuclear proteins and DNA, which intern may cause neurotoxicity (Li and Zhang 2012b). Even in small concentrations, they are a threat to the environment and human health because they are not metabolized by the body (non-biodegradable) and accumulate in the soft tissues. When they become lodged in the brain the brain does not function normally, causing neurological symptoms and cognitive disorders. Environmental exposures of these exogenous metal ions are associated with significantly increased risk of brain injury.

In contrast to the brain grey matter that is made up of neuronal cell bodies, the brain white matter consists mostly of glial cells and myelinated nerve fibers. The latter is formed by oligodendrocytes, a type of glial cells that wrap around nerve fibers forming a specialized membrane differentiation, the so-called myelin sheath. The brain white matter injury is complex and involves numerous processes, including: energy failure, loss of cell ion homeostasis, acidosis, excitotoxicity, free radical-mediated toxicity, generation of arachidonic acid products, activation of glial cells, and disruption of the blood–brain barrier (BBB) (Woodruff et al. 2011). These are interrelated and coordinated events, which can lead to apoptosis or neuronal death. Glial cells function primarily as the physical and metabolic supports for neurons. However, glial cells present the cellular and the molecular mechanisms to communicate with neurons (and among themselves). Astrocytes perform many functions essential for normal neuronal activity, including the uptake of glutamate, the release of glutamine, the buffering of K^+ and H^+, and water transport (Aschner et al. 1998; Garcia et al. 2006). Therefore, white matter, long thought to be a passive tissue, actively affects how the brain learns and dysfunctions. Astrocytes have also high levels of glutathione (GSH) compared to neurons, and provide protection against oxidative stress (Watts et al. 2005). Furthermore, by producing and secreting a variety of neurotrophins, growth factors, cytokines, extracellular matrix proteins, proteoglycans and cholesterol, astrocytes provide a major support for neurons by fostering their survival, proliferation, differentiation, neurite outgrowth, and synaptogenesis (Sawada et al. 1994; Smith and Strunz 2005; Giordano et al. 2009; Dodla et al. 2010; Chen et al. 2011). This abundance of functions of astrocytes has led to the emerging notion that these glial cells may be a primary target in the neurotoxicity (Aschner and Costa 2005). Indeed, by interfering with astrocytic functions, heavy metals may ultimately and indirectly cause neuronal toxicity. The aim of this chapter is to overview current knowledge of the effect of metal dyshomeostasis on the brain white matter.

23.2 Dyshomeostasis of Endogenous Heavy Metals

Perturbed homeostasis of metal ions in neurological conditions has been well recognized for several decades (Farber 1981; Raichle 1983). The intracellular concentration of metal ions is tightly regulated through transports, homeostasis, compartmentalization, and binding to designated cell constituents. Under an unwelcome event, they could escape out of the control mechanism and interact with other protein sites, leading to malfunctioning of cells. For example, considerable evidence has emerged regarding the role of Fe, Zn, Mg, Cu, Mn, or Se in neurotoxicity as well as neuroprotection following neurological disorders (Li and Zhang 2012a, b). The excessive metals are capable of generating reactive free radicals, thus affecting oxidative stress and the intracellular redox potential.

Zn imbalance has been proposed as another cause for neurotoxicity (Bush 2003; Frederickson et al. 2005; Sensi et al. 2009). Particularly, considerable evidence has emerged regarding the role of Zn neurotoxicity following ischemic stroke. These studies demonstrate that neurons give rise to the accumulation of intracellular Zn in focal brain ischemia (Galasso and Dyck 2007), with the highest accumulation in CA1 region of hippocampus, the region most vulnerable to excitotoxic damage (Wei et al. 2004; Stork and Li 2006, 2009). Ischemia-driven Zn rises are the result of a combined process of Zn influx and Zn release from intracellular stores, and synaptically released Zn permeates postsynaptic neurons through NMDAR-associated channels and VGCCs (Frederickson et al. 2005; Sensi et al. 2009). The excessive zinc accumulation in extracellular space can inhibit the glutamate uptake by glutamate transporters (Suh et al. 2007). The contribution of Zn to ischemic damage has been further clarified that Zn increase is associated with a loss of plasma membrane permeability and with mitochondrial Zn uptake and depolarization. Neurons possess a pool of intracellularly releasable Zn that is bound by cytosolic metallothioneins or contained within intracellular organelles such as mitochondria, vesicles, and lysosomes (Colvin et al. 2010; Hwang et al. 2008). Recent study suggests that Zn is sequestered into thapsigargin/IP3-sensitive stores and is released upon agonist stimulation (Stork and Li 2010). Zn overload induces neuronal death by physical injury to the mitochondria (Medvedeva et al. 2009; Sensi et al. 2009). Activation of 12-lipoxygenase and mitogen-activated protein kinase (MAPK) by excessive Zn contribute to the toxicity of liberated Zn to neurons and oligodendrocytes (Zhang et al. 2007).

Changes in Fe metabolism in the brain have long been associated with neurodegenerative diseases (Zecca et al. 2004). Fe homeostasis is involved in many metabolic processes, including the storage and transport of oxygen, electron transport, and oxidation–reduction reactions, as well as DNA synthesis. In the white matter, Fe is normally present in patches of intensely iron-stained oligodendrocytes and astrocytes (Burdo et al. 1999). Fe functions in the formation and/or maintenance of the myelin sheet and may play a role in the pathology of myelin diseases (LeVine and Macklin 1990; LeVine 1991; Morath and Mayer-Proschel 2001). Iron deficiency leads to hypomyelination both in humans and animal models, and the

neurological sequelae of hypomyelination are significant (Todorich et al. 2009, 2011). Excess iron might be a mediator of oligodendrocyte cell death in periventricular white matter following hypoxia in the neonatal brain (Rathnasamy et al. 2011). Iron is potentially toxic to oligodendrocyte progenitors due to its high intracellular levels and its ability to catalyze oxidant-producing reactions. Elevated levels of free iron contribute to dopamine-induced toxicity in oligodendrocyte progenitors by the increased expression of the stress protein heme oxygenase-1 (HO-1), nuclear condensation, and caspase-3 activation (Hemdan and Almazan 2006). Therefore, there is a critical relationship between oligodendrocyte development, myelin production, and iron bioavailability.

Experimental and clinical data implicate Fe in brain injury after stroke (Selim and Ratan 2004; Carbonell and Rama 2007). The application of MRI to estimate Fe content within the hematoma found that hematoma Fe content correlates with the relative perihematoma edema volume (Lou et al. 2009). Ferritin-bound ferric iron is released after being reduced to ferrous iron, a process that is facilitated by superoxide, acidosis, and nitric oxide (Galaris et al. 2008; Kurz et al. 2008), which are abundant during cerebral ischemia. As unbound Fe gains access to the extracellular space, its uptake by neuronal cells is paradoxically enhanced by increased level of intracellular Fe (Perez de la Ossa et al. 2010). Fe-dependent oxidative stress in the penumbra can lead to necrosis and further neurological deterioration following ischemic stroke. Therefore, excess of Fe should be considered pathological in the ischemic brain (Selim and Ratan 2004). Aneurysmal subarachnoid hemorrhage (SAH) is a serious disease causing high morbidity and mortality during early and delayed period. Increased brain Fe levels or Fe overload contribute to brain edema, oxidative injury, and brain atrophy after SAH (Gu et al. 2009). The administration of appropriate "labile Fe" chelating agents, preferentially prior to reperfusion, might improve the efficacy of any therapeutic strategy (Galaris et al. 2008).

Mn is an essential trace element required for normal development and function as well as normal biochemical activities in the brain. Mn in the brain is found at higher concentrations in astrocytes than in neurons, can be cytotoxic to astrocytes. Therefore, Mn is preferentially deposited in astrocytes because of the presence of high capacity transporters in these cells (Aschner et al. 1992, 1999). Such preferential accumulation suggests that astrocytes may be more vulnerable to Mn toxicity than other neural cells. Mn directly affects glial cells and neurons by impairing energy metabolism and increasing the formation of reactive oxygen species (ROS) (Milatovic et al. 2009). Specifically, Mn is sequestered by mitochondria where it inhibits oxidative phosphorylation and increases matrix calcium, ultimately driving the production of excess intracellular ROS (Gunter et al. 2006b). Therefore, Mn may exert its toxic effects with glutamate oxidative stress and glial inflammation (Chen et al. 2006). Mn activates glial cells, increasing proinflammatory mediator expression, which causes neuroinflammation, release of proinflammatory cytokines, breaching of BBB permeability, and leukocyte invasion (Filipov et al. 2005; Tjalkens et al. 2006; Lee et al. 2012).

Mn exerts important functions in metabolic and redox homeostasis. However, increasingly concerns are rising about the Mn exposure of humans and related

neurotoxic effects (Erikson et al. 2007). Excessive accumulation of Mn in humans results in neurobehavioral deficits such as hyperactivity, neurological syndrome similar to chronic Parkinson's disease, including hypokinesia, rigidity, and muscle tremors, as well as headache, insomnia, hallucinations, and emotional instability (Bowler et al. 1999; Dobson et al. 2004). Both psychological and motor deficits are attributable to Mn deposition in the brain and subsequent disruption of basal ganglia circuitry, with globus pallidus, substantia nigra pars reticulata, and striatum being the primary targets (Bird et al. 1984; Aschner et al. 2007a). There are similarities between Mn-exposure and ischemia induced glutamate excitotoxicity in increased extracellular glutamate levels and depletion of cellular ATP (Erikson et al. 2007), and increasing concerns about the Mn exposure of humans and related neurotoxic effects (Erikson et al. 2007; Michalke et al. 2007).

Copper (Cu) is an essential trace element in the brain that can be toxic at elevated levels. Cu accumulation is a suspected etiology in several neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, Wilson's disease, and prion-induced disorders (Bush 2003; Tiffany-Castiglioni et al. 2011). Cu overload is toxic and induces cytotoxicity by increasing the intracellular Cu concentration and producing oxidative stress (Bush 2003; Brown 2004; Tiffany-Castiglioni et al. 2011). Since not only copper deficiency but also excess of copper can seriously affect cellular functions, the cellular copper metabolism is tightly regulated. Astrocytes are a proposed depot in the brain for Cu and other metals, and appear to play a pivotal role in the copper metabolism. The capacity of astrocytes to efficiently accumulate extracellular copper will help to protect other brain cells against copper-induced toxicity (Scheiber and Dringen 2013). The high antioxidative potential of astrocytes as well as their ability to upregulate their storage capacity for copper upon copper exposure are likely to contribute to the reported resistance of astrocytes against copper toxicity. With their strategically important localization between capillary endothelial cells and neuronal structures they are ideally positioned to transport copper from the BBB to provide essential copper to neurons and other cells in the brain (Scheiber and Dringen 2013). Cell culture studies also indicate that astrocytes are more resistant to Cu-induced cytotoxicity than are neurons (Reddy et al. 2008; Scheiber et al. 2010), as astrocytes are the primary repository of Cu in the brain, astrocytes may protect neurons from Cu toxicity.

Cobalt (Co) is an essential trace element in humans, playing a critical role in the production of vitamin B12 (hydroxycobalamin) and other cobalamines. Its deficiency can result in the development of pernicious anemia and the increase in the risk of developmental abnormalities and growth failure in infants (Stabler and Allen 2004). However, excessive level of Co can also be harmful as Co is cytotoxic to many cell lines and can induce cell death by apoptosis and necrosis (Olivieri et al. 2001). Co was used to induce ischemic pre-conditioning in vivo (Shukla et al. 2011; Jiang et al. 2012). Although the exact mechanisms of how Co exerts its toxic effects are not fully understood, different mechanisms have been proposed. Co can induce mitochondrial damage with release of apoptogenic factors, and can cause DNA strand breaks due to its capacity to produce ROS, which are highly reactive against DNA and other biomolecules (De Boeck et al. 2003). Se is an essential micronutrient

necessary for normal growth and function of the brain. The physiological role of Se remained unraveled, but seleno-proteins or -enzymes playing important roles in various processes of redox signaling (Savaskan et al. 2003). Se deficiency is detrimental to cellular functions mediated by these protein/enzymes, leading to increased oxidative stress and adversely affecting neuronal cell survival. Studies have demonstrated that selenium is required for the normal morphological development of oligodendrocytes, and Selenium deficiency may predispose oligodendrocytes to demyelinating injury (Gu et al. 1997). Selenium deficiency may specifically inhibit the progression from immature to mature oligodendrocytes (Gu et al. 1997). Early administration of selenium may improve neurological outcome after neurotoxic insults (Reisinger et al. 2009).

23.3 Action of Exogenous Heavy Metals

Exogenous metal dyshomeostasis, although generally no known useful physiological role associated with them, has attracted the interest of researchers investigating the etiology of a variety of neurological conditions. Chronic heavy metal (Cd, Pb, Hg, As, Ni) contaminations are becoming an emerging epidemic and pose a major worldwide health problem. Cd, as widely used in paints and in batteries, is a carcinogen and induces tumors in many human organs. The brain is especially vulnerable to Cd exposure-induced damage, and Cd entry into the CNS may be associated with severe neurodegenerative disorders such as behavioral defects, amyotrophic lateral sclerosis (ALS), Alzheimer's disease, and Parkinsonism symptoms (Okuda et al. 1997; Bar-Sela et al. 2001; Panayi et al. 2002). Cd toxicity can directly affect brain white matter as neurochemical changes and brain lesions in white matter were reported in experimental animals (Fern et al. 1996). Oligodendrocytes appear to be one of direct targets of Cd insult (Almazan et al. 2000; Hossain et al. 2009). As a recognized neurotoxin, Cd exerts its toxic effects by the perturbation of cellular redox balance, and subsequent reduction of the total brain antioxidant status, the inhibition of astrocyte glutamate transporters (Cookson and Pentreath 1996; Im et al. 2006; Liu et al. 2008). Cd is also found to reduce intracellular glutathione levels, mitochondrial injury and to increase ROS in both astrocytes and oligodendrocytes (Almazan et al. 2000; Hossain et al. 2009).

Pb is one of the most widespread toxicants in the environment, and its neurotoxicities on neuronal tissue are widely known. As the sites of Pb deposition in the central nervous system, Pb tends to accumulate in glial cells, causing glial injury that may precede its neuronal toxic effects. Experimental acute Pb intoxication has been shown to be associated with astrocyte swelling with the increase of water permeability (Gunnarson et al. 2005). Exposure to Pb results in an increase available glutamate in extracellular space by the inhibition glutamate uptake into astrocytes, and inhibition of glutamine synthetase activity (Engle and Volpe 1990; Sierra and Tiffany-Castiglioni 1991; Ronnback and Hansson 1992; Struzynska et al. 2005). The accumulation of glutamate in extracellular space triggers the cellular stress responses initiated by glutamate-induced excessive Ca influx. Pb-induced oxidative

stress in glial cells is an important mechanism in Pb-induced neurotoxicity. Acute exposure to Pb can induce HSP70 synthesis in astrocytes and non-pyramidal neurons in the rat hippocampus (Selvin-Testa et al. 1997). The perturbation of galactolipid metabolism by Pb cause myelin deficits. Several studies revealed that the prolonged exposure of low concentration of Pb caused approximately 50 % reduction in levels of the galactolipid biosynthetic transferases in oligodendrocytes (Deng et al. 2001; Deng and Poretz 2001a, b). Galactolipids are expressed during differentiation of oligodendrocyte lineage cells and accumulate in myelin.

Chronic Hg contaminations are becoming an emerging epidemic and pose a major worldwide health problem. It is well established that astrocytes play a pivotal role in the etiology of Hg-induced neurotoxicity as Hg preferentially accumulates in astrocytes (Aschner et al. 2007b). Different chemical forms of Hg account for the various degrees of toxicities by Hg. For example, methylmercury (MeHg) is more toxic and more permeable to BBB than other forms ($HgCl_2$, HgS) (Clarkson 1983). Astrocytic predisposition to be damaged by MeHg offers a potential explanation for its neurotoxicity, as astrocytes are a target for Hg accumulation in the cortex and cerebellum. Selective impairment of glutamate transport by Hg may represent a critical early pathogenetic feature of Hg-induced neurotoxicity (Aschner et al. 2007b). The accumulation of glutamate in the extracellular space maintains a sustained membrane depolarization, Ca influx into neurons, and excessive generation of ROS, suggesting that Hg-induced neuronal toxicity is secondary to disturbances in astrocytes.

Arsenic (As) is the most common cause of acute heavy metal poisoning and is number 1 on the priority list of hazardous substances, the so-called top 20 list released by Agency for Toxic Substances and Disease Registry. As-induced toxicity is released into the environment by the smelting process of metals, as well as by the manufacturing of chemicals. Therefore, its contamination of drinking water is becoming a major worldwide public health problem such as hypertension, diabetes mellitus, carotid atherosclerosis, ischemic stroke (Wang et al. 2007). Chronic exposure to As at nonlethal levels can result in a variety of outcomes including cognitive impairment (Kapaj et al. 2006; Hamadani et al. 2011). Nickel (Ni) may be as injurious as Pb; exposure to Ni has been related to a variety of neurological symptoms that may attribute to its action in glutamatergic receptors such as NMDA receptor channels (Gavazzo et al. 2011).

23.3.1 Mechanisms for Heavy Metal Induced White Matter Damages

23.3.1.1 Glutamate Excitotoxicity

During excitatory synaptic transmission, glutamate is released at a high concentration into the extracellular space, and normal physiological levels are maintained by re-uptake of glutamate through neuronal or glial glutamate transporters. However, extracellular glutamate overload occurs in neuronal injury or toxic chemical

exposure. Methylmercury (MeHg) has been shown to preferentially accumulate in astrocytes, inhibit astrocytic glutamate uptake, and stimulate the efflux of excitatory amino acids (Aschner et al. 2007b). Cd inhibits the glutamate transporters expression in astrocytes that suppressed the astrocytic glutamate uptake activity (Liu et al. 2008). Hg inhibit glutamate and aspartate uptake in astrocytes (Allen et al. 2001a, b), increasing glutamate concentration in the extracellular space. Inhibition of glutamate uptakes by MeHg may be linked to voltage-sensitive calcium channels as evidenced by the lack of glutamate uptake inhibition by MeHg in calcium-free medium or in the presence of channel blockers (Kim and Choi 1995). Exposure to Pb results in an increase available glutamate in extracellular space by the inhibition glutamate uptake into astrocytes, and inhibition of glutamine synthetase activity (Engle and Volpe 1990; Sierra and Tiffany-Castiglioni 1991; Ronnback and Hansson 1992; Struzynska et al. 2005). The accumulation of glutamate in extracellular space triggers the cellular stress responses initiated by membrane depolarization and excessive Ca influx.

Selective impairment of glutamate transport by these heavy metals may represent a critical early pathogenetic feature of metal-induced neurotoxicity. The accumulation of glutamate in the extracellular space maintains a sustained membrane depolarization, Ca influx into neurons, and excessive generation of ROS, suggesting that Hg-induced neuronal toxicity is secondary to disturbances in astrocytes.

Excessive stimulation of glutamatergic receptors induces alterations in the concentration of ions and membrane excitability, leading to neurotoxicity. There is a negative relationship between Cu or Zn homeostasis and NMDA receptor activity. Potentially paradoxical actions of Cu or Zn are that both ions also block GABA(A) receptor-mediated current. Adding to already "touchy" situation, most of the glutamatergic synapses in the cerebral cortex co-release Zn (maybe Cu too) along with glutamate (Bush 2003; Frederickson et al. 2005; Sensi et al. 2009). Mn may exert its neurotoxic effects by facilitating the release of excessive amounts of glutamate into the extracellular space (Erikson et al. 2007). Mn preferentially accumulates in astrocytes where it is known to impair glutamate transporter function (Hazell and Norenberg 1997; Erikson and Aschner 2002). There is compelling evidence of glutamate-mediated excitotoxicity in Mn neurotoxicity, and the involvement of astrocytic glutamate transporters in this process (Aschner et al. 1992; Hazell and Norenberg 1997). Mn decreases astrocytic glutamate uptake from the synapse, effectively increasing the chances of glutamate-mediated excitotoxicity to surrounding neurons.

23.3.1.2 Oxidative Stress

Antioxidants provide cellular defense against ROS, with GSH constituting the most important and abundant component. Both neurons and astrocytes contain GSH; however, astrocytes contain higher concentrations of GSH than neurons (Kranich et al. 1996; Sagara et al. 1996). MeHg-induced neurotoxicity have shown to deplete astrocytic GSH and, subsequently, to generate ROS (Choi et al. 1996; Sanfeliu et al. 2001). The astrocytes provide a critical precursor cysteine to neurons for GSH synthesis

(Kranich et al. 1996; Sagara et al. 1996; Shanker and Aschner 2001). Therefore, the availability of cysteine from glial cells becomes rate limiting for synthesis of neuronal GSH. A recent study suggests that *N*-acetyl cysteine treatment reduces mercury-induced neurotoxicity (Falluel-Morel et al. 2012). Pb-induced oxidative stress in glial cells is an important mechanism in Pb-induced neurotoxicity. Acute exposure to Pb can induce HSP70 synthesis (Selvin-Testa et al. 1997), and heme oxygenase-1 (HO-1) in astrocytes, but not in neurons (Cabell et al. 2004). HSP70 and HO-1 are inducible proteins induced in response to stress such as oxidative stress and toxic chemicals. Other exogenous heavy metal ions (Cd, Ni As) are also linked with Ca dyshomeostasis, oxidative stress, and mitochondrial dysfunction (Finney and O'Halloran 2003; Im et al. 2006; Modi and Katyare 2009; Houston 2011).

The accumulation of endogenous heavy metals such as Zn, Fe, and Cu are associated with oxidative stress. ROS/PKC-alpha/Ras/Raf/ERK and ROS/Src/Ras/Raf/ERK signaling and cPLA(2) were actively involved in zinc-induced astrocyte damage (Liao et al. 2011). Energy failure and initial oxidative stress such as nitric oxide production may elicit the dysregulation homeostasis of metal ions, which in turn exert its toxic effects by the perturbation of cellular redox balance, inhibition of oxidative DNA repair systems, alteration in signal transduction, further stimulation in the production of ROS. These metals may accumulate in or be taken up by mitochondria and produces mitochondrial membrane permeability transition, inhibits respiratory complex I, and causes cytochrome c release following stroke (Bush 2003; Frederickson et al. 2005; MacDonald et al. 2006; Galaris et al. 2008).

At the cellular level, Mn preferentially accumulates in mitochondria, where it disrupts oxidative phosphorylation and increases the generation of ROS, causes mitochondrial dysfunction, including the inhibition of enzymes of TCA cycle, and a reduction in the activities of the electron transport chain, ultimately resulting in ATP depletion (Zheng et al. 1999; Zwingmann et al. 2004; Gunter et al. 2006a). Some of these mitochondrial events are significantly blocked by antioxidants, suggesting the involvement of oxidative stress in the mechanism of mitochondrial dysfunction (Rao and Norenberg 2004). Se may act as an antioxidant either after incorporation into selenoproteins or directly as in the case of selenite (Savaskan et al. 2003; Reisinger et al. 2009). In the case of Zn, Cu or Mn, they are also intrinsic factors for neuron survival, and in low amounts, is an active neuroprotectant against neurotoxic cell death. This protective effect is assumed to be mediated in part through antioxidant enzymes of superoxide dismutase (SOD; Zn, Cu, or Mn are cofactors), and the antagonism of glutamatergic receptors (NMDA receptor by Zn) activation (Frederickson et al. 2005; Erikson et al. 2007). In general, it is widely accepted that excessive increases of these metal ions in neurological disorders is detrimental.

23.3.1.3 Cerebral Edema

Cerebral edema with cellular swelling is a common consequence of brain hypoxia, trauma and intoxication and represents a risk for further morbidity and permanent brain damage. Aquaporins are water channels that allow rapid osmotically driven

water transport across cell membranes. Among them, Aquaporin 4 is the most abundantly expressed aquaporin within the brain (Nielsen et al. 1997; Vajda et al. 2002; Gunnarson et al. 2005). Gunnarson et al. reported that the exposure of Pb increased AQP4 water permeability in an astrocyte cell line transiently transfected with AQP4 and in rat hippocampus astroglial cells in primary culture (Gunnarson et al. 2005). Mn exposure was also shown to induce astrocyte swelling and such swelling may be caused by oxidative stress and/or mPT (Rama Rao et al. 2007). Astrocyte swelling by Mn may represent an important aspect of manganese neurotoxicity. As aquaporin-4 (AQP4) is important in the mechanism of astrocyte swelling, Mn increased AQP4 protein expression in the plasma membrane of cultured astrocytes (Rao et al. 2010). Hg has been shown to preferentially accumulate in astrocytes and implicated in producing astrocytic swelling (Nicchia et al. 2000).

23.3.1.4 Blood–Brain Barrier Dysfunction

BBB isolates the brain from the systemic blood circulation and forms a restrictive barrier against toxins and foreign compounds. The BBB is formed by endothelial cells and partly by astrocytes. Therefore, glial cells form an important part of the BBB. In fact, most of the outer area of the endothelial cells is covered with foot processes of glial cells. Many studies have reported an important role of Fe in supporting the generation of ROS which affect BBB permeability by activation of matrix metalloproteinases, particularly matrix metalloproteinase-9, leading to degradation of vascular basement membrane collagen and modulation of tight junction protein complexes (Perez de la Ossa et al. 2010; Selim and Ratan 2004). Ischemic stroke and hypoxic stress disrupt the BBB, which subsequently aggravate brain tissue damage. Following BBB disruption, the ferritin and the free Fe can enter the penumbra, leading to necrosis and further neurological deterioration following ischemic stroke. Mn or Hg exposures are associated with the loss of BBB integrity (Toimela et al. 2004; Erikson et al. 2007; Nayak et al. 2011). Cd also increases permeability of the BBB in rats (Shukla et al. 1996). Pb exposure has also been associated with leakage or disruption of the BBB in vitro, probably by acting directly on the barrier structure to increase the permeability (Balbuena et al. 2010).

23.4 Summary

White matter damages results from the interaction of complex pathophysiological processes in many neurological conditions. While the mechanisms triggering white matter injury are only partially understood, both oligodendrocytes and astrocytes are particularly sensitive to either the dyshomeostasis of endogenous heavy metal ions or the exposures of toxic heavy metals. Although the dyshomeostasis of metals is certainly not the only trigger of the neurological disorders, therapeutic interventions aimed at restoring metal homeostasis remain strong candidates. For example,

a chelator may serve as a protective therapeutic agent for reducing Zn overload that occurs following ischemia or other insults (Calderone et al. 2004; Barkalifa et al. 2009; Li 2012). Se is a potent protective agent for neurons through the expression of selenoproteins, which may be a potential therapeutic target of neurological disorders (Savaskan et al. 2003; Reisinger et al. 2009). Therefore, a deeper knowledge about the mechanisms of white matter injury mediated by heavy metals will open opportunities for the development of therapies to treat neurological disorders.

References

Allen JW, Mutkus LA, Aschner M (2001a) Methylmercury-mediated inhibition of 3H-D-aspartate transport in cultured astrocytes is reversed by the antioxidant catalase. Brain Res 902:92–100

Allen JW, Shanker G, Aschner M (2001b) Methylmercury inhibits the in vitro uptake of the glutathione precursor, cystine, in astrocytes, but not in neurons. Brain Res 894:131–140

Almazan G, Liu HN, Khorchid A, Sundararajan S, Martinez-Bermudez AK, Chemtob S (2000) Exposure of developing oligodendrocytes to cadmium causes HSP72 induction, free radical generation, reduction in glutathione levels, and cell death. Free Radic Biol Med 29:858–869

Aschner M, Costa LG (2005) The role of glia in neurotoxicity. CRC Press, Boca Raton, FL

Aschner M, Gannon M, Kimelberg HK (1992) Manganese uptake and efflux in cultured rat astrocytes. J Neurochem 58:730–735

Aschner M, Conklin DR, Yao CP, Allen JW, Tan KH (1998) Induction of astrocyte metallothioneins (MTs) by zinc confers resistance against the acute cytotoxic effects of methylmercury on cell swelling, Na+ uptake, and K+ release. Brain Res 813:254–261

Aschner M, Vrana KE, Zheng W (1999) Manganese uptake and distribution in the central nervous system (CNS). Neurotoxicology 20:173–180

Aschner M, Guilarte TR, Schneider JS, Zheng W (2007a) Manganese: recent advances in understanding its transport and neurotoxicity. Toxicol Appl Pharmacol 221:131–147

Aschner M, Syversen T, Souza DO, Rocha JB, Farina M (2007b) Involvement of glutamate and reactive oxygen species in methylmercury neurotoxicity. Braz J Med Biol Res 40:285–291

Balbuena P, Li W, Magnin-Bissel G, Meldrum JB, Ehrich M (2010) Comparison of two blood-brain barrier in vitro systems: cytotoxicity and transfer assessments of malathion/oxon and lead acetate. Toxicol Sci 114:260–271

Barkalifa R, Hershfinkel M, Friedman JE, Kozak A, Sekler I (2009) The lipophilic zinc chelator DP-b99 prevents zinc induced neuronal death. Eur J Pharmacol 618:15–21

Bar-Sela S, Reingold S, Richter ED (2001) Amyotrophic lateral sclerosis in a battery-factory worker exposed to cadmium. Int J Occup Environ Health 7:109–112

Bird ED, Anton AH, Bullock B (1984) The effect of manganese inhalation on basal ganglia dopamine concentrations in rhesus monkey. Neurotoxicology 5:59–65

Bowler RM, Mergler D, Sassine MP, Larribe F, Hudnell K (1999) Neuropsychiatric effects of manganese on mood. Neurotoxicology 20:367–378

Brown DR (2004) Role of the prion protein in copper turnover in astrocytes. Neurobiol Dis 15:534–543

Burdo JR, Martin J, Menzies SL, Dolan KG, Romano MA, Fletcher RJ, Garrick MD, Garrick LM, Connor JR (1999) Cellular distribution of iron in the brain of the Belgrade rat. Neuroscience 93:1189–1196

Bush AI (2003) Copper, zinc, and the metallobiology of Alzheimer disease. Alzheimer Dis Assoc Disord 17:147–150

Cabell L, Ferguson C, Luginbill D, Kern M, Weingart A, Audesirk G (2004) Differential induction of heme oxygenase and other stress proteins in cultured hippocampal astrocytes and neurons by inorganic lead. Toxicol Appl Pharmacol 198:49–60

Calderone A, Jover T, Mashiko T, Noh KM, Tanaka H, Bennett MV, Zukin RS (2004) Late calcium EDTA rescues hippocampal CA1 neurons from global ischemia-induced death. J Neurosci 24:9903–9913

Carbonell T, Rama R (2007) Iron, oxidative stress and early neurological deterioration in ischemic stroke. Curr Med Chem 14:857–874

Chen CJ, Ou YC, Lin SY, Liao SL, Chen SY, Chen JH (2006) Manganese modulates pro-inflammatory gene expression in activated glia. Neurochem Int 49:62–71

Chen J, Costa LG, Guizzetti M (2011) Assessment of cholesterol homeostasis in astrocytes and neurons. Methods Mol Biol 758:403–414

Choi BH, Yee S, Robles M (1996) The effects of glutathione glycoside in methyl mercury poisoning. Toxicol Appl Pharmacol 141:357–364

Clarkson TW (1983) Mercury. Annu Rev Public Health 4:375–380

Colvin RA, Holmes WR, Fontaine CP, Maret W (2010) Cytosolic zinc buffering and muffling: their role in intracellular zinc homeostasis. Metallomics 2:306–317

Cookson MR, Pentreath VW (1996) Protective roles of glutathione in the toxicity of mercury and cadmium compounds to C6 glioma cells. Toxicol In Vitro 10:257–264

De Boeck M, Kirsch-Volders M, Lison D (2003) Cobalt and antimony: genotoxicity and carcinogenicity. Mutat Res 533:135–152

Deng W, Poretz RD (2001a) Lead alters the developmental profile of the galactolipid metabolic enzymes in cultured oligodendrocyte lineage cells. Neurotoxicology 22:429–437

Deng W, Poretz RD (2001b) Lead exposure affects levels of galactolipid metabolic enzymes in the developing rat brain. Toxicol Appl Pharmacol 172:98–107

Deng W, McKinnon RD, Poretz RD (2001) Lead exposure delays the differentiation of oligodendroglial progenitors in vitro. Toxicol Appl Pharmacol 174:235–244

Dobson AW, Erikson KM, Aschner M (2004) Manganese neurotoxicity. Ann N Y Acad Sci 1012:115–128

Dodla MC, Mumaw J, Stice SL (2010) Role of astrocytes, soluble factors, cells adhesion molecules and neurotrophins in functional synapse formation: implications for human embryonic stem cell derived neurons. Curr Stem Cell Res Ther 5:251–260

Engle MJ, Volpe JJ (1990) Glutamine synthetase activity of developing astrocytes is inhibited in vitro by very low concentrations of lead. Brain Res Dev Brain Res 55:283–287

Erikson K, Aschner M (2002) Manganese causes differential regulation of glutamate transporter (GLAST) taurine transporter and metallothionein in cultured rat astrocytes. Neurotoxicology 23:595–602

Erikson KM, Thompson K, Aschner J, Aschner M (2007) Manganese neurotoxicity: a focus on the neonate. Pharmacol Ther 113:369–377

Falluel-Morel A, Lin L, Sokolowski K, McCandlish E, Buckley B, DiCicco-Bloom E (2012) N-acetyl cysteine treatment reduces mercury-induced neurotoxicity in the developing rat hippocampus. J Neurosci Res 90:743–750

Farber JL (1981) The role of calcium in cell death. Life Sci 29:1289–1295

Fern R, Black JA, Ransom BR, Waxman SG (1996) Cd(2+)-induced injury in CNS white matter. J Neurophysiol 76:3264–3273

Filipov NM, Seegal RF, Lawrence DA (2005) Manganese potentiates in vitro production of proinflammatory cytokines and nitric oxide by microglia through a nuclear factor kappa B-dependent mechanism. Toxicol Sci 84:139–148

Finney LA, O'Halloran TV (2003) Transition metal speciation in the cell: insights from the chemistry of metal ion receptors. Science 300:931–936

Frausto da Silva JJR, Williams RJP (2001) The biological chemistry of the elements: the inorganic chemistry of life. Oxford University Press, New York

Frederickson CJ, Koh JY, Bush AI (2005) The neurobiology of zinc in health and disease. Nat Rev Neurosci 6:449–462

Galaris D, Skiada V, Barbouti A (2008) Redox signaling and cancer: the role of "labile" iron. Cancer Lett 266:21–29

Galasso SL, Dyck RH (2007) The role of zinc in cerebral ischemia. Mol Med 13:380–387
Garcia SJ, Gellein K, Syversen T, Aschner M (2006) A manganese-enhanced diet alters brain metals and transporters in the developing rat. Toxicol Sci 92:516–525
Gavazzo P, Tedesco M, Chiappalone M, Zanardi I, Marchetti C (2011) Nickel modulates the electrical activity of cultured cortical neurons through a specific effect on N-methyl-D-aspartate receptor channels. Neuroscience 177:43–55
Giordano G, Pizzurro D, VanDeMark K, Guizzetti M, Costa LG (2009) Manganese inhibits the ability of astrocytes to promote neuronal differentiation. Toxicol Appl Pharmacol 240:226–235
Gu J, Royland JE, Wiggins RC, Konat GW (1997) Selenium is required for normal upregulation of myelin genes in differentiating oligodendrocytes. J Neurosci Res 47:626–635
Gu Y, Hua Y, Keep RF, Morgenstern LB, Xi G (2009) Deferoxamine reduces intracerebral hematoma-induced iron accumulation and neuronal death in piglets. Stroke 40:2241–2243
Gunnarson E, Axehult G, Baturina G, Zelenin S, Zelenina M, Aperia A (2005) Lead induces increased water permeability in astrocytes expressing aquaporin 4. Neuroscience 136:105–114
Gunter KK, Aschner M, Miller LM, Eliseev R, Salter J, Anderson K, Gunter TE (2006a) Determining the oxidation states of manganese in NT2 cells and cultured astrocytes. Neurobiol Aging 27:1816–1826
Gunter TE, Gavin CE, Aschner M, Gunter KK (2006b) Speciation of manganese in cells and mitochondria: a search for the proximal cause of manganese neurotoxicity. Neurotoxicology 27:765–776
Hamadani JD, Tofail F, Nermell B, Gardner R, Shiraji S, Bottai M, Arifeen SE, Huda SN, Vahter M (2011) Critical windows of exposure for arsenic-associated impairment of cognitive function in pre-school girls and boys: a population-based cohort study. Int J Epidemiol 40: 1593–1604
Hanna R, Doudna JA (2000) Metal ions in ribozyme folding and catalysis. Curr Opin Chem Biol 4:166–170
Hazell AS, Norenberg MD (1997) Manganese decreases glutamate uptake in cultured astrocytes. Neurochem Res 22:1443–1447
Hemdan S, Almazan G (2006) Iron contributes to dopamine-induced toxicity in oligodendrocyte progenitors. Neuropathol Appl Neurobiol 32:428–440
Hossain S, Liu HN, Nguyen M, Shore G, Almazan G (2009) Cadmium exposure induces mitochondria-dependent apoptosis in oligodendrocytes. Neurotoxicology 30:544–554
Houston MC (2011) Role of mercury toxicity in hypertension, cardiovascular disease, and stroke. J Clin Hypertens (Greenwich) 13:621–627
Hwang JJ, Lee SJ, Kim TY, Cho JH, Koh JY (2008) Zinc and 4-hydroxy-2-nonenal mediate lysosomal membrane permeabilization induced by H2O2 in cultured hippocampal neurons. J Neurosci 28:3114–3122
Im JY, Paik SG, Han PL (2006) Cadmium-induced astroglial death proceeds via glutathione depletion. J Neurosci Res 83:301–308
Jiang H, Liu F, Yang H, Li Y (2012) Effects of cobalt nanoparticles on human T cells in vitro. Biol Trace Elem Res 146:23–29
Kapaj S, Peterson H, Liber K, Bhattacharya P (2006) Human health effects from chronic arsenic poisoning—a review. J Environ Sci Health A Tox Hazard Subst Environ Eng 41:2399–2428
Kim P, Choi BH (1995) Selective inhibition of glutamate uptake by mercury in cultured mouse astrocytes. Yonsei Med J 36:299–305
Kranich O, Hamprecht B, Dringen R (1996) Different preferences in the utilization of amino acids for glutathione synthesis in cultured neurons and astroglial cells derived from rat brain. Neurosci Lett 219:211–214
Kurz T, Terman A, Gustafsson B, Brunk UT (2008) Lysosomes in iron metabolism, ageing and apoptosis. Histochem Cell Biol 129:389–406
Lee E, Yin Z, Sidoryk-Wegrzynowicz M, Jiang H, Aschner M (2012) 15-Deoxy-delta12,14-prostaglandin J(2) modulates manganese-induced activation of the NF-kappaB, Nrf2, and PI3K pathways in astrocytes. Free Radic Biol Med 52:1067–1074

LeVine SM (1991) Oligodendrocytes and myelin sheaths in normal, quaking and shiverer brains are enriched in iron. J Neurosci Res 29:413–419
LeVine SM, Macklin WB (1990) Iron-enriched oligodendrocytes: a reexamination of their spatial distribution. J Neurosci Res 26:508–512
Li YV (2012) Zinc overload in stroke. In: Li YV, Zhang JH (eds) Metal ion in stroke. Springer, New York, pp 167–189
Li YV, Zhang JH (2012a) Metal ion in stroke. Springer, New York
Li YV, Zhang JH (2012b) Metal ions in stroke pathophysiology. In: Li YV, Zhang JH (eds) Metal ion in stroke. Springer, New York, pp 1–13
Liao SL, Ou YC, Lin SY, Kao TK, Pan HC, Chang CY, Lai CY, Lu HC, Wang WY, Chen CJ (2011) Signaling cascades mediate astrocyte death induced by zinc. Toxicol Lett 204:108–117
Liu YP, Yang CS, Tzeng SF (2008) Inhibitory regulation of glutamate aspartate transporter (GLAST) expression in astrocytes by cadmium-induced calcium influx. J Neurochem 105: 137–150
Lou M, Lieb K, Selim M (2009) The relationship between hematoma iron content and perihematoma edema: an MRI study. Cerebrovasc Dis 27:266–271
MacDonald JF, Xiong ZG, Jackson MF (2006) Paradox of Ca2+ signaling, cell death and stroke. Trends Neurosci 29:75–81
Medvedeva YV, Lin B, Shuttleworth CW, Weiss JH (2009) Intracellular Zn2+ accumulation contributes to synaptic failure, mitochondrial depolarization, and cell death in an acute slice oxygen-glucose deprivation model of ischemia. J Neurosci 29:1105–1114
Michalke B, Berthele A, Mistriotis P, Ochsenkuhn-Petropoulou M, Halbach S (2007) Manganese species from human serum, cerebrospinal fluid analyzed by size exclusion chromatography-, capillary electrophoresis coupled to inductively coupled plasma mass spectrometry. J Trace Elem Med Biol 21(suppl 1):4–9
Milatovic D, Zaja-Milatovic S, Gupta RC, Yu Y, Aschner M (2009) Oxidative damage and neurodegeneration in manganese-induced neurotoxicity. Toxicol Appl Pharmacol 240:219–225
Modi HR, Katyare SS (2009) Cadmium exposure-induced alterations in the lipid/phospholipids composition of rat brain microsomes and mitochondria. Neurosci Lett 464:108–112
Morath DJ, Mayer-Proschel M (2001) Iron modulates the differentiation of a distinct population of glial precursor cells into oligodendrocytes. Dev Biol 237:232–243
Nayak P, Sharma SB, Chowdary NV (2011) Augmentation of aluminum-induced oxidative stress in rat cerebrum by presence of pro-oxidant (graded doses of ethanol) exposure. Neurochem Res 35:1681–1690
Nicchia GP, Frigeri A, Liuzzi GM, Santacroce MP, Nico B, Procino G, Quondamatteo F, Herken R, Roncali L, Svelto M (2000) Aquaporin-4-containing astrocytes sustain a temperature- and mercury-insensitive swelling in vitro. Glia 31:29–38
Nielsen S, Nagelhus EA, Amiry-Moghaddam M, Bourque C, Agre P, Ottersen OP (1997) Specialized membrane domains for water transport in glial cells: high-resolution immunogold cytochemistry of aquaporin-4 in rat brain. J Neurosci 17:171–180
Okuda B, Iwamoto Y, Tachibana H, Sugita M (1997) Parkinsonism after acute cadmium poisoning. Clin Neurol Neurosurg 99:263–265
Olivieri G, Hess C, Savaskan E, Ly C, Meier F, Baysang G, Brockhaus M, Muller-Spahn F (2001) Melatonin protects SHSY5Y neuroblastoma cells from cobalt-induced oxidative stress, neurotoxicity and increased beta-amyloid secretion. J Pineal Res 31:320–325
Panayi AE, Spyrou NM, Iversen BS, White MA, Part P (2002) Determination of cadmium and zinc in Alzheimer's brain tissue using inductively coupled plasma mass spectrometry. J Neurol Sci 195:1–10
Perez de la Ossa N, Sobrino T, Silva Y, Blanco M, Millan M, Gomis M, Agulla J, Araya P, Reverte S, Serena J, Davalos A (2010) Iron-related brain damage in patients with intracerebral hemorrhage. Stroke 41:810–813
Raichle ME (1983) The pathophysiology of brain ischemia. Ann Neurol 13:2–10
Rama Rao KV, Reddy PV, Hazell AS, Norenberg MD (2007) Manganese induces cell swelling in cultured astrocytes. Neurotoxicology 28:807–812

Rao KV, Norenberg MD (2004) Manganese induces the mitochondrial permeability transition in cultured astrocytes. J Biol Chem 279:32333–32338
Rao KV, Jayakumar AR, Reddy PV, Tong X, Curtis KM, Norenberg MD (2010) Aquaporin-4 in manganese-treated cultured astrocytes. Glia 58:1490–1499
Rathnasamy G, Ling EA, Kaur C (2011) Iron and iron regulatory proteins in amoeboid microglial cells are linked to oligodendrocyte death in hypoxic neonatal rat periventricular white matter through production of proinflammatory cytokines and reactive oxygen/nitrogen species. J Neurosci 31:17982–17995
Reddy PV, Rao KV, Norenberg MD (2008) The mitochondrial permeability transition, and oxidative and nitrosative stress in the mechanism of copper toxicity in cultured neurons and astrocytes. Lab Invest 88:816–830
Reisinger J, Hollinger K, Lang W, Steiner C, Winter T, Winter A, Mori M, Lindorfer A, Kiblbock D, Siostrzonek P (2009) Does early administration of selenium improve neurological outcome after cardiac arrest? Am J Emerg Med 27:176–181
Ronnback L, Hansson E (1992) Chronic encephalopathies induced by mercury or lead: aspects of underlying cellular and molecular mechanisms. Br J Ind Med 49:233–240
Sagara J, Makino N, Bannai S (1996) Glutathione efflux from cultured astrocytes. J Neurochem 66:1876–1881
Sanfeliu C, Sebastia J, Ki SU (2001) Methylmercury neurotoxicity in cultures of human neurons, astrocytes, neuroblastoma cells. Neurotoxicology 22:317–327
Savaskan NE, Brauer AU, Kuhbacher M, Eyupoglu IY, Kyriakopoulos A, Ninnemann O, Behne D, Nitsch R (2003) Selenium deficiency increases susceptibility to glutamate-induced excitotoxicity. FASEB J 17:112–114
Sawada J, Kikuchi Y, Shibutani M, Mitsumori K, Inoue K, Kasahara T (1994) Induction of metallothionein in astrocytes by cytokines and heavy metals. Biol Signals 3:157–168
Scheiber IF, Dringen R (2013) Astrocyte functions in the copper homeostasis of the brain. Neurochem Int 62(5):556–565
Scheiber IF, Schmidt MM, Dringen R (2010) Zinc prevents the copper-induced damage of cultured astrocytes. Neurochem Int 57:314–322
Selim MH, Ratan RR (2004) The role of iron neurotoxicity in ischemic stroke. Ageing Res Rev 3:345–353
Selvin-Testa A, Capani F, Loidl CF, Lopez EM, Pecci-Saavedra J (1997) Prenatal and postnatal lead exposure induces 70 kDa heat shock protein in young rat brain prior to changes in astrocyte cytoskeleton. Neurotoxicology 18:805–817
Sensi SL, Paoletti P, Bush AI, Sekler I (2009) Zinc in the physiology and pathology of the CNS. Nat Rev Neurosci 10:780–791
Shanker G, Aschner M (2001) Identification and characterization of uptake systems for cystine and cysteine in cultured astrocytes and neurons: evidence for methylmercury-targeted disruption of astrocyte transport. J Neurosci Res 66:998–1002
Shukla D, Saxena S, Purushothaman J, Shrivastava K, Singh M, Shukla S, Malhotra VK, Mustoori S, Bansal A (2011) Hypoxic preconditioning with cobalt ameliorates hypobaric hypoxia induced pulmonary edema in rat. Eur J Pharmacol 656:101–109
Shukla A, Shukla GS, Srimal RC (1996) Cadmium-induced alterations in blood-brain barrier permeability and its possible correlation with decreased microvessel antioxidant potential in rat. Hum Exp Toxicol 15:400–405
Sierra EM, Tiffany-Castiglioni E (1991) Reduction of glutamine synthetase activity in astroglia exposed in culture to low levels of inorganic lead. Toxicology 65:295–304
Smith GM, Strunz C (2005) Growth factor and cytokine regulation of chondroitin sulfate proteoglycans by astrocytes. Glia 52:209–218
Stabler SP, Allen RH (2004) Vitamin B12 deficiency as a worldwide problem. Annu Rev Nutr 24:299–326
Stork CJ, Li YV (2006) Intracellular zinc elevation measured with a “calcium-specific” indicator during ischemia and reperfusion in rat hippocampus: a question on calcium overload. J Neurosci 26:10430–10437

Stork CJ, Li YV (2009) Rising zinc: a significant cause of ischemic neuronal death in the CA1 region of rat hippocampus. J Cereb Blood Flow Metab 29(8):1399–1408
Stork CJ, Li YV (2010) Zinc release from thapsigargin/IP3-sensitive stores in cultured cortical neurons. J Mol Signal 5:5
Struzynska L, Chalimoniuk M, Sulkowski G (2005) Changes in expression of neuronal and glial glutamate transporters in lead-exposed adult rat brain. Neurochem Int 47:326–333
Suh SW, Aoyama K, Alano CC, Anderson CM, Hamby AM, Swanson RA (2007) Zinc inhibits astrocyte glutamate uptake by activation of poly(ADP-ribose) polymerase-1. Mol Med 13: 344–349
Tiffany-Castiglioni E, Hong S, Qian Y (2011) Copper handling by astrocytes: insights into neurodegenerative diseases. Int J Dev Neurosci 29:811–818
Tjalkens RB, Zoran MJ, Mohl B, Barhoumi R (2006) Manganese suppresses ATP-dependent intercellular calcium waves in astrocyte networks through alteration of mitochondrial and endoplasmic reticulum calcium dynamics. Brain Res 1113:210–219
Todorich B, Pasquini JM, Garcia CI, Paez PM, Connor JR (2009) Oligodendrocytes and myelination: the role of iron. Glia 57:467–478
Todorich B, Zhang X, Connor JR (2011) H-ferritin is the major source of iron for oligodendrocytes. Glia 59:927–935
Toimela T, Maenpaa H, Mannerstrom M, Tahti H (2004) Development of an in vitro blood-brain barrier model-cytotoxicity of mercury and aluminum. Toxicol Appl Pharmacol 195:73–82
Vajda Z, Pedersen M, Fuchtbauer EM, Wertz K, Stodkilde-Jorgensen H, Sulyok E, Doczi T, Neely JD, Agre P, Frokiaer J, Nielsen S (2002) Delayed onset of brain edema and mislocalization of aquaporin-4 in dystrophin-null transgenic mice. Proc Natl Acad Sci U S A 99:13131–13136
Wang CH, Hsiao CK, Chen CL, Hsu LI, Chiou HY, Chen SY, Hsueh YM, Wu MM, Chen CJ (2007) A review of the epidemiologic literature on the role of environmental arsenic exposure and cardiovascular diseases. Toxicol Appl Pharmacol 222:315–326
Watts LT, Rathinam ML, Schenker S, Henderson GI (2005) Astrocytes protect neurons from ethanol-induced oxidative stress and apoptotic death. J Neurosci Res 80:655–666
Wei G, Hough CJ, Li Y, Sarvey JM (2004) Characterization of extracellular accumulation of Zn2+ during ischemia and reperfusion of hippocampus slices in rat. Neuroscience 125:867–877
Woodruff TM, Thundyil J, Tang SC, Sobey CG, Taylor SM, Arumugam TV (2011) Pathophysiology, treatment, and animal and cellular models of human ischemic stroke. Mol Neurodegener 6:11
Zecca L, Youdim MB, Riederer P, Connor JR, Crichton RR (2004) Iron, brain ageing and neurodegenerative disorders. Nat Rev Neurosci 5:863–873
Zhang Y, Aizenman E, DeFranco DB, Rosenberg PA (2007) Intracellular zinc release, 12-lipoxygenase activation and MAPK dependent neuronal and oligodendroglial death. Mol Med 13:350–355
Zheng W, Zhao Q, Slavkovich V, Aschner M, Graziano JH (1999) Alteration of iron homeostasis following chronic exposure to manganese in rats. Brain Res 833:125–132
Zwingmann C, Leibfritz D, Hazell AS (2004) Brain energy metabolism in a sub-acute rat model of manganese neurotoxicity: an ex vivo nuclear magnetic resonance study using [1-13C]glucose. Neurotoxicology 25:573–587

Chapter 24
Anesthesia and White Matter Injury

Phillip Vlisides and Zhongcong Xie

24.1 Introduction

Since the first public demonstration of ether anesthesia in 1846, the exact mechanisms of anesthesia as well as any potentially neurotoxic effects of anesthetics remain to be clearly defined. With roughly 234 million people receiving anesthesia care for surgery annually worldwide (Weiser et al. 2008), it remains imperative to elucidate potentially neurotoxic effects of anesthetics and to administer them in the safest way possible. There is growing preclinical evidence that various anesthetic agents leave different cellular and molecular signatures of neurotoxicity in animal and cell-culture models. This may be of concern in certain populations with a potentially vulnerable cognitive cache, such as the young and the elderly. Additionally, anesthetics may in fact directly modulate the pathophysiologic substrates of various central nervous system (CNS) disease entities. In particular, different anesthetic agents and approaches may affect clinical outcomes for those with CNS disease such as Alzheimer's disease (AD), stroke, and traumatic brain injury (TBI), though much clinical work remains to consistently test this hypothesis.

Though laboratory data have generated the hypothesis that different anesthetic medications may have varying degrees of neurotoxicity, the clinical relevance of such a postulate has yet to be determined. In this chapter, we will describe both the intrinsic properties of anesthetic-mediated neurotoxicity as well as the mechanisms by which anesthetics may directly influence the pathophysiology of various CNS diseases. Both preclinical and clinical evidence to date on these subjects will be reviewed and discussed.

P. Vlisides, M.D. • Z. Xie, M.D., Ph.D. (✉)
Department of Anesthesia, Critical Care, and Pain Medicine,
Massachusetts General Hospital, Boston, MA, USA
e-mail: zxie@partners.org

S. Baltan et al. (eds.), *White Matter Injury in Stroke and CNS Disease*, Springer Series in Translational Stroke Research 4, DOI 10.1007/978-1-4614-9123-1_24,

24.2 Neurotoxicity of Anesthetics

As mentioned above, there is a growing concern over the potential for intrinsic neurotoxic side effects of anesthetics. Specifically, these concerns rest largely in populations with neuronal susceptibility to toxicity, particularly infants, children, and the elderly. Below, we will discuss two populations—the young and the elderly—in which deleterious neurologic effects of anesthetics may be of particular concern.

24.2.1 Anesthesia and Neurodevelopment

Many preclinical studies have alluded to the potentially neurotoxic effects of anesthetics on the young brain and neurodevelopment (Jevtovic-Todorovic et al. 2003; Sun 2010). Specifically, both isoflurane and sevoflurane have been shown to induce apoptosis (Lu et al. 2010; Satomoto et al. 2009; Yang et al. 2008; Zhang et al. 2010), and growth of rat hippocampal neural precursors have also been inhibited by isoflurane (Sall et al. 2009). Numerous studies of anesthetics and neurodevelopment have been analyzed in different animal species. Rodents exposed to both isoflurane and sevoflurane in utero have demonstrated impaired behavior, learning, and memory (Palanisamy et al. 2011; Zheng et al. 2013). Isoflurane has also been demonstrated to induce neuroapoptosis in the developing brain of mice, rats, and rhesus monkeys along with ketamine (Sun 2010; Creeley and Olney 2010). Desflurane, a newer volatile anesthetic agent, has also been recently demonstrated to induce neuroapoptosis and impair working memory in neonatal mice (Kodama et al. 2011). Different studies, however, have shown that desflurane might not induce neurotoxicity and impairment of learning and memory in young mice (Shen et al. 2013). Exactly how this anesthetic-induced neurodegeneration plays a role in the ultimate cytoarchitecture of developing brains (if at all) remains unclear. Enough concern has been generated, however, to perform more preclinical studies and to begin clinical investigation of anesthetic exposure to the developing human brain.

To examine the question of anesthetic exposure in the young and cognitive development, a number of observational studies have been carried out to analyze this relationship. Multiple retrospective studies have demonstrated a link between multiple anesthetic exposures and subsequent learning disabilities later in life (Wilder et al. 2009; Flick et al. 2011; DiMaggio et al. 2011). These studies do have the caveat, however, of being retrospective in nature, and multiple confounders are present. In fact, other studies have failed to find any association. Data from the Netherlands Twin Registry demonstrate that children exposed to anesthesia before 3 years of age had no increased association with cognitive problems compared to their sibling twin unexposed to anesthesia (Bartels et al. 2009). These twin pairs did, however, have a lower overall educational achievement level and more cognitive problems compared to twin pairs not exposed to anesthesia. An alternative hypothesis, which the

study brings forth, is that children who require anesthesia and surgical services early in life may be more predisposed to the development of learning disorders later in life. The cause of these learning disorders, however, may not be the anesthetic exposure per se. Ultimately, randomized, controlled, prospective trials will be needed to further answer this question. At present, two large-scale, multicenter trials are ongoing which will hopefully further answer these questions (Sun 2010).

24.2.2 Anesthesia and Cognitive Dysfunction

On the other end of the age spectrum, great efforts are being undertaken to examine if anesthetic exposure may facilitate cognitive dysfunction in the elderly. Notably, it has been suggested that anesthetics may exacerbate AD pathology in numerous animal models (Xie et al. 2006, 2008; Eckenhoff et al. 2004), though this will be discussed in further detail below. Multiple studies have demonstrated memory impairment in aged rats weeks after anesthetic exposure, with isoflurane and nitrous oxide being implicated in particular (Culley et al. 2004, 2007). Isoflurane has also been suggested to induce mitochondrial dysfunction and impairment of learning and memory in mice (Zhang et al. 2012b). Other data demonstrate that isoflurane increases hippocampal inflammatory cytokine expression and spatial-memory impairment weeks after exposure (Lin and Zuo 2011). Of note, however, when studied months later, rats exposed to isoflurane demonstrated no signs of neurodegeneration or cognitive dysfunction (Stratmann et al. 2010), pointing to the possibility that anesthetic-induced neurotoxicity may be a transient phenomenon which resolves over time.

Although some studies have suggested that postoperative cognitive dysfunction (POCD) may not last long (5 % at 6 months and 1 % at 12 months) (Abildstrom et al. 2000; Williams-Russo et al. 1995), there is a consensus that even if POCD only lasts for a short period of time, it can still have a major impact on the quality of life of elderly people and their caregivers. Short-term POCD can still negatively affect the post-discharge functioning of patients, such as taking medications, providing self-care, and so forth, which could ultimately cause other adverse health outcomes (Leung and Sands 2009). Likewise, POCD both at hospital discharge and 3 months after discharge has been associated with increased mortality (Monk et al. 2008). Ultimately, clinical studies have also been warranted to examine the question of anesthesia-induced cognitive dysfunction in the elderly (Zhang et al. 2012a).

Clinical data have also been accumulating with regard to the risk factors for POCD. Unfortunately, many confounders surface while trying to study POCD. Though the type of anesthetic exposure is often the focus of such studies, factors such as age, coincident medical illness, patient demographics, type of surgery, perioperative course, and lack of clear consensus as to the exact definition of POCD all make for a difficult course while trying to study anesthetics and POCD (Monk et al. 2008; Newman et al. 2007). Despite challenges, many studies have been undertaken in attempt to assess the effects of anesthesia on cognitive dysfunction in the elderly.

To date, studies comparing regional anesthesia and general anesthesia have not largely demonstrated differences in POCD incidence (Rasmussen et al. 2003; Newman et al. 2007). A retrospective study analyzing patients with major illness and those who underwent surgery revealed that neither non-demented nor mildly demented patients demonstrated an accelerated long-term decline compared to controls (Avidan et al. 2009). This was shown after modeling to plot cognitive trajectory 2 years prior to the "event" as well as for several years afterwards. With regard to comparing anesthetic agents, many small, underpowered studies exist that do not show differences in rates of POCD (Rasmussen et al. 2006; Coburn et al. 2007; Rortgen et al. 2010; Hocker et al. 2009). This has also recently been re-demonstrated when comparing propofol and isoflurane in a prospective, randomized trial; preoperative cognitive status was in fact significantly associated with postoperative delirium (POD) (Monk et al. 2011). Differences in POCD incidence have, however, recently been demonstrated in a small pilot study comparing desflurane and isoflurane (Zhang et al. 2012a). More clinical data will be needed to further support or refute these notions. Whether or not this paradigm shifts in patients with specific disease states (i.e., AD) is also unclear. Clinical data will also be needed to assess if certain anesthetic agents play a role in altering cognitive function in patients with certain CNS diseases, which will be discussed further below.

24.3 Anesthetics and CNS Disease

We have described above some of the preclinical and clinical data that exist describing the potential intrinsic neurotoxic side effects of anesthetics. Below, we will discuss how certain anesthetic agents and approaches may specifically interact with and modify preexisting CNS disease. Ultimately, with more data, clinical guidelines may one day be developed to guide decision-making with regard to anesthetic approaches in patients with specific CNS disease pathologies.

24.3.1 Anesthetics and Alzheimer's Disease Pathophysiology

Perhaps most concerning is the possibility that anesthetics may in fact accelerate AD pathophysiology in CNS white matter. Currently, there are about 8.5 million people with AD who undergo surgery each year worldwide (Zhang et al. 2012b; Thies and Bleiler 2011; Silbert et al. 2011), and that number is expected to continue to grow (Alzheimer's Association 2012). The main mechanisms by which anesthetics are thought to contribute to AD pathology include β-amyloid protein (Aβ)-mediated toxicity (Eckenhoff et al. 2004; Xie et al. 2006, 2008; Whittington et al. 2011) and tau protein hyperphosphorylation with resulting neurofibrillary tangle (NFT) formation (Planel et al. 2007, 2009; Whittington et al. 2011; Dong et al. 2012). Indeed, both isoflurane and sevoflurane have been shown to enhance Aβ production

and Aβ-mediated apoptosis (Xie et al. 2006, 2008; Dong et al. 2009; Lu et al. 2010). Propofol, an intravenous anesthetic, has been shown to induce tau protein hyperphosphorylation with resulting NFT formation (Whittington et al. 2011). Isoflurane has also been implicated in this pathophysiology as well when accompanied by anesthesia-induced hypothermia (Planel et al. 2008, 2009), though this has recently been demonstrated under normothermic conditions as well (Dong et al. 2012). Finally, sevoflurane has also recently been demonstrated to induce tau phosphorylation in mice (Le Freche et al. 2012).

Interestingly, a newer inhalational anesthetic agent, desflurane has been associated with different cellular and molecular effects on cell cultures, and in some cases, even different outcomes in terms of learning and memory in animals. Desflurane has recently been shown to not cause Aβ secretion or amyloid protein processing in human cell cultures (Zhang et al. 2008). Desflurane is also associated with less mitochondrial damage in mouse cell cultures and less learning and memory impairment in mice compared to isoflurane (Zhang et al. 2012b). Additionally, increased cerebrospinal fluid (CSF) levels of Aβ have recently been noted in humans exposed to isoflurane compared to those exposed to desflurane (Zhang et al. 2013). Whether or not these findings are of clinical significance has yet to be determined, although a recent pilot study with small sample size (15 patients per group) has shown that surgery under isoflurane anesthesia, but not desflurane anesthesia, may be associated with cognitive function decline (Zhang et al. 2012a).

Ultimately, more basic science investigation is needed to further explore and elucidate the mechanisms by which anesthetics exert their effects on preexisting AD pathology. As described above, different anesthetic agents seem to leave different cellular and molecular signatures in animal models with AD pathology. Whether or not these signatures are clinically significant remains to be determined. Clinical studies examining various anesthetic agents and their differential effects on AD pathology and trajectory are likely needed to substantiate preclinical evidence to date. Specifically, randomized, controlled, prospective trials comparing various anesthetic agents in patients with AD may demonstrate differences in cognitive trajectory and outcomes among different anesthetic agents.

24.3.2 Anesthesia and Cerebral Ischemia

A number of studies have examined the notion that volatile anesthetic agents may afford neuroprotection during periods of ischemia. In neonatal piglets undergoing low-flow cardiopulmonary bypass (CPB) and deep hypothermic circulatory arrest (DHCA), use of desflurane was associated with less histologic damage and improved neurologic outcomes compared to the use of fentanyl and droperidol (Loepke et al. 2002; Kurth et al. 2001). Isoflurane has been reported to be associated with decreased electroencephalographic signs of ischemia compared to older agents in patients undergoing carotid endarterectomy (Michenfelder et al. 1987; Messick et al. 1987). Various animal stroke models have also demonstrated reduced infarct size and

Table 24.1 Proposed benefits of general and local anesthesia for endovascular management of acute stroke

General anesthesia	Local anesthesia/sedation
Immobility	Neurologic assessments possible
Strict BP control	Less abolishment of cardiovascular reflexes
Controlled anesthesia induction	Avoid risks of general anesthesia
Airway control	No increased hemorrhage rate[a]
?Hypothermic benefits	?Fewer complications[a]
?Neuroprotection[b]	?Better outcomes[a]

[a]Suggested based on retrospective studies (Jumaa et al. 2010; Davis et al. 2012)
[b]As discussed in the text, anesthetic neuroprotection during periods of ischemia has not been conclusively demonstrated clinically
? = Postulated, though unproven, benefit to each anesthetic approach

neurologic damage with isoflurane preconditioning (Liu et al. 2006; Kapinya et al. 2002a, b; Blanck et al. 2000; Li et al. 2013).

Unfortunately, anesthetic ischemic neuroprotection in the clinical setting has been difficult to demonstrate. Trials examining jugular venous oxygen saturation ($SjvO_2$) during cardiac surgery have not shown a consistent protection from desaturation among various types of anesthetics (Kadoi et al. 2003; Nandate et al. 2000; Souter et al. 1998). In a study directly comparing patients anesthetized with either isoflurane or propofol during cardiac surgery, there were no differences in neurologic outcome between groups, though the sample size (20 patients) was small (Kanbak et al. 2004). Perhaps part of the issue is that patients undergoing surgery during which cerebral ischemia may occur (i.e., cardiac surgery) will receive anesthetics for the surgery itself; the question then becomes whether or not certain anesthetic agents afford more neuroprotection compared to others. Though laboratory studies demonstrate the possibility of ischemic neuroprotection with specific, individual agents when studied, no single anesthetic agent to date has been clinically shown to better protect against ischemic insult compared to others.

With regard to clinical management of ischemic stroke, there is emerging controversy as to when general anesthesia should be instituted prior to endovascular treatment of ischemic stroke. Proponents of general anesthesia argue that strict blood pressure control, inhibition of patient movement (especially during angiography and intracranial catheter placement), mild hypothermia induced by general anesthesia, and obviating the potential need to convert to general anesthesia in a crisis situation may lead to improved patient outcomes (Brekenfeld et al. 2010). Those who generally favor local anesthesia with sedation have argued that recent trials have demonstrated neither increased cerebral hemorrhage (i.e., surrogate for vessel perforation from catheter placement) in patients receiving sedation compared to general anesthesia, nor a high rate of conversion from sedation to general anesthesia (Gupta 2010; Abou-Chebl et al. 2010; Jumaa et al. 2010). These comparisons have been summarized in Table 24.1. Further, studies thus far have demonstrated worse outcomes in those managed with general anesthesia for endovascular stroke treatment (Davis et al. 2012; Jumaa et al. 2010), though patients who received general anesthesia also seemed to be sicker at baseline in these studies. As the controversy

persists, anesthetic decision-making at present likely remains on a case-by-case basis depending on the clinical scenario.

24.3.3 Traumatic Brain Injury

In addition to the primary, mechanical insult sustained from TBI, subsequent secondary brain injury results from deleterious inflammation as well as metabolic and cellular changes from the TBI (McIntosh et al. 1996). As such, many preclinical investigations have been undertaken with the goal of better understanding TBI pathophysiology. Among those investigations are studies examining different anesthetic agents and their potential for modulating this secondary pathophysiologic cascade. Specific anesthetic agents have been shown to have varied effects in animal and cell-culture models of TBI. For example, propofol has recently been studied in a cell-culture model of TBI. Propofol was shown to attenuate microglial cell-mediated release of the proinflammatory markers tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and nitric oxide (NO) when exposed to an increased ambient pressure (30 mmHg, aimed to mimic increased intracranial pressure [ICP]) (Yu et al. 2011). Phagocytosis was also limited when cells were exposed to propofol. The authors suggest that this propofol-induced modulation of microglial phagocytic activity and cytokine release may alleviate neuroinflammation associated with TBI. Additionally, propofol was also shown to be protective in mice hippocampal cell cultures when exposed to mechanical TBI (Rossaint et al. 2009). Cell cultures exposed to propofol demonstrated a dose-dependent decrease in total and secondary tissue trauma. In similar mice models of TBI, use of isoflurane to induce controlled cortical impact (i.e., the TBI insult) was associated with smaller contusion and better neurologic outcome (Luh et al. 2011). Rats exposed to isoflurane in an experimental model of TBI also had the best cognitive recovery compared to six other anesthetic agents (Statler et al. 2006). Perhaps conversely, sevoflurane was associated with decreased brain edema formation and less blood–brain barrier (BBB) disruption compared to isoflurane in a similar model of TBI in mice (Thal et al. 2012). Though some conflicting data exist on anesthetic-mediated effects on neuroinflammation and BBB integrity in laboratory models of TBI, the idea that anesthetic medications differentially affect inflammatory cascades, BBB integrity, and total tissue trauma during TBI is an intriguing premise. Certainly, more data are needed to further clarify and define inhalational and intravenous anesthetics' effects on TBI pathophysiology.

Clinically, very few data are available on outcomes related to different anesthetic approaches to TBI cases. In addition to experimental evidence that propofol may afford some degree of neuroprotection in the TBI setting, propofol has also clinically been shown to produce positive effects on cerebral physiology—notably maintained cerebral pressure, decreased cerebral blood flow (CBF), decreased cerebral metabolic rate ($CMRO_2$), and decreased ICP (Petersen et al. 2003; Kaisti et al. 2002; Ludbrook et al. 2002). Thus, propofol seems like an attractive choice as an anesthetic

agent for intra-operative TBI management. Unfortunately, however, few data exist regarding the effects of various anesthetics on TBI outcomes. Nevertheless, an anesthetic approach that optimizes the patient's cerebral physiology and adherence to TBI management guidelines remains paramount (Sharma and Vavilala 2012; Bratton et al. 2007).

24.3.4 Anesthetics and Neuroinflammation

An additional finding is that anesthetic agents may cause a modulation of neuroinflammation. This phenomenon is of particular interest, as neuroinflammation has been demonstrated to induce various cognitive deficits in both animal models and humans (Wang et al. 2012; Yirmiya and Goshen 2011; Dantzer et al. 2008). Presently, there is a growing focus that the deleterious effects on learning and memory may be more detrimental than previously perceived. As such, anesthetic-mediated modulation of neuroinflammation may conceivably affect clinical outcomes in pathologic situations associated with neuroinflammation.

Recent studies have shown both isoflurane (Wu et al. 2012) and sevoflurane (Shen et al. 2013; Zheng et al. 2013) may induce neuroinflammation and lead to cognitive impairment. A particular realm of concern lies with the effects of inflammation on learning and memory. In mice, the cytokine IL-1β has been shown to impair memory via the α-a5-subunit-containing gamma-aminobutyric acid type A (α-5$GABA_A$) receptor subunit (Wang et al. 2012). Activation of this same receptor is a mechanism by which isoflurane induces memory deficits in mice after anesthesia (Zurek et al. 2012). Thus, treatment of an inverse α-5$GABA_A$ agonist may plausibly prevent inflammatory- or anesthetic-induced memory deficits via α-5$GABA_A$ activation. This has indeed been shown in mice, where IL-1β-induced memory deficits were prevented in mice after α-5$GABA_A$ gene deletion or inverse agonist administration (Wang et al. 2012). Additionally, α-5$GABA_A$ inverse agonists have also prevented isoflurane-induced memory deficits (Zurek et al. 2012; Saab et al. 2010). These findings present an interesting pathway by which pharmacologic intervention (i.e., administration of an α-5$GABA_A$ inverse agonist) may prevent anesthetic-induced memory deficits. This of course, however, would need to be substantiated with clinical trials.

24.4 Conclusions

As outlined above, anesthetic agents could play central role in clinical care of patients with neurologic disease. For patients on both ends of the age spectrum, anesthetics may potentially harm the cognitive function in these potentially vulnerable patients. While compelling laboratory studies have hinted at this theme, ongoing clinical trials will hopefully shed light on the clinical relevance of this possibility.

With regard to anesthetic care in patients with preexisting neurologic disease, we have also seen from various laboratory studies that anesthetics may modulate the pathophysiology of various neurologic disease states. Further clinical investigation needs to be completed to substantiate this claim, especially in the arenas of patients with Alzheimer's disease, stroke, and TBI. Lastly, anesthetic agents may also affect the cellular and molecular responses of neuroinflammation within the CNS. Certain deleterious endpoints of neuroinflammation, such as deficits in learning and memory, may be mitigated by targeted pharmacologic intervention. Therefore, it is possible that anesthetics may induce learning and memory impairments via neuroinflammation. Therapeutic strategies based on these findings may also be used to prevent cognitive injury (i.e., memory deficits) induced from anesthesia, which is a paradigm that has been demonstrated in the laboratory. Ultimately, this is an exciting time in the fields of neuroscience and anesthesiology. Certainly, there is much to discover within this realm and more work to be done to better understand the effects of anesthesia on the CNS.

References

Abildstrom H, Rasmussen LS, Rentowl P, Hanning CD, Rasmussen H, Kristensen PA, Moller JT (2000) Cognitive dysfunction 1–2 years after non-cardiac surgery in the elderly. ISPOCD group. International Study of Post-Operative Cognitive Dysfunction. Acta Anaesthesiol Scand 44(10):1246–1251

Abou-Chebl A, Lin R, Hussain MS, Jovin TG, Levy EI, Liebeskind DS, Yoo AJ, Hsu DP, Rymer MM, Tayal AH, Zaidat OO, Natarajan SK, Nogueira RG, Nanda A, Tian M, Hao Q, Kalia JS, Nguyen TN, Chen M, Gupta R (2010) Conscious sedation versus general anesthesia during endovascular therapy for acute anterior circulation stroke: preliminary results from a retrospective, multicenter study. Stroke 41(6):1175–1179. doi:10.1161/STROKEAHA.109.574129, STROKEAHA.109.574129 [pii]

Alzheimer's Association (2012) 2012 Alzheimer's disease facts and figures. Alzheimers Dement 8(2):131–168. doi:10.1016/j.jalz.2012.02.001, S1552-5260(12)00032-5 [pii]

Avidan MS, Searleman AC, Storandt M, Barnett K, Vannucci A, Saager L, Xiong C, Grant EA, Kaiser D, Morris JC, Evers AS (2009) Long-term cognitive decline in older subjects was not attributable to noncardiac surgery or major illness. Anesthesiology 111(5):964–970. doi:10.1097/ALN.0b013e3181bc9719

Bartels M, Althoff RR, Boomsma DI (2009) Anesthesia and cognitive performance in children: no evidence for a causal relationship. Twin Res Hum Genet 12(3):246–253. doi:10.1375/twin.12.3.246, 10.1375/twin.12.3.246 [pii]

Blanck TJ, Haile M, Xu F, Zhang J, Heerdt P, Veselis RA, Beckman J, Kang R, Adamo A, Hemmings H (2000) Isoflurane pretreatment ameliorates postischemic neurologic dysfunction and preserves hippocampal Ca2+/calmodulin-dependent protein kinase in a canine cardiac arrest model. Anesthesiology 93(5):1285–1293

Bratton SL, Chestnut RM, Ghajar J, McConnell Hammond FF, Harris OA, Hartl R, Manley GT, Nemecek A, Newell DW, Rosenthal G, Schouten J, Shutter L, Timmons SD, Ullman JS, Videtta W, Wilberger JE, Wright DW (2007) Guidelines for the management of severe traumatic brain injury. XI. Anesthetics, analgesics, and sedatives. J Neurotrauma 24(suppl 1):S71–S76. doi:10.1089/neu.2007.9985

Brekenfeld C, Mattle HP, Schroth G (2010) General is better than local anesthesia during endovascular procedures. Stroke 41(11):2716–2717. doi:10.1161/STROKEAHA.110.594622, STROKEAHA.110.594622 [pii]

Coburn M, Baumert JH, Roertgen D, Thiel V, Fries M, Hein M, Kunitz O, Fimm B, Rossaint R (2007) Emergence and early cognitive function in the elderly after xenon or desflurane anaesthesia: a double-blinded randomized controlled trial. Br J Anaesth 98(6):756–762. doi:10.1093/bja/aem103, aem103 [pii]

Creeley CE, Olney JW (2010) The young: neuroapoptosis induced by anesthetics and what to do about it. Anesth Analg 110(2):442–448. doi:10.1213/ANE.0b013e3181c6b9ca, ANE.0b013e3181c6b9ca [pii]

Culley DJ, Baxter MG, Crosby CA, Yukhananov R, Crosby G (2004) Impaired acquisition of spatial memory 2 weeks after isoflurane and isoflurane-nitrous oxide anesthesia in aged rats. Anesth Analg 99(5):1393–1397; table of contents. doi:10.1213/01.ANE.0000135408.14319.CC 99/5/1393 [pii]

Culley DJ, Raghavan SV, Waly M, Baxter MG, Yukhananov R, Deth RC, Crosby G (2007) Nitrous oxide decreases cortical methionine synthase transiently but produces lasting memory impairment in aged rats. Anesth Analg 105(1):83–88. doi:10.1213/01.ane.0000266491.53318.20, 105/1/83 [pii]

Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW (2008) From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci 9(1):46–56. doi:10.1038/nrn2297, nrn2297 [pii]

Davis MJ, Menon BK, Baghirzada LB, Campos-Herrera CR, Goyal M, Hill MD, Archer DP (2012) Anesthetic management and outcome in patients during endovascular therapy for acute stroke. Anesthesiology 116(2):396–405. doi:10.1097/ALN.0b013e318242a5d2

DiMaggio C, Sun LS, Li G (2011) Early childhood exposure to anesthesia and risk of developmental and behavioral disorders in a sibling birth cohort. Anesth Analg 113(5):1143–1151. doi:10.1213/ANE.0b013e3182147f42, ANE.0b013e3182147f42 [pii]

Dong Y, Zhang G, Zhang B, Moir RD, Xia W, Marcantonio ER, Culley DJ, Crosby G, Tanzi RE, Xie Z (2009) The common inhalational anesthetic sevoflurane induces apoptosis and increases beta-amyloid protein levels. Arch Neurol 66(5):620–631. doi:10.1001/archneurol.2009.48, 66/5/620 [pii]

Dong Y, Wu X, Xu Z, Zhang Y, Xie Z (2012) Anesthetic isoflurane increases phosphorylated tau levels mediated by caspase activation and Abeta generation. PLoS One 7(6):e39386. doi:10.1371/journal.pone.0039386, PONE-D-12-09437 [pii]

Eckenhoff RG, Johansson JS, Wei H, Carnini A, Kang B, Wei W, Pidikiti R, Keller JM, Eckenhoff MF (2004) Inhaled anesthetic enhancement of amyloid-beta oligomerization and cytotoxicity. Anesthesiology 101(3):703–709, 00000542-200409000-00019 [pii]

Flick RP, Katusic SK, Colligan RC, Wilder RT, Voigt RG, Olson MD, Sprung J, Weaver AL, Schroeder DR, Warner DO (2011) Cognitive and behavioral outcomes after early exposure to anesthesia and surgery. Pediatrics 128(5):e1053–e1061. doi:10.1542/peds.2011-0351, peds.2011-0351 [pii]

Gupta R (2010) Local is better than general anesthesia during endovascular acute stroke interventions. Stroke 41(11):2718–2719. doi:10.1161/STROKEAHA.110.596015, STROKEAHA.110.596015 [pii]

Hocker J, Stapelfeldt C, Leiendecker J, Meybohm P, Hanss R, Scholz J, Bein B (2009) Postoperative neurocognitive dysfunction in elderly patients after xenon versus propofol anesthesia for major noncardiac surgery: a double-blinded randomized controlled pilot study. Anesthesiology 110(5):1068–1076. doi:10.1097/ALN.0b013e31819dad92

Jevtovic-Todorovic V, Hartman RE, Izumi Y, Benshoff ND, Dikranian K, Zorumski CF, Olney JW, Wozniak DF (2003) Early exposure to common anesthetic agents causes widespread neurodegeneration in the developing rat brain and persistent learning deficits. J Neurosci 23(3):876–882, 23/3/876 [pii]

Jumaa MA, Zhang F, Ruiz-Ares G, Gelzinis T, Malik AM, Aleu A, Oakley JI, Jankowitz B, Lin R, Reddy V, Zaidi SF, Hammer MD, Wechsler LR, Horowitz M, Jovin TG (2010) Comparison of

safety and clinical and radiographic outcomes in endovascular acute stroke therapy for proximal middle cerebral artery occlusion with intubation and general anesthesia versus the nonintubated state. Stroke 41(6):1180–1184. doi:10.1161/STROKEAHA.109.574194, STROKEAHA.109.574194 [pii]

Kadoi Y, Saito S, Kunimoto F, Goto F, Fujita N (2003) Comparative effects of propofol versus fentanyl on cerebral oxygenation state during normothermic cardiopulmonary bypass and postoperative cognitive dysfunction. Ann Thorac Surg 75(3):840–846

Kaisti KK, Metsahonkala L, Teras M, Oikonen V, Aalto S, Jaaskelainen S, Hinkka S, Scheinin H (2002) Effects of surgical levels of propofol and sevoflurane anesthesia on cerebral blood flow in healthy subjects studied with positron emission tomography. Anesthesiology 96(6):1358–1370, 00000542-200206000-00015 [pii]

Kanbak M, Saricaoglu F, Avci A, Ocal T, Koray Z, Aypar U (2004) Propofol offers no advantage over isoflurane anesthesia for cerebral protection during cardiopulmonary bypass: a preliminary study of S-100beta protein levels. Can J Anaesth 51(7):712–717, 51/7/712 [pii]

Kapinya KJ, Lowl D, Futterer C, Maurer M, Waschke KF, Isaev NK, Dirnagl U (2002a) Tolerance against ischemic neuronal injury can be induced by volatile anesthetics and is inducible NO synthase dependent. Stroke 33(7):1889–1898

Kapinya KJ, Prass K, Dirnagl U (2002b) Isoflurane induced prolonged protection against cerebral ischemia in mice: a redox sensitive mechanism? Neuroreport 13(11):1431–1435

Kodama M, Satoh Y, Otsubo Y, Araki Y, Yonamine R, Masui K, Kazama T (2011) Neonatal desflurane exposure induces more robust neuroapoptosis than do isoflurane and sevoflurane and impairs working memory. Anesthesiology 115(5):979–991. doi:10.1097/ALN.0b013e318234228b

Kurth CD, Priestley M, Watzman HM, McCann J, Golden J (2001) Desflurane confers neurologic protection for deep hypothermic circulatory arrest in newborn pigs. Anesthesiology 95(4):959–964

Le Freche H, Brouillette J, Fernandez-Gomez FJ, Patin P, Caillierez R, Zommer N, Sergeant N, Buee-Scherrer V, Lebuffe G, Blum D, Buee L (2012) Tau phosphorylation and sevoflurane anesthesia: an association to postoperative cognitive impairment. Anesthesiology 116(4):779–787. doi:10.1097/ALN.0b013e31824be8c7

Leung JM, Sands LP (2009) Long-term cognitive decline: is there a link to surgery and anesthesia? Anesthesiology 111(5):931–932. doi:10.1097/ALN.0b013e3181bc988f

Li H, Yin J, Li L, Deng J, Feng C, Zuo Z (2013) Isoflurane postconditioning reduces ischemia-induced nuclear factor-kappaB activation and interleukin 1beta production to provide neuroprotection in rats and mice. Neurobiol Dis. doi:10.1016/j.nbd.2012.12.014, S0969-9961(13)00005-3 [pii]

Lin D, Zuo Z (2011) Isoflurane induces hippocampal cell injury and cognitive impairments in adult rats. Neuropharmacology 61(8):1354–1359. doi:10.1016/j.neuropharm.2011.08.011, S0028-3908(11)00344-3 [pii]

Liu Y, Xiong L, Chen S, Wang Q (2006) Isoflurane tolerance against focal cerebral ischemia is attenuated by adenosine A1 receptor antagonists. Can J Anaesth 53(2):194–201, 53/2/194 [pii]

Loepke AW, Priestley MA, Schultz SE, McCann J, Golden J, Kurth CD (2002) Desflurane improves neurologic outcome after low-flow cardiopulmonary bypass in newborn pigs. Anesthesiology 97(6):1521–1527, 00000542-200212000-00026 [pii]

Lu Y, Wu X, Dong Y, Xu Z, Zhang Y, Xie Z (2010) Anesthetic sevoflurane causes neurotoxicity differently in neonatal naive and Alzheimer disease transgenic mice. Anesthesiology 112(6):1404–1416. doi:10.1097/ALN.0b013e3181d94de1

Ludbrook GL, Visco E, Lam AM (2002) Propofol: relation between brain concentrations, electroencephalogram, middle cerebral artery blood flow velocity, and cerebral oxygen extraction during induction of anesthesia. Anesthesiology 97(6):1363–1370, 00000542-200212000-00006 [pii]

Luh C, Gierth K, Timaru-Kast R, Engelhard K, Werner C, Thal SC (2011) Influence of a brief episode of anesthesia during the induction of experimental brain trauma on secondary brain damage and inflammation. PLoS One 6(5):e19948. doi:10.1371/journal.pone.0019948, PONE-D-11-02382 [pii]

McIntosh TK, Smith DH, Meaney DF, Kotapka MJ, Gennarelli TA, Graham DI (1996) Neuropathological sequelae of traumatic brain injury: relationship to neurochemical and biomechanical mechanisms. Lab Invest 74(2):315–342

Messick JM Jr, Casement B, Sharbrough FW, Milde LN, Michenfelder JD, Sundt TM Jr (1987) Correlation of regional cerebral blood flow (rCBF) with EEG changes during isoflurane anesthesia for carotid endarterectomy: critical rCBF. Anesthesiology 66(3):344–349

Michenfelder JD, Sundt TM, Fode N, Sharbrough FW (1987) Isoflurane when compared to enflurane and halothane decreases the frequency of cerebral ischemia during carotid endarterectomy. Anesthesiology 67(3):336–340

Monk TG, Weldon BC, Garvan CW, Dede DE, van der Aa MT, Heilman KM, Gravenstein JS (2008) Predictors of cognitive dysfunction after major noncardiac surgery. Anesthesiology 108(1):18–30. doi:10.1097/01.anes.0000296071.19434.1e, 00000542-200801000-00007 [pii]

Monk TG, McDonagh D, Martin G, Warren L, Attix DK (2011) Preoperative cognition, not the type of anesthesia, predicts postoperative delirium. Abstract presented at the meeting of the American Society of Anesthesiologists, Chicago, IL

Nandate K, Vuylsteke A, Ratsep I, Messahel S, Oduro-Dominah A, Menon DK, Matta BF (2000) Effects of isoflurane, sevoflurane and propofol anaesthesia on jugular venous oxygen saturation in patients undergoing coronary artery bypass surgery. Br J Anaesth 84(5):631–633

Newman S, Stygall J, Hirani S, Shaefi S, Maze M (2007) Postoperative cognitive dysfunction after noncardiac surgery: a systematic review. Anesthesiology 106(3):572–590, 00000542-200703000-00023 [pii]

Palanisamy A, Baxter MG, Keel PK, Xie Z, Crosby G, Culley DJ (2011) Rats exposed to isoflurane in utero during early gestation are behaviorally abnormal as adults. Anesthesiology 114(3):521–528. doi:10.1097/ALN.0b013e318209aa71

Petersen KD, Landsfeldt U, Cold GE, Petersen CB, Mau S, Hauerberg J, Holst P, Olsen KS (2003) Intracranial pressure and cerebral hemodynamic in patients with cerebral tumors: a randomized prospective study of patients subjected to craniotomy in propofol-fentanyl, isoflurane-fentanyl, or sevoflurane-fentanyl anesthesia. Anesthesiology 98(2):329–336, 00000542-200302000-00010 [pii]

Planel E, Richter KE, Nolan CE, Finley JE, Liu L, Wen Y, Krishnamurthy P, Herman M, Wang L, Schachter JB, Nelson RB, Lau LF, Duff KE (2007) Anesthesia leads to tau hyperphosphorylation through inhibition of phosphatase activity by hypothermia. J Neurosci 27(12):3090–3097. doi:10.1523/JNEUROSCI.4854-06.2007, 27/12/3090 [pii]

Planel E, Krishnamurthy P, Miyasaka T, Liu L, Herman M, Kumar A, Bretteville A, Figueroa HY, Yu WH, Whittington RA, Davies P, Takashima A, Nixon RA, Duff KE (2008) Anesthesia-induced hyperphosphorylation detaches 3-repeat tau from microtubules without affecting their stability in vivo. J Neurosci 28(48):12798–12807. doi:10.1523/JNEUROSCI.4101-08.2008, 28/48/12798 [pii]

Planel E, Bretteville A, Liu L, Virag L, Du AL, Yu WH, Dickson DW, Whittington RA, Duff KE (2009) Acceleration and persistence of neurofibrillary pathology in a mouse model of tauopathy following anesthesia. FASEB J 23(8):2595–2604. doi:10.1096/fj.08-122424, fj.08-122424 [pii]

Rasmussen LS, Johnson T, Kuipers HM, Kristensen D, Siersma VD, Vila P, Jolles J, Papaioannou A, Abildstrom H, Silverstein JH, Bonal JA, Raeder J, Nielsen IK, Korttila K, Munoz L, Dodds C, Hanning CD, Moller JT (2003) Does anaesthesia cause postoperative cognitive dysfunction? A randomised study of regional versus general anaesthesia in 438 elderly patients. Acta Anaesthesiol Scand 47(3):260–266, 057 [pii]

Rasmussen LS, Schmehl W, Jakobsson J (2006) Comparison of xenon with propofol for supplementary general anaesthesia for knee replacement: a randomized study. Br J Anaesth 97(2):154–159. doi:10.1093/bja/ael141, ael141 [pii]

Rortgen D, Kloos J, Fries M, Grottke O, Rex S, Rossaint R, Coburn M (2010) Comparison of early cognitive function and recovery after desflurane or sevoflurane anaesthesia in the elderly: a double-blinded randomized controlled trial. Br J Anaesth 104(2):167–174. doi:10.1093/bja/aep369, aep369 [pii]

Rossaint J, Rossaint R, Weis J, Fries M, Rex S, Coburn M (2009) Propofol: neuroprotection in an in vitro model of traumatic brain injury. Crit Care 13(2):R61. doi:10.1186/cc7795, cc7795 [pii]

Saab BJ, Maclean AJ, Kanisek M, Zurek AA, Martin LJ, Roder JC, Orser BA (2010) Short-term memory impairment after isoflurane in mice is prevented by the alpha5 gamma-aminobutyric acid type A receptor inverse agonist L-655,708. Anesthesiology 113(5):1061–1071. doi:10.1097/ALN.0b013e3181f56228, 00000542-201011000-00018 [pii]

Sall JW, Stratmann G, Leong J, McKleroy W, Mason D, Shenoy S, Pleasure SJ, Bickler PE (2009) Isoflurane inhibits growth but does not cause cell death in hippocampal neural precursor cells grown in culture. Anesthesiology 110(4):826–833. doi:10.1097/ALN.0b013e31819b62e2

Satomoto M, Satoh Y, Terui K, Miyao H, Takishima K, Ito M, Imaki J (2009) Neonatal exposure to sevoflurane induces abnormal social behaviors and deficits in fear conditioning in mice. Anesthesiology 110(3):628–637. doi:10.1097/ALN.0b013e3181974fa2

Sharma D, Vavilala MS (2012) Perioperative management of adult traumatic brain injury. Anesthesiol Clin 30(2):333–346. doi:10.1016/j.anclin.2012.04.003, S1932-2275(12)00039-0 [pii]

Shen X, Dong Y, Xu Z, Wang H, Miao C, Soriano SG, Sun D, Baxter MG, Zhang Y, Xie Z (2013) Selective anesthesia-induced neuroinflammation in developing mouse brain and cognitive impairment. Anesthesiology 118(3):502–515. doi:10.1097/ALN.0b013e3182834d77

Silbert B, Evered L, Scott DA, Maruff P (2011) Anesthesiology must play a greater role in patients with Alzheimer's disease. Anesth Analg 112(5):1242–1245. doi:10.1213/ANE.0b013e3182147f5b, 112/5/1242 [pii]

Souter MJ, Andrews PJ, Alston RP (1998) Propofol does not ameliorate cerebral venous oxyhemoglobin desaturation during hypothermic cardiopulmonary bypass. Anesth Analg 86(5):926–931

Statler KD, Alexander H, Vagni V, Dixon CE, Clark RS, Jenkins L, Kochanek PM (2006) Comparison of seven anesthetic agents on outcome after experimental traumatic brain injury in adult, male rats. J Neurotrauma 23(1):97–108. doi:10.1089/neu.2006.23.97

Stratmann G, Sall JW, Bell JS, Alvi RS, May L, Ku B, Dowlatshahi M, Dai R, Bickler PE, Russell I, Lee MT, Hrubos MW, Chiu C (2010) Isoflurane does not affect brain cell death, hippocampal neurogenesis, or long-term neurocognitive outcome in aged rats. Anesthesiology 112(2):305–315. doi:10.1097/ALN.0b013e3181ca33a1, 00000542-201002000-00014 [pii]

Sun L (2010) Early childhood general anaesthesia exposure and neurocognitive development. Br J Anaesth 105(suppl 1):i61–i68. doi:10.1093/bja/aeq302, aeq302 [pii]

Thal SC, Luh C, Schaible EV, Timaru-Kast R, Hedrich J, Luhmann HJ, Engelhard K, Zehendner CM (2012) Volatile anesthetics influence blood–brain barrier integrity by modulation of tight junction protein expression in traumatic brain injury. PLoS One 7(12):e50752. doi:10.1371/journal.pone.0050752, PONE-D-12-18390 [pii]

Thies W, Bleiler L (2011) 2011 Alzheimer's disease facts and figures. Alzheimers Dement 7(2):208–244. doi:10.1016/j.jalz.2011.02.004, S1552-5260(11)00036-7 [pii]

Wang DS, Zurek AA, Lecker I, Yu J, Abramian AM, Avramescu S, Davies PA, Moss SJ, Lu WY, Orser BA (2012) Memory deficits induced by inflammation are regulated by alpha5-subunit-containing GABAA receptors. Cell Rep 2(3):488–496. doi:10.1016/j.celrep.2012.08.022, S2211-1247(12)00262-8 [pii]

Weiser TG, Regenbogen SE, Thompson KD, Haynes AB, Lipsitz SR, Berry WR, Gawande AA (2008) An estimation of the global volume of surgery: a modelling strategy based on available data. Lancet 372(9633):139–144. doi:10.1016/S0140-6736(08)60878-8, S0140-6736(08)60878-8 [pii]

Whittington RA, Virag L, Marcouiller F, Papon MA, El Khoury NB, Julien C, Morin F, Emala CW, Planel E (2011) Propofol directly increases tau phosphorylation. PLoS One 6(1):e16648. doi:10.1371/journal.pone.0016648

Wilder RT, Flick RP, Sprung J, Katusic SK, Barbaresi WJ, Mickelson C, Gleich SJ, Schroeder DR, Weaver AL, Warner DO (2009) Early exposure to anesthesia and learning disabilities in a population-based birth cohort. Anesthesiology 110(4):796–804. doi:10.1097/01.anes.0000344728.34332.5d

Williams-Russo P, Sharrock NE, Mattis S, Szatrowski TP, Charlson ME (1995) Cognitive effects after epidural vs general anesthesia in older adults. A randomized trial. JAMA 274(1):44–50

Wu X, Lu Y, Dong Y, Zhang G, Zhang Y, Xu Z, Culley DJ, Crosby G, Marcantonio ER, Tanzi RE, Xie Z (2012) The inhalation anesthetic isoflurane increases levels of proinflammatory TNF-alpha, IL-6, and IL-1beta. Neurobiol Aging 33(7):1364–1378. doi:10.1016/j.neurobiolaging.2010.11.002, S0197-4580(10)00476-8 [pii]

Xie Z, Dong Y, Maeda U, Alfille P, Culley DJ, Crosby G, Tanzi RE (2006) The common inhalation anesthetic isoflurane induces apoptosis and increases amyloid beta protein levels. Anesthesiology 104(5):988–994, 00000542-200605000-00015 [pii]

Xie Z, Culley DJ, Dong Y, Zhang G, Zhang B, Moir RD, Frosch MP, Crosby G, Tanzi RE (2008) The common inhalation anesthetic isoflurane induces caspase activation and increases amyloid beta-protein level in vivo. Ann Neurol 64(6):618–627. doi:10.1002/ana.21548

Yang H, Liang G, Hawkins BJ, Madesh M, Pierwola A, Wei H (2008) Inhalational anesthetics induce cell damage by disruption of intracellular calcium homeostasis with different potencies. Anesthesiology 109(2):243–250. doi:10.1097/ALN.0b013e31817f5c47, 00000542-200808000-00013 [pii]

Yirmiya R, Goshen I (2011) Immune modulation of learning, memory, neural plasticity and neurogenesis. Brain Behav Immun 25(2):181–213. doi:10.1016/j.bbi.2010.10.015, S0889-1591(10)00521-0 [pii]

Yu G, Dymond M, Yuan L, Chaturvedi LS, Shiratsuchi H, Durairaj S, Marsh HM, Basson MD, Yu G, Dymond M, Yuan L, Chaturvedi LS, Shiratsuchi H, Durairaj S, Marsh HM, Basson MD (2011) Propofol's effects on phagocytosis, proliferation, nitrate production, and cytokine secretion in pressure-stimulated microglial cells. Surgery 150(5):887–896. doi:10.1016/j.surg.2011.04.002, S0039-6060(11)00163-2 [pii]

Zhang B, Dong Y, Zhang G, Moir RD, Xia W, Yue Y, Tian M, Culley DJ, Crosby G, Tanzi RE, Xie Z (2008) The inhalation anesthetic desflurane induces caspase activation and increases amyloid beta-protein levels under hypoxic conditions. J Biol Chem 283(18):11866–11875. doi:10.1074/jbc.M800199200, M800199200 [pii]

Zhang Y, Dong Y, Wu X, Lu Y, Xu Z, Knapp A, Yue Y, Xu T, Xie Z (2010) The mitochondrial pathway of anesthetic isoflurane-induced apoptosis. J Biol Chem 285(6):4025–4037. doi:10.1074/jbc.M109.065664, M109.065664 [pii]

Zhang B, Tian M, Zhen Y, Yue Y, Sherman J, Zheng H, Li S, Tanzi RE, Marcantonio ER, Xie Z (2012a) The effects of isoflurane and desflurane on cognitive function in humans. Anesth Analg 114(2):410–415. doi:10.1213/ANE.0b013e31823b2602, ANE.0b013e31823b2602 [pii]

Zhang Y, Xu Z, Wang H, Dong Y, Shi HN, Culley DJ, Crosby G, Marcantonio ER, Tanzi RE, Xie Z (2012b) Anesthetics isoflurane and desflurane differently affect mitochondrial function, learning, and memory. Ann Neurol 71(5):687–698. doi:10.1002/ana.23536

Zhang B, Tian M, Zheng H, Zhen Y, Yue Y, Li T, Li S, Marcantonio ER, Xie Z (2013) Effects of anesthetic isoflurane and desflurane on human cerebrospinal fluid abeta and tau level. Anesthesiology. doi:10.1097/ALN.0b013e31828ce55d

Zheng H, Dong Y, Xu Z, Crosby G, Culley DJ, Zhang Y, Xie Z (2013) Sevoflurane anesthesia in pregnant mice induces neurotoxicity in fetal and offspring mice. Anesthesiology 118(3):516–526. doi:10.1097/ALN.0b013e3182834d5d

Zurek AA, Bridgwater EM, Orser BA (2012) Inhibition of alpha5 gamma-aminobutyric acid type A receptors restores recognition memory after general anesthesia. Anesth Analg 114(4):845–855. doi:10.1213/ANE.0b013e31824720da, ANE.0b013e31824720da [pii]

About the Editors

Selva Baltan, M.D., Ph.D. is an Associate Professor of Molecular Medicine at the Department of Neurosciences at the Cleveland Clinic. Her research focuses on mechanisms of brain cell damage following stroke in white matter in a region-specific and age-specific manner. Currently she is interested in the role of protein acetylation and mitochondrial dynamics in white matter stroke which has expanded her interests to neurodegenerative diseases such as Multiple Sclerosis and Alzheimer's disease that involve white matter.

S. Thomas (Tom) Carmichael is a neurologist and neuroscientist in the Department of Neurology at the David Geffen School of Medicine at UCLA. Dr. Carmichael is Professor and Vice Chair in the Department, with active laboratory and clinical interests in stroke and neurorehabilitation and how the brain repairs from injury. He received his M.D. and Ph.D. degrees from Washington University School of Medicine in 1993 and 1994, and completed a Neurology residency at Washington University School of Medicine, serving as Chief Resident in 1997–1998. Dr. Carmichael was a Howard Hughes Medical Institute postdoctoral fellow at UCLA from 1998 to 2001, studying mechanisms of axonal sprouting, with a clinical emphasis on neurorehabilitation and stroke. He has been on the UCLA faculty since 2001. Dr. Carmichael's laboratory studies the molecular and cellular mechanisms of neural repair after stroke and other forms of brain injury. This research focuses on the processes of axonal sprouting and neural stem cell and progenitor responses after stroke, and on neural stem cell transplantation. Dr. Carmichael is an attending physician on the Neurorehabilitation and Stroke clinical services at UCLA.

Dr. Carmichael has published important papers on stroke recovery that have defined mechanisms of plasticity and repair. These findings include the fact that the stroke produces stunned circuits that limit recovery, but can be restored to normal functioning with newly identified experimental drugs. His work has identified a novel brain "growth program" that is activated by stroke and leads to the formation of new connections. These studies have also identified how this growth program changes with age and how specific molecules in the aged brain block the formation of new connections and of recovery.

S. Baltan et al. (eds.), *White Matter Injury in Stroke and CNS Disease*, Springer Series in Translational Stroke Research 4, DOI 10.1007/978-1-4614-9123-1,

This and other works have led to new directions in stroke therapeutics, including therapies with stem cell and tissue engineering applications. Dr. Carmichael is in the midst of stroke stem cell development applications with the FDA and with biotechnology companies.

Dr. Carlos Matute (1956; Zaragoza, Spain) is Professor of Neuroscience at the University of País Vasco. He graduated in Sciences and got his Ph.D. degree at the University of Zaragoza. Postdoctoral states included the Brain Research Institute (Zurich, Switzerland; 1983–1986), and the Department of Psychobiology at UC Irvine (1987–1990). He has been Visiting Professor at Max-Plank Institute (Göttingen, Germany) and at Einstein College of Medicine (New York, USA).

His main research interest is the understanding of the molecular and cellular basis of neurodegeneration and neuroprotection. Recent work carried in Dr. Matute´s laboratory has served to understand some of the mechanisms involved in neuronal cell death and to find out novel molecules with protective properties such as antioxidants. He has also been interested in the neurobiology of glial cells. He and his colleagues discovered that oligodendrocytes are highly vulnerable to excitotoxic signals mediated by glutamate and ATP receptors which can be activated during tissue damage, inflammation, and other pathological conditions including ischemia and multiple sclerosis. His laboratory also worked out some of the mechanisms leading to oligodendrocyte death as a consequence of excitotoxic insults. This includes activation of several types of ionotropic glutamate receptors and loss of glutamate homeostasis as a consequence of inflammation in different experimental paradigms. He also observed that some of the alterations operating in experimental studies occur also in animal models of multiple sclerosis and in postmortem nervous tissue from patients suffering from this disease. Collectively, this research has generated new knowledge about neuronal and oligodendrocyte death which will serve to develop new therapeutic ideas to treat diseases of the white matter.

Carlos Matute has published over 150 papers and book chapters.

Guohua Xi, M.D. received his medical degree from Zhejiang Medical University, China, and had his postdoctoral training at the University of Cincinnati and the University of Michigan. Currently he is Richard C. Schneider Research Professor, Professor of Neurosurgery and Associate Director of Crosby Neurosurgical Laboratories at the University of Michigan. He has published 108 peer-reviewed papers and 75 book chapters and serves as a reviewer on NIH ANIE Study Section. He is also on several editorial boards including *Stroke* and the *Journal of Cerebral Blood Flow and Metabolism*. His research is supported by NIH/AHA and his research interests are: (1) the mechanisms of brain injury after hemorrhagic stroke; (2) iron chelation therapy for intracerebral hemorrhage; and (3) the mechanisms of preconditioning-induced tolerance to brain injury.

John H. Zhang M.D., Ph.D., is Professor of Neurosurgery, Anesthesiology, Neurology, and Physiology, Director of Basic Science Research in the Departments of Neurosurgery and Anesthesiology, Vice-Chair for Basic Science Department at the Loma Linda University School of Medicine. He has research interests in Cerebral Vascular Disorders, Animal Model of Neurological Diseases, and Medical Gas Research fields. Dr. Zhang is the Editor-in-Chief of Translational Stroke Research and Medical Gas Research. Dr. Zhang published 11 books on subjects related to Stroke and other Neurological Disorders.

Index

A

Adenosine triphosphate (ATP), 42, 44, 69, 96, 119, 205, 230, 282, 389, 390, 392–394, 397, 413, 417, 436, 438, 440, 442, 445, 447, 453, 493, 527, 538, 540–542, 544–546, 557, 561

Alzheimer's disease (AD), 45, 72, 172, 182, 207, 232, 257, 291, 294, 414, 433, 446, 507, 547, 557, 558, 569, 572–573

AMPA receptors, 42, 43, 45, 83, 117, 118, 120, 121, 123, 157, 158, 208, 378, 395, 417, 437, 438, 443, 444, 452

Amyloid precursor protein (APP), 41, 145, 188, 227–228, 294, 295, 342, 359, 446, 474, 475, 478, 512, 513

Anesthesia, 182, 197, 569–577

Angiotensin converting enzyme inhibitor (ACEi), 301, 303, 515

Angiotensin receptor blocker (ARB), 301

Animal model, 9, 28, 53–61, 65, 73, 81–83, 85, 111, 115, 116, 121, 126, 128, 131, 143, 144, 146–159, 170, 198–205, 210, 211, 220–222, 227, 233, 239, 241, 244, 245, 258, 259, 322, 373, 408, 416, 423, 444, 461–493, 523, 549, 555, 571, 573, 576

βAPP. *See* Beta amyloid precursor protein (βAPP)

Astrocyte(s), 5, 39, 85, 118, 145, 170, 182, 200, 232, 257, 274, 283, 324, 376, 414, 434, 467, 507, 538, 554

Axoglial unit, 521, 523, 525–528, 530, 531

Axon(s), 3, 40, 76, 93, 109, 155, 171, 181, 203, 226, 257, 273, 281, 321–364, 374, 410, 433, 464, 506, 521, 539

Axonal damage, 26, 41–43, 100, 102, 144, 171, 182, 183, 185, 188–191, 206, 209, 210, 227–228, 257, 274, 336, 338, 341, 342, 345, 386, 411, 412, 415–416, 441, 444, 453, 470, 490, 513, 524, 527, 528

Axonal injury, 42, 93, 95, 96, 98, 102, 110, 126, 152, 155, 181, 183, 184, 188, 189, 191–192, 209, 227, 228, 241, 274, 322, 323, 327, 336, 340, 341, 350, 355, 357–361, 407, 414, 420, 421, 423, 512, 513, 521–531, 542

B

Beta amyloid precursor protein (βAPP), 183, 188, 189

Beta-amyloid protein, 572, 573

Blood–brain barrier, 23, 24, 61, 68, 155, 203, 222, 274, 300, 324, 342, 356, 407, 410, 414, 420–422, 443, 447, 476, 524, 537, 554, 562, 575

Brain inflammation, 153, 154, 481–486

Brain injury, 39, 91–102, 109, 143, 169, 191, 202, 230, 257, 271, 283, 321, 433, 509, 524, 545, 553, 569

C

CADASIL, 53–61, 72, 73, 77, 85

Cell death, 43–45, 47, 69, 111, 113, 114, 116, 119, 120, 125–131, 152, 154, 173, 198, 200, 202, 204, 205, 210, 211, 236, 239, 241, 245, 246, 259, 260, 326, 359, 390, 412, 438, 441, 442, 447, 453, 463, 510, 511, 527, 529, 545, 556, 557, 561

S. Baltan et al. (eds.), *White Matter Injury in Stroke and CNS Disease*, Springer Series in Translational Stroke Research 4, DOI 10.1007/978-1-4614-9123-1,

Cerebral blood flow (CBF), 42, 57–59, 70, 147, 182, 200, 273, 470, 471, 509, 515, 575
Cerebral ischemia, 40, 41, 43, 46, 83, 145, 181–192, 198–200, 202, 204–207, 210, 211, 227, 236, 237, 244, 246, 271, 274, 414, 474, 482, 486, 507, 509, 512–515, 529, 556, 573–575
Cerebral palsy (CP), 109, 143, 175, 203, 471, 509
Cerebrovascular disease, 40, 45, 70, 183, 442, 514, 521
Circulating biomarkers, 274
Cognition, 72, 85, 191, 285, 303, 326
Compound action potential, 11–13, 42, 174, 336–338, 375, 376
Conduction velocities, 5, 7, 8, 11–14, 333, 336
2',3'-Cyclic nucleotide 3'-phosphodiesterase (CNPase), 41, 153, 476

D
Delayed cerebral injury, 274–278
Dementia, 40, 55, 67, 70, 71, 73, 74, 78, 94, 95, 169, 181, 182, 282, 284, 286, 289, 303, 359, 375, 442, 446, 489, 506, 521, 523
Dementia with Lewy bodies (DLB), 282–284, 288
Demyelination, 26, 40, 58, 68, 171, 182, 228, 257, 291, 359, 405, 434, 470, 514, 539
Diabetes mellitus type 2 (DM2), 298, 299, 301, 303, 304
Diffuse axonal injury (DAI), 93, 95, 96, 98, 102, 191–192, 201, 322, 326–328, 333, 341, 361, 362
Diffusion imaging, 97–102
Diffusion tensor imaging (DTI), 68, 76–78, 80, 85, 95–102, 181, 186, 187, 220, 301, 302, 327, 333, 362, 522
Diffusion-weighted imaging (DWI), 70, 71, 80, 81, 172, 185–188, 271, 272, 301
Dynamic contrast-enhanced, 68, 79

E
Early brain injury, 271–273
Edaravone, 182, 189, 191, 206, 209, 511, 513, 515
Excitotoxicity, 42–45, 83, 96, 111, 112, 116–118, 120–122, 126, 130, 131, 145, 157–158, 173, 204, 207, 221, 230, 236, 261, 287, 303, 377, 380, 382, 384, 385, 388–390, 392, 393, 395, 397, 415–417, 440–447, 450, 452, 453, 510, 524, 554, 557, 559–560
Experimental intracerebral hemorrhage, 219–246
Extracellular matrix, 221, 240, 261, 263, 274, 322, 357–361, 554

G
Gap junctions, 18–22, 24, 25, 27, 28, 173
Glia, 6, 17, 21–26, 154, 172, 181, 226, 257, 263, 281–283, 287, 304, 387, 397, 433–443, 446, 449, 540, 543, 545
Global cerebral ischemia, 181–192, 198–200, 202, 204–206, 513, 514
Glutamate, 11, 42, 83, 96, 111, 157, 174, 204, 229, 261, 375, 411, 435, 484, 510, 527, 546, 554
Glutamate transporter, 117–120, 125, 204, 377, 378, 382, 384, 388, 389, 392, 417, 440, 442, 444, 555, 558–560
Gray matter to white matter (GWR) X-ray attenuation ratio, 185

H
Heavy metal, 287, 553–563
High angular resolution diffusion imaging, 100–102
Hydrocephalus, 273–275
Hyperlipidemia, 69, 297, 304
Hypertension, 69, 73, 74, 85, 86, 131, 181, 183, 278, 285, 294, 297–304, 463, 477, 523, 559
Hypocapni, 182, 183
Hypoperfusion, 55, 58, 61, 69, 70, 72, 81, 85, 146, 158, 181–183, 186, 191, 273, 287, 509, 512, 514–516, 528
Hypothermia, 46–48, 118, 185, 241, 244, 573, 574
Hypoxia–ischemia (HI), 47, 119, 121, 124, 127, 143, 144, 146–149, 208–210, 442–443, 466, 471, 489, 510, 511

I
Inflammatory response, 68, 85, 146, 148, 152–155, 182, 188, 206, 233–238, 240, 241, 243, 275, 297, 359, 407, 410, 464, 477, 483, 492, 493, 510, 529, 530
Innate immune cells, 476, 483

Ion channels, 9, 11, 123, 350, 353, 416, 434, 435, 527, 541, 542
Ischemia, 11, 39, 57, 68, 110, 143, 169–175, 181–192, 197–211, 227, 259, 271, 357, 375, 414, 433, 462, 507, 522, 545, 555, 573
Ischemic stroke, 39–48, 55, 59, 73, 185, 220, 230, 234, 244, 245, 374, 461–493, 512, 555, 556, 559, 562, 574

L
Lacuna infarct, 169–173, 175
Leukoaraiosis, 55, 57, 59, 61, 68, 74, 75, 78, 220, 375, 523
Lipopolysaccharide (LPS), 151, 414, 484

M
Magnetic resonance imaging (MRI), 3–5, 55, 56, 67, 68, 70, 71, 73–75, 77–83, 85, 91–102, 149, 151, 152, 155, 172, 181, 185, 186, 188, 203, 220, 222, 229, 271, 273, 291, 299–301, 303, 327, 406, 407, 422, 423, 463, 484, 522, 547, 556
Matrix, 81, 202, 205–206, 221, 240, 261, 263, 274, 322, 357, 358, 360, 361, 436, 482, 545, 554, 556, 562
Medication, 230, 301, 304, 569, 571, 575
Memantine, 45, 123, 124, 184, 185, 207, 437, 443
Metal ion, 553–555, 561, 562
Metalloproteinases, 81, 85, 202, 205–206, 209, 240, 357, 562
Microglia, 68, 112, 145, 171, 182, 200, 228, 257, 283, 358, 377, 411, 432, 465, 507, 537, 575
Microglia/macrophages, 85, 145, 154, 233, 246, 260, 478–481, 485, 487–493, 508
Mitochondria, 27, 42, 69, 96, 130, 169, 182, 287, 346, 385, 416, 436, 506, 528, 537–549, 555, 571
Modifiable risk factors, 298–304
Multiple sclerosis (MS), 10, 46, 67, 68, 169, 205, 232, 235, 257, 259–265, 281, 283, 291, 300, 303, 357, 359, 394, 405–423, 433, 439, 443–445, 486, 523, 527, 547, 548
Myelin, 3, 40, 67, 110, 144, 171, 181, 202, 226, 257, 273, 281, 321–364, 374, 405, 433, 464, 506, 521, 538, 554
Myelinated axons, 3–14, 23, 24, 27, 28, 40, 42, 172, 174, 181, 183, 321–364, 380, 384, 434, 449, 466, 467, 471, 472, 512, 539, 540
Myelin basic protein (MBP), 15, 41, 120, 147, 153, 157, 183, 205, 228, 380, 420, 467, 472, 473, 512
Myelin damage, 41, 293, 298, 300–302, 384, 446, 472, 473, 488

N
Neonatal hypoxia–ischemia, 47, 124, 127, 146–148, 210, 466, 471, 489, 511
Neural network synchrony, 283, 289
Neurodegeneration, 205, 232, 238, 408, 410–412, 414, 415, 418, 419, 439, 489, 570, 571
Neuroinflammation, 240, 414, 423, 556, 575–577
Neuroprotection, 45–46, 113–114, 127, 130–131, 184, 244, 342–346, 348, 359, 361, 447, 511, 528, 531, 537–549, 553, 555, 573–575
Neurotoxicity, 43–46, 246, 382, 414, 463, 464, 490, 492, 553–555, 557–562, 569–572
Neutrophils, 231, 233, 238–240, 245, 465, 476–478, 480–483, 485–487, 492
NG2 cells, 283, 284, 286, 293
NMDA receptors, 27, 42–45, 48, 96, 112–114, 117, 123–124, 157, 158, 182, 184, 204, 207, 208, 378, 380, 387, 436, 437, 439, 442–444, 449, 559–561
Notch3, 54–61, 73
Nuclear factor-kappaB, 235–237

O
Obesity, 297, 298, 304, 477
Oligodendrocyte, 6, 39, 69, 109, 144, 170, 181, 197, 230, 257, 281, 282, 321, 376, 410, 434, 464, 506, 522, 537, 554
Oligodendrocyte progenitor, 41, 257, 415, 418, 420, 440, 442, 507, 509–512, 556
Oxidative stress, 96, 112, 114, 125, 127, 129, 174, 182, 184, 191, 203, 204, 206, 208, 234–237, 241, 286, 287, 385, 418, 419, 482, 505–516, 543, 547, 554–558, 560–562

P
Parkinson's disease (PD), 111, 112, 157, 169, 281, 414, 547, 557
Periventricular leukomalacia (PVL), 40, 109–112, 114–118, 120, 126–131, 144, 145, 152, 154–156, 158, 185, 203, 207, 375, 377, 382, 442, 443, 509

Periventricular white matter injury (PWMI), 143, 144, 146, 158, 273, 274, 506, 509–511
Plasma proteins, 155, 226, 227, 230–232, 234, 235
Positron emission tomography (PET), 152
Premyelinating oligodendrocytes (preOLs), 144–147, 149, 150, 154, 158, 510
Progressive lesion development, 523–525
Proteolipid protein (PLP), 15, 153, 155, 258, 264, 420

R
Recovery, 26, 46–48, 71, 77, 81, 95, 99, 100, 126, 169, 181, 187, 188, 201, 203, 210, 221, 228, 238, 258, 272, 322, 326, 327, 333, 335, 338–341, 345, 357–361, 364, 377, 382, 386–388, 392, 394–397, 405, 407, 408, 412, 477, 491, 525, 526, 529–531, 538, 553, 575
Regeneration, 46, 47, 257, 261–263, 435, 529, 530
Renin angiotensin system (RAS), 297, 299–302, 561
Rodent stroke models, 39, 209

S
Selective vulnerability, 109–131, 208, 333
Small vessel disease, 55, 70, 72–74, 78, 85, 169, 181, 186, 521, 523
Stem cell therapy, 47, 48
Subarachnoid hemorrhage, 271–278, 464, 470, 556

T
Toll-like receptor 4 (TLR-4), 154, 156, 240–241, 263
Transient brain ischemia, 437, 474
Traumatic, 10, 46, 91–102, 169, 183, 220, 257, 283, 321–364, 433, 545, 569, 575
Traumatic brain injury, 46, 91–102, 169, 191–192, 230, 257, 283, 433, 446, 545, 569, 575–576
T2-weighted MRI, 71, 85, 422

U
Ultrastructure, 40, 327–335, 355, 356, 363
Unmyelinated axons, 6–8, 292, 322–326, 328–331, 333–337, 341, 344–346, 348–350, 353–355, 357, 358, 361–364, 378, 380, 395

V
Vascular cognitive impairment, 71, 73, 78, 85
Vascular dementia, 40, 71, 78, 169, 181, 182, 359, 442, 489, 506, 521, 523
Very low birth weight (VLBW), 143, 509

W
Wallerian degeneration, 96, 98, 183, 184, 191, 220, 326, 333, 408, 525, 529
White matter
  damage, 26–28, 40–42, 45, 56, 69–81, 85, 91–102, 151, 153, 156, 157, 164, 174, 181, 184, 188, 203–205, 207, 210, 220, 222, 241, 298–300, 302–304, 405–423, 442, 446, 464–476, 481, 485–493, 509, 559–562
  stroke, 257–261, 263, 265, 383, 521–531
White matter hyperintensities (WMH), 55, 73, 75, 77, 181, 299, 302–304, 522
White matter lesions (WML), 73, 74, 76–78, 81, 115, 149, 150, 154–157, 167, 171, 172, 181, 182, 186, 187, 191, 197, 198, 259, 260, 291, 299, 406, 422, 489, 509, 514–516