Keiji Wada *Editor*

Neurodegenerative Disorders as Systemic Diseases

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ISBN 978-4-431-54540-8 ISBN 978-4-431-54541-5 (eBook)
DOI 10.1007/978-4-431-54541-5

Library of Congress Control Number: 2015954481

Springer Tokyo Heidelberg New York Dordrecht London

Printed on acid-free paper

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

Preface

This book focuses on a new concept of neurodegeneration for readers in a broad range of fields. To date, neurodegeneration, or neurodegenerative disorder, has been studied in the context of neuroscience and neurology. Classically, neuronal cell death was a hallmark of neurodegenerative disorders. The cell-autonomous mechanism was extensively studied in cell death. However, it has become evident that neural dysfunction is more important in the pathophysiology of neurodegenerative disorders. Additionally, it has become evident that communication among neurons, glial cells, and vessels is important for brain function and that the network among the brain and peripheral tissues/organs plays a crucial role in neural information processes. Namely, neurodegenerative disorders should be considered systemic diseases, and accordingly, neurodegeneration or neurodegenerative disorder is now a topic even in fields outside neuroscience and neurology.

This paradigm shift implies that neurodegenerative disorder must be approached by combining neurology, psychiatry, endocrinology, immunology, metabolism, and other disciplines. The shift also implies that the environment and brain function must be included in studies of the disorder. Thus, the shift is likely related to changes in medical care for neurodegenerative disorders as well. Besides classical pharmacotherapy, technological advances now allow the use of various approaches to improve the quality of life of patients suffering from such disorders.

Contributing researchers describe their philosophy and current research, and review articles related to the theme "neurodegenerative disorders as systemic diseases" are included. I believe that a comprehensive book to approach neurodegenerative disorder as a systemic disease is needed. I hope this book provides readers with a fresh approach to the disorder, paving the way for new research and improved quality of health care for patients.

Kodaira, Japan — Keiji Wada

Contents

Part I
A New Concept of Neurodegeneration in Biology and Pathophysiology

Chapter 1
Conformational Disease and RNA Disease Theory in the Context of Neurodegenerative Diseases

Tomohiko Ishihara, Masatoyo Nishizawa, and Osamu Onodera

Abstract Neurodegenerative diseases cause slowly progressive loss of specific systems of the nervous tissues and show unique neuronal inclusions. During the last 30 years, the causative genes of many hereditary neurodegenerative diseases and the components of most of their characteristic inclusions have been identified. However, the molecular mechanisms responsible for neuronal degeneration have not been fully clarified. Here we review the history of neurodegenerative disorders and current explanations for their molecular pathogenesis – conformational disease theory and RNA disease theory – which attempt to explain the vulnerability of neurons to these disorders in terms of their unique cell features.

Keywords System selectivity • Propagation • Conformational disease • Proteinopathy • Prion • RNA-binding protein • RNA disease • Ribostasis • RNP granules

1.1 Introduction: How the Concept of Neurodegenerative Disease was Established

Neurodegenerative diseases including amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and multiple system atrophy (MSA) cause progressive loss of neurons. Each of these disorders has its own unique signature of slowly progressive neurological deterioration affecting specific systems of the nervous tissues and showing unique neuronal inclusions.

In 1817 J. Parkinson was the first to recognize so-called "shaking palsy" as a specific disease. Subsequently, this became known as "Parkinson's disease" (PD). PD is characterized by tremor and akinesia (Parkinson 2002). His work heralded the

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K. Wada (ed.), *Neurodegenerative Disorders as Systemic Diseases*,
DOI 10.1007/978-4-431-54541-5_1

general concept of neurodegenerative diseases showing unique neurological features. However, it was not until the discovery of ALS that neurological symptoms unique to a disease were recognized as being attributable to involvement of specific systems of the nervous tissues. In 1869 J.M. Charcot revealed that selective neuronal degeneration in the spinal cord caused progressive muscle weakness and atrophy (Charcot and Joffroy 1869). The condition was later termed "amyotrophic lateral sclerosis" (ALS). In 1911 A. Alzheimer reported the presence of unique structures in the intra- and extracellular spaces of the brain in patients with a progressive form of dementia, later termed "Alzheimer's disease" (AD) (Alzheimer 1911). In the following year F.H. Lewy characterized eosinophilic round inclusions in the brain of PD patients, which were later termed "Lewy bodies" (Lewy 1912). These pioneering studies led to the fundamental concept that neurodegenerative disorders are each associated with characteristic inclusions in the affected tissues. Subsequent studies of neurodegenerative disease have investigated the molecular mechanisms associated with the inclusions unique to each type. Although the components of these inclusions in almost all neurodegenerative disorders have been identified during the last 30 years, the molecular mechanisms of neurodegeneration have not been fully elucidated. Here we review the history of research into the molecular pathogenesis of neurodegenerative disease.

1.2 Proteinopathy in Neurodegenerative Disease

Components of the unique inclusions found in non-hereditary neurodegenerative diseases comprise mostly five types: amyloid β (Aβ), tau, α-synuclein, fused in sarcoma (FUS), and TAR DNA-binding protein 43 kDa (TDP-43) (Table 1.1). The discovery of mutations in each of the genes that encode the aggregated proteins

Table 1.1 Typical neurodegenerative diseases and proteins that accumulate in conformational diseases

		Symptom				
		Dementia			MND	ExPy
Accumulated protein	α-Synuclein	DLB				PD, MSA
	Aβ		AD			
	Tau		AD	FTD		PSP, CBD
	TDP-43			FTD	ALS	
	FUS			FTD	ALS	

Note: Correlations among the typical neurodegenerative diseases, their symptoms, and accumulated proteins are given in this table. Note that a single protein can accumulate in several diseases and that different proteins are able to accumulate in the same disease

(Abbr) *AD* Alzheimer's disease; *ALS* Amyotrophic lateral sclerosis; *CBD* Corticobasal degeneration; *DLB* Dementia with Lewy bodies; *ExPy* Extrapyramidal disease; *FTD* Frontotemporal dementia; *MSA* Multiple system atrophy; *MND* Motor neuron disease; *PD* Parkinson's disease; *PSP* Progressive supranuclear palsy

found in patients with familial neurodegenerative disorders has lent weight to the contention that these inclusions play a primary role in disease pathogeneses (Goate et al. 1991; Hardy and Selkoe 2002; Hutton et al. 1998; Rademakers et al. 2004; Polymeropoulos et al. 1997; Eschbach and Danzer 2014; Yokoseki et al. 2008; Vance et al. 2009; Lagier-Tourenne et al. 2010). Identification of the component proteins makes it possible to classify neurodegenerative disorders according to the type of component proteins – proteinopathy – rather than the system involved. α-Synuclein, which is the main component of the Lewy body in the PD brain (Spillantini et al. 1997; Wakabayashi et al. 1997), also accumulates in dementia with Lewy bodies and MSA. Although the clinical spectra of these disorders differ, collectively they are now designated as α-synucleinopathies (Takeda et al. 2006). Similarly to AD, progressive supranuclear palsy, corticobasal degeneration, and a type of frontotemporal dementia (FTD) all form tau protein inclusion bodies, collectively known as "tauopathies" (Spillantini and Goedert 2013).

The components of ubiquitin-positive inclusions in ALS and FTD were not identified until 2006. In 2006 TDP-43 was finally identified as a component of ubiquitin-positive inclusions in ALS and FTD (Neumann et al. 2006; Arai et al. 2006), suggesting the concept of TDP-43 proteinopathy (Neumann et al. 2007). The system and cell type involved in each disorder are correlated with the unique clinical phenotype (Fig. 1.1). For example, the behavior variant of FTD (bvFTD), which is characterized by prominent early personality or behavioral changes associated with frontotemporal lobar degeneration (Rascovsky et al. 2011), shows inclusions comprising several different proteins. Of 66 cases of bvFTD, 42 % had tau-immunopositive inclusions, 30 % had TDP-43-immunopositive inclusions, and 13 % had FUS-immunopositive inclusions (Chare et al. 2014).

1.3 The Initiation of Conformational Disease

Evidence for inclusion bodies defining specific neurodegenerative disorders has led to the concept of conformational disease caused by proteins unfolding (Carrell and Lomas 1997) such that they lose their normal three-dimensional structure and develop a tendency to aggregate. Basically, the proposed process responsible for aggregate formation is one in which the conformation of a monomeric protein is altered through abnormal phosphorylation, truncation, and/or change to a β-sheet structure. These proteins then form dimers, followed by oligomers and fibrils, which are insoluble (Barghorn et al. 2004).

Several mechanisms have been proposed for initiation of the disease process. First, in hereditary neurodegenerative disease, the mutant form of the disease-related protein acquires aggregability. Polyglutamine diseases have been well studied as models for conformational disease (Orr and Zoghbi 2007). Polyglutamine diseases are hereditary neurodegenerative diseases that include HD, spinocerebellar ataxia (SCA) 1, 2, 3, 6, 7, and 17, dentatorubral-pallidoluysian atrophy (DRPLA), and spinal and bulbar muscular atrophy (SBMA). They are

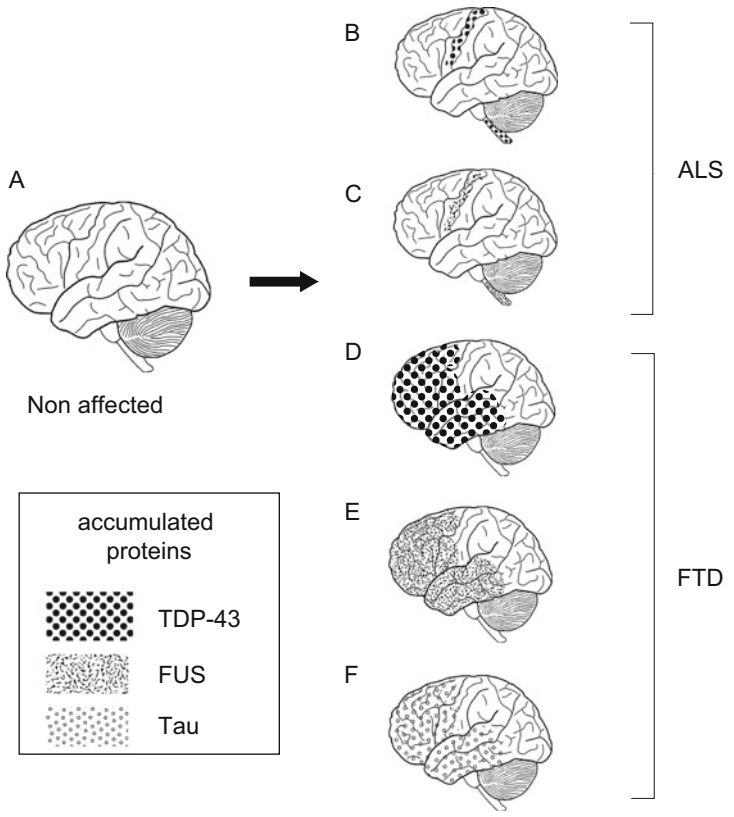

Fig. 1.1 Abnormal protein accumulation in conformational disease: (*A*) non-affected brain; (*B*, *C*) ALS-affected brains with (*B*) TDP-43 or (*C*) FUS protein inclusions in the pyramidal tract, motor cortex, and spinal cord; (*C–E*) FTD-affected brains with (*D*) TDP-43, (*E*) FUS, or (*F*) tau protein; (*B–E*) abnormal protein can be deposited in multiple lesions and can bring about several different diseases; (*D–F*) several proteins can accumulate in one particular system independently and can produce a clinically indistinguishable disease

caused by expansion of a polyglutamine stretch in the causative gene (Orr and Zoghbi 2007; Takahashi et al. 2010). In these disorders, inclusions that are immunopositive for the expanded polyglutamine stretch are observed in the affected neurons (Nagai et al. 2007). Although there is no functional relevance in these causative proteins, the length of the polyglutamine stretch is closely correlated with age at disease onset and disease severity (Nagai et al. 2007; Igarashi et al. 1992; Gatchel and Zoghbi 2005), as well as with aggregation properties both *in vitro* and *in vivo* (Takahashi et al. 2010; Nagai et al. 2007; Chai et al. 1999). These facts indicate that the aggregability of the mutant protein determined by the degree of expansion of the polyglutamine stretch is directly associated with the molecular pathogenesis of polyglutamine disease. It has been proposed that the expanded polyglutamine stretch undergoes conversion to a misfolded β-sheet

conformation, resulting in formation of inclusion bodies through the assembly of oligomeric intermediates. Indeed, the mutated α-synuclein associated with PD also show increased aggregability (Eschbach and Danzer 2014; Lagier-Tourenne et al. 2010; Johnson et al. 2009; Austin et al. 2014), indicating the existence of a common mechanism for neurodegenerative disorders.

Another proposed mechanism of pathogenesis is one in which aggregation results from an increase in the amount of causative protein or peptide. This is analogous to the production of inclusions in hereditary neurodegenerative diseases resulting from the increasing number of genes. An increase in the number of α-synuclein genes causes familial PD (Singleton et al. 2003), and a point mutation of the gene can increase the amount of causative peptide. Moreover, increase in the number of *APP* genes and mutations in *APP* increases the production of Aβ and causes familial AD (Goate et al. 1991; Hardy and Selkoe 2002). Finally, perturbations in the proportion of messenger RNA (mRNA) splicing variants lead to production of inclusions (Goedert and Spillantini 2006). Alternative splicing of tau generates six isoforms containing either three or four microtubule-binding repeats in approximately equal proportions (Spillantini and Goedert 2013). Furthermore, mutation of *MAPT*, which encodes tau protein, alters the proportion of splicing variants and causes tau aggregation in several disorders (Rademakers et al. 2004; Spillantini and Goedert 2013). However, in sporadic cases the mechanism initiating the change to an aggregation-prone protein form remains unclear.

Conformational change may also occur as a secondary or tertiary disease mechanism. In AD there are two main inclusions: senile plaques and neurofibrillary tangles. A major component of the former is Aβ protein (Hardy and Selkoe 2002), and the major component of the latter is tau protein (Iqbal and Grundke-Iqbal 2006). The Aβ oligomer promotes tau phosphorylation and causes aggregation (Tomiyama et al. 2010). These facts argue for AD possibly being, primarily, a disease involving Aβ deposition and, secondarily, a tauopathy. Although tau protein is involved in neuronal cell death, it is considered from this viewpoint to have little influence on system selectivity.

1.4 The Propagation of Prion-like Protein in Neurodegenerative Disease

It has been proposed that the pathological misfolding of disease-associated proteins occurs autonomously and independently in each cell, indicating that the spread of pathological change is a consequence of these regional changes (Goedert et al. 2010; Prusiner 2012). However, a pathological feature of neurodegenerative disorders has disproved this hypothesis. Braak et al. (2003) proposed that α-synuclein inclusions in PD patients form in two regions: (1) from the dorsal vagal nucleus to the medulla oblongata and midbrain, or (2) from the olfactory bulb finally spreading to the prefrontal cortex, cingulate gyrus, and mesial temporal lobe.

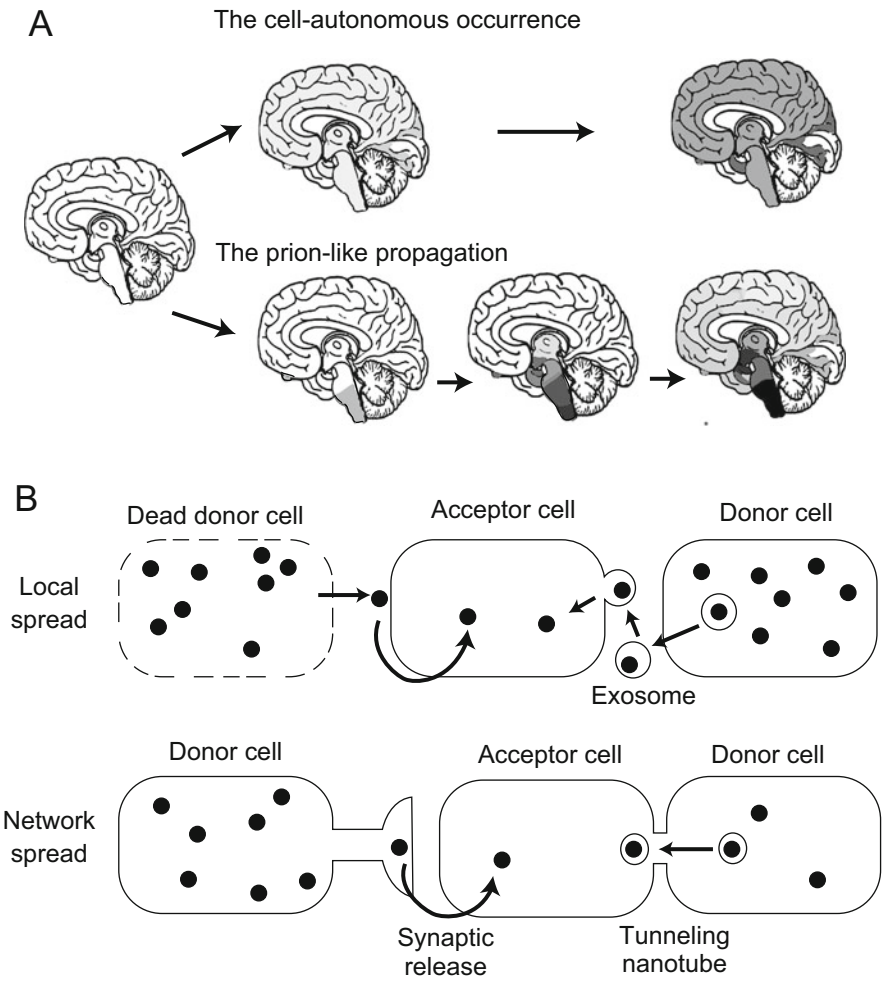

Fig. 1.2 Propagation of prion-like protein: (*A*) conventional supposition in which misfolded protein occurs autonomously and independently in each cell; (*B*) prion-like propagation theory of neurodegenerative disease, which contends that accumulation of misfolded protein begins in a specific location in the brain and progresses continuously. (This figure is based on Goedert et al. 2010 and Frost and Diamond 2010)

In order to explain the propagation of pathological misfolding of disease-associated proteins in a restricted area of the nervous system, the prion hypothesis has been proposed (Goedert et al. 2010) (Fig. 1.2A).

Prion disease is a lethal neurological disorder caused by proteinaceous infectious particles (prions) (Prusiner 1998). Normal prion protein (PrP^{c}) is expressed systemically and is relatively highly expressed in the central nervous system (Bendheim et al. 1992). Abnormal prion protein (PrP^{sc}), which is insoluble and rich in β-sheet structure, is derived from PrP^{c} through a conformational change

without any amino acid alteration. The PrP^{sc} replicates itself in an autocatalytic chain reaction (Cohen et al. 1994; Lansbury 1994). The prion hypothesis expands the concept of pathologically infectious misfolded protein to other proteinopathies. This theory contends that the pathological misfolded protein is transferred to other cells, where it induces a conformational change in normally folded protein, resulting in spread of the pathological protein through the central nervous system. Indeed, transmission of pathological misfolded α-synuclein has been reported in the brain of PD patients who have received transplanted fetal neurons. α-Synuclein inclusions have been found in these transplanted fetal neurons, suggesting that pathological misfolded protein has been transmitted to them (Li et al. 2008). Moreover, transmission of misfolded protein has been proven in animal models of α-synucleinopathy (Luk et al. 2012; King et al. 2012; Li et al. 2013) and tauopathy (Clavaguera et al. 2009).

The prion-like propagation hypothesis raises the possibility that protein with a prion-like domain may cause neurodegenerative disease. The prion-like domain in RNA-binding protein has been attracting attention as a mechanism that may account for initiation of inclusion body formation in neurodegenerative diseases. King et al. (2012) have computationally predicted prion-like domains in 21,873 human proteins and actually confirmed their presence in 246 proteins (Couthouis et al. 2011). Of these 246 proteins, 49 are speculated to be RNA-binding types (Li et al. 2013), including *TARDBP* (King et al. 2012), *FUS* (King et al. 2012; Li et al. 2013), *TAF15* (Couthouis et al. 2011), and *EWSR2* (Couthouis et al. 2012), which are associated with neurodegenerative disease. Kim et al. (2013) have hypothesized that RNA-binding proteins with prion-like domains may cause human disease. They found pathogenic mutations of heterogeneous nuclear ribonucleoproteins (hnRNPs) A2B1 and A1 in these domains in families with inherited motor neuron disease. Moreover, causative mutations in *TARDBP* and *FUS* have frequently been observed in the prion domain. They enhance the prion-like properties of the protein (Lagier-Tourenne et al. 2010; Lattante et al. 2013). These findings lend weight to the hypothesis that prion-like propagation may underlie the molecular pathogenesis of neurodegenerative disorders.

Although accumulating evidence supports the prion-like propagation hypothesis, several issues remain to be clarified. For example, transmission has not been proven in many human neurodegenerative disorders. Transmission from tissues containing PrP^{sc} has been demonstrated in kuru (Manuelidis et al. 2009), iatrogenic prion disease (Brown et al. 2012), and variant Creutzfeldt–Jakob disease (vCJD) in humans (Will et al. 1996). Transmission from tissue containing pathological misfolded α-synuclein and Aβ proteins in humans is still unclear (Irwin et al. 2013). Pituitary growth hormone (c-hGH) extracted from postmortem brain is known to cause iatrogenic CJD (Brown et al. 2012) with a frequency of 0.4 % (29 cases/7700 recipients) in the United States (Brown et al. 2012). On the other hand, in a follow-up study of 6190 patients out of 7700 (average age 37.3 years, mean follow-up 27.2 years), none developed AD or PD (Irwin et al. 2013). The frequency of PD and AD is significantly higher than that of CJD. Moreover, tau, Aβ, and α-synuclein can accumulate in the pituitary gland (Irwin et al. 2013). Thus,

c-hGH extracted from postmortem brain might contain these misfolded proteins. These facts indicate that the infectivity of Aβ and α-synuclein is markedly lower than that of PrP^{sc}.

Another issue is how pathological misfolded protein is transmitted to other cells. In prion disease, PrP^{sc} spreads to the whole brain without any marked system selectivity (Parchi et al. 1999). To explain the progression of neurodegenerative disease in terms of the prion theory, it is necessary to clarify the mechanism responsible for selective propagation. Local-spread and network-spread pathways have been proposed for transcellular propagation (Frost and Diamond 2010). The former pathway is regulated by the release of aggregated proteins into the extracellular space after cell death or the release of exosomes through exocytosis (Frost and Diamond 2010; Fevrier et al. 2004). The network-spread pathway is regulated by the trans-synaptic mechanism (Frost and Diamond 2010) or through the nanotube tunnel system that links cells (Gousset et al. 2009) (Fig. 1.2B). The network-spread pathway may play an important role in explaining the system selectivity of individual neurodegenerative disorders. However, it is still unclear how such disorders stop spreading in the brain. In some cases of ALS, TDP-43 pathology is observed in the pyramidal system as well as the frontotemporal lobe, indicating that TDP-43 pathology starts in the former and then spreads to the latter. However, Nishihira et al. (2008) have reported that TDP-43 pathology in some cases of ALS is restricted to the pyramidal system, even in those showing a long clinical course. Thus, disease duration cannot explain difference in the pattern of TDP-43 pathology. Therefore, although the prion hypothesis is attractive as an explanation of the system selectivity of neurodegenerative disorders, based on our current knowledge it is still insufficient.

1.5 How Conformational Disease Causes Cell Death

Protein or peptide with a pathological conformation induce cell death or dysfunction through a mechanism that involves gain of toxicity or loss of function (Winklhofer et al. 2008). The gain-of-toxicity mechanism results from inclusion bodies acquiring a pathogenetic nature that causes dysfunction and cell death, whereas the loss-of-function mechanism involves loss of the protein's normal function through conformational change, with resulting deleterious effects on the cell. These mechanisms are not contradictory concepts and both mechanisms might cause disease in some cases. For example, aggregation of tau inhibits normal axonal transport in tauopathy, resulting in dysfunction and cell death (gain of toxicity) (Stokin et al. 2005), whereas it also decreases the amount of normal tau, thus impairing normal function of the protein in microtubule stabilization (loss of function) (Yoshiyama et al. 2013; Weingarten et al. 1975). Although the gain-of-toxicity hypothesis holds that inclusion bodies are cytotoxic, recent studies have highlighted the importance of oligomer in the process of cell dysfunction or cell death (Takeda et al. 2006; Takahashi et al. 2010; Nagai et al. 2007; Sawada et al. 2004).

1.6 Diseases Caused by Alteration of RNA

In contrast with conformational disease, several neurodegenerative disorders are caused by alteration of RNA (O'Rourke and Swanson 2009; Cooper et al. 2009). In one such type of disease, disease-associated mutated RNAs bind to proteins and alter their function. Expansion of nucleotide repeats in the untranslated region causes neurodegenerative diseases including SCA 8, 10, 12, 31, 36, and myotonic dystrophy type 1 (Todd and Paulson 2010; de Leon and Cisneros 2008). An expansion of GGGGCC repeats in the first intron of C9orf72 has been shown to be a common cause of familial/sporadic ALS and frontotemporal lobar dementia (FTLD) (DeJesus-Hernandez et al. 2011; Konno et al. 2013). Nucleotide repeat expansion disease can show a pathogenetic mechanism that reflects both conformational disease and RNA disease: (1) the proteins that bind specifically to the unique sequence are trapped in an abnormal mRNA repeat, causing loss of protein function (Lee and Cooper 2009); (2) the mRNA repeat enhances the expression of other proteins through activation of an unknown mechanism (Lee and Cooper 2009); and (3) the nucleotide repeat in the untranslated region can express homopolymeric expansion proteins in all three reading frames, regardless of the start codon (repeat-associated non-ATG translation) (Cleary and Ranum 2013; Zu et al. 2011) (Fig. 1.3).

The unknown mechanism is associated with RNA metabolism in several ways, including transcription, splicing, editing, polyadenylation, transportation,

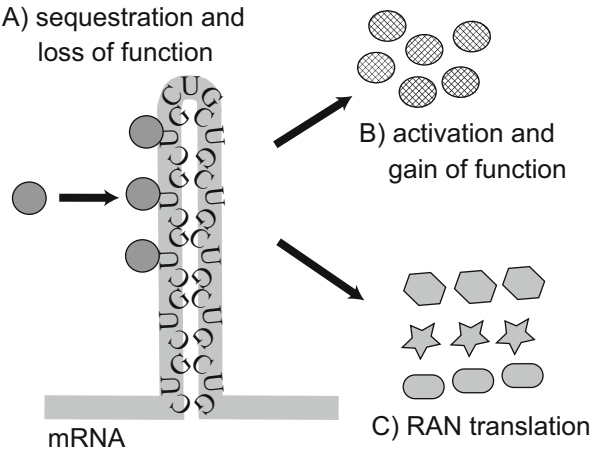

Fig. 1.3 The pathological mechanism responsible for expansion of nucleotide repeats in the untranslated region. The expanded nucleotide forms a hairpin-like structure. A CUG expansion is shown as an example: (*A*) RNA-binding protein is trapped specifically in the repeat expansion; (*B*) the repeat induces an increase in the expression level of other proteins through an unknown mechanism; (*C*) the repeat in the untranslated region can initiate transcription, and three forms of the protein are expressed regardless of the start codon (This figure is based on Lee and Cooper (2009))

translation, and degradation of mRNA (Cooper et al. 2009). Homeostasis of RNA is termed "ribostasis" (Li et al. 2013; Ramaswami et al. 2013). We first review diseases associated with aberrant pre-mRNA splicing, which has been detected in many hereditary neurodegenerative disorders, including myotonic dystrophy type 1 and type 2 as well as fragile X-associated tremor/ataxia syndrome (FXTAS) (Licatalosi and Darnell 2006; Ling et al. 2013; Sellier et al. 2010). Most of these diseases are caused by sequestration of splicing factors in an expanded repeat motif in RNA.

1.7 Gems and Motor Neuron Disease

However, the causative protein for spinal muscular atrophy – survival of motor neuron (SMN) – has a specific function in the pre-mRNA machinery. SMN forms inclusions in the nucleus known as "Gems," which are the sites of assembly and maturation of small nuclear ribonucleoprotein (snRNP) (Morris 1783; Coady and Lorson 2011; Dundr 2012). snRNP is an essential component of the splicing machinery (Will and Luhrmann 2005). In the assembly of snRNP, SMN first forms a dimer and binds directly to Gemin 2, 3, and 8 and indirectly to Gemin 4, 5, 6, 7, and unrip protein (Neuenkirchen et al. 2008). The SMN complex then binds to the Sm complex and uridine-rich small nuclear RNAs (U snRNAs) and transports them into the nucleus (Burghes and Beattie 2009). Gems are inclusions where additional proteins are assembled on snRNPs and U snRNAs are modified, consequently forming a spliceosome. Spliceosomes regulate the splicing of pre-mRNA and are classified as major or minor, according to consensus sequences of acceptor and donor sites for pre-mRNA splicing (Will and Luhrmann 2005). Although the major class of spliceosomes regulates most pre-mRNA splicing, minor spliceosomes also play an important role in regulating the splicing or global speed of pre-mRNA processing (Turunen et al. 2013). In spinal muscular atrophy, the number of Gems and U snRNA in the minor spliceosomes are markedly decreased, resulting in aberrant alternative splicing (Burlet et al. 1998; Gabanella et al. 2007; Shan et al. 2010; Zhang et al. 2008; Lotti et al. 2012).

TDP-43 also co-localizes with Gems (Wang et al. 2002; Tsuiji et al. 2013). The amount of TDP-43 protein affects the number of Gems (Shan et al. 2010; Ishihara et al. 2013). It has been found that Gems are decreased in affected tissue in ALS (Tsuiji et al. 2013; Ishihara et al. 2013). Furthermore, U12 snRNA, belonging to the minor spliceosome class, is decreased in tissue with TDP-43 pathology, but not without (Ishihara et al. 2013). Finally, immunohistochemical analysis has revealed that the amount of snRNP belonging to minor spliceosomes is decreased in spinal

motor neurons in ALS patients (Ishihara et al. 2013). These findings are consistent with previous results obtained using an SMN-reduced mouse model (Zhang et al. 2008). However, another group has reported that an increase in U snRNAs and snRNPs subtypes is accompanied by a decrease in the number of Gems in tissues affected by ALS (Tsuiji et al. 2013). Therefore, the type of alteration of U snRNA and snRNPs occurring in ALS is still unclear.

1.8 Stress Granules and RNP Granules in Motor Neuron Disease

Several RNA-binding proteins have been identified as causative genes for motor neuron disease (Couthouis et al. 2011, 2012; Li et al. 2013; Iguchi et al. 2013). These proteins are frequently observed in cytoplasmic ribonucleoprotein (RNP) granules and stress granules (SGs), which have a role in the triage and degradation of RNA (Ramaswami et al. 2013; Kiebler and Bassell 2006). They are composed of mRNAs and RNA-binding proteins (King et al. 2012; Li et al. 2013; Ramaswami et al. 2013), suggesting that SGs play an important role in the pathogenesis of neurodegenerative disorders (Kiebler and Bassell 2006; Guil et al. 2006; Dormann et al. 2010; Anderson and Kedersha 2008). Indeed, pathological alleles of these RNA-binding proteins, such as FUS and TDP-43, facilitate the formation of SGs (Li et al. 2013; Kim et al. 2013; Wolozin 2012). Notably, disease mutations promote excess incorporation of hnRNPA2 and hnRNPA1 into stress granules and drive the formation of cytoplasmic inclusions in animal models that recapitulate human pathology (Kim et al. 2013). Thus, dysregulated polymerization caused by a potent mutant in a prion-like domain of these RNA-binding proteins can initiate degenerative disease (Kim et al. 2013). In addition, ataxin-2 increases the risk of ALS by modifying the function of SGs (Elden et al. 2010). Ataxin-2 showing intermediate expansion of the polyglutamine tract facilitates the formation of SGs, followed by inhibition of SG degradation after exposure to stress (Farg et al. 2013).

These results raise the possibility that neurodegeneration can be brought about by increasing the formation of SGs (Li et al. 2013). Several hypotheses have been proposed to explain how aggregated proteins in RNP granules inhibit normal RNA metabolism: (1) by binding to mRNA and inhibiting its expression (Nevins et al. 2003); (2) by inhibiting the protective response of SG to stress (McDonald et al. 2011; Aulas et al. 2012); and (3) by being sequestered into SG, followed by consequent loss of their own function (Aulas et al. 2012) (Fig. 1.4).

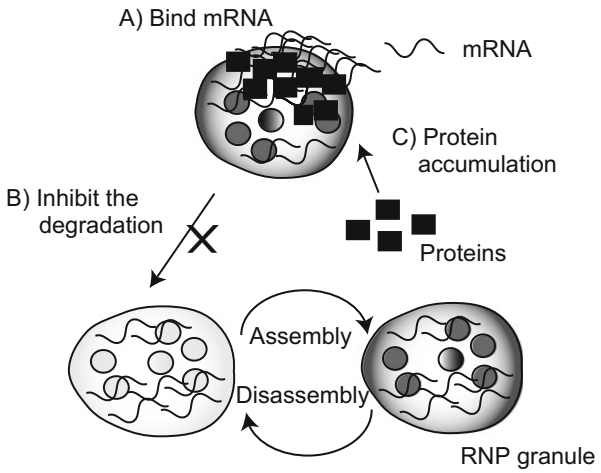

Fig. 1.4 Pathological consequences of expansion of nucleotide repeats in the untranslated region. Several specific proteins and RNA form RNP granules, which can be synthesized and degraded rapidly in response to the state of the cell: (*A*) aggregated proteins bind to specific mRNA and inhibit its expression; (*B*) aggregated proteins inhibit the degradation and synthesis of RNP granules; (*C*) aggregation of the protein causes loss of its own function

1.9 Perspective

This chapter has reviewed the history of neurodegenerative disease and current concepts of the molecular pathogenesis of neurodegenerative disorders: conformational disease theory and RNA disease theory. These hypotheses are not mutually exclusive, and a combination of both might occur in several diseases (Ramaswami et al. 2013; Ling et al. 2013). Further studies of both mechanisms may lead to the development of therapeutic strategies that could theoretically include removing accumulated protein (Nicoll et al. 2003; Davtyan et al. 2013), inhibiting its accumulation (Crouch et al. 2011), regulating pre-mRNA splicing (Cooper et al. 2009), and controlling RNP granule assembly (Ramaswami et al. 2013). These mechanisms also explain the vulnerability of neurons in neurodegenerative disorders to unique features of neurons (Ramaswami et al. 2013): terminally differentiated and non-dividing cells. These features may contribute to the accumulation of unfolded proteins. In addition, neurons possess higher numbers of RNP granules in their dendrites and axons, suggesting that neurons might be vulnerable to RNP alteration. Finally, the tight connectivity of synapses may facilitate the spread of misfolded proteins or RNP granules to adjacent neurons (Ramaswami et al. 2013; Brundin et al. 2010) (Fig. 1.5).

The prion-like propagation hypothesis may explain the mechanism for system-selective spread of disease-associated pathological protein in neurodegenerative diseases. However, it is not fully understood why each disease involves a selected system. The causative genes for hereditary neurodegenerative disease are often

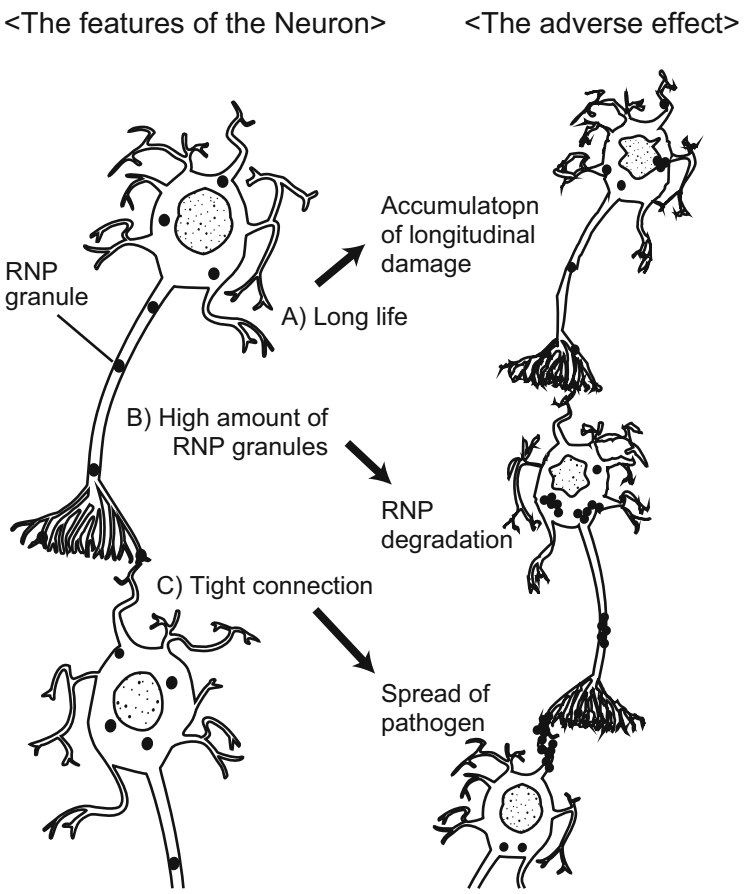

Fig. 1.5 Features of neurons and adverse effects of protein accumulation: (*A*) the long life of neurons makes them vulnerable to accumulation of longitudinal damage, and this results in neurodegeneration; (*B*) this neuron has high numbers of stress granules and RNP granules, which could cause degeneration through degradation and accumulation; (*C*) tight connections between other neurons facilitate spread of pathogenic protein (This figure is based on Ramaswami et al. 2013)

expressed ubiquitously and may not explain system selectivity in each case. For example, although TDP-43 is expressed ubiquitously, *TARDBP* mutation, which encodes the TDP-43 protein, causes familial ALS (Yokoseki et al. 2008; Lagier-Tourenne et al. 2010). Interestingly, most cases of *TDP-43* mutation do not show the FTD phenotype, which is categorized as a TDP-43 proteinopathy (Lagier-Tourenne et al. 2010; Borroni et al. 2009). In contrast, *progranulin* mutation causes FTD with TDP-43 proteinopathy, but not ALS (Mackenzie 2007). Therefore, the system selectivity of each disorder can be explained by the specific causative gene, although it remains to be clarified why the selected system is rendered vulnerable to initiation.

The molecular mechanism responsible for initiation of sporadic neurodegenerative disease is still not clear, although genome-wide analysis has identified several genes that are involved (Tsuji 2010; McMillan et al. 2014). Specific genotypes of *APOE* and a haplotype of *MAPT* carry a high risk of AD and tauopathy, respectively (Goedert et al. 2010; Strittmatter et al. 1993; Takei et al. 2009). However, most patients with neurodegenerative disorders do not have these high-risk genotypes or haplotypes. Further elucidation of the pathogenesis of sporadic neurodegenerative diseases may lead to the development of new strategies for their treatment or prevention.

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Chapter 2
Brain–Peripheral Organ Communication

Masayuki Sekiguchi

Abstract Communication between the brain and internal organs is likely utilized to maintain homeostasis in the body of an animal. Consistently, converging evidence suggests that the brain receives information not only from the outside but also from the inside of the body. The perception of information from the inside of the body is called "interoception," which includes a sense of the condition of internal organs such as the lungs, the heart, and the organs of the digestive system, as well as a sense of internal milieu such as pH and temperature of body fluid. This perception and subsequent response of the brain provide one of the processes in brain–internal organ communications. Recent findings have shown that interoception plays more pivotal roles than have been thought. For example, it has been revealed that interoception of the condition of the liver plays pivotal roles in health and disease. The liver is the body's center of metabolism, and the condition of this organ is likely to influence brain functions. The main purpose of this chapter is to understand how the brain and internal organs interact, mainly from the point of view of interoception. This is the reason why the liver is taken as an example of an internal organ and knowledge of the interaction between the brain and this organ is mainly described.

Keywords Interoception • Humoral factor • Vagus nerve • Nucleus of the solitary tract • The insular cortex • The liver

2.1 Introduction

It is little wonder that the brain and internal organs influence each other. Such communication is likely utilized to maintain homeostasis in the body of an animal. Consistently, converging evidence suggests that the brain receives information not only from the outside but also from the inside of the body (Craig 2002). The perception of information from the inside of the body is called "interoception"

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K. Wada (ed.), *Neurodegenerative Disorders as Systemic Diseases*,
DOI 10.1007/978-4-431-54541-5_2

(Damasio and Carvalho 2013), which includes a sense of the condition of internal organs such as the lungs, the heart, and the organs of the digestive system, as well as a sense of internal milieu such as the pH and temperature of body fluid (Craig 2002). Therefore, perception and subsequent response of the brain constitute one of the processes in brain–internal organ communications. On the other hand, the perception of information from the outside of the body is called 'extraception', which is a sense of the outside provided by hearing, sight, touch, taste, or smell (Damasio and Carvalho 2013). This sense is captured by respective sensory neurons, converted to an electrical signal, and transferred to the central nervous system (CNS). The brain responds to both extraceptive and intraceptive information, and the consequence of these senses appears not only in reflex changes but also long-lasting changes in behavior (Dantzer et al. 2008). Extraception is of course the main means of perception for the activity of an organism, but recent findings have shown that interoception plays more pivotal roles than previously thought. For example, it has been revealed that interoception of the condition of the liver plays pivotal roles in health and disease (Dantzer et al. 2008; D'Mello and Swain 2011; Yamada and Katagiri 2007). The liver is the body's center of metabolism, and the condition of this organ is likely to influence brain function.

The main purpose of this chapter is to understand how the brain and internal organs interact, mainly from the point of view of interoception. This is the reason why the liver is taken as an example of an internal organ and knowledge of the interaction between the brain and this organ is mainly described.

2.2 Interoception Using Afferent Vagus Nerves

2.2.1 Vagus Nerve

The brain and internal organs are known to communicate with each other in two different ways: neuronal and humoral communication (Dantzer et al. 2008; D'Mello and Swain 2011; Yamada and Katagiri 2007). In the neuronal mechanism, one of the neurons used in this communication is the vagus nerve, the 10th cranial nerve (Fig. 2.1A), and another is the sympathetic nerve (Fig. 2.1B, see Sect. 2.5). The vagal trunk is made up of two different fibers, one is a preganglionic efferent fiber originated in the dorsal motor nucleus of the vagus and nucleus ambiguus in the brainstem, which is projected to the ganglion near each target organ. This efferent fiber occupies approximately 27 % of total fibers (3000/11,000 fibers) in vagus subdiaphragmatic trunks in the case of rats (Berthoud and Neuhuber 2000). The other is a major afferent fiber originated in the jugular and nodose ganglia in the cervix, where one axon end is projected to the CNS and the other end is projected to each internal target organ (Berthoud and Neuhuber 2000). The afferent fiber occupies the remaining 73 % of total vagus fibers (8000/11,000 fibers) in the case of rats (Berthoud and Neuhuber 2000). The efferent fiber acts to regulate the

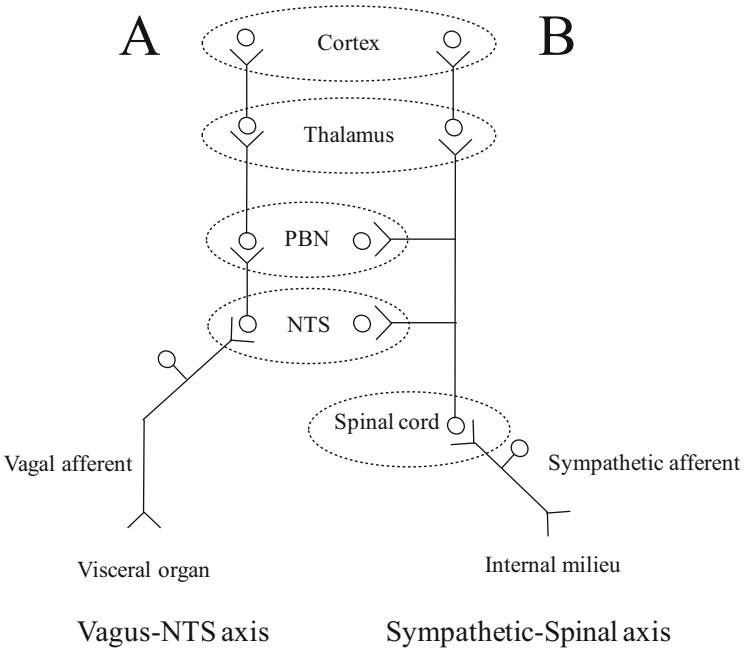

Fig. 2.1 A schematic diagram of two neural routes that mediate peripheral organ–brain interactions: (*A*) The afferent vagus–nucleus tract solitarii (NTS) axis. Information on the visceral organs is transmitted to the parabrachial nucleus (PBN) via the NTS, which is then transferred to the thalamus (the projection site is the medial portion of the ventroposterior thalamic complex), and finally to the cerebral cortex (mainly a posterior part of the insular cortex). (*B*) The sympathetic–spinal axis. Sympathetic afferents, in response to nociception related to mechanical, chemical, or thermal stimulation, transmit information to particular spinal neurons in the dorsal horn of the gray matter, and these neurons directly project to thalamic neurons in another part to which the vagus–NTS axis projects (the lateral part of the "shell" of the ventroposterior thalamic complex). Spinal neurons also project to the NTS and PBN by means of axon collaterals. The posterior insular cortex is the main projecting site of such thalamic neurons

activity of internal organs, and the afferent fiber transmits information about the organ to the brain. It is estimated that approximately 80 % of fibers in the main vagus trunk are unmyelinated (Hoffman and Schnitzlein 1961; Foley and DuBois 2004), and that most of the remaining 20 % of fibers are poorly myelinated (Friede and Samorajski 1967). This tendency is particularly strong in the vagal branches (Prechtl and Powley 1990). High content of unmyelinated fibers is a characteristic feature of the vagus nerve.

The common hepatic branch of the vagus diverges from the left subdiaphragmatic vagus trunk passing along the esophagus (Berthoud and Neuhuber 2000; Puizillout 2005), immediately caudal to the diaphragm. The common hepatic branch is made up of about 3000 fibers, and afferent fibers occupy 2200 of them. There are only 200 efferent fibers, the remaining 600 are non-vagal adventitial fibers such as sympathetic postganglion or dorsal root afferent fibers (Prechtl and

Powley 1990). The common hepatic branch of fibers further diverge and they project to the liver, bile duct, portal vein, and paraganglia (Berthoud et al. 1992); the remaining fibers form a gastro-duodenum branch (Berthoud and Neuhuber 2000; Puizillout 2005; Berthoud et al. 1992). The heterogeneity of the fibers in the common hepatic branch is important as described below. At least in the rat liver, vagal afferent axons terminate at the connective tissue surrounding intrahepatic triads, and their primary target is likely not to be the liver parenchyma (Berthoud and Neuhuber 2000; Berthoud et al. 1992). However, it seems that there is a species difference with respect to the target site (Metz and Forssmann 1980).

Generally, it is thought that vagal afferent fibers project to neurons in the nucleus tractus solitarii (NTS), the projection site in the CNS (Paton 1999). From the NTS, there is a direct connection to several brain stem nuclei; projection to the parabrachial nucleus (PBN) is particularly important as a relay pathway to the thalamus (Berthoud and Neuhuber 2000). It is thought that glutamate is a principal neurotransmitter released from vagal afferent terminals in the modulation of respiration, cardiovascular reflexes, gastrointestinal dynamics, and information about types, quantity, and location of nutrients such as lipid, carbohydrate, and protein (Hornby 2001; Travagli et al. 2006; Zheng et al. 2005; Ritter 2011; Julio-Pieper et al. 2011; Kline 2008). The mechanism in which only one kind of transmitter, glutamate, classifies information with different modalities is not yet understood, but one possible explanation is that afferent fibers originated in one internal organ project to particular NTS neurons with similar function and projection. However, a viscerotopic distribution of postsynaptic NTS neurons with different functions is not known (Paton 1999). For example, physiologically characterized neurons responding to distinct afferent modalities are very close neighbors and form neither laminar structure nor cluster (Paton 1999). On the other hand, it is known that viscerotopic distribution can be found in the same modality: for example, gustatory afferents project in the rostral part of NTS but gastrointestinal afferents localize more caudally (Altschuler et al. 1989). In addition to glutamate, neuromodulators that uniquely regulate vagal afferent from particular internal organs have been found. These are substance P (Sekizawa et al. 2003; de Lartigue 2014), nitric oxide and GABA in the case of regulation of cardiovascular functions, tachykinin and neurokinin A in the case of regulation of respiration, and cocaine and amphetamine regulated transcript (CART) and melanin concentrating hormone (MCH) in the control of food intake (de Lartigue and Raybould 2011; de Lartigue 2014).

Similarly to vagus afferent fibers innervating other organs, the CNS axon of the vagus nerve innervating the liver is known to project to the NTS (Rogers and Hermann 1983) in the rat. Detailed projecting sites are the left subnucleus gelatinosus, the medial division of the left solitary nucleus, and the left lateral edge of the area postrema (Rogers and Hermann 1983). Another study, however, could not find any axonal labeling in the NTS after injection of a transganglionic tracer, cholera toxin β-subunit–horseradish peroxidase, into the hepatic nerve, ileocolic vein, portal vein wall, hepatic hilus, and hepatic parenchyma (Shin and Loewy 2009). An early electrophysiological study in the rat suggests that stimulation of the hepatic branch elicits responses in the left medulla with long latencies (Adachi

1981, 1984). The reason for long latencies is thought to be hepatic vagal branches comprising large numbers of non-medullated fibers (Adachi 1984). There are no data on the neurotransmitter of the vagal afferent innervating the liver.

Vagotomy has frequently been used to assess whether particular biological changes are mediated by the vagus nerve. Vagotomy of the common hepatic branch, in particular, has been extensively used, but care needs to be taken when interpreting the results from this method, largely due to heterogeneity of this branch (as mentioned earlier). In addition, the existence of another vagus branch that innervates the liver through the dorsal (right) subdiaphragmatic vagus trunk has been reported (Jancso et al. 1977; Niijima 1983; Berthoud et al. 1992; Phillips et al. 1997), which makes the interpretation of hepatic branch vagotomy more difficult.

It is known that capsaicin destroys unmyelinated afferents of both dorsal root and nodose ganglions (Jancso et al. 1977; Nagy et al. 1981; Chung et al. 1990; Holzer 1991; Carobi 1996) via the VR-1 vanilloid receptor (Michael and Priestley 1999). In the case of the vagus nerve innervating the liver in the rat, neonatal capsaicin treatment is known to completely destroy the smallest fibers (up to 180 μm^2) innervating the left and median lobes and the right and caudate lobes of the liver (Carobi and Magni 1985). In addition, similar treatment reduced the number of neurons (up to 300 μm^2) (Carobi and Magni 1985). Although there is no guarantee that all vagus afferents are capsaicin sensitive (Berthoud et al. 1997), capsaicin treatment may be an effective means of making vagus afferents innervating the liver inactive.

2.2.2 Chemical Substances That Change the Activity of the Vagal Hepatic Branch

As a result of neurons transmitting their activity by generating electrical spikes, the chemical substance that facilitates or suppresses vagal nerve spike activity is a candidate interoception mediator. There are several endogenous chemical substances that facilitate or suppress the spontaneous firing of this nerve, such as those listed in the following subsections (see also Table 2.1). It is important to keep in mind that the studies described in the following subsections are conducted using the common hepatic branch of the vagus nerve. This nerve bundle is composed not only of nerves that innervate the hepatoportal region but also nerves that innervate outside this region, mainly the gastrointestinal region (Horn and Friedman 2004), as mentioned earlier. Therefore, the possibility that activity may originate in fibers other than those from hepatoportal regions cannot be completely excluded.

Amino Acids When injected into the hepatic portal vein, some amino acids cause the firing rate in the hepatic branch of the vagus nerve to increase, although it takes 60 min or more to reach the maximal response (Niijima and Meguid 1995). Amino

Table 2.1 Endogenous compounds that modulate electrical activity of the common hepatic branch of the vagus nerve in rodents

Compound	Action	Route	Reference
Alanine, arginine, histidine,	+	PV (10 mM)	Niijima and Meguid (1995)
leucine, lysine, serine,			
tryptophan, valine			
Cysteine, glycine,	_	PV (10 mM)	Niijima and Meguid (1995)
isoleucine, methionine,			
proline, threonine			
Linoleic acid	+	JI (1 ml/45 s)	Randich et al. (2001)
	+	PV (1.5 mg)	Randich et al. (2001)
D-glucose	_	PV (<100 mg/kg)	Niijima (1982)
	_	DI (5 %, 5 ml)	Niijima (1982)
		[glucose] in PV↑	
Insulin	+	IV	Niijima (1984)
Glucagon	_	IV	Niijima (1984)
GLP-1	+	PV (0.2 pmol)	Nishikawa et al. (1996)
CCK-8	+	IV (2 μg/kg)	Cox and Randich (1997)
IL-1β	+	PV (100 pg)	Niijima (1996)
Somatostatin	+	PV (3.05 pmol)	Nakabayashi (1997)

(Abbr) *DI* Duodenum infusion; *IV* intravenous; *JI* Jejunal infusion; *PV* portal vein

acids with such enhancing activity are alanine, arginine, histidine, leucine, lysine, serine, tryptophan, and valine. On the other hand, cysteine, glycine, isoleucine, methionine, proline, and threonine suppress the activity of vagus nerves (Niijima and Meguid 1995). The mechanism by which these amino acids elicit their activity is unclear but amino acid sensors on the vagus nerve are supposed to exist (Niijima and Meguid 1995).

Fatty Acids It is known that linoleic acid injected into the hepatic portal vein increases hepatic vagus activity (Randich et al. 2001). Linoleic acid is an essential fatty acid and omega-6 polyunsaturated fatty acid. The action of linoleic acid on the vagus nerve is much slower than that of CCK-8 (2 μg/kg injected into the hepatic portal vein), in that it takes more than 30 min to reach the maximum response in the case of linoleic acid but less than 10 s in the case of CCK-8.

D-Glucose The rate of afferent discharges recorded from the hepatic branch of the vagus nerve in the guinea pig is dose-dependently decreased by injection of D-glucose in the hepatic portal vein. Similar injection of other sugars, D-mannose, D-fructose, D-galactose, L-glucose, D-xylose, or D-arabinose, failed to induce changes in the discharge rate (Niijima 1982). Therefore, it is known that there is an inverse relationship between the activity of glucose-sensitive hepatic vagal afferents and glucose concentrations.

Insulin and Glucagon Consistent with the results of glucose (mentioned above), the rate of discharge of hepatic vagal afferents in the rat is facilitated following

administration of insulin and inhibited after application of glucagon (Niijima 1984). The so-called "hepatoportal glucose sensor," the structure of which is not completely defined, is thought to be located upstream of the hepatic hilus and negative for the involvement of hepatocytes (Niijima 1984; Adachi et al. 1984). It is reported, on the other hand, that hypoglycemic signals in the portal vein are mediated by sympathetic afferents, because celiac-superior mesenteric ganglionectomy (but not hepatic branch vagotomy) abolishes the consequence of hypoglycemic signals (Fujita and Donovan 2005).

Glucagon-Like Peptide-1 (GLP-1) A truncated form of GLP-1 administered into the hepatic portal vein (0.2 pmol) facilitates hepatic vagal afferent activity. Since the administration of 4.0 pmol elicited a similar facilitation, it seems that 0.2 pmol GLP-1 is sufficient to being about maximal response (Nishikawa et al. 1996). On the other hand, a similarly administered glucose-dependent insulinotropic polypeptide does not facilitate hepatic vagal afferent activity (Nishikawa et al. 1996).

Cholecystokinin-8 (CCK-8) CCK-8 (2 μg/kg) also increases hepatic vagal afferent activity (15 of the 28 fibers tested, 54 %) as a result of intravenous administration via the CCK_A receptor (Cox and Randich 1997). The response to CCK-8 injected in this way also occurs very quickly (within 10 s).

Interleukin-1β (IL-1β) Injection of IL-1β (an inflammatory factor) into the hepatic portal vein facilitates firing of the hepatic vagus nerve in the rat. One shot of 100 pg IL-1β elicited gradual increase in spike frequency over 60 min (Ek et al. 1998). Intravenous injection of IL-1β also activates vagal afferents. It can be demonstrated that somata and/or fibers of sensory neurons of the vagus nerve express receptors to IL-1 and prostaglandin E2, and that circulating IL-1 stimulates vagal sensory activity via both prostaglandin-dependent and prostaglandin-independent mechanisms (Ek et al. 1998).

Somatostatin (SS) SS (3.05 pmol, a physiological dose) injected into the rat portal vein increases the spike discharge rate in hepatic vagal afferents (Nakabayashi 1997).

2.2.3 Interoception in Metabolism

A likely indicator of brain–liver interaction is when metabolism in the liver is altered (Fig. 2.2A). For example, liver-specific disruption of peroxisome proliferator-activated receptor γ (PPARγ) in leptin-deficient obese mice prevents hepatic steatosis, but increases peripheral adiposity to aggravate diabetic phenotypes in the mouse (Matsusue et al. 2005). In contrast, tissue-specific overexpression of PPARγ in the liver induces severe hepatic steatosis, but peripheral adiposity is rather reduced as a result of enhanced lipolysis (Uno et al. 2006). A putative mechanism underlying these homeostatic changes between the liver and adipose tissues is participation of the autonomic nervous system. Namely,

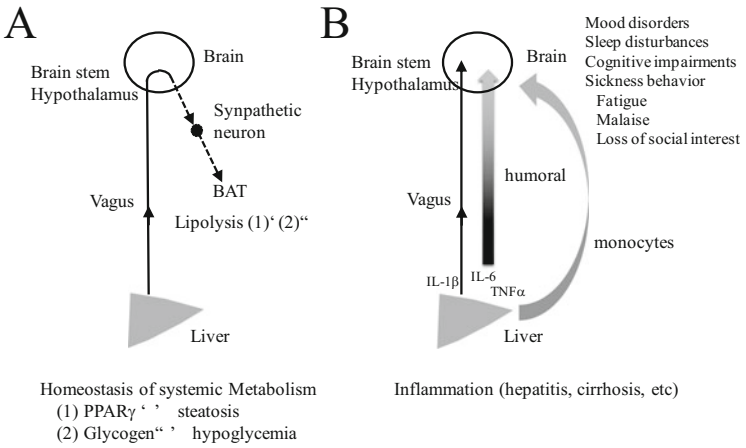

Fig. 2.2 Two potential pathways for liver–brain communication: (*A*) Homeostatic control of systemic metabolism. Overexpression of PPARγ in the liver induces steatosis of the liver, which facilitates lipolysis in brown adipose tissue (BAT) (1). On the contrary, decrease in liver glycogen reduces lipolysis such that lipids accumulate in BAT (2). These two homeostatic changes are mediated by vagus afferent fibers from the liver to the brain and sympathetic neurons from the brain to the BAT. (*B*) Effects of liver inflammation (in hepatitis or cirrhosis) on brain function. The vagus nerve innervating the liver can be activated by proinflammatory cytokines such as IL-1β, which may follow a neuronal route to influence the brain. Moreover, inflammation of the liver induces the release of cytokines IL-1β, IL-6, and TNFα into body fluid. These cytokines react with receptors on endothelial cells in the brain. Furthermore, an immune cell (a monocyte) transmigrates into the brain in response to initial activation of brain-resident microglia to produce a potent monocyte chemoattractant (MCP-1). Such liver–brain communications can induce changes in neural network activity in the brain. Changes in behavior that are induced result in mood disorders, sleep disturbance, cognitive impairment, and fatigue

information is transmitted from the liver to the brain via neuronal and humoral factors, and then the brain controls adipose tissues using efferent sympathetic nerves. Indeed, the increase of lipolysis in brown adipose tissue (BAT) by overexpression of PPARγ in the liver is abolished by hepatic branch vagotomy (Uno et al. 2006). Furthermore, high-fat feeding induced hepatic glucokinase upregulation, and hepatic glucokinase gene overexpression dose-dependently decreased adaptive thermogenesis by downregulating thermogenesis-related genes in the BAT (Tsukita et al. 2012). This inter-tissue (liver-to-BAT) system is mediated by the afferent vagus from the liver to the brain and sympathetic efferents from the brain to the BAT (Tsukita et al. 2012). The liver–brain–adipose neural axis is found to have an important role in switching the fuel source from glycogen to triglycerides under prolonged fasting conditions (Izumida et al. 2013).

2.2.4 *Interoception in Inflammation*

Another likely indicator of brain–liver interaction is the appearance of chronic liver diseases such as hepatitis and cirrhosis (Fig. 2.2B). Chronic inflammation of the liver is a disorder that is frequently associated with sickness behavior (fatigue, malaise, loss of appetite, loss of social interest, and so on) and alteration in mood state (D'Mello et al. 2009; Nguyen et al. 2007; Swain 2006; Swain and Maric 1995; Capuron and Miller 2011; Isik et al. 2007; Jones et al. 2006). In these disorders, significant pathological CNS tissue damage is basically absent (Forton et al. 2002, 2008; Pollak et al. 2003). A mechanism that explains CNS symptoms in periphery-originated diseases satisfactorily is that incorrect information about the state of peripheral organs is transmitted to the brain. It is thought that both neuronal and humoral factors are responsible for this and that vagus nerves are main players in neuronal factors (Goehler et al. 2000). In fact, the common hepatic branch of the vagus nerve can respond to proinflammatory cytokine IL-1β (as mentioned above) (Niijima 1996; Ek et al. 1998). The vagus nerve can be shown to express cytokine receptors such as the IL-1 receptor (Ek et al. 1998). In addition, endotoxin lipopolysaccharide (LPS), a gram-negative bacterial cell wall component, is a potent inducer of TNFα, IL-1β, and IL-6 (Dantzer et al. 2008; Capuron and Miller 2011; Lebbar et al. 1986; Vallieres and Rivest 1997; Laye et al. 2000). It is known that plasma endotoxin is elevated in patients with chronic liver disease (Yamamoto et al. 1994; Swain 2006). Therefore, cytokines released peripherally in response to bacterial endotoxin are thought to be important in communication between the brain and the liver through the vagus nerve. Consistently, in LPS-induced peritoneal inflammation, the NTS to which vagus afferents are projected, and subsequent regions to which NTS neurons project abundantly – the PBN and the hypothalamic paraventricular nucleus – are positive when assessed with expression of immediate early gene c-fos (Wan et al. 1993; Goehler et al. 2000). The intraperitoneal injection of IL-1β not only induces c-fos expression in the brain but also alters social exploratory behavior in the rat (Bluthe et al. 1996). It is known that changes in both c-fos expression and behavior can be reduced by subdiaphragmatic vagotomy (Bluthe et al. 1996).

Brain regions activated by inflammation were elucidated by getting healthy male volunteers (some of whom received typhoid vaccination or saline) to conduct a Stroop task during functional magnetic resonance imaging. Typhoid but not saline injection produced a robust inflammatory response and a significant increase in fatigue, confusion, and impaired concentration in participants. Performance of the Stroop task under inflammation-activated brain conditions involved the participation of the brainstem, thalamus, amygdala, cingulate, and bilateral mid and anterior insula in afferent interoception. Activity changes in these regions can be confirmed to be a result of inflammation (Harrison et al. 2009).

Dantzer et al. (2008) is an excellent review on the relation between inflammation and sickness behavior. The authors show that decompensation of sickness behavior could be a risk factor for mood disorders.

2.3 The Insular Cortex as the Destination of Interoception

A large part of the information collected by interoception generates the reflex to maintain the body's homeostasis. The hypothalamus controls a vast array of these physiological processes including sleep/wake cycles, sexual behavior and reproduction, and metabolic control such as thermoregulation, energy intake/expenditure, glucose metabolism, lipid metabolism, and food and water intake. In many cases, sympathetic neurons play a central role in these reflex reactions. There are excellent reviews on the role that sympathetic neurons and the hypothalamus play in homeostatic control (Saper 2002; Kalsbeek et al. 2014). To avoid duplication, I would like to introduce here another brain structure, the insular cortex, as a major ascending destination of interoception.

The insular cortex is part of the cerebral cortex and is located deep in the lateral sulcus in humans. In rodents, it surrounds the rhinal fissure and is between the primary/secondary somatosensory and piriform cortices in the most anterior–posterior levels (Paxinos and Franklin 2001). The insular cortex is normally divided into three subdivisions (Cechetto and Saper 1987): the agranular insular cortex (AI), which surrounds the rhinal fissure; the granular insular cortex (GI), which is located just ventral to the secondary somatosensory cortex; and the dysgranular insular cortex (DI), which is between the AI and GI. Each subdivision is thought to involve sensory information, in particular. For example, the AI is thought to be involved in nociceptive and autonomic information processing (Cechetto and Saper 1987; Jasmin et al. 2003; Burkey et al. 1996, 1999); the GI plays a crucial role in modulating visceral function (Yamamoto et al. 1981, 1984); and the DI participates in gustatory processing (Cechetto and Saper 1987). It is known that ascending visceral afferents, which project from ventroposterolateral (VPL) and ventroposteromedial (VPM) parvicellular (pc) nuclei of the thalamus and from the PBN, are localized in the GI and DI (Allen et al. 1991). On the other hand, integrated limbic afferents from the infralimbic cortex and the mediodorsal nucleus of the thalamus project to the AI (Allen et al. 1991). The convergence of visceral and limbic information may be used by the insular cortex to integrate autonomic response with ongoing behavior and emotion.

2.4 Vagus Nerve Stimulation

Stimulation of the vagus nerve has been clinically utilized in the treatment of epilepsy and depression (Beekwilder and Beems 2010; Bonaz et al. 2013). The first attempt at vagus stimulation using an implanted electrode in humans was reported in 1988 for the treatment of drug-resistant epilepsy (Penry and Dean 1990). In 1997, vagus nerve stimulation was approved by the Food and Drug Administration (FDA) in the United States as an adjunctive treatment for drug-resistant seizures, and then in 2005 for depression. It can be demonstrated that the

activation of vagus afferents suppresses seizure in rats (Krahl et al. 1998, 2001). The latter therapy is based on mood improvement in patients with epilepsy receiving vagus nerve stimulation (Groves and Brown 2005), but its effectiveness has not yet been confirmed by meta-analysis because of insufficient data (Martin and Martin-Sanchez 2012). Vagus nerve stimulation is effected by wrapping an electrode around the left vagus nerve in the neck (Reid 1990).

The precise mechanism underlying vagus nerve stimulation in epilepsy and depression is obscure, but it is postulated that vagus nerve stimulation modulates the activity of the following neuronal networks via the NTS (Bonaz et al. 2013): (1) activation of the thalamus and thalamocortical pathway; (2) suppression of the amygdala and hippocampus; and (3) increased norepinephrine and serotonin release in widespread cerebral regions. The locus coeruleus (LC), the main origin of noradrenergic projection, mediates at least some of the effects of vagus nerve stimulation in attenuating seizures because LC lesions suppress the antiepileptic effect of vagus nerve stimulation (Krahl et al. 1998). The LC is directly connected to the NTS. Activity mapping using c-fos in rats shows that positive signals are increased in the amygdala, cingulate, LC, and hypothalamus in response to antiepileptic stimulation of the vagus nerve (Naritoku et al. 1995). In humans, functional neuroimaging studies suggest that the thalamus, cerebellum, orbitofrontal cortex, limbic system, hypothalamus, and medulla exert long-term changes in their activity in response to vagus stimulation (Lomarev et al. 2002; Chae et al. 2003).

In addition, vagus stimulation can be shown to have effects on memory. Vagus stimulation given immediately after training enhances retention performance on inhibitory avoidance tasks (Clark et al. 1995). This enhancement in memory performance is not mediated by efferent fibers, but by afferent fibers (Clark et al. 1998). An explanation for this phenomenon is that memory storage processes may be influenced by peripheral activation of the vagus nerve. The concept that electrical stimulation of the vagus can enhance memory was found to be true of humans too: namely, vagus nerve stimulation administered after learning significantly enhances retention of word recognition memory in patients enrolled in clinical studies evaluating the capacity of vagus stimulation to control epilepsy (Clark et al. 1997, 1999).

The effect of vagus stimulation on memory has recently been expanded to extinction learning of fear memory. Fear memory is an emotional memory and is very important for the survival of an organism (LeDoux 2000). However, since excess fear memory limits an individual's behavior, there is an innate mechanism that suppresses fear memory. Extinction learning is one such mechanism (Myers and Davis 2006; Quirk and Mueller 2008), and inhibitory control of the amygdala by the medial prefrontal cortex via inhibitory neurons in the amygdala is thought to be the underlying process (Likhtik et al. 2008). For extinction learning of fear memory, there needs to be persistent re-exposure of the individual (who acquired fear) to contextual or sensory cues that induce fear memory (conditioned stimulus). Vagus nerve stimulation during this re-exposure can be demonstrated to enhance extinction learning potently (Pena et al. 2013). The exact mechanism behind this

effect is not understood. However, norepinephrine released by vagus stimulation, as mentioned earlier, is likely to be involved in the consolidation of extinction memory since it participates in memory consolidation (Roozendaal and McGaugh 2011) and reconsolidation (Debiec and LeDoux 2006). Because extinction learning is thought to be a biological mechanism or an experimental model of exposure therapy, a behavioral cognitive therapy (McNally 2007), combining vagus stimulation and exposure therapy may be effective for the therapy of particular psychiatric disorders in which fear is a causative factor (Milad et al. 2006).

2.5 Another Neuronal Route of Interoception

Afferent fibers of sympathetic neurons are also thought to play a pivotal role in interoception (Saper 2002, Fig. 2.1B). These sympathetic neurons project to dorsal horn sensory neurons in the spinal cord, and these spinal neurons directly project to the thalamic nuclei (the intralaminar nuclei and the shell area along the ventral and lateral margins of the VPMpc nucleus) (Horie and Yokota 1990; Kobayashi 1998; Koyama et al. 1998) by sending collateral projections to brain structures such as the NTS (Menetrey and Basbaum 1987; Gamboa-Esteves et al. 2001) and the PBN (Hylden et al. 1989). Therefore, information through this sympathetic nerve and the vagal nerve may converge to integrate these two senses in the NTS and PBS. There is a dense projection to the posterior insular cortex from the lateral portion of the VPMpc nucleus (Casey et al. 1996; Craig et al. 2000).

Information on the part played by spinal sympathetic afferent fibers in interoception came about as a result of reports from patients with spinal cord transections. For example, patients with spinal cord transections cannot report abdominal warmth or discomfort of acid reflux after eating a hot meal, but they can feel vague fullness mediated by the vagus nerve (Strauther et al. 1999; Juler and Eltorai 1985). In general, spinal sympathetic afferent fibers are thought to transfer visceral nociception related to mechanical, chemical, or thermal stimulation (Adelson et al. 1997; Ammons 1992). Moreover, it seems these afferent fibers are the main means of transmitting this kind of nociception to the level of conscious perception. For example, it is well known that sympathetic afferent fibers of greater splanchnic nerves transmit visceral pain via the spinal cord, which is important clinically. On the other hand, vagal afferents are generally not considered to be involved in nociception and pain (Berthoud and Neuhuber 2000). However, there is growing evidence to show that vagal afferents play a complex role in these modalities. For example, electrical stimulation of vagal afferents often elicits excitation of spinothalamic tract neurons in the upper cervical spinal cord (Fu et al. 1992; Chandler et al. 1996). A tracing study has demonstrated that primary vagal afferents project to the upper cervical spinal cord (McNeill et al. 1991). It is believed that vagal afferents function in the affective and emotional aspects of pain rather than in sensory discrimination (Traub et al. 1996).

2.6 Future Study

This chapter has provided a partial overview of communications between the brain and peripheral organs (in particular, those by the vagus nerve in the hepatoportal region). Since these two tissues also communicate with each other using humoral factors, identification of the means of interaction should be carried out carefully. Nevertheless, the presence of the vagus nerve and sympathetic spinal nerve system, which connects the brain and peripheral organs, is significant to understand homeostasis in the whole body. It seems that this homeostasis is important not only for maintenance of healthy conditions but also for pathogenesis of diseases. Indeed, the so-called "Braak's hypothesis" (Braak et al. 2003) can be applied to types of Parkinson's disease, in which degeneration of dopaminergic neurons in the substantia nigra pars compacta occurs in the midbrain. Braak's hypothesis postulates that protein accumulation in the enteric nervous system of the gastrointestinal tract occurs first and is then spread to the brain through the vagus nerve to trigger Parkinson's disease. To assess the authenticity of such a hypothesis, the accumulation of findings on brain–peripheral organ communication will rapidly increase in importance.

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Chapter 3
The Brain–Immune Network in Spinal Cord Injury

Masaki Ueno and Toshihide Yamashita

Abstract Diseases or injuries in the central nervous system (CNS) often cause robust immune responses, which significantly affect the recovery process. Here we review recent knowledge about brain–immune system interactions, which occur during degenerative and reparative processes, and focus mainly on spinal cord injury (SCI). Immune system–brain inflammatory responses involve multiple cell types that originate in the bloodstream and reside in the brain. Studies indicate that these cells have bidirectional destructive and supportive effects on the repair of damaged neural tissue after SCI. These opposing roles likely depend on the types of cells and their state of activation. Further detailed investigations on the mechanisms and function of their interactions are required to ultimately reduce the toxicity and enhance the trophic effects of the immune system. This would lead to the development of novel strategies to enhance recovery after SCI. The recent discovery of neural circuits that directly regulate immune responses has further highlighted brain–immune system communication. In this regard, signals from the brain to the immune system should also be considered to understand the whole pathology of SCI. In this review, we aim to emphasize that cell–cell and system–system interactions are important concepts for understanding the complex reactions that occur in the degenerating CNS.

Keywords Central nervous system • Spinal cord injury • Regeneration • Immune reaction • Neuron • Glia • Inflammation

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K. Wada (ed.), *Neurodegenerative Disorders as Systemic Diseases*,
DOI 10.1007/978-4-431-54541-5_3

Abbreviations

AD	Autonomic dysreflexia
CIDS	CNS injury-induced immunodepression
CNS	Central nervous system
HPA	Hypothalamo-pituitary-adrenal
NE	Norepinephrine
PVN	Paraventricular nucleus
ROS	Reactive oxygen species
SCI	Spinal cord injury
SNS	Sympathetic nervous system
TBI	Traumatic brain injury

3.1 Introduction

The central nervous system (CNS) regulates most of the functions that we need to survive, ranging from motor, sensory, and cognitive systems to involuntary autonomic functions. Many kinds of degenerative diseases in the CNS cause cell death or degeneration of neurons and related glial cells such as oligodendrocytes (myelin) and astrocytes. This generally occurs in specific brain regions, depending on the type of CNS disease/injury, and destroys neural circuits engaged in specific functions. Thus, it ultimately results in functional deficits and severely affects quality of life. Importantly, the damage to neural tissue is accompanied (or sometimes preceded) by immune responses, and this profoundly influences disease progression and recovery. It could be said that the outcome of immune responses is one of the critical processes that determines the severity of CNS diseases. The CNS is historically considered to be an immune privileged system (Galea et al. 2007; Shechter et al. 2013). In brain physiology, the infiltration of immune cells and immune reactions are suppressed mainly by the blood–brain barrier (BBB), which is composed of endothelial cells that are interconnected by tight junctions and ensheathed by astroglial endfeet (Shechter et al. 2013). According to this view, the entry of immune cells into neural tissue and their responses are classically considered to have a destructive effect on neural tissues. However, recent findings indicate that immune components sometimes play a beneficial role in tissue function and recovery. Research in recent decades, in particular, has shown that immune responses have bidirectional roles in disease progression (i.e., both detrimental and supporting roles in the healing process) (Popovich and Longbrake 2008). The results of these studies are often considered controversial by researchers, suggesting that immune responses are more complex than we realize. Differences in cell types and their activation states as well as disease conditions (and sometimes the methods employed in experiments) could lead to opposing conclusions and account for these discrepancies. Understanding the complex role of immune–nervous system

interactions is indispensable for developing therapeutic strategies, and further detailed investigations are required from this perspective.

Although most studies focus on the effects of immune responses to the nervous system, as described above, we also have to think about another aspect of their interaction: the functions of the neural system that can sense inflammatory responses and ultimately regulate them. This has recently been proposed as an "inflammatory reflex" that is likely similar to the autonomic functions classically known to regulate and maintain physiological homeostasis in organs of the cardiovascular, urinary, and digestive systems (Tracey 2009). This reflex system, which the brain uses to regulate immune responses, is assumed to maintain proper homeostatic inflammatory responses in order to repair lesions. Importantly, it is possible that abnormal regulation of the immune reaction can develop, given that this system is generally used in inflammatory responses occurring throughout the body, and some parts of this immune-regulating neural circuit are damaged in CNS diseases. The immune dysfunction caused by an abnormal reflex would further affect progress of the disease. Hence, it would be difficult to predict and understand the processes involved in CNS diseases without thinking about this brain–immune system reaction.

This bidirectional brain–immune interaction would undoubtedly be achieved by chemical or molecular transmission. Identifying the key molecular signals that mediate this communication will aid in understanding complex interactions and in finding a therapeutic target. Interestingly, recent studies have shown many examples of molecules that were originally found to work in either the nervous or immune system, but also function in the other system. For example, neurotransmitters and axon guidance molecules in the nervous system have also been shown to participate in functions of the immune system (Takamatsu and Kumanogoh 2012; Tracey 2009). These findings imply that both systems not only physically interact, but can also communicate by sharing common molecules. This viewpoint provides us with important clues to understanding their interactions.

Interactions between the immune and nervous systems are possibly involved in various CNS diseases such as degenerative brain diseases (e.g., Alzheimer's disease and Parkinson's disease), autoimmune diseases (e.g., multiple sclerosis), stroke, and trauma (e.g., traumatic brain injury, TBI and spinal cord injury, SCI). Although a number of studies have reported the interaction of both systems, in this review we would like to focus on SCI. The spinal cord is a central part of the CNS that mediates signals between the peripheral nervous system and higher order brain regions. Motor, sensory, and autonomic functions particularly rely on spinal neural networks. SCI is mainly caused by trauma from vehicle accidents, sporting injuries, and falls, producing incomplete or complete injuries. The injury destroys the neural circuit, leading to partial or complete loss of function, which dramatically decreases the quality of life of patients. Unfortunately, there are so far no effective treatments that can recover the function, mostly due to the lack of regenerative capacity of the CNS. The final goal for therapies would be to reconstruct the circuit as closely as possible to a normal one. Importantly, a body of evidence has demonstrated that immune responses influence the regenerative responses of the nervous system in

multiple ways, which can be either detrimental or supportive to neural tissue. Various types of cells with different levels of activation participate in this inflammatory cascade. On the other hand, SCI frequently disrupts the neural circuit of the inflammatory reflex that further modulates actions of the immune system. Thus, SCI involves many complicated aspects of the brain–immune interaction and provides us with an ideal model to consider these forms of communication and their outcomes on recovery. Apparently, their interaction could be the key process determining the degree of recovery after SCI.

In this review, we would like to introduce recent knowledge on brain–immune interactions in SCI. First, we will describe the kinds of inflammatory cells and molecules that emerge from injury in the spinal cord, and how the immune reactions affect the repair and regenerative processes. Then, we will review the opposite nervous–immune system regulation that further influences the recovery process. We would like to argue that revealing the whole interaction between both systems would pave the way to establishing a novel therapeutic strategy for SCI.

3.2 Responses of the Immune System and Their Effect on the Nervous System After SCI

3.2.1 General Inflammatory Cascade After SCI

Although the initial degree of injury determines the damage in the neural circuit and the functional deficit (Hill et al. 2009), the secondary damage or healing process has a further impact on this. This is promoted by the inflammatory cascade (i.e., hemorrhage, ischemia, immune responses, edema, and scar formation, as seen in various injured organs). These events generally support the repair processes of tissue, but sometimes excessive responses can have devastating effects. In fact, degeneration of neuronal and glial cells by necrotic and apoptotic cell death, axon degeneration, and demyelination, which all destroy the neural circuit, can be initiated by toxic factors and pro-inflammatory cytokines secreted by infiltrating inflammatory cells.

Here, we first would like to describe the patterns of immune responses in SCI (Fig. 3.1). The BBB is initially damaged by the injury, followed by the invasion of inflammatory cells. This process can be evaluated by horseradish peroxidase (HRP) injection into the bloodstream, which extravasates for up to 1–2 weeks after SCI in rodents (Noble and Wrathall 1989; Whetstone et al. 2003). The secondary peak seen in mice might be related to monocyte and neutrophil infiltration (Lee et al. 2011; Popovich et al. 1996a). Pro-inflammatory cytokines such as TNF-α and IL-1β can further facilitate BBB permeability and destruction, suggesting that these cytokines regulate the stability of BBB and entry of leukocytes (Schnell et al. 1999b). Elevated expression of matrix metalloproteinase-9 (MMP-9) also plays a role in the dysfunction of the BBB (Noble et al. 2002).

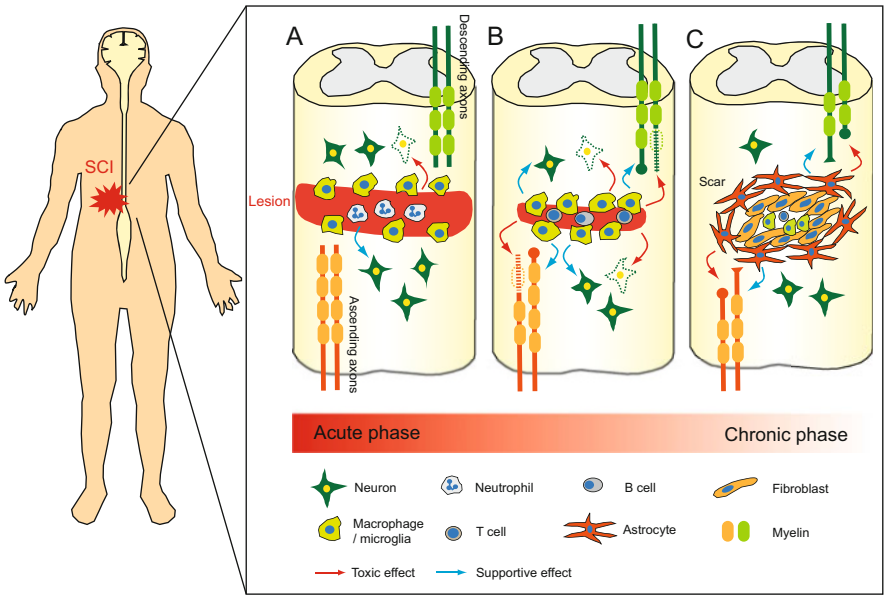

Fig. 3.1 Immune response in SCI (immune system–brain interaction): (*A*) following initial injury, neutrophils and macrophages (monocytes) invade the lesion by activating resident microglia; (*B*) then, T and B cells engaged in adaptive immunity also infiltrate; (*C*) finally, activated astrocytes and fibroblasts further delineate the lesion to form glial and fibrotic scars. Each cell component has been shown to have a detrimental (*red arrows*) or supportive effect (*blue arrows*) on neural tissue and regrowth of axons during the healing process (see the text for details)

Although the inflammatory responses are somewhat different among the species (human, rats, and mice) and among strains within the same species (Fleming et al. 2006; Kigerl et al. 2006; Popovich et al. 1997), neutrophils are the first cells to invade the lesion, starting within the first few hours or days (Schnell et al. 1999a). Monocytes of hematopoietic origin then infiltrate within a week after SCI, with concomitant activation of resident microglial cells (Donnelly and Popovich 2008). Thereafter, the entry of lymphocytes (T and B cells) is observed in the lesion within a period of weeks to months (Fleming et al. 2006; Kigerl et al. 2006; Sroga et al. 2003). Injury signals such as adenosine triphosphate (ATP), danger-associated molecular patterns (DAMPs: heat shock proteins, HMGB1, and peroxiredoxins) and cytokines (IL-1β and TNF-α) emanate from CNS resident cells to recruit and activate circulating leukocytes (de Rivero Vaccari et al. 2012; Shichita et al. 2012). Since these cells often stay in the lesion for months, SCI could be perceived as a chronic inflammatory disease.

Other components such as glial cells (astrocytes) and fibroblasts are further involved in the repair process to create scar tissue (Silver and Miller 2004), following the entry of leukocytes derived from hematopoietic cells. Astrocytes are activated; they proliferate, and migrate or extend their processes to surround the lesion and create a glial scar. This separates the inflammatory and intact regions

and helps limit the expansion of inflammation, reconstruct the BBB, and form scar tissue within 1–2 weeks (Okada et al. 2006; Wanner et al. 2013). In the core of the lesion, fibroblasts eventually accumulate and create a fibrotic scar, to fill the necrotic gap between intact neural tissues (Herrmann et al. 2010; Zukor et al. 2013).

As overviewed here, multiple players are involved in the healing process. The following sections summarize how each cellular player influences neighboring neural tissue and the recovery process.

3.2.2 Neutrophils

Neutrophils are the first responders to inflammation that occurs as a result of infections and injuries (Wright et al. 2010) (Fig. 3.1A). In fact, they start appearing in the acute phase, 3–24 h after SCI, and their numbers peak at around day 3 in mice and at 8 h in rats (Kigerl et al. 2006; Mawhinney et al. 2012; Stirling and Yong 2008; Taoka et al. 1997b). In most species, neutrophils have disappeared within the first week (Donnelly and Popovich 2008), but they persist for several months in mice (Kigerl et al. 2006; Mawhinney et al. 2012). They generally have bactericidal properties for engaging in the defense of host tissue and function via phagocytosis and the release of anti-bacterial granules into extracellular space. These granules contain reactive oxygen species (ROS), lysosomal enzymes (myeloperoxidase MPO, elastase, MMP-9), and pro-inflammatory cytokines (Wright et al. 2010). Hence, it is conceivable that this defensive strategy can simultaneously damage neighboring healthy bystander tissue as well. Indeed, it is suggested that neutrophils damage neural tissue after SCI. Inhibiting the invasion of neutrophils by leukocyte depletion, treatment with prostaglandin E2, anti-P-selectin, anti-ICAM1, or anti-CD11d antibodies to inhibit endothelial cell–neutrophil interaction, or iloprost (a synthetic analog of prostacyclin), and knockout of MMP-9 are all correlated with reduced entry of neutrophils and enhanced recovery of motor functions and histopathological events (Gris et al. 2004; Hamada et al. 1996; Naruo et al. 2003; Noble et al. 2002; Taoka et al. 1997a, b). Inhibiting the release of these factors using elastase inhibitors, and knockout of MPO, are also correlated with reduced tissue damage and recovery (Kubota et al. 2012; Taoka et al. 1998). Each modulation that reduces damage by neutrophils is well correlated with decreases in cell death, BBB permeability, production of enzymes, ROS, and pro-inflammatory cytokines, suggesting a destructive role for neutrophils. However, the actual role of neutrophils should still be carefully evaluated, since in most cases these experiments do not specifically target neutrophils. For example, ICAM-1 and P-selectin knockout (KO) mice show better recovery and tissue sparing than antibody treatments, but they also reduce macrophage/microglial invasion (Farooque et al. 1999, 2001). P2X4 knockout and anti-CD11d antibody treatments also reduce the number of neutrophils concomitantly with monocytes/macrophages and improve behavioral recovery (de Rivero Vaccari et al. 2012; Gris et al. 2004).

In contrast to their detrimental role, recent reports demonstrate that the selective depletion of neutrophils using anti-Ly6G antibody alone has no effect on recovery, whereas depletion of both neutrophils and macrophages improves recovery (Lee et al. 2011). In another study, there was a conflicting finding that depletion of neutrophils using anti-Ly6G antibody worsens pathological processes and recovery (Stirling et al. 2009). Although the reasons for these controversies are yet unknown, the timing of treatment and the specificity of the antibody to neutrophils should be further examined.

From the reports described above, it can be assumed that neutrophils are required for the initial repair process, but the excessive responses frequently seen in SCI are detrimental to tissue sparing. In addition, both neutrophils and macrophages, which are the main players in innate immune responses, are suggested to have close molecular and functional relationships in the healing processes.

3.2.3 Microglia and Macrophages/Monocytes

Macrophages derived from circulating hematogenous monocytes subsequently enter the lesion, either during the period extending from hours to 3 days after SCI or coincidentally with neutrophils (Fleming et al. 2006; Popovich et al. 1997; Stirling and Yong 2008) (Fig. 3.1A). Their numbers peak at around day 7 (Kigerl et al. 2006). They are $CD45^{high}$, $CD11b^{+}$, $GR1^{+/-}$, and $F4/80^{+}$ cells (Pineau et al. 2010; Stirling and Yong 2008). They often stay in the lesion for weeks (Okada et al. 2006; Popovich et al. 1997). The time taken for a 50 % reduction from maximum cell numbers is 55 days in rat SCI (compared with 1.2 days for neutrophils and 1.5 days for lymphocytes), suggesting that macrophages/microglia are the main players engaging in chronic inflammation in the lesion (Pruss et al. 2011).

Microglia are the resident tissue macrophages in the CNS. After injury, microglia can be easily and rapidly activated by injury signals in the brain. For example, injury-released ATP induces migration or extension of processes toward an ATP gradient (Davalos et al. 2005; Popovich and Longbrake 2008). Recent genetic fate–mapping studies indicate that microglia are derived from specific precursors in the yolk sac during early embryonic stages and enter the brain thereafter (Ginhoux et al. 2010; Ueno and Yamashita 2014). Under physiological conditions, circulating monocytes are not recruited to the brain to become microglia (Ajami et al. 2007), and they are a different population even in SCI or an experimental autoimmune encephalitis (EAE) model (Ajami et al. 2011; Shechter et al. 2009). Hence, microglia and macrophages/monocytes should be considered as different populations when we study their roles in SCI. Since microglia and macrophages share common molecular profiles, discriminating between them is still however technically difficult. High CD45 expression in hematogenous monocytes and its low expression in microglia could be a suitable marker to distinguish them, but activated amoeboid microglia can exhibit high CD45 expression and need

careful interpretation (David and Kroner 2011; Thawer et al. 2013). Fortunately, recent studies have developed novel methods to discriminate between them, and their specific roles are being revealed. Bone marrow chimeras, parabiosis, and transgenic mice (*Lys*-EGFP mice expressing EGFP in macrophage/monocytes but not in microglia) have been developed for this purpose (Ajami et al. 2011; Mawhinney et al. 2012; Shechter et al. 2009).

Classical experiments have also been conducted by using depletion and transplantation of microglia/macrophages to understand their role in SCI, although these techniques might not clearly distinguish those two populations. Experiments have indicated different detrimental and beneficial effects on recovery from injury. Activated macrophages were seen close to demyelinating areas, suggesting they have toxic properties in SCI (Blight 1985). Indeed, reduction of monocyte/macrophage infiltration and their inactivation using silica, anti-CD11d antibody, and minocycline all improve recovery (Blight 1994; Gris et al. 2004; Stirling et al. 2004). Selective depletion of circulating monocytes using liposomal clodronate can also preserve neural tissue and enhance recovery (Popovich et al. 1999). The secretion of pro-inflammatory cytokines and other biomolecules (MMP-9, iNOS, and superoxide) can be attributed to their toxic role. Indeed, microglia/macrophages express IL-1β and TNF-α within a day (Longbrake et al. 2007; Pineau and Lacroix 2007), and IL-1 receptor antagonist, soluble tumor necrosis factor receptor (TNFR), TNFR1 KO mice, and TNF-α antibody all reduce injury-induced cell death and improve recovery after SCI (Ferguson et al. 2008; Genovese et al. 2008; Nesic et al. 2001). It can be assumed that appropriate activation of macrophages/microglia is critically important in order to control increased toxicity. Indeed, neuron–microglia/macrophage interactions via Cx3cl1–Cx3cr1 and CD200–CD200R have been shown to suppress the abnormal activation of microglia under physiological conditions. Disruption of this signaling aberrantly activates microglia/macrophages and destroys neural tissue (Cardona et al. 2006; Hoek et al. 2000).

In contrast, some other studies have reported contradictory results. Administration of liposomal clodronate alone does not affect the recovery process (Lee et al. 2011). Furthermore, transplantation of macrophages pre-exposed to peripheral nerves promotes recovery after SCI (Rapalino et al. 1998). The same group used elegant transgenic and chimeric methods to show that adoptive transfer of monocytes promotes recovery, whereas depletion of CD11c-DTR^{+} chimeric monocytes by injection of diphtheria toxin worsens spontaneous recovery (Shechter et al. 2009). In ischemic brain injury, ablation of presumptive resident microglia in CD11b HSVTK mice further damages the lesion (Lalancette-Hebert et al. 2007). These reports are consistent with an immune-regulatory and supportive role for microglia/macrophages in SCI.

These controversial results imply the existence of subtypes of macrophages and microglia. Macrophages have recently been categorized into classically activated pro-inflammatory M1 and alternatively activated anti-inflammatory M2 subtypes (David and Kroner 2011; Sica and Mantovani 2012). This classification might also apply to microglia. Such diversity may explain the contradicting results among

experiments. The Th1 cytokine IFN-γ (interferon gamma) can shift the balance toward the M1 state, which produces pro-inflammatory cytokines (IL-12, IL-23, IL-1β, and TNF-α) and ROS and nitrogen species. On the other hand, Th2 cytokines such as IL-4 lead to M2 differentiation, which inhibits the production of pro-inflammatory cytokines and increases interleukin-10 (IL-10) and transforming growth factor-beta (TGF-β), as well as other M2 markers like Arginase 1. Recent detailed analyses of M1/M2 phenotypes in SCI have revealed the existence of both M1 and M2 populations. M1 and subsequent M2 populations are observed in SCI (Kigerl et al. 2009; Thawer et al. 2013). Intriguingly, M1 and M2 monocytes/macrophages are recruited along different routes to the lesion site: M1 cells from the adjacent leptomeninges via chemokine (C-C motif) ligand 2 (CCL2) signaling and M2 cells from choroid plexus–cerebrospinal fluid (Shechter et al. 2013). M1 activation is predominant in the chronic phase and may prolong inflammation, which would lead to toxic effects on neurons (Kigerl et al. 2009). ROS, nitric oxide (NO), cytokines (TNF-α, IL-1β), and MMPs secreted by M1 macrophages would potentially be toxic to neural tissue (Block et al. 2007). On the other hand, M2-like IL-10 and arginase-expressing macrophages are recruited in the margin of the lesion, and they are likely to contribute to the healing process (Shechter et al. 2009). Microglia/macrophages can express growth factors such as brain-derived neutrotrophic factor (BDNF), glial-derived neutrotrophic factor (GDNF), insulin-like growth factor 1 (IGF1), and NT-3 in the developing brain and even within the lesion of SCI (Batchelor et al. 1999; Elkabes et al. 1996; Nakajima and Kohsaka 2004; Rolls et al. 2008; Ueno et al. 2013). Those factors are possibly protective in damaged tissue, but their role and characterization in terms of M1/M2 cells have not yet been clearly examined.

The primary role of microglia/macrophages is phagocytosis. After injury, cellular debris, especially myelin debris, is thought to be one of the key factors inhibiting axonal regeneration (Geoffroy and Zheng 2014). Hence, phagocytosis by microglia/macrophage would be important in this context. Indeed, clearance of degenerating axons by macrophage/microglia appears to be important for subsequent axonal regeneration, at least *in vitro* (Tanaka et al. 2009). *In vivo*, microglia in the acute phase and subsequent invading macrophages engage in phagocytosis after SCI (Greenhalgh and David 2014). However, it seems that macrophages/microglia are less efficient at clearance within the CNS compared with the peripheral nervous system (Avellino et al. 1995; Lazarov-Spiegler et al. 1996; Lotan and Schwartz 1994), which may explain why axonal regeneration is limited in the CNS. Hence, an increase in phagocytic activity will potentially be beneficial as a therapy.

In addition to their immune-modulatory and phagocytic effects, macrophages/microglia appear to have a direct role in the regenerative processes of neural circuits, especially in axons. Inhibition of microglia/macrophage activation by minocycline treatment can reduce axonal dieback (Kitayama et al. 2011; Stirling et al. 2004). It has been reported that macrophages inhibit axonal elongation (Busch et al. 2009; Horn et al. 2008), and M1 macrophages and microglia inhibit axonal growth *in vitro* (Kigerl et al. 2009; Kitayama et al. 2011). Microglia can express axon guidance molecules, such as Slit, Netrin (Wehrle et al. 2005), semaphorin 7A

(SEMA7A) (Pasterkamp et al. 2003), and repulsive guidance molecule a (RGMa) (Kitayama et al. 2011) in the injured CNS, which could affect axonal remodeling. We have reported that RGMa expressed in activated microglia inhibits axonal growth *in vitro*, and minocycline treatment can inhibit RGMa expression resulting in reduced axonal dieback (Kitayama et al. 2011). These results strongly suggest that microglia/macrophages can affect the status of axonal regeneration. Interestingly, microglia can also expresses axonal growth factors such as IGF1 (Ueno et al. 2013), thrombospondin (Chamak et al. 1994, 1995), and neurotrophin-3 (NT-3) (Elkabes et al. 1996), suggesting that they may be able to facilitate axon growth under some conditions. However, there is still no direct evidence *in vivo* that these factors in macrophages/microglia affect axon regeneration. It will be particularly important to know which state of microglia/macrophage activation produces inhibitory or supportive factors for axonal growth, and whether these factors modulate regenerative responses. Recently developed knock-in mice (e.g., the *Cx3cr1*–Cre and *Cx3cr1*–CreER lines specifically expressed in microglia; Goldmann et al. 2013), will be ideal tools for understanding the role of each molecule more clearly *in vivo*.

Although numerous points remain unclear, M2-skewed cells are in general likely to be supportive for tissue repair in SCI. This raises the possibility that treatments that enhance M2 polarization will promote recovery. In this context, strategies that replenish appropriate microglia/macrophages and stimulate axonal regeneration and repair have been evaluated (Bouhy et al. 2006; Lazarov-Spiegler et al. 1996; Rapalino et al. 1998), and preclinical trials in which autologous activated macrophages are injected into injured spinal cord (ProCord) have been conducted as Phase II studies. Although it is a promising idea, some reports indicate that transplantation of M2 macrophages decreases the number of M2 in the lesions of mice, suggesting that the environment there polarizes the cells to M1 and limits the efficiency of therapy (Kigerl et al. 2009). Further evaluation in terms of M1/M2 polarization and molecular mechanisms is needed in order to utilize macrophage/microglia as a therapy. M2-polarizing drugs could be an alternative way, but it may be more effective to combine the methods to modify the lesion milieu.

3.2.4 T Lymphocytes

T cells show a different pattern of infiltration among species after SCI: biphasic invasion in the first and fourth weeks in rats and a gradual increase toward 10 weeks in mice (Sroga et al. 2003) (Fig. 3.1B). T cells are major components in the adaptive immune response and several types of T lymphocytes – $CD8^+$, $CD4^+$ (Th1, Th2, and Th17), and regulatory $CD4^+$ T cells – are involved here (Liblau et al. 2013). $CD4^+$ T cells are composed of: Th1 cells which activate macrophages and $CD8^+$ T cells by IL-2, IFN-γ, and TNF-α; Th2 cells which promote activation and maturation of B cells by IL-4, 5, and 13; Th17 cells which release IL-17, 21, and 22 and activate or recruit neutrophils; and $Foxp3^+$ regulatory T cells which are thought to

limit immune responses. CD8-expressing T cells secrete toxic factors, such as perforin, granzymes, and cytokines to directly destroy abnormal cells, such as infected and tumor cells.

The role of T cells in recovery after SCI are again controversial (Popovich and Jones 2003). Many studies indicate that T cells are detrimental to neural tissue and recovery from SCI. *In vitro*, T cells have a toxic effect on neurons (Giuliani et al. 2003). Reduction of T cells in nude rats (*RAG1*$^{-/-}$, *RAG2*$^{-/-}$) or nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice, in which maturation of both T and B cells is blocked, and on anti-CXCL10 antibody treatment, which inhibits the recruitment of T cells, ameliorate secondary damage and enhance recovery after SCI or TBI (Fee et al. 2003; Gonzalez et al. 2003; Luchetti et al. 2010; Potas et al. 2006; Wu et al. 2012). It has been demonstrated that T cells are activated by CNS antigens and persist in the tissue (Hickey et al. 1991). Some lines of evidence indicate that these auto-reactive T cells are also toxic. For example, myelin basic protein (MBP)-reactive CD4$^+$ T cells exacerbate the lesion and functional deficits after SCI (Jones et al. 2002, 2004; Popovich et al. 1996b).

In contrast, neuroprotective roles have also been reported. For example, auto-reactive T cells are likely to promote recovery in CNS injuries and degenerative diseases such as Alzheimer's disease, and the "protective autoimmunity" concept has emerged (Schwartz and Raposo 2014). Indeed, adoptive transfer of myelin-reactive T cells and vaccination with auto-antigen (MBP or Nogo-A) has been shown to protect tissue and improve locomotor function after SCI (Hauben et al. 2000, 2001a, b). These approaches are attractive as therapeutic strategies, but careful interpretation is required since conflicting results have also been reported. These controversies may be attributed to the multiple types of T cells. We recently demonstrated that adoptive transfer of Th1-conditioned lymphocytes, but not Th2 or Th17 cells, could enhance the recovery of motor function after SCI (Ishii et al. 2012, 2013). Although the mechanism underlying this beneficial role is still unclear, expression of IL-10 and neurotrophic factors in transferred T cells and the presence of IL-10 expressing activated microglia/macrophages can be correlated with recovery.

The role of regulatory T cells is still under investigation. One report suggests that loss of regulatory T cells exacerbates SCI (Marcondes et al. 2005), but this needs further evaluation. As for Th17 cells, recent reports demonstrate their deleterious effects in EAE and stroke models (Kebir et al. 2007; Shichita et al. 2009). In SCI, transfer of Th17-conditioned cells worsens behavioral recovery in the acute phase (Ishii et al. 2012), but the detailed function and mechanisms are still unknown. Understanding the roles of each T cell subtype in the pathology of SCI and how these populations can be regulated could lead to the establishment of therapeutic strategies.

Reports have proposed further direct roles for T cells in regeneration. In co-culture experiments, activated natural killer (NK) and CD8$^+$ T cells inhibit neurite outgrowth without detectable neuronal apoptosis in a contact-dependent but antigen-independent manner, whereas activated CD4$^+$ T cells promote neurite outgrowth (Pool et al. 2012). Interestingly, activated T cells can produce

neurotrophic factors such as BDNF, NT-3, and GDNF (Ishii et al. 2012, 2013; Kerschensteiner et al. 1999), which could be neuroprotective and facilitate regeneration. We reported that Th1 cells, but not Th2 or naive T cells, can enhance axonal elongation *in vitro*, and that SEMA4A and IFN-γ are involved in this process (Ishii et al. 2010). Semaphorins were originally described as axon guidance molecules in the nervous system, but it has been revealed that they are largely expressed in immune cells and have diverse functions in immune responses (Takamatsu and Kumanogoh 2012). It will be intriguing to examine whether these immune-derived semaphorins affect regenerative responses in SCI.

In conclusion, further analysis is needed on the roles T cells play in axon regeneration, especially *in vivo*. It should be noted in this case that both indirect and direct effects of T cells could contribute to resilience. For example, transfer of Th1 cells may slightly enhance the regenerative response of axons, but simultaneous activation of macrophages/microglia may directly affect regeneration (Ishii et al. 2012, 2013). *In vitro*, T cells can enhance the neuroprotective properties of microglia and astrocytes (Garg et al. 2008; Shaked et al. 2005). Thus, the complex interactions among multiple types of immune cells, and neural cells, need careful investigation.

3.2.5 B Lymphocytes

B cells activated by extracellular antigens produce cytokines and antibodies, which target the injured/infected cells that are removed by macrophages. In SCI, B cells appear after a week and chronically persist in the lesion (Ankeny et al. 2006; Schnell et al. 1999a) (Fig. 3.1B). The role of B cells in the repair process remains controversial. In particular, auto-antibodies produced post injury are a focus of debate. Auto-antibodies to MBP, monosialotetrahexosylganglioside (GM1) gangliosides, and myelin-associated glycoprotein (MAG), have actually been detected in human SCI patients (Hayes et al. 2002; Mizrachi et al. 1983; Skoda et al. 2006). Even in mouse SCI, B cells are activated in lymphoid tissues such as those of the spleen, and antibodies are produced against antigens from both inside and outside the CNS (Ankeny et al. 2006, 2009). Although the mechanisms underlying the activation of auto-reactive B (and T) cells are still unclear, cells showing self-recognition are likely to exist as a suppressive mechanism even under physiological conditions (Schwartz and Raposo 2014). Regarding the negative effects, B cell knockout mice show increased amounts of spared tissue and better functional recovery than wild-type (WT) mice (Ankeny et al. 2009). Furthermore, injection of antibodies produced in SCI mice into the spinal cord causes dramatic loss of neuronal cells and aberrant inflammation by macrophages/microglia, accompanied by hind limb paralysis (Ankeny et al. 2009). These results indicate the dark side of B cells and auto-reactive antibodies. As already discussed regarding T cell protective autoimmunity, self-reactive antibodies may play some beneficial roles in recovery, however. For example, B cells produce anti-myelin proteins, which are

generally thought to inhibit axonal regeneration. Pre-immunization with spinal cord homogenates promotes axonal regeneration without detrimental immune responses (Huang et al. 1999). Myelin-reactive antibodies are thought to mediate these effects, since antisera containing these antibodies can block inhibition of neurite outgrowth by myelin *in vitro*.

3.2.6 Glial and Fibrotic Scar

Following inflammatory reaction, other components such as astrocytes and fibroblasts become involved in the creation of scar tissue (Fig. 3.1C). Obviously, activation of astrocytes and the formation of a glial scar are required for proper recovery, since ablation or inactivation of astrocytes using genetically modified mice severely exacerbates inflammation and secondary damage, and affects the recovery process (Faulkner et al. 2004; Okada et al. 2006). They play a role in separating the inflammatory response from neighboring intact neural tissue (Wanner et al. 2013). At the expense of this healing role, they are classically considered a principal barrier to axonal regeneration by expressing chondroitin sulfate proteoglycans (CSPGs) (Silver and Miller 2004). Recent reports, however, suggest that there are several subtypes of astrocytes, some of which can support recovery. For example, TGF-α overexpression increases the numbers of astrocytes that are supportive of axon growth (White et al. 2011), and nuclear factor kappaB (NF-κB) inhibition in astrocytes facilitates the healing process, resulting in reduced lesion volume (Brambilla et al. 2005). Transplantation of astrocytes differentiated from embryonic precursor cells improves recovery and promotes the regeneration of axons (Davies et al. 2006).

Fibroblasts also accumulate in the core of the lesion, where they stabilize the damaged tissue. Although it was believed that these cells are mainly derived from meningeal cells, recent studies using transgenic mice and lineage tracing have demonstrated that the majority of the cells are derived from $Glast^+$ or $Col1\alpha1^+$ pericytes or perivascular cells (Goritz et al. 2011; Soderblom et al. 2013). Importantly, ablation of these populations using Glast-Rasless mice leads to the formation of a large cavity, indicating that fibrotic scar tissue is indispensable for repair (Goritz et al. 2011). On the other hand, the fibroblastic scar is now considered to be the major barrier to axonal regeneration. In the adult CNS, many inhibitory molecules such as myelin-associated proteins and astrocyte-derived CSPGs in the local environment are thought to inhibit axonal regeneration. Moreover, loss of the intrinsic capacity of neurons to regrow axons has been recognized as a second factor impeding regeneration in the adult CNS (Liu et al. 2011). Deletion of *Pten*, which usually suppresses the mammalian target of rapamycin (mTOR) and AKT pathways, can reactivate this intrinsic signal and induce robust regeneration of axons post SCI (Liu et al. 2010; Zukor et al. 2013), most likely by overcoming most of the inhibitory molecules in the local environment. However, even under these conditions, axons can only grow along bridging astrocytes in the scar, but not over

the fibronectin^{+} fibrotic tissue (Zukor et al. 2013). This strongly suggests that the fibrotic scar is the ultimate barrier to regeneration. Hence, modulation of this structure without weakening the process of repair will be needed to enhance regeneration. From this point of view, an increase in bridging growth-supportive astrocytes to accelerate the process of scar formation (Brambilla et al. 2005; Davies et al. 2006; White et al. 2011), and direct reprogramming of scar cells (Su et al. 2014) are expected to be promising approaches. Transplanting neural cells could be an alternative way to fill the lesion gap, which would simultaneously reconstruct the neural connection (Abematsu et al. 2010; Lu et al. 2012). In addition, these transplanted cells might be able to modulate immune function and lead to better recovery (Pluchino et al. 2005; Uccelli et al. 2007). Immune cells would also affect scar formation, possibly via M2 macrophages/microglia expressing TGF-β. This scar–immune interaction also needs to be examined in more detail.

3.2.7 Targeting Immune to Neural Actions: Future Directions

Since multiple players including immune and glial cells engage in repair processes by communicating with each other, research such as that described here on the role of each cell type and their interactions (immune–immune, immune–glia, immune–nervous) should be undertaken. The glucocorticosteroid methylprednisolone is the only agent widely used in the clinic to suppress the global immune reaction post SCI, but the effect of this treatment is still doubtful and controversial (Jones et al. 2005; Popovich and Longbrake 2008). Thus, it is critically important to understand the complicated neurotoxic and reparative effects of the inflammatory response in order to identify target molecules or cells. In general, a limited inflammatory response would be beneficial for repairing the tissue, but prolonged and excessive inflammatory responses are thought to be deleterious. Hence, therapeutic strategies that accelerate the immune and repair process, and make the lesion smaller, would be the best options for tissue sparing and the reorganization of neural circuits that will lead to efficient recovery.

3.3 CNS Regulation of the Immune System Affects the Recovery Process Post SCI

3.3.1 CNS Regulation of the Immune System

Regarding the brain–immune interaction in CNS diseases and injuries, we also have to consider the nervous system that autonomically regulates immune responses and

further influences the recovery process. It has already been highlighted that the immune system is regulated by the nervous system (Fig. 3.2A). The state of the immune system is sensed by the autonomic nervous system via the afferent vagus nerve (Goehler et al. 2000; Tracey 2009), or directly by CNS neurons that are mainly found in circumventricular organs lacking a BBB (Buller 2001) and in the medial preoptic area, via pro-inflammatory cytokines (TNF-α, IL-1β, etc.). Such signals received by the CNS generally cause fever and anti-inflammatory immunosuppressive reactions. In fact, intracerebral or peripheral administration of these cytokines is perceived by neurons and induces physiological changes such as fever, decreased activity, and activation of the stress response by the hypothalamo–pituitary–adrenal (HPA) axis (Tracey 2009). Signals received in the CNS are processed into three output pathways: the HPA axis, sympathetic nervous system (SNS), and parasympathetic nervous system.

In the HPA axis, the signals are first relayed to the paraventricular nucleus (PVN) in the hypothalamus (Haddad et al. 2002). PVN neurons then release

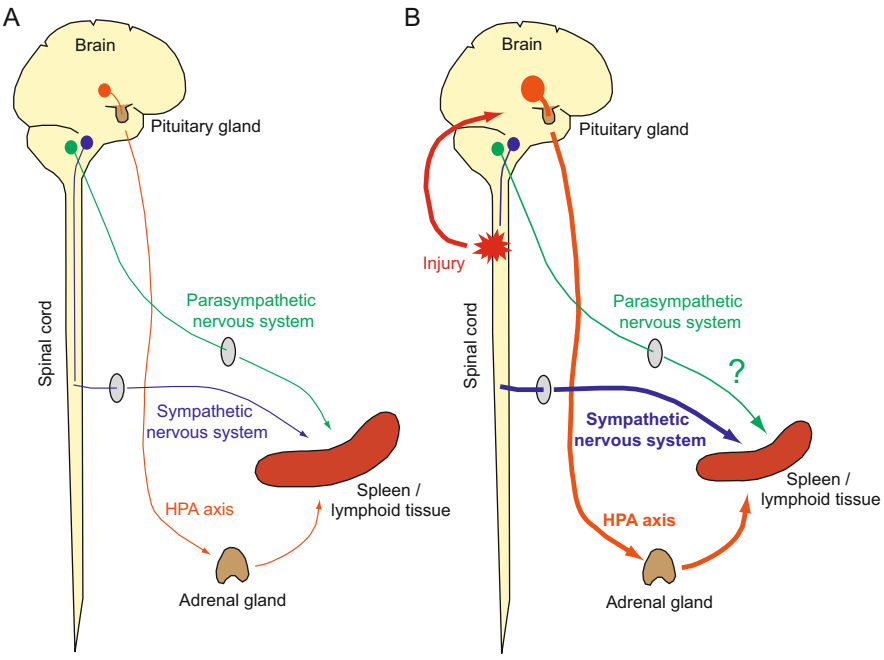

Fig. 3.2 CNS regulation of immune system (brain–immune system interaction): (*A*) three output pathways – HPA axis (*orange*), sympathetic nervous system (SNS; *blue*), and parasympathetic nervous system (*green*) – are known to be immunosuppressive in regulating immune system homeostasis; (*B*) following SCI, injury signals (*red*) are transmitted to the CNS and activate the HPA axis. If the SNS circuit between the brain and spinal cord is destroyed, the local SNS circuit innervating the spleen (or lymphoid tissue) is also activated. Both systems suppress immune reaction. CIDS might be caused by excess response of these inflammatory reflex circuits. The parasympathetic nervous system has not been examined for this condition yet (see the text for details)

corticotropin-releasing factor (CRF), which stimulates the release of adrenocorticotropic hormone (ACTH) in the anterior lobe of the pituitary gland. ACTH further stimulates glucocorticoid secretion in the adrenal gland. Glucocorticoid has a global immunosuppressive effect, by suppression of cytokines (IL-1β, TNF-α, IL-8), prostaglandin, and NO production. It also has apoptotic effects on T lymphocytes and decreases antigen representation.

The SNS is known to innervate lymphoid tissues (thymus, bone marrow, spleen, lymph nodes) and releases norepinephrine (NE) (Felten 2000). NE is able to inhibit pro-inflammatory responses (IFN-γ in Th1 cells; TNF-α and IL-1β in monocytes; NK cell activity), and increase IL-10 expression in monocytes (Meisel et al. 2005). In the parasympathetic nervous system, the immune signals received are reflexively transmitted to peripheral organs through the vagus nerve, and this circuit also has an immunosuppressive role. Indeed, stimulation of the vagus nerve can suppress TNF-α release in peripheral organs, with increased acetylcholine in the spleen. Acetylcholine decreases the release of TNF-α, IL-1β, IL-6, and IL-18 from macrophages (Borovikova et al. 2000). Acetylcholine-synthesizing T cells mediate this vagus nerve–initiated immune suppression (Rosas-Ballina et al. 2011).

3.3.2 Immune Suppression Post CNS Injuries

Infections are a major cause of morbidity and mortality in patients with CNS injuries such as stroke, TBI, and SCI (Meisel et al. 2005). Although these infections occur through invasive treatment, aspiration, and bladder dysfunction, it has been recognized that secondary immunodeficiency caused by CNS injuries (CNS injury-induced immunodepression syndrome, CIDS) underlies the increased susceptibility to infections (Meisel et al. 2005). In SCI, infections such as pneumonia and urinary tract infections are frequently seen in about 28–38 % cases, and about 50 % of deaths are due to infection (Meisel et al. 2005). The immune system is actually suppressed in this condition; the functions of lymphocytes and monocytes are somehow impaired. Immune suppression and vulnerability to infection are frequently observed in stroke, TBI, and SCI patients, depending on the lesion site and volume (Campagnolo et al. 2000; Hagen et al. 2010; Quattrocchi et al. 1992; Riegger et al. 2009). Immune suppression can also be detected in stroke or SCI in rodent models (Offner et al. 2006; Oropallo et al. 2012; Zhang et al. 2013).

Although the mechanism behind CIDS still remains unknown, two putative mechanisms should be considered (Fig. 3.2B). First, the neural systems that engage in the control of inflammation – the HPA axis, SNS, or parasympathetic nervous system – could be over-activated and might lead to excessive immune depression, causing CIDS. The second possibility is that the immune-regulating circuits themselves are disrupted by CNS injuries and are over-activated. This neurogenic anti-inflammatory mechanism can be implicated by the fact that CIDS is often seen in CNS injuries in a region-specific manner. For example, immune depression evaluated by impaired phagocytosis of neutrophils is observed in cervical SCI patients,

but not in SCI below T10 (Campagnolo et al. 1997). In this case, it is possible that the sympathetic reflex circuit located at the thoracic level above T10 could be enhanced by cervical SCI leading to immune depression, but not by lower SCI.

Indeed, both mechanisms are likely to be involved in immune suppression in a mouse SCI model. In a comparison between T3 and T9 SCI, both SCI models increase circulating corticosterone, whereas only T3 SCI increases the amount of NE in the spleen (Lucin et al. 2007). This suggests that activation of the HPA axis is initiated by general injury signals perceived by the CNS, and NE may be increased by local SNS activation caused by region-specific disruption of sympathetic circuitry. These immune-suppressive responses reduce T and B cell numbers, with increased apoptosis and antibody production, and induction of splenic atrophy (Lucin et al. 2007). Glucocorticoid and NE synergistically induce apoptosis in splenocytes, T cells, and B cells, but this can be partially rescued by treatment with antagonists (Lucin et al. 2009).

Although detailed mechanisms underlying neurogenic CIDS are still unknown, abnormal regulation of autonomic systems could be involved. A circuit from higher brain regions to the spinal cord controls the autonomic, sympathetic, and parasympathetic systems. Hence, destruction of this spinal circuit by SCI can have an impact on many aspects of homeostatic functions that are regulated by these systems (e.g., cardiovascular, bladder and bowel regulation, thermoregulation, and sexual function; Inskip et al. 2009). Autonomic dysreflexia (AD), in particular, is often observed in patients with SCI, starting with severe episodic hypertension and accompanied by bradycardia, sweating, and headaches (Inskip et al. 2009). AD mostly occurs in SCI at higher levels in the cord but not at levels below the spinal circuit of the SNS. Therefore, AD is thought to be due to excessive sympathetic system activation in a spinal cord that has lost proper control from higher brain regions. AD is often evoked by sensory stimulation from the bladder and bowel whose spinal circuits are located below the injury level. Indeed, experiments in rodents suggest that abnormal SNS activation and reorganization below the lesion could cause this symptom (Cameron et al. 2006; Hou et al. 2008).

Interestingly, it has recently been demonstrated that such sympathetic activation also leads to immune depression (Zhang et al. 2013). AD and immune depression have both been induced by T3 but not T9 SCI in mice, implying a similar induction mechanism. Since the spinal sympathetic circuits innervating the spleen are located in T4–8 (Cano et al. 2001), T3 SCI separates this spinal circuit from higher brain centers and possibly enhances the spinal sympathetic reflex. Indeed, experimental activation of this reflex circuit has induced both AD and immune suppression, with elevations of NE and corticosterone. Thus, the SNS reflex circuit is revealed as being related to CIDS. It is still unclear how the circuit is altered or how it causes immune depression. Furthermore, the function of the parasympathetic nervous system in immune suppression remains to be explored in this case.

3.3.3 Targeting Neural to Immune Actions

As described here, brain–immune reactions further complicate the mechanism of disease progression in SCI. Features of inflammatory responses in SCI are determined not only by factors locally regulating the immune response in the lesion, but also changes in the immune-regulating neural circuit triggered by CNS injuries. Thus, we need to think about the bidirectional interactions of both systems when investigating the mechanism behind disease and developing a therapeutic method. So far, there are no direct treatments addressing CIDS (Meisel et al. 2005), but clinical and experimental data indicate that careful treatment of infection and immune responses will be required. Methylprednisolone should also be used more carefully since it suppresses global immune response. Ultimately, to inhibit such abnormal immune depression caused by the destruction of neural circuits, a regenerative approach to reconnect higher brain centers with the spinal cord will be of primary importance to preserve homeostatic immune responses as well as other motor and sensory functions in SCI.

Acknowledgments Work undertaken in the Yamashita lab was supported by a grant for Core Research for Evolutional Science and Technology (CREST) from the Japan Science and Technology Agency (JST) and a Grant-in-Aid for Scientific Research (S) from the Japan Society for the Promotion of Sciences (JSPS 25221309). M.U. was supported by a Postdoctoral Fellowship for Research Abroad from the JSPS and by the Precursory Research for Embryonic Science and Technology (PRESTO) program from the JST.

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Part II
Changes in Clinical Evaluation and New Biomarkers

Chapter 4
Parkinson's Disease; Neurodegeneration as Systemic Disease

Chi-Jing Choong, Hisae Sumi-Akamaru, and Hideki Mochizuki

Abstract Parkinson's disease (PD) is a progressive motor disorder characterized by selective dopaminergic neuronal degeneration in the substantia nigra (SN) accompanied by intraneuronal α-synuclein containing inclusions called "Lewy bodies." Nowadays, PD can also be described as a heterogeneous multisystem neurodegenerative disease since α-synuclein deposits are not restricted to the central nervous system but also found in peripheral nerves. Braak staging, which depends on the regional distribution of α-synuclein deposits, can be used to understand disease progression before the appearance of motor disturbance. Non-motor symptoms, such as olfactory dysfunction (hyposmia), rapid eye movement (REM) sleep behavior disorder (RBD), autonomic dysfunction, and depression that precede the onset of motor dysfunction are thought to be related to deposition of α-synuclein in lower brainstem or peripheral nerves, which occur earlier than in the SN. Combining non-motor symptoms and imaging such as metaiodobenzylguanidine (MIBG) cardiac scintigraphy and dopamine transporter imaging with single-photon emission computed tomography (DAT-SPECT) could be helpful for early diagnosis of PD. α-Synuclein containing inclusions in the brain is a pathological hallmark of PD, but its biological significance remains controversial as there are several other types of familial PD without α-synuclein deposition. As a result of relatively low specificity, neither plasma nor cerebrospinal fluid α-synuclein are presently reliable markers of PD. Instead, α-synuclein in solid tissue samples of the enteric and autonomic nervous systems offer potential as a surrogate marker of brain synucleionopathy.

Keywords Parkinson's disease • Biomarkers • Premotor • Neuroimaging • Alpha synuclein • Lewy body pathology

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K. Wada (ed.), *Neurodegenerative Disorders as Systemic Diseases*,
DOI 10.1007/978-4-431-54541-5_4

4.1 Introduction

In "An essay on the shaking palsy," James Parkinson first described six cases of paralysis agitans that showed characteristic involuntary tremor, upper bodies bent in forward manner, and diminished muscle strength, without sensory loss (Parkinson 1817). Charcot saluted Parkinson for his early observations, but stated that the syndrome would be better named Parkinson's disease (PD) because the patients were neither markedly weak nor were they necessarily troubled with tremor (Charcot 1872). In the 1960s, the focus on motor symptoms and signs dramatically intensified following the discovery that they are due to selective loss of dopaminergic cells in the substantia nigra (SN). Resting tremor, rigidity, bradykinesia/akinesia, and postural instability are the cardinal motor features that occur only after considerable neurologic damage has occurred and symptoms could be improved by administration of the dopamine precursor L-dopa. Additional clinical features include masked facial expression, drooling, slurred speech, shuffling gait, micrographia, and reduced arm swing while walking. Traditionally, diagnosis of PD has been made based on the presence of these motor symptoms. The Unified Parkinson's Disease Rating Scale (UPDRS) is the most commonly used scale in the clinical setting to evaluate the severity of PD symptoms. Other commonly used scales for evaluation of PD patients are the Hoehn and Yahr Staging Scale and the Schwab and England Activities of Daily Living (ADL) Scale.

Non-motor symptoms often precede the onset of motor dysfunction. In a retrospective study, pre-PD patients were reported to have more central nervous system (CNS), psychologic, musculoskeletal, and cardiovascular symptoms and hence made more visits to general practitioners than control subjects during the prodromal phase. The term "prodromal" is used to refer to the phase lasting years prior to the onset of overt parkinsonism (Gonera et al. 1997). Ever since Langston (2006) described the complexity of early non-motor PD symptoms including rapid eye movement (REM) sleep behavior disorder (RBD), hyposmia, constipation, and depression, it has become well known that PD is a systemic disease, in which various organs are affected (Siderowf and Lang 2012). In addition, cardiac sympathetic denervation occurs in virtually all PD patients and is progressive over the course of the disease. Patients of different gender and age at onset present clinically different non-motor symptoms. Male PD patients complain of problems having sex and taste/smelling difficulties much more frequently than female PD patients. Late-onset PD is characterized by more olfactory and sensory symptoms, autonomic symptoms, sleep disorders, dementia, and psychosis than early-onset PD (Picillo et al. 2013; Zhou et al. 2013).

Novel discoveries of biomarkers during preclinical (premotor) and clinical (motor) stages have simplified differential diagnosis, prognosis, and treatment of PD. Premotor biomarkers including hyperechogenicity of the SN, olfactory and autonomic dysfunction, depression, and REM sleep disorder may be noticed at the preclinical stage (Postuma et al. 2012). Neuroimaging biomarkers – positron emission tomography (PET), single-photon emission computed tomography

(SPECT), magnetic resonance imaging (MRI) – and neuropsychological deficits can facilitate differential diagnosis. Promising biomarkers include fluid biomarkers, pathological forms of α-synuclein, *DJ-1*, amyloid β and tau in cerebrospinal fluid (CSF), patterns of gene expression, protein profiling in the blood, and CSF samples – all of which are currently under investigation.

4.2 Genetic and Lewy Body Pathology

To date, several genetic mutations have been linked to increased incidence of PD. Representative genes include *SNCA*, *parkin*, *PINK1*, *DJ-1*, *LRRK2*, *ATP13A2*, *UCHL1*, *PLA2G6*, *FBXO7*, *MAPT*, *HLA-DRB5*, *BST1*, *GAK*, *ACMSD*, *STK39*, *MCCC1*/*LAMP3*, *SYT11*, *CCDC62*/*HIP1R*, *VPS35*, *PITX3*, *EIF4G1*, and *Omi*/*HTRA2* (Chartier-Harlin et al. 2011; Guo et al. 2011; Vilariño-Güell et al. 2011; Zimprich et al. 2011; Xiromerisiou et al. 2010; International Parkinson Disease Genomics Consortium et al. 2011).

Poulopoulos et al. (2012) recently published a review of the pathological patterns of all PD-related mutation carriers. Some 19 autopsies of α-synuclein mutation carriers, 49 of *LRRK2* mutation carriers, 9 of *parkin* mutation carriers, 1 of a *PINK1* mutation carrier, and 86 of glucocerobrosidase mutation carriers have been carried out in recent years. Most autopsies of α-synuclein, *LRRK2 G2019S*, *PLA2G6*, and glucocerebrosidase mutation carriers demonstrated Lewy body pathology, as opposed to *parkin* and *LRRK2* non-*G2019S* mutation carriers. However, marked variability was observed even among carriers of identical mutations. Thus, Lewy pathology may not be a reliable indicator of PD cellular dysfunction in a genetically defined patient population.

4.3 Braak Staging of Lewy Body Pathology

Lewy body pathology can be observed in central and peripheral nervous system structures. The main component protein of Lewy bodies has been shown to be α-synuclein (Spillantini et al. 1997). Whether Lewy bodies and Lewy neurites are themselves neurotoxic or bystanders of another primary pathophysiological process is still debated (Jellinger 2009), but their occurrence marks the presence of the disease. To consider the clinical course and pathomechanism of PD, Braak staging of PD brain pathology (Braak et al. 2003) provides useful information about the distribution of Lewy bodies or α-synuclein deposition. In this hypothesis, α-synuclein deposits begin at the lower brainstem or olfactory bulb, spread rostrally, arrive at the parkinsonism-causing SN, and finally reach the cerebral cortex. Braak staging has attracted much attention, as it seems to explain the clinical course of PD from the prodromal phase to PD with dementia. However, predictive validity remains obscure as a result of numerous observations of a population of aged

individuals showing brain synucleinopathy ranging up to Braak stages 4–6 at postmortem despite having no neurological signs (Burke et al. 2008). Furthermore, there is no relationship between Braak stage and disease severity or duration; peripheral pathology is also not described in Braak staging (Parkkinen et al. 2005; Parkkinen et al. 2011).

4.4 Propagation Hypothesis of α-Synuclein

Several recent experimental findings and clinical observations have suggested that prion-like aggregation transmission contributes to the anatomical spread of Lewy pathology associated with PD.

This theory first surfaced when a series of autopsies of PD patients, who had received transplants of healthy embryonic neurons more than a decade earlier, developed characteristic α-synuclein and ubiquitin-positive Lewy bodies in a subset (2–5 % over 5 years) of grafted neurons (Brundin et al. 2008; Kordower et al. 2008a, b; Li et al. 2008, 2010). Studies on PD patients dying sooner (within 1–5 years of transplant surgery) did not reveal any protein aggregates in grafted neurons, showing that Lewy bodies develop slowly or with a long delay in previously healthy embryonic neurons. These data suggest host–graft disease propagation.

Evidence that α-synuclein moves between neurons has been found in both *in vitro* and *in vivo* studies. Lee et al. (2008) reported that extracellular fibrillar and non-fibrillar oligomeric aggregates were internalized into neuronal cells via endocytosis while monomeric α-synuclein directly translocated across the plasma membrane. Desplats et al. (2009) co-cultured two populations of SH-SY5Y cells, one overexpressing myc-tagged α-synuclein (donor cells) and the other labeled with fluorescein without α-synuclein overexpression (acceptor cells). After 24 h, transmission of myc-tagged α-synuclein from donor cells was detected in acceptor cells accompanied by the formation of juxtanuclear inclusion bodies, with increasing numbers directly correlated with increasing expression levels in donor cells. Cell–cell transmission of α-synuclein occurred without potential involvement of membrane leakage but appeared more robust with cell death (Yasuda et al. 2013). Another study reported that green fluorescent protein (GFP)-tagged α-synuclein oligomers added to culture media can be internalized by primary cortical neurons engineered to express α-synuclein tagged with red fluorescent protein and induce the formation of inclusion bodies in the cytoplasm of recipient cells, suggesting that internalized protein can act as a catalyst to recruit endogenous α-synuclein into aggregates (Danzer et al. 2009).

Mouse neural stem cells tagged with GFP were reported to develop intracellular α-synuclein immunoreactivity and occasional α-synuclein-positive inclusion bodies when injected into the hippocampus of α-synuclein transgenic mice (Desplats et al. 2009). These studies suggest that host-derived α-synuclein can enter transplanted neural cells and trigger α-synuclein aggregation and deposition, analogous to the findings in grafted PD patients.

Taken together, the results from human autopsies, cell cultures, and transgenic mice suggest that intercellular α-synuclein transfer can contribute to the spread of neuropathology in PD, which could explain why the pathology progresses stereotypically in accordance with Braak stages.

4.5 Non-motor Signs in Parkinson Disease

4.5.1 Rapid Eye Movement (REM) Sleep Behavior (RBD)

RBD is parasomnia manifested by vivid dreams associated with dream enactment behavior as a result of loss of normal atonia during REM sleep. Findings from animal and human studies have suggested that lesions or dysfunction in REM sleep and motor control circuitry in pontomedullary structures cause REM sleep behavior disorder phenomenology (Boeve et al. 2013). In most cases, evidence for the existence of RBD comes from bed partner comments. Polysomnography can discriminate RBD from non-REM parasomnia or sleep apnea (Postuma et al. 2012). Cohort studies from sleep disorder clinics report that 40–65 % RBD patients develop neurodegenerative syndrome in 10 years, and the median latency between RBD onset and the diagnosis of PD ranges from 12 to 14 years (Schenck et al. 1996; Schenck and Mahowald 2003). In a clinicopathological study, 94 % of 170 neurodegenerative disorders associated with RBD were synucleinopathies including Lewy body disease and multiple system atrophy (Boeve et al. 2013), indicating a high RBD–synucleinopathy association. The extremely high risk and long latency of intervention makes RBD, a marker of prodromal PD and RBD patients, the ideal candidate for neuroprotective therapy against synucleinopathy. Decreased striatal dopamine transporter uptake and SN hyperechogenicity have been found to be risk markers of synucleinopathy in RBD patients (Iranzo et al. 2010).

4.5.2 Olfactory Dysfunction (Hyposmia)

Many PD patients show a decline in odor identification and discrimination, the degree of which correlates with disease duration and severity (Doty et al. 1992; Hawkes et al. 1997; Deeb et al. 2010; Siderowf et al. 2005; Pearce et al. 1995). Most patients are unaware of hyposmia as it is not disabling. However, the diagnostic utility of olfactory dysfuction has gradually gained prominence and appropriate means of examining smell sense have been set up. The University of Pennsylvania Smell Identification Test (UPSIT) and the German Sniffin' Sticks Test are popular examination methods. Baba et al. (2012) reported PD patients with severe hyposmia demonstrated more pronounced cognitive decline and severe brain

atrophy over a 3-year observation period, hence highlighting the predictive importance of olfactory testing for identifying dementia in PD. Likewise, olfactory dysfunction also occurs in Alzheimer's disease (AD) and increases with severity of dementia (Kovacs 2004). Severe hyposmia is predictive but not specific of PD. In some patients, hyposmia might be dysfunction of odor memory, possibly due to hippocampal dysfunction. Olfaction is reported to be impaired in *SNCA*, *parkin*, *PINK1*, *LRRK2*, *ATP13A2*, and glucocerebrosidase gene (GBA) mutations related to PD (Silveira-Moriyama et al. 2010; Saunders-Pullman et al. 2010). Initiation of Lewy body pathology distribution in the olfactory bulb (Sengoku et al. 2008) may reflect the relationship between hyposmia and PD and the potential for olfactory dysfuction to be used as a biomarker of PD.

4.5.3 Autonomic Dysfunction

The purpose of the autonomic system is to maintain homeostasis of human body. The autonomic system is divided into the sympathetic noradrenergic system which controls circulation, the parasympathetic nervous system (PNS) the main nerve of which is the vagus, the sympathetic cholinergic system which mediates sweating and thermoregulation, and the enteric nervous system (ENS). Lewy pathology in PNS and ENS suggests a wide distribution of affected lesions in PD. Dysautonomia in PD includes constipation, urinary urgency and frequency, drooling, orthostatic intolerance, erectile dysfunction, and altered sweating (Goldstein 2003; Ziemssen et al. 2011).

4.5.4 Constipation

Constipation, a well-known symptom of PD, was in fact part of James Parkinson's original description of the disorder and can appear more than 20 years before the onset of motor signs. Numerous causes of constipation have been postulated in PD including inadequate hydration and/or dehydration, the presence of Lewy bodies in the myenteric plexus of the colon, and uncoordinated control of bowel muscles. The Honolulu Heart Program, which involved 6790 men aged 51–75 years, showed that men who averaged less than one bowel movement per day were 2.7 times more likely to develop PD than men who had one bowel movement per day (Abbott et al. 2001). Dopaminergic defect of the ENS was also observed in PD patients with chronic constipation (Singaram et al. 1995). Retrospective analysis of routine colon biopsies has recently shown α-synuclein deposits in the ENS 2–5 years before the clinical onset of PD, making them a useful and accessible biomarker (Shannon et al. 2012).

4.5.5 Cardiac Sympathetic Denervation

Cardiac sympathetic denervation, another symptom of autonomic dysregulation, is virtually universal in patients with PD. More than 40 neuroimaging studies have reported early and progressive loss of sympathetic noradrenergic nerves in patients with PD. There may be a connection between cardiac sympathetic denervation and other symptoms of autonomic dysfunction, particularly orthostatic hypotension. However, it should be noted that about one half of patients with PD who do not have orthostatic hypotension also show evidence of loss of noradrenergic innervation (Goldstein et al. 2005). From the results of standard MRI scans, cerebroventricular enlargement or cortical atrophy has been observed in 92 % of PD patients with orthostatic hypotension and just over 22 % in PD patients without orthostatic hypotension, suggesting an association among Lewy body dementia, PD, and orthostatic hypotension (Kim et al. 2012; Thaisetthawatkul et al. 2004). Orthostatic hypotension can be a risk factor of dementia in PD patients. Baroreflex sensitivity and heart rate variability, which are stimulated by the PNS, have been reported to be significantly decreased in PD patients (Schmidt et al. 2009; Friedrich et al. 2010).

Cardiac sympathetic denervation seems to have linked etiologically with α-synucleinopathy because patients with familial PD, as a result of the gene encoding α-synuclein mutating or from triplication of the normal gene, have low myocardial concentrations of 6-[18F]fluorodopamine-derived radioactivity. A significant reduction in the cardiac uptake of metaiodobenzylguanidine (MIBG) during [123I]MIBG myocardial scintigraphy irrespective of disease severity, disease duration, treatment, and pre-existing dysautonomic signs has been observed in PD patients and dementia with Lewy bodies (DLB) patients but not with most multiple system atrophy (MSA) patients (Orimo et al. 2005; Goldstein 2007; Mitsui et al. 2006). It hence provides useful diagnostic information in differentiating PD from other neurological diseases. Baroreflex–cardiovagal failure and cardiac sympathetic denervation may occur before onset of movement disorder, suggesting that such neurocardiological testing might provide a biomarker for detecting presymptomatic or early PD and for following responses to putative neuroprotective treatments.

Iijima et al. (2010) assessed cardiac sympathetic function and olfactory sensitivity in 40 non-demented patients with idiopathic PD and age-matched controls by means of MIBG uptake and the Odor Stick Identification Test for the Japanese, respectively. Results showed that the olfactory system degenerated in parallel with cardiac sympathetic nervous system innervation in early-PD patients, but that the two systems may degenerate at different rates in patients with more advanced PD. Olfactory testing combined with MIBG myocardial scintigraphy has potential to be a diagnostic indicator of PD.

4.6 *In Vivo* Molecular Imaging in PD Diagnosis

4.6.1 SPECT

Degeneration of the nigrostriatal system causes denervation in the striatum with dopamine loss ranging from 44 % to 98 % (Rajput et al. 2008), which significantly correlates with the loss of dopaminergic SN neurons (Bernheimer et al. 1973). Tyrosine hydroxylase (TH) is the rate-limiting enzyme of dopamine synthesis, and dopamine transporter (DAT) is a protein located on the presynaptic side of the dopaminergic synapses that transport dopamine out of the synaptic cleft and back into presynaptic axons for either reuse or degradation to control the effect of dopamine. DAT immunoreactivity in the striatum is inversely correlated with the total α-synuclein burden but not with Lewy body counts in the SN, which supports the concept of synaptic dysfunction and impairment of axonal transport by α-synuclein aggregation (Kovacs et al. 2008). At the time of motor symptom onset, the extent of striatal dopamine marker loss exceeds that of dopaminergic SN neurons. Note that the previous belief that PD motor symptoms first appear when more than 50 % of dopaminergic SN neurons have been lost has recently been changed by the suggestion that only around 30 % of dopaminergic SN neurons have been lost but 50–60 % of their axon terminals have been lost at the time of first diagnosis of PD.

In January 2011, the Food and Drug Administration (FDA) approved 123I-ioflupane, a cocaine analog of high affinity and relatively good selectivity for DAT, to be used in DAT-SPECT imaging to detect loss of functional dopaminergic neuron terminals in the striatum of patients with clinically uncertain parkinsonian syndromes including idiopathic PD, MSA, and progressive supranuclear palsy (PSP). The extent to which DAT binding is reduced is related to disease severity. In early PD, there is usually an asymmetrical pattern of reduced DAT binding starting in the dorsal putamen contralateral to the clinically most symptomatic body side, gradually progressing anteriorly and ipsilaterally as the disease becomes more severe. Activity in the caudate nucleus is relatively preserved in early disease but will also decline with advancing disease. On the other hand, in atypical parkinsonian syndromes (MSA, PSP), there usually will be a more symmetrical decrease in DAT binding and relatively more involvement of the caudate nucleus; however, there is too much overlap to reliably differentiate between PD and these other conditions.

Nevertheless, DAT-SPECT imaging is useful to differentiate these parkinsonian syndromes from essential tremor and dystonic tremor, psychogenic parkinsonism, and neuroleptic-induced parkinsonism since each of these conditions would produce normal DAT scans. DAT-SPECT can also be used to differentiate DLB that typically shows bilateral loss of DAT binding from other dementias that show normal DAT binding (Janssen 2012).

Berendse and Ponsen (2009) evaluated the efficacy of combining olfactory testing and labeled DAT-SPECT imaging as a means of identifying patients with premotor PD. Of 361 asymptomatic first-degree relatives of patients with

parkinsonism, unexplained hyposmia was found to be associated with a 12.5 % risk of PD onset within 5 years. Poor performance on odor discrimination carried the highest risk of later PD. An important baseline was that every first-degree relative who later developed PD had both hyposmia and abnormally low levels of striatal DAT binding. Berendse and Ponsen (2009) noted that this two-step approach (i.e., olfactory testing and DAT-SPECT) might serve to diagnose premotor-stage PD. However, this technique is not practical at present because the low positive predictive value of hyposmia would necessitate carrying out an excessive number of DAT and SPECT scans in healthy individuals.

4.6.2 Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS)

A T1-weighted MRI technique could potentially be used to follow disease progression in PD. The technique was used in a recent study to assess changes in the size and signal of the SN of PD patients compared with controls. Patients with advanced disease showed greater size reduction than those in early-phase PD, suggesting stage-dependent reduction in PD as a putative marker of neuromelanin loss (Schwarz et al. 2011).

Ziegler et al. (2013) utilized multispectral structural MRI to measure the volume of the substantia nigra pars compacta (SNc) and the cholinergic basal forebrain in PD patients and matched controls. Hoehn and Yahr stage 1 PD patients showed significantly decreased SNc volume and those in stage 3 showed additional volume loss. In contrast, basal forebrain volume loss occurred later in the disease and there was a significant decrease in basal forebrain volume in patients with Hoehn and Yahr stage 2 or 3 PD.

Low N-acetyl-aspartate in the CNS indicates neuronal loss. The study by Camicioli et al. (2007) showed that PD patients exhibit selective magnetic resonance spectroscopy (MRS) decrease of N-acetyl-aspartate in the presupplementary motor area but not in the anterior cingulate gyrus or posterior cingulate, consistent with neuronal dysfunction. This warrants further examination as a biomarker for PD.

4.6.3 Positron Emission Tomography (PET)

In vivo PET imaging employing specific 18F-dopa and 18F-fluorodeoxyglucose (FDG) can provide very precise measurements of brain regional dopaminergic neurotransmission and mitochondrial bioenergetics, respectively, in the PD brain. Hence this non-invasive dynamic approach provides an exact estimate of disease progression and confirms the clinical diagnosis of PD (Eggers et al. 2014). Lewy

body cortical and limbic involvement is seen in advanced PD and can be detected using 18FDG-PET as an abnormality in the levels of resting brain metabolism in these regions. Additionally, widespread microglial activation can be detected in PD using PET (Brooks and Pavese 2011).

The serotonergic system appears to be remarkably well preserved in early PD, but is affected in advanced PD. Using PET imaging, Guttman et al. (2007) found that brain serotonin transporter (SERT) activity, a marker of serotonergic neurons, was lower in PD than that in controls, with significant changes in the orbitofrontal cortex, caudate, putamen, and midbrain but only a slight reduction in the dorsolateral prefrontal cortex (an area implicated in major depression). These data suggest that loss of brain regional serotonergic innervation might be a common feature of advanced PD.

Early PD diagnosis may be facilitated by combining non-motor and neuroimaging biomarkers. Goldstein et al. (2010) evaluated 23 patients with sporadic PD, using the UPSIT to quantify olfaction and PET with radiological markers to measure cardiac noradrenergic innervation and striatal dopaminergic innervation. Baroreflex–cardiovagal gain and baroreflex–sympathoneural function were also assessed. The results demonstrated that, independent of parkinsonism or striatal dopaminergic denervation, anosmia was associated with baroreflex failure and cardiac noradrenergic denervation, suggesting an association between cardiac dysfunction in PD and hyposmia, both of which may be present in PD prior to the onset of motor dysfunction.

4.6.4 Sonography

Striatal iron content is significantly increased in PD patients and correlates with the severity of clinical symptomatology (Ye et al. 1996). Iron content can be detected using an ultrasonic diagnostic procedure in which the echo of ultrasonic waves increases with the concentration of iron.

In more than 90 % of patients with idiopathic PD, hyperechogenicity of the SN is found to be a typical, stable sign using transcranial sonography (TCS). Animal experiments provided the first evidence of an association between SN hyperechogenicity and increased tissue iron content. There then followed studies revealing the same association in the human brain. Multivariate analysis carried out using postmortem brains from normal subjects showed a significant positive correlation between the echogenic area of the SN and the concentration of iron, H-ferritins, and L-ferritins but a significant negative correlation between echogenicity and the neuromelanin content of the SN. In PD, loss of neuromelanin and increase of iron is typically observed in SN. As SN hyperechogenicity is typical for PD or subjects with preclinical impairment of the nigrostriatal system, TCS enables the detection of increased iron and decreased neuromelanin levels at the SN, even before the clinical manifestation of PD (Berg 2006).

4.6.5 Potential Body Fluid-based and Tissue-based Biomarkers

Cerebrospinal fluid (CSF) is an excellent means of identifying biomarkers for neurological diseases affecting the CNS because it remains in direct contact with the CNS and reflects the neurochemical state under different physiological and pathological conditions. In addition to CSF, the protein content of blood, serum, plasma, saliva, and urine can be analyzed (Sharma et al. 2013).

DJ-1 is a multifunctional protein that participates in transcriptional regulation, antioxidative stress reaction, as well as chaperone, protease, and mitochondrial regulation (Ariga et al. 2013). DJ-1 deletions and point mutations associated with its loss of function have been shown to cause autosomal recessive PD. Previous studies have shown that DJ-1 levels were significantly increased in the CSF and plasma of sporadic PD patients when compared with controls. Upregulation of CSF DJ-1 in the early stages of PD (Hoehn and Yahr 1–2) differed from that in the advanced stages of PD (Hoehn and Yahr 3–4). On the other hand, plasma DJ-1 levels in the advanced stages of PD were higher than those in the early stages of PD, demonstrating that plasma DJ-1 was correlated with disease severity in PD. Plasma DJ-1 levels were also significantly higher in DLB than both controls and early-stage PD patients. These findings suggest that CSF DJ-1 could be a possible biomarker for early sporadic PD and plasma DJ-1 could be a useful biomarker for the evaluation of disease severity in PD and possibly in other Lewy body diseases (Waragai et al. 2006, 2007). However, Hong et al. (2010) reported lower CSF DJ-1 levels in PD patients versus controls. Conflicting results from these studies may be the result of differences in applied assays and/or the significant effect of blood contamination (Hong et al. 2010).

Lin et al. (2012) measured total DJ-1 and its isoforms in the whole blood of PD patients and controls in a search for potential biomarkers of PD. Seven DJ-1 isoforms were detected, and modifications in the 4-hydroxy-2-nonenal levels in the plasma of late-stage PD patients were considered candidate biomarkers.

Aside from protein levels, Kumaran et al. (2009) reported region-specific decreases of DJ-1 mRNA levels in the putamen, frontal cortex, parietal cortex, and cerebellum in PD patients (approximately 30–60 %) compared with controls as well as a preponderance of acidic isoelectric point (pI) isoforms of DJ-1 monomers in PD-vulnerable regions, suggestive of differential post-translational modifications. Such a change in the levels in CSF, plasma, and brain tissue may be the result of active transport like secretion.

Studies have demonstrated reduced CSF α-synuclein in patients with advanced PD-type diseases (Kroksveen et al. 2011; Mollenhauer and Trenkwalder 2009; Mollenhauer 2014). The diagnostic sensitivity of CSF α-synuclein ranged from 70 % to 93 %, but specificity was much lower when distinguishing PD from other movement disorders. Combinations with other α-synuclein species and other CSF markers need to be considered to improve diagnostic performance. A proteomic study reported that the CSF levels of AD biomarkers – amyloid beta (Aβ) 1-42, total

tau, and phosphorylated tau (phospho-tau) 181 – were potential diagnostic and prognostic markers of early-stage PD. The phospho-tau 181/Aβ(1-42) ratio showed significant differences between PD and control groups (Maarouf et al. 2013). Another study showed that the Aβ(1-42), total tau, and phosphorylated tau values of a group of patients with PD were similar to those of controls, whereas total tau/α-synuclein and phosphorylated tau/α-synuclein ratios showed the lowest values (Aerts et al. 2012; Parnetti et al. 2011). Combining α-synuclein with AD biomarkers helps to differentiate PD from other neurological disorders and might help improve PD with dementia/DLB versus AD in differential diagnosis (Mollenhauer et al. 2011).

In the same way that phosphorylated tau protein is more specific for AD, post-translational modifications of α-synuclein may increase the diagnostic accuracy for PD. α-Synuclein in Lewy bodies has been shown to be phosphorylated (at S87, S129, or Y125), ubiquitinated (K12, K21, K23), truncated (at its C-terminus), and oxidized (by tyrosine nitration). Apart from the monomeric α-synuclein species mentioned above, oligomeric and post-translationally modified α-synuclein can be detected in CSF. However, the extent to which monomeric and oligomeric α-synuclein levels and post-translational modifications reflect the condition of α-synuclein in the CNS or whether it correlates with disease progression or severity remains unknown (Schmid et al. 2013).

A recent preliminary study of salivary α-synuclein and DJ-1 suggested that these proteins could be potential diagnostic markers of PD. Salivary α-synuclein levels tended to decrease while DJ-1 levels tended to increase in PD patients compared with the control group. α-Synuclein levels appeared to be negatively correlated with the severity of motor symptoms, while DJ-1 levels did not correlate well with UPDRS motor scores (Devic et al. 2011). Submandibular salivary gland biopsies taken to detect Lewy-type α-synucleinopathy may be useful as a confirmation of PD diagnosis. Hilton et al. (2014) reported an accumulation of extensively phosphorylated α-synuclein within mucosal and submucosal nerve fibers and ganglia in the biopsy samples of PD patients. These samples were taken up to 8 years prior to the onset of motor symptoms. All patients with positive biopsies showed early autonomic symptoms and all controls were negative. This study demonstrates that accumulation of α-synuclein in the gastrointestinal tract is a highly specific finding that could be used to confirm a clinical diagnosis of PD.

Malek et al. (2014) published a systematic review of available evidence from 49 studies to determine the utility of α-synuclein as a peripheral biomarker of PD. Peripheral tissues such as colonic mucosa showed a sensitivity of 42–90 % and a specificity of 100 %; submandibular salivary glands showed a sensitivity and specificity of 100 %; skin biopsy showed 19 % sensitivity and 80 % specificity at detecting α-synuclein pathology. CSF α-synuclein showed 71–94 % sensitivity and 25–53 % specificity at distinguishing PD from controls. Plasma α-synuclein showed 48–53 % sensitivity and 69–85 % specificity. Neither plasma α-synuclein nor CSF α-synuclein were found to be reliable markers of PD. This contrasted with α-synuclein in solid tissue samples of the enteric and autonomic nervous system, which were found to offer some potential as surrogate markers of brain synucleinopathy.

Low levels of plasma apolipoprotein A1 have been reported to correlate with earlier PD onset in symptomatic patients and with reduced DAT density in asymptomatic patients. These data suggest that plasma apolipoprotein A1 may be a new biomarker for PD risk (Swanson et al. 2015; Qiang et al. 2013).

ATP13A2 is a lysosome-specific transmembrane ATPase protein of unknown function whose point mutation has been linked to familial PD cases. Dehay et al. (2012) found that ATP13A2 levels were decreased in dopaminergic nigral neurons from postmortem brains of sporadic PD patients. Interestingly, ATP13A2 was detected as a component of Lewy bodies in these patients.

A panel of five genes in blood comprising p19 S-phase kinase-associated protein 1A, huntingtin-interacting protein-2, aldehyde dehydrogenase family 1 subfamily A1, 19S proteasomal protein PSMC4, and heat shock 70 kDa protein 8 yielded a sensitivity and specificity in detecting PD of 90.3 and 89.1, respectively. These findings provide evidence that the panel can be used to diagnose early/mild PD and possibly even asymptomatic PD (Molochnikov et al. 2012).

Other potential PD candidate biomarkers include ceruloplasmin, chromogranin B, apolipoprotein h, oxidatively modified superoxide dismutase 1, lysosomal enzyme beta-glucocerebrosidase, as well as proteins related to protein aggregation and Lewy body formation, tissue transglutaminase, and osteopontin (da Costa et al. 2011; Choi et al. 2005; Beavan and Schapira 2013; Wilhelmus et al. 2011; Ribner et al. 2011). Furthermore, a number of proteins involved in inflammation and glial activation including interleukin-1 beta, interleukin-6, brain-derived neurotrophic factor, tissue inhibitor of matrix metalloproteinase-1, tumor necrosis factor-alpha and beta-2-microglobulin are also candidate PD biomarkers (Mogi et al. 1994a, b, 1995; Mogi and Nagatsu 1999; Nagatsu et al. 2000).

The candidacy of some potential biomarkers is based on single finding. Hence, the need for validation of potential CSF biomarkers in pathologically confirmed cohorts to account for interlaboratory variability between multiple centers. The list will undoubtedly grow in the near future as progress is made in proteomics strategy and subsequent validation of identified proteins. Furthermore, additional proteins are likely to emerge as a result of new knowledge on the pathogenetic processes involved in PD (van Dijk et al. 2010).

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Chapter 5
Clinical Systems Neuroscience

Takashi Hanakawa

Abstract Clinical systems neuroscience is advocated here as an academic discipline for clinicians and scientists who are, or will be, involved in research related to neurological, neurosurgical, and psychiatric disorders. Clinical systems neuroscience concerns neural functions as an operation of a system, not of a simple collection of elements. It deals with issues situated at the intersection between clinical practice and neuroscience at the system, cognitive, and behavioral levels. The recent emergence of non-invasive brain mapping, brain stimulation, and brain–machine interface techniques constitutes the technical background of clinical systems neuroscience. Clinical systems neuroscience is about to depart from being a method for understanding the pathophysiology of neuropsychiatric disorders to a clinical tool supporting diagnosis and treatment.

Keywords Neurology • Neurosurgery • Psychiatry • Systems neuroscience • Cognitive neuroscience • Behavioral neuroscience • Neuroimaging • Neurophysiology • Brain stimulation • Brain–machine interface

5.1 Principles Underlying Clinical Systems Neuroscience

In this chapter the author proposes adoption of clinical systems neuroscience, a discipline situated at the intersection between clinical medicine and neuroscience at the systems, cognition, and behavior levels. Clinical systems neuroscience concerns malfunctions resulting from neural systems disease related to movement, sensory perception, imagery, memory, attention, language, learning, cognition, attention, emotion, social interaction, and decision making. In neuroscience, high-level brain functions such as cognition, language, and decision making have been the subject matter of cognitive/behavioral neuroscience. In the author's opinion, however, the buildup of knowledge has made the distinction between systems neuroscience and cognitive/behavioral neuroscience unclear in terms of neural correlates (Hanakawa 2011), computational principles (Grush 2004), and probably the level of

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K. Wada (ed.), *Neurodegenerative Disorders as Systemic Diseases*,
DOI 10.1007/978-4-431-54541-5_5

organization. More importantly, at least from the clinician–scientist point of view, an isolated set of knowledge specific to each neuroscience sub-discipline falls short of being of use in clinical settings because problems existing in a neurological patient often span from systems neuroscience to cognitive/behavioral neuroscience. Therefore, the author proposes a discipline that covers basic knowledge and methodologies which can act as a bridge connecting systems, cognitive, and behavioral neuroscience for clinicians and scientists (e.g., neurologists, neurosurgeons and psychiatrists, neuroradiologists, and rehabilitation therapists who have scientific and inquisitive minds). Clinical systems neuroscience would provide a *lingua franca* for clinicians who are interested in cutting-edge research, neuroscientists who are interested in malfunctions of the nervous system, and engineers who want to develop innovative systems for patients with neuropsychiatric disorders.

The remarkable development of computer science, informatics, and information technology (IT) in recent decades has supported the non-invasive measurement and analysis of brain activity both offline and online, and has greatly contributed to the emergence of clinical systems neuroscience. Multidimensional neuroimaging (Hanakawa 2010), the integration of multimodal neuroimaging in both space and time, combines clinical neurophysiology, neuroimaging, neurostimulation, and neuro-IT methodologies, and has the potential to become a very powerful tool for clinical systems neuroscience.

5.2 Clinical Systems Neuroscience: Past

Several clinical and academic disciplines have joined together to form clinical systems neuroscience. Clinically, knowledge garnered from clinical experience is the foundation of clinical neuroscience. The discovery of an association between loss of speech (motor aphasia) and a left inferior frontal lesion by Paul Broca in 1861 marked the beginning of the understanding that cognitive functions could be located to specific convolutions of the brain. This epoch-making discovery was followed by various lesion studies that associated a cognitive function with a specific region of the brain: for instance, memory studies inspired by the case of patient H.M. who suffered from bilateral hippocampal lesions (Corkin 2002) and behavioral studies inspired by the case of Phineas Gage who suffered from a medial prefrontal lesion (Damasio et al. 1994). The lineage of clinical systems neuroscience can be traced back to these lesion studies in neurology and cognitive neuropsychology. Note that the brains or skulls of these historical patients have often been preserved, which has facilitated later examination using modern neuroimaging techniques (Damasio et al. 1994; Dronkers et al. 2007). Functional neurosurgery is another important basis for clinical systems neuroscience. Invaluable knowledge about the functional organization of the cerebral cortex resulted from Wilder Penfield's electrical stimulation of the brain of patients who were awake during neurosurgery. Recordings from, and stimulations of, the human brain using surgically implanted electrodes provided important opportunities both for understanding

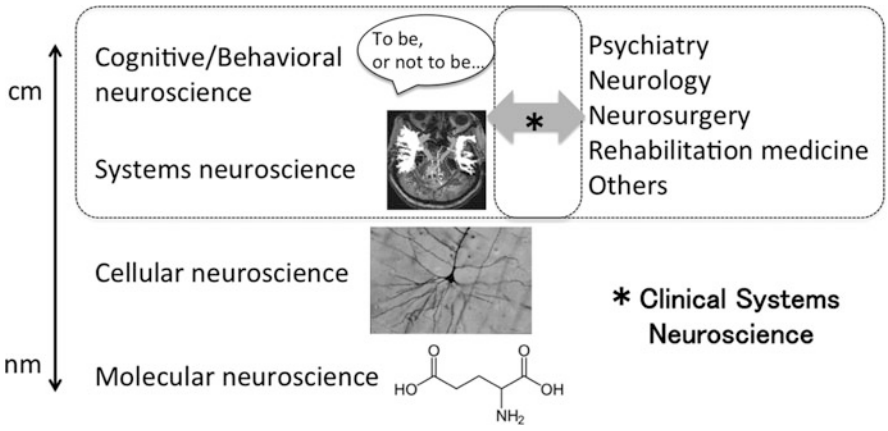

Fig. 5.1 Clinical systems neuroscience consists of multi-layered sub-disciplines with different spatial scales: molecular neuroscience (nanometer), cellular neuroscience (micrometer), systems neuroscience and cognitive/behavioral neuroscience (millimeter to centimeter). Advances in non-invasive techniques that examine brain functions have made it easier for clinicians to ask meaningful scientific questions at the systems, cognitive, and behavioral neuroscience levels. Neuroscientists, in turn, can explore a new discipline for basic research, one that holds the potential to provide solutions to unanswered questions in clinical medicine. Clinical systems neuroscience has come to the fore as a result of mutual interactions between neuroscience and clinical medicine, as distinct from unidirectional translation from basic research to clinical medicine

systems in the human brain and for developing new therapeutic methods. From a technical perspective, clinical neurophysiology is a medical specialty that has used electrophysiological methods such as electroencephalography (EEG) and electromyography (EMG) to record spontaneous and evoked responses in both the peripheral and central nervous systems. Clinical neurophysiology uses such recording techniques to diagnose patients with nervous system disorders and to clarify their pathophysiology. Clinical neurophysiology partially overlaps with clinical systems neuroscience. Another technical background is provided by neuroradiology, a sub-specialty of radiology, which focuses on diagnosing abnormalities in the central and peripheral nervous systems using magnetic resonance imaging (MRI) and computed tomography (CT). MRI is one of the most powerful tools subserving clinical systems neuroscience. Moreover, nuclear medicine is another medical sub-discipline that provides other useful technologies, such as positron emission tomography (PET), for clinical systems neuroscience.

Systems neuroscience is a sub-discipline of basic neuroscience that studies the brain and spinal cord as systems – not as elements – from the anatomical, functional, and computational viewpoint often at the level of neural circuits (Fig. 5.1). Systems neuroscience typically, but not exclusively, studies how an organism perceives stimuli from the external environment, makes a decision, and translates the decision into behaviors. Neuroanatomical, neurophysiological, and more recently neuroimaging techniques are the typical technologies applied to systems

neuroscience. Cognitive neuroscience is based on both psychology and neuroscience, and concerns the neurobiological substrates of cognitive abilities and mental processes. Neuroimaging techniques have enabled non-invasive measurements of brain activity during various mental processes in humans and have greatly contributed to advances in cognitive neuroscience. Behavioral neuroscience puts more weight on behavioral aspects than cognitive neuroscience in the study of non-human animal species. These sub-disciplines of neuroscience partially overlap each other. Moreover, as mentioned earlier, clinical problems often extend from systems neuroscience to cognitive/behavioral neuroscience. This is the reason the author believes the concept of systems neuroscience should be extended to cover issues typically handled by cognitive/behavioral basic neuroscience. Clinical systems neuroscience can begin when such an extended version of systems neuroscience is applied to clinically oriented questions, using such methodologies as non-invasive physiology, imaging, stimulation, and neuro-IT.

5.3 Clinical Systems Neuroscience: Present

Clinical systems neuroscience examines the functional neuroanatomy of the nervous systems in living humans, especially that of patients with neurological, neurosurgical, psychiatric, or other systemic diseases involving the nervous system, and does so using non-invasive methods. However, functional neurosurgery is an exception. Currently available tools such as neuroprostheses are based on electrophysiological recordings, neuroimaging, brain stimulation, and neuro-IT. The technical details and application of each method to each functional system are beyond the scope of this chapter. Hence, the chapter restricts itself to briefly summarizing the technical background and characteristics of every tool that has played a part in the emergence of clinical systems neuroscience. Likewise, discussion of every application to every neuropsychiatric disorder is beyond the scope of this chapter. Therefore, typical applications of each tool to just a few diseases are described with the objective of solving problems in clinical systems neuroscience.

5.3.1 Electrophysiology

5.3.1.1 Electroencephalography (EEG)

Since its discovery by Hans Berger in 1929, EEG has been widely used as a clinical or scientific method to monitor ongoing brain activity. Signals acquired using a scalp electroencephalogram (also abbreviated as EEG) are believed to reflect a summation of local field potentials (LFPs). LFPs recorded by EEG are considered to reflect mostly excitatory postsynaptic potentials (EPSPs) and partly inhibitory postsynaptic potentials (IPSPs) generated in the apical dendrites of pyramidal cells.

Hence, an EEG mainly comprises inputs into a large population of cortical neurons which, when synchronized, may produce oscillatory EEG patterns that can be recorded from surface EEG.

A scalp EEG is recorded by means of an electroencephalograph in conjunction with electrodes attached to the scalp, often arranged according to the International 10–20 System of Electrode Placement (Fp1, Fp2, F3, F4, C3, C4, P3, P4, O1, O2, F7, F8, T3, T4, T5, T6, Fz, Cz, Pz, A1, and A2). The letters F, T, P, and O represent the frontal, temporal, parietal, and occipital lobes, respectively. The letter C refers to electrodes close to the central sulcus, corresponding to the primary motor and somatosensory cortices. The letter A refers to the earlobes. The letter z (zero) refers to electrodes placed on the midline. Even numbers and odd numbers refer to electrode positions on the right and left hemispheres, respectively. For research purposes, electrode caps with denser arrays of electrodes (e.g., 64, 128, and 256 electrodes) are available.

An EEG often reveals conspicuous oscillatory activity. Oscillatory EEG activities are classified into α (8–15 Hz), β (16–31 Hz), γ (>32 Hz), δ (<4 Hz), and θ (4–7 Hz) ranges or bands according to their frequency. Resting-state EEG activity is dominated by α-range oscillations over the parieto (P) to occipital (O) electrodes (posterior dominant rhythm) in healthy subjects. The posterior dominant rhythm is suppressed by eye opening (visual inputs) and by reduced arousal levels. Although it is unclear how posterior dominant rhythms are generated and modulated, functional MRI (fMRI) combined with EEG has started to shed light on the modulation mechanisms of the posterior dominant rhythm at rest (Omata et al. 2013). Other important rhythms are sensorimotor rhythms (SMRs), or mu rhythms; they are α-band oscillations recorded over the central (C) electrodes. SMRs are considered to reflect the idling state of the motor system since movement suppresses them. However, since SMRs are also suppressed by motor imagery or motor intention, their amplitude is now used as a feature to detect the motor intention of patients who cannot actually move (i.e., the brain–machine interface).

The normal oscillatory activities observed in EEG recording are collectively called "background EEG activity." Diffuse slowing and/or reduction of background EEG activity indicates diffuse impairment of brain functions (diffuse encephalopathy), especially when accompanied by disturbance of consciousness. The complete loss of EEG background activity is termed "electrocerebral inactivity or silence," which is seen in complete and irreversible brain functions like brain death. Local slowing or reduction of EEG background activity suggests focal abnormality of brain functions resulting from, for example, space-occupying lesions and ischemic lesions.

Detecting epileptiform discharges using EEG is an indispensable process in the diagnosis of epilepsy. Epileptiform discharges are distinctive waves or complexes lasting several tens of milliseconds to a few hundred milliseconds, often called "spikes" or "sharp waves," which stand out from background activity. Physiologically, an epileptiform discharge corresponds to a paroxysmal depolarization shift (which is abnormal) and to a large EPSP occurring in a group of neurons in epileptic foci.

The advantages of scalp EEG as a tool of clinical systems neuroscience include non-invasiveness, fine temporal resolution, and prevalence in its use in research and clinical institutes all over the world. Conversely, the shortcomings of scalp EEG include poor spatial resolution/localization and inaccessibility to deep-brain structures. Poor localization can be improved by source localization techniques, which have been developed with the aid of anatomical/functional MRI (Yoshimura et al. 2012) and modeling methods (Michel et al. 2004). The development of these techniques, coupled with other visualization technology, has made EEG an even more powerful tool in basic and clinical systems neuroscience than before. A good example is the brain–machine interface using SMRs or evoked potentials such as P300, as will be discussed later. Furthermore, EEG (coupled with other methodologies) is an emerging method that takes advantage of the high temporal resolution of EEG and supplements spatial localization with other imaging methods. For example, simultaneous EEG and neuroimaging, especially fMRI, is an emerging method used to localize epileptic foci (Chaudhary et al. 2013).

5.3.1.2 Intracranial Recording

A number of invasive electrophysiological techniques have been established for clinical application. Electrocorticography (ECoG) is one such technique in which EEG can be recorded from an array of electrodes placed over the surface of the cerebral cortex. Typically, arrays of platinum electrodes are placed subdurally by means of surgery. ECoG has been the most reliable and powerful tool for localizing epileptic foci in patients with intractable epilepsy who are candidates for surgical removal of these foci. Another advantage of ECoG is that it can map functional areas of the cerebral cortex beneath electrode arrays such that surgical removal of the tissue does not damage functionally important (called "eloquent") brain regions. This mapping can be carried out by electrical stimulation of the brain by means of electrodes or by recording evoked potentials in response to cognitive, motor, and sensory tasks. Brain mapping with ECoG provides complementary information when used with other brain-mapping tools such as fMRI (Hanakawa et al. 2001). Moreover, ECoG may clinically serve to bring about a high-performance brain–machine interface (BMI) (Yanagisawa et al. 2012) since the signal-to-noise ratio of ECoG is superior to that of scalp EEG.

Another clinically important type of intracranial electrophysiological recording can be obtained from electrodes implanted for deep-brain stimulation (DBS) to treat movement disorders. Recordings from DBS electrodes placed in the subthalamic nucleus or the globus pallidus have provided invaluable information about the pathophysiology of Parkinson's disease. In particular, it has been shown that β-range oscillation is enhanced in the subthalamic nucleus and high-frequency stimulation suppresses the oscillation, thereby inducing clinical benefit in Parkinson's disease (Kuhn et al. 2008).

5.3.1.3 Evoked Potentials and Event-related Potentials

Triggered averaging of EEG signals time-locked to repetitive sensory stimuli allows evoked potentials to be recorded by means of both scalp and intracranial recordings. Similarly, it is possible to record averaged EEG potentials time-locked to particular cognitive or motor events called "event-related potentials" (ERPs).

Sensory evoked potentials (SEPs) are evoked responses in the peripheral nerves, spinal cord, and brain following electrical stimulation of peripheral nerves such as the median nerve and the tibial nerve. SEPs are used to detect damage to the posterior column–medial lemniscus sensory system, which conveys touch, vibration, and proprioception. In patients with multiple sclerosis, sensory-evoked responses can be delayed or diminished. Conversely, patients with cortical myoclonus often show giant SEPs reflecting cortical hyperexcitability (Rothwell et al. 1984). Visual evoked potentials (VEPs) are responses to flash stimuli or visual pattern stimuli, and are recorded over occipital EEG electrodes. Abnormal VEPs may result from dysfunctions of the visual system including the visual nerves, optic tracts, lateral geniculate, optic radiation, and primary visual cortex. Short-latency auditory evoked potentials (AEPs) are often called "brainstem auditory evoked potentials" (BAEPs). BAEPs typically show seven positive peaks within 10 ms of click sounds. BAEPs are used to test brainstem function.

P300 is a positive ERP recorded approximately 300 ms after presentation of rare stimuli in an oddball paradigm in which frequent and rare stimuli are randomly presented and a participant is asked to count the number of rare stimuli. P300 can be applied to a type of BMI called the "P300 speller", which detects brain activity related to the selection of stimuli such as letters (Farwell and Donchin 1988). Mismatch negativity is an ERP, also based on an oddball paradigm, which follows rare stimuli regardless of whether a participant pays attention to the stimuli or not. Mismatch negativity is reduced in patients with chronic schizophrenia (Nagai et al. 2013).

Movement-related cortical potentials (MRCPs) or Bereitschaftpotentials (BPs) are slow cortical potentials that precede voluntary movements. MRCPs/BPs are thought to originate from the supplementary motor area and primary motor cortex. Contingent negative variations (CNVs) are slow negative EEG potentials observed when a participant is anticipating an event of interest (Walter et al. 1964). CNVs are influenced by attention to an event.

5.3.1.4 Magnetoencephalography (MEG)

Much like EEG, the main source of MEG signals is synchronous postsynaptic currents in the pyramidal neurons of the cerebral cortex (Hari and Salmelin 2012). These electric currents accompany an electromagnetic field, which is theoretically detectable by coils outside the body. In contrast with the distorted electrical signals measured by scalp EEG as a consequence of poorly conducting skull

bones, the magnetic field measured by MEG is not affected by the skull. MEG therefore shows great potential to localize the source of synaptic activity precisely. Although the electromagnetic field in the brain is very small (10–100 fT), the development of superconducting quantum interference devices (SQUIDs) has made detection of the electromagnetic field in the human brain possible (Cohen 1972). A helmet-type, whole scalp–covering MEG system with more than 300 SQUID sensors is currently available.

Note that MEG and EEG complement each other. The sensitivity of MEG to electromagnetic fields is different from that of EEG in that it depends on the direction of currents. MEG is most sensitive to currents tangential to the skull (i.e., postsynaptic activity occurring in the banks of cerebral gyri buried within the sulci/fissures), whereas EEG has sensitivity to signals from the depth of the brain and from the cerebral convexity. With these limitations in mind, electromagnetic source modeling that can provide information on the timing and direction of current flow is of great help in interpreting MEG data. The most straightforward approach has been to use an equivalent current dipole (ECD) model. A single ECD or multiple ECDs can be modeled. In minimum-norm estimates and minimum-current estimates (MNE/MCE), many small dipoles are placed throughout the cortex and their strengths are estimated as a function of time (Lin et al. 2006). In the beamforming procedure (Hillebrand et al. 2005), the volume of the brain is scanned by sequential application of spatial filters to pass activity from a specific brain area with maximum gain, while suppressing activity from other areas. Despite such progress, the currently available source analysis procedure cannot circumvent the inverse problem (i.e., the source of the electromagnetic field cannot be uniquely solved).

MEG has been applied to solving questions in basic systems neuroscience and cognitive/behavioral neuroscience including sensory perception, movement, language, social interaction, and development/learning. MEG can pinpoint the cortical generators of various evoked and event-related potentials that had been studied with EEG. MEG can also be applied to studying oscillatory brain activity and interareal connectivity. In the clinical domain, MEG plays an important role in epilepsy and preoperative mapping (Stufflebeam et al. 2009). MEG has also been applied to pathophysiological studies in movement disorders (Mima et al. 1998) and schizophrenia (e.g., mismatch field).

5.3.1.5 Assessment of Peripheral Nervous Systems and Muscles

Conventional electrophysiology techniques used to study functions of the peripheral nervous system and muscles are of importance, alone or in combination, to assess the peripheral functions of motor, sensory, and autonomic systems. It is also important to combine their use with modern neuroimaging and neurostimulation techniques.

Needle EMG is an important technique used to detect motor unit potentials. Active denervation can be diagnosed by detecting fibrillation potentials and

positive sharp waves during muscle relaxation. Surface EMG is used to assess gross muscle activities. Integrated EMG has often been used to quantify gross muscle activities since it is proportional to isometric tension. Surface MEG is also used to record muscle activities evoked by electrical or magnetic stimulation of peripheral nerves and the motor cortex (nerve conduction studies, F-waves, H-reflex, etc.). It is possible to record surface EMG during neuroimaging (Hanakawa et al. 2002, 2003a), and during simultaneous brain stimulation and neuroimaging (Hanakawa et al. 2009; Shitara et al. 2011, 2013). Surface EMG helps assess the gross pattern of involuntary movement such as tremor, myoclonus, chorea, and dystonia. Therefore, neuroimaging studies on the pathophysiology of involuntary movement depend on surface EMG being monitored and recorded simultaneously and the data used as a covariate in the imaging analysis for proper interpretation of imaging findings.

5.3.2 Neuroimaging

Clinical systems neuroscience substantially relies on developing neuroimaging techniques that emerged in the final two decades of the twentieth century. Functional neuroimaging techniques include PET, single-photon emission computed tomography (SPECT), near-infrared spectroscopy (NIRS), and MRI. These techniques allow functional, neurochemical, and/or anatomical information to be obtained from healthy and diseased human brains non-invasively. These methods are occasionally called "brain-mapping techniques" since they are used to identify which parts of the brain represent a function of interest and then to create functional maps of the brain. The philosophy underlying such techniques is the assumption that a spatially segregated part of the brain code controls one or more distinct brain functions (functional segregation). Researchers in the field most often use Talairach and Tournoux's (1988) coordinate system for the coordinates of brain maps. The system was originally (Talairach and Tournoux 1988) developed to assist neurosurgeons plan and execute stereotaxic surgery. A surface-based coordinate system has also been proposed to map the cerebral cortex (Dale et al. 1999; Fischl et al. 1999). However, functional segregation may not account for all the control mechanisms of cognition and behavior. It is important here to test the hypothesis that functions of the brain may primarily arise from interactions across different brain regions (functional integration). Note that functional segregation and functional integration are not mutually exclusive. Hence, it may be worth considering both types of control mechanisms whenever attempts are made to explain a particular ability of the brain before jumping to a conclusion.

5.3.2.1 Magnetic Resonance Imaging (MRI)

MRI is a medical imaging technique that makes use of nuclear magnetic resonance (NMR), in which nuclei in a magnetic field absorb and emit electromagnetic

radiation. Nuclei with an intrinsic magnetic moment and angular momentum (such as ^{1}H and ^{13}C) can be tested with NMR, but currently available medical MRI systems almost exclusively use ^{1}H because of the high prevalence of ^{1}H protons in the body. Protons behave like microscopic magnets. Inside a strong static magnetic field, some protons are aligned parallel to the magnetic field and some are aligned anti-parallel to it. The number of parallel protons and anti-parallel protons are not equal; there are slightly more parallel protons than anti-parallel protons. This difference (approximately 2×10^{15} protons in a $1 \times 1 \times 5$ mm^3 voxel) produces a magnetization vector, which is a vectorial summation of all the magnetic protons in the voxel. 'Voxel' here refers to a unit in a regular grid in three-dimensional space, and is a unit of MRI acquisition. Now, protons have a resonance frequency of 128 MHz at 3 T (tesla). When a 128 MHz electromagnetic pulse is emitted from a transmit coil, protons absorb it and start to resonate in a 3 T MRI system. By creating a magnetic gradient and modulating electromagnetic pulses, MRI can tag the position of a voxel at which resonated protons emit electromagnetic radiation. Moreover, when the electromagnetic pulse disappears, the resonated protons start to emit electromagnetic radiation during a process that relaxes back toward the original condition. This is called the "relaxation process" during which electromagnetic radiation from protons can be detected by receiver coils.

There are a number of tissue factors influencing the relaxation process: longitudinal relaxation time (T1, spin–lattice) and transverse relaxation time (T2, spin–spin). T2 assumes a homogeneous magnetic field in the environment; however, a biological environment has magnetic inhomogeneity. For example, magnetic inhomogeneity may result from deoxy-hemoglobin (deoxy-Hb), which is a paramagnetic substance in red blood cells. The transverse relaxation time of tissue in the presence of magnetic inhomogeneity is called T2*, which is shorter than T2.

Conventional brain structural MRIs – such as T1-weighted, T2-weighted, proton density, and fluid-attenuated inversion recovery (FLAIR) images – have been of great importance in neuroradiological diagnosis of disorders. These images can detect atrophy, ischemic lesions, hemorrhages, space-occupying lesions, inflammation, metal deposition, and so forth. Clinical as well as scientific studies have made use of three-dimensional T1-weighted MRIs with fast spoiled gradient echo (FSPGR) or magnetization prepared rapid gradient echo (MPRAGE) sequences for volumetry, voxel-based morphometry (VBM), tensor-based morphometry (TBM), and cortical thickness measurement (Fig. 5.2). Gray matter volume or cortical thickness, as studied with these methods, has been shown to correlate with many aspects of human abilities (Hosoda et al. 2013). Recently, VBM has also been used as a method to elucidate neuroplasticity associated with development as well as use-dependent neuroplasticity associated with various kinds of learning and training in adults (Hosoda et al. 2013; Tanaka et al. 2013a). Gray matter/cortical thickness is also informative in clinical studies of dementia, movement disorders, and psychiatric disorders (Namiki et al. 2008; Yamada et al. 2007; Garraux et al. 2004, 2006).

Emerging MRI methodologies comprise a number of diffusion-weighted MRI (DWI) techniques (Fig. 5.2, *Middle*) such as diffusion tensor imaging (DTI),

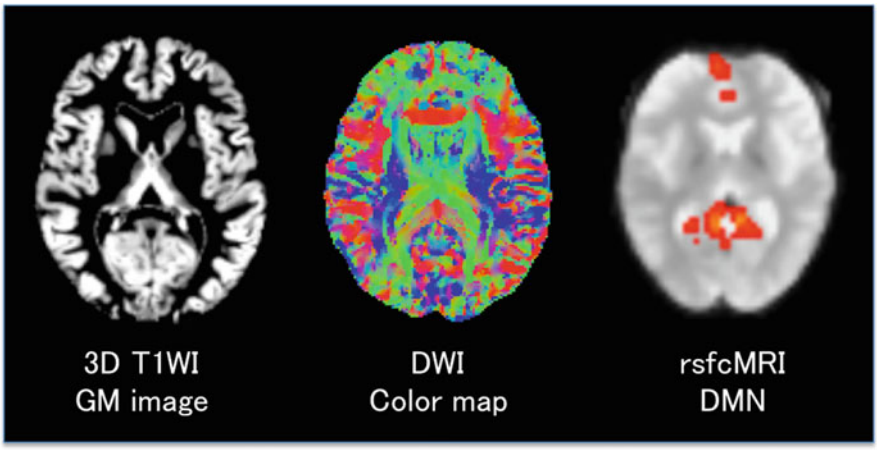

Fig. 5.2 Three representative images indicating different information obtained with MRI from a healthy volunteer. *Left*. A gray matter (GM) segmented image from a three-dimensional T1-weighted image. GM images have been used for VBM analysis of GM volume correlated with a cognitive function or differences in GM volume across groups. *Middle*. A diffusion-weighted image (DWI) measures the diffusion of water molecules. Assuming that the diffusion of water molecules reflects the orientation of fibers in white matter (WM), DWI can yield, for example, a color map simulating voxel-wise predominance of WM fiber orientations (*red* = right–left fiber orientation; *green* = anterior–posterior; *blue* = superior–inferior). *Right*. Resting-state functional connectivity MRI (rsfcMRI) shows the medial prefrontal area and posterior cingulate area (*red*) corresponding to parts of the default mode network (DMN). An independent component analysis (ICA) was used to separate different resting-state networks (RSNs) in an rsfcMRI dataset without *a priori* hypothesis. The DMN (*red*) is overlain onto a gradient-echo echo planar image used for acquisition of rsfcMRI data

diffusion kurtosis imaging (DKI), diffusion spectral imaging (DSI), and q-space imaging (QSI). DWI can be used to visualize the diffusion of protons, especially those of water molecules. It has had a major impact on clinical practice in detecting early stroke (Moseley et al. 1990). DTI-derived images – such as fractional anisotropy (FA) and mean diffusivity (MD)/apparent diffusion coefficient (ADC) – allow quantitative analysis of changes in water diffusion associated with morphological change and damage to brain tissue. Moreover, diffusion-based fiber tractography has proven to be useful in assessing macroanatomical fiber connections in both healthy and diseased people. For example, DWI-based tractography is used to determine the dominant hemisphere (Matsumoto et al. 2008) and guide neurosurgical procedures (Okada et al. 2006). It is also possible to assess longitudinal changes in DWI-derived parameters resulting from neuroplastic changes associated with learning (Hosoda et al. 2013).

Blood oxygenation level–dependent (BOLD) fMRI is a functional neuroimaging method that uses deoxy-Hb as an internal contrast agent (Ogawa et al. 1992). To date, it has mostly been carried out using T2*-weighted MRI such as gradient-echo echo planar imaging (GE-EPI). BOLD fMRI uses T2* signals as a surrogate measure of synaptic/neuronal activations (see the "Neurometabolic–Hemodynamic

Coupling" section later in the chapter). For more than two decades, BOLD fMRI has been the most widely and intensively used neuroimaging/brain-mapping tool, despite the cost of establishing and maintaining an MRI facility. There are several reasons for this. First, BOLD fMRI has the finest spatial resolution (typically, a few millimeters) of all the currently available non-invasive brain-mapping tools. Moreover, it provides the most accurate localization in the whole brain from the cortical surface to the deep nuclei and cerebellum. Nowadays, spinal cord fMRI is also feasible. fMRI has a time resolution (typically, a few seconds) which is sufficient to capture BOLD signal time courses after a brief stimulus. Second, structual MRI taken together with fMRI can obtain a variety of structural information with high spatial resolution as described above (such as three-dimensional T1-weighted images). Third, BOLD fMRI is capable of studying not only functional segregation but also functional integration (functional connectivity and effective connectivity) by using either psychophysiological interactions (Friston et al. 1997; Abe et al. 2007; Aso et al. 2010) or dynamic causal modeling (Friston et al. 2003). Task-related fMRI has been applied to an enormous number of issues in systems/cognitive/behavioral neuroscience: sensory perception (Ban et al. 2013; Yamamoto et al. 2008; Aso et al. 2007; Sawamoto et al. 2000), movement (Hanakawa et al. 2003a, 2006, 2008a; Toma et al. 1999), language (Nakamura et al. 2000a, 2002; Crinion et al. 2006; Hosoda et al. 2011), imagery (Hanakawa et al. 2003a, 2008b), attention (Nakamura et al. 2000b), cognition (Hanakawa et al. 2002, 2003b, c; Abe et al. 2007; Kasahara et al. 2013), decision making (Fukui et al. 2005), and social interaction (Shinozaki et al. 2007; Fukui et al. 2006). Similarly, task fMRI has been applied to a variety of disorders involving the central nervous system (Tanaka et al. 2012; Oga et al. 2002; Bohlhalter et al. 2006). Note, however, that interpretation of task fMRI results is often difficult because task difficulty and task performance are often significantly different between a healthy group and a patient group. To circumvent this problem, recent attention has been focused to resting-state functional connectivity MRI (rsfcMRI) in which participants undergo no particular task (Fox and Raichle 2007; Smith et al. 2013). This variation of fMRI depends on ultra-slow fluctuations (0.01–0.1 Hz) of BOLD signals, which perhaps reflect changes in synaptic/neural activities occurring spontaneously across the nodes of a functional network. Thus, without any tasks, rsfcMRI can identify motor, visual, attention, auditory, and default mode network (DMN) systems (Raichle et al. 2001) (Fig. 5.2, *Right*). For example, abnormalities in rsfcMRI have been identified in dementia, schizophrenia, mood disorders, epilepsy, and movement disorders (e.g., Greicius 2008).

As a result of fMRI having a powerful magnetic field, it has previously been difficult to acquire fMRI data simultaneously with other recording techniques. However, technical advances have made it possible to combine fMRI with many other techniques such as transcranial magnetic stimulation (TMS) (Hanakawa et al. 2009; Shitara et al. 2011, 2013) and EEG (Omata et al. 2013). In particular, simultaneous EEG–fMRI has the potential to become an important tool in clinical research of epilepsy (Chaudhary et al. 2013).

5.3.2.2 Positron Emission Tomography (PET)

PET is a nuclear medicine imaging technique that uses a positron-emitting radionuclide-labeled tracer. It can create a three-dimensional functional image of the body including the brain. The labeled tracer is typically injected into the bloodstream, where the tracer is distributed throughout the body according to its biochemical characteristics. The positron-emitting radionuclide emits a positron, which travels a short distance (~1 mm) in tissue and interacts with an electron. When the two particles meet, they annihilate each other and emit annihilation radiation (a pair of 511 keV gamma photons emitted in opposite directions). This pair of gamma photons simultaneously reaches scintillators on the scanner and produces a burst of light, which is detected by photomultiplier tubes. Detection of this coincidence event allows PET to accurately localize the distribution of the tracer in the brain. After collecting many coincidence events, a tomographic reconstruction method is applied to yield a three-dimensional image dataset.

A shortcoming of PET is radiation exposure. Another is the expensive cost of a PET facility, which is typically equipped with a PET scanner(s), a cyclotron, and a laboratory for tracer synthesis. This is because many positron-emitting radionuclides used for brain PET have short lives, and therefore tracers must be synthesized just before use. Nevertheless, PET is a powerful and indispensible functional imaging tool for both the basic and clinical domains of systems neuroscience. One of the strengths PET has over other imaging modalities is its high signal-to-noise ratio. Another is the capability of PET to obtain specific molecular information depending on the tracer in use (so-called "molecular imaging"). Brain PET images using ^{18}F-labeled fluorodeoxyglucose (^{18}F-FDG) reflect glucose metabolism levels associated with regional neural/synaptic activities. Reduced levels of ^{18}F-FDG indicate pathological changes in brain metabolism associated with neuropsychiatric disorders such as epilepsy (Takaya et al. 2006) and Alzheimer's disease (Weiner et al. 2010). Moreover, the development of tracers binding to β-amyloid, such as Pittsburgh compound B (PIB), has dramatically changed PET diagnosis of Alzheimer's disease, preclinical Alzheimer's disease, and mild cognitive impairment (MCI) (Weiner et al. 2010). The development of new amyloid tracers is currently ongoing. Other tracers detecting neuroaggregates (e.g., tau) in the human brain are also close to being approved for clinical application.

PET also allows neurotransmitter imaging of many types: dopamine receptors (^{11}C-raclopride and ^{18}F-fallypride), enzymes related to dopamine synthesis (^{18}F-dopa), acetylcholine synthesis (^{11}C-methylpiperidin propionate), and serotonin transporters (^{11}C-DASB).

^{15}O-labeled water is distributed in the brain in proportion to regional cerebral blood flow (rCBF). Changes in rCBF are connected with changes in neural/synaptic activities (as will be discussed later in Sect. 5.3.3). Thus, brain PET images of $H_2{}^{15}O$ distribution during a particular task can identify brain regions neurally activated during the task in healthy (Hanakawa et al. 2002; Lerner et al. 2009) and diseased people (Lerner et al. 2004, 2007; Sawamoto et al. 2007). Such PET

activation studies are also possible to test dopamine release during a particular task. Internally released dopamine competes with externally administered ^{11}C-raclopride for dopamine receptor D2 binding. Therefore, reduced ^{11}C-raclopride uptake during an experimental task compared with a control task indicates increased dopamine release in the experimental task (Sawamoto et al. 2008).

Silicon avalanche photodiodes have replaced conventional photomultiplier tubes in recent PET systems. Since they function within a strong magnet, it is now possible to combine PET and MRI as a hybrid PET/MRI system using a single scanner device.

5.3.2.3 Single-photon Emission Tomography (SPECT)

SPECT is another nuclear medicine imaging technique that uses a gamma-emitting radioisotope. A SPECT machine is usually equipped with multiple gamma cameras to acquire multiple two-dimensional images from multiple directions. After image acquisition, a tomographic reconstruction method is applied to yield a three-dimensional dataset. Alhough SPECT has lower spatial resolution than PET, it is less expensive.

SPECT has been used to measure brain perfusion using ^{99m}Tc-HMPAO (hexamethylpropylene amine oxime) or ^{99m}Tc-ECD (ethyl cysteinate dimer). Perfusion SPECT can detect changes in diffuse perfusion abnormality in dementia. It can also detect focal changes in cerebral vascular disorders. Perfusion SPECT has a particular application in functional neuroimaging. PAO and ECD are fixed within the brain a few minutes post injection using the so-called "snapshot method." Therefore, SPECT provides a unique opportunity to study mechanisms underlying walking difficulty in neurological disorders. Patients with Parkinson's disease show underactivation of the supplementary motor areas as they walk (Hanakawa et al. 1999a), whereas they show overactivity in the lateral premotor cortex during paradoxical improvement of walking under appropriate visual guidance (Hanakawa et al. 1999b). A follow-up study into vascular parkinsonism replicated dysfunctions in supplementary motor areas and compensatory activity in the lateral motor cortex during parkinsonian walking (Iseki et al. 2010).

SPECT with ^{123}I-ioflupane (FP-CIT SPECT) can now be used for dopamine transporter (DAT) imaging to visualize nigrostriatal dopaminergic nerve terminals. Parkinson's disease and dementia with Lewy bodies show reduced DAT signals in the striatum (putamen and caudate nucleus).

5.3.2.4 Near-infrared Spectroscopy (NIRS)

NIRS uses the near-infrared region of the electromagnetic spectrum (700–1400 nm) and measures absorbance at specific wavelengths corresponding to oxyhemoglobin (oxy-Hb) and deoxy-Hb. NIRS devices come with a pair of emitters and a probe detector attached to the scalp. Near-infrared light in the 700–900 nm range of the

spectrum penetrates the scalp and skull, and then reaches the surface of the brain. In this range of the spectrum, oxy-Hb and deoxy-Hb are strong absorbers of light. Therefore, NIRS can measure the relative concentration of oxy-Hb and deoxy-Hb from the brain surface. The detector typically measures reflected (backscattered) light in which photons may take a variety of paths, often modeled as an ellipsoid in shape. The distance between the emitters and the detector (typically 2–3 cm) determines the size of the ellipsoid. The modified Beer– Lambert law is used to calculate micromolar-level changes in oxy-Hb and deoxy-Hb tissue.

Task-induced changes in oxy-Hb and deoxy-Hb are considered to reflect synaptic/neuronal activity according to the principle of neurometabolic–hemodynamic coupling (see next section). Therefore, much like fMRI, functional NIRS (fNIRS) is possible by measuring oxy-Hb and deoxy-Hb as surrogate markers of synaptic/neuronal activity changes correlated with a task. Although this assumption has been confirmed by a few simultaneous fMRI–fNIRS studies (Sato et al. 2013), it is also known that fNIRS is strongly influenced by hemodynamic changes in the scalp (Takahashi et al. 2011; Kirilina et al. 2012). Hence, every effort should be made to remove the effects of changes in scalp blood flow such that fNIRS data can be accurately interpreted. Temporal resolution of fNIRS is carried out in a matter of seconds because of fNIRS dependence on hemodynamic signals, although sampling of fNIRS can be carried out in a matter of milliseconds. Other limitations of fNIRS are difficulty in measuring deep-brain structures and low spatial information.

On the other hand, fNIRS has obvious advantages over other neuroimaging methods: it is completely safe, it is non-invasive, setting it up is relatively easy, and there is no need for rigid restraint of head and body. Such advantages can best be exemplified by its application to infants (Taga et al. 2003). Another example is its application to studying cortical motor area activity during walking (Miyai et al. 2001).

fNIRS is a tool of clinical systems neuroscience, whose application to developmental neurology and pediatrics is particularly warranted, considering the thin skulls of the pediatric population and thereby easy penetration of near-infrared light to the brain. Assessment of brain activity during walking has been employed to examine the pathophysiology of disturbances in patients with walking difficulty (Miyai et al. 2002). Other important applications of fNIRS could be to restless patients or to critically ill patients. Lastly, convenience in the use of fNIRS may bring about wide clinical applications in daily practice. Note that psychiatrists in Japan have started to apply fNIRS to everyday clinical diagnosis of psychiatric disorders (Takizawa et al. 2014).

*5.3.3 Neurometabolic–*Hemodynamic Coupling

Many neuroimaging methods measure signals derived from hemodynamic changes as surrogate markers of neural/synaptic activity. It is widely accepted that hemodynamic signals faithfully reflect neural and synaptic activity, as a result of putting

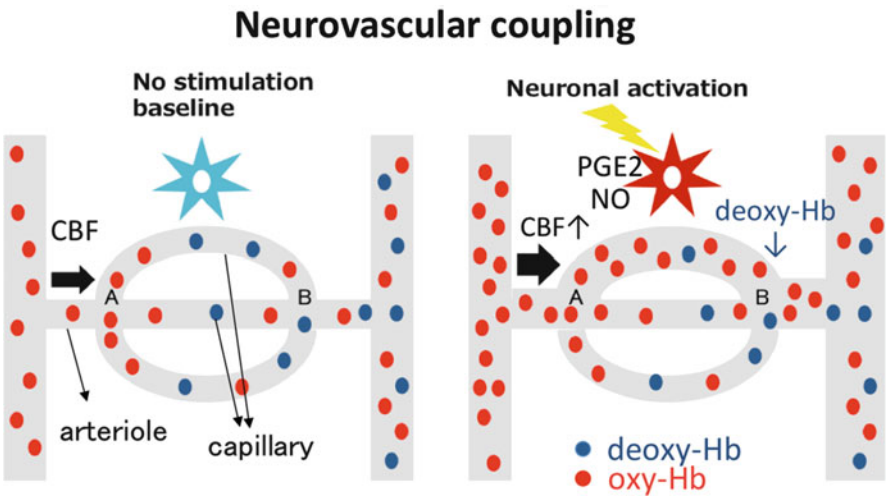

Fig. 5.3 Neurovascular coupling. Compared with a no-stimulation baseline condition, increased synaptic and neural activities result in releases of neurotransmitters such as glutamate. Glutamate induces vaso-effective messengers including nitric oxide (NO) and prostaglandin E2 (PGE2). PGE2 dilates capillaries through actions on pericytes and NO dilates arterioles through actions on smooth muscles, resulting in increased CBF. Although neuronal activation consumes oxygen, thereby converting oxy-Hb to deoxy-Hb, excessive CBF has the effect of diluting deoxy-Hb. The series of processes by which synaptic/neuronal activities give rise to hemodynamic changes is called "neurovascular coupling," and is the basis of functional neuroimaging such as fMRI and fNIRS

the emphasis on LFPs closely related to synaptic activity (Logothetis et al. 2001). However, interpretation of the results obtained with the neuroimaging method described above depends on the relationship among neuronal/synaptic activity, metabolic changes, and hemodynamic changes (neurometabolic–hemodynamic coupling) occurring at the molecular and cellular level being understood.

Neurons maintain a negative membrane potential (−70 mV) at rest. A neuron can generate an action potential on demand, when depolarized above a threshold by EPSP. Both action and synaptic potentials are generated by the inflow of cations, primarily sodium. Once depolarized, a neuron needs to pump sodium out of the cell to restore resting membrane potential. Na+/K+ ATPase brings this about by converting its energy onto adenosine triphosphate (ATP). It is believed that the brain almost exclusively depends on the metabolism of glucose for the generation of ATP. In fact, the brain consumes ~20 % of the glucose required for the whole body. These events lead to the quite reasonable assumption that regional glucose consumption is tightly coupled with local neural and synaptic activity (Heeger and Ress 2002).

Local neural/synaptic activity also increases nearby cerebral blood flow (Fig. 5.3). rCBF increases in proportion with local glucose consumption. Although it remains unclear how cerebral blood flow is regulated in response to neural/synaptic activity, two possible mechanisms have been proposed to date. The

conventional view is that a messenger such as nitric oxide (NO) first relaxes the smooth muscles of arterioles and enlarges their diameters, leading to blood flow increases. Capillaries and venules are passively enlarged (much like a balloon) in response to blood flow increases (Buxton et al. 1998). Very recently, however, Hall et al. (2014) proposed a different view. They believe that synaptic/neural activity–dependent glutamate release first results in the relaxation of pericytes of capillaries through the actions of a messenger. The key messenger in this process is assumed to be prostaglandin E2 (PGE2), which is known to be a pivotal mediator in the neuroimmune system and neuroendocrine system of the brain. Pericyte-mediated blood flow increase also requires NO to inhibit synthesis of a vasoconstrictor substance (20-hydroxyeicosatetraenoic acid). Capillaries dilate faster than arterioles and account for 84 % of blood flow increase. This capillary-first theory may bring a paradigm shift in the understanding of neural/synaptic activity and hemodynamic changes.

Despite the precise regulation mechanisms of neurovascular coupling, blood flow increase in response to neural/synaptic activity exceeds the demand for oxygen consumption. This leads to dilution of deoxy-Hb, which is the intrinsic MRI contrast medium representing the level of blood oxygenation. Deoxy-Hb is paramagnetic and any increases in its level have the effect of decreasing the homogeneity of local magnetic fields. Therefore, deoxy-Hb can be used as an internal contrast agent to reflect neural activity–dependent blood flow changes (the so-called BOLD signals previously discussed). Synaptic/neuronal activations can be indirectly detected by T2*-weighted MRI (the so-called BOLD fMRI previously discussed) (Ogawa et al. 1992).

5.3.4 Neurostimulation

5.3.4.1 Transcranial Magnetic Stimulation (TMS)

TMS is a widely applied non-invasive brain stimulation technique (Hallett 2007). Before its development, transcranial electrical stimulation (TES) was the technique of choice to generate a high-voltage electric shock over the primary motor cortex (Merton and Morton 1980). Although TES can produce a brief muscle response, the motor-evoked potential (MEP), TES is quite painful. Instead of directly applying an electric current over the scalp, TMS generates a rapidly changing magnetic field by passing a brief electric current through a coil (Barker et al. 1985). Since magnetic fields easily penetrate the scalp and skull without attenuation, it induces electric currents (so-called "eddy currents") in the brain. In this way eddy currents in the brain activate a group of neurons beneath the coil. When applied over the primary motor cortex, TMS can induce muscle twitching (MEP) with little or no pain.

Differently shaped TMS coils stimulate the brain in different ways. A round coil induces the strongest electromagnetic fields near the circumference of the coil (no electromagnetic field at the center of the coil). A figure of eight–shaped

coil produces a more focal electromagnetic field at the intersection of the two round components.

TMS of the primary motor cortex provides indispensable knowledge to researchers working in the fields of basic systems neuroscience and clinical motor neuroscience. Central conduction time is the time it takes neural signals to travel from the primary motor cortex to the motor neuron pool in the spinal cord. Subtracting peripheral conduction time from MEP latency yields central conduction time. Measurement of central conduction time may be useful in clinical diagnosis of movement disturbance. TMS of different parts of the primary motor cortex such as the motor cortical homunculus induces MEPs in different parts of the body. Such motor mapping has been proven to be useful in clinical neuroscience, for instance, to show that reorganization of motor somatotopy may underlie the pathophysiology of phantom limb pain syndrome (Hanakawa 2012). Motor cortical excitability (Hallett 2007) can be measured through single-pulse TMS of the primary motor cortex, which provides information on resting and active motor thresholds, recruitment curves, and silent periods. Paired-pulse TMS of the motor cortex allows measurement of short intracortical inhibition (SICI), short intracortical facilitation (ICF), and long intracortical inhibition (LICI). When two coils are placed over the bilateral motor cortices, interhemispheric inhibition (IHI) can be measured. IHI reflects inhibition of the primary motor cortex in one hemisphere after stimulating the primary motor cortex in the opposite hemisphere in a timely manner.

TMS is an important tool in the study of non-motor systems as well. TMS of visual areas may cause visual experiences (phosphenes or moving phosphenes) or disrupt visual experiences (scotoma). In the study of other sensory and cognitive systems, interruption of ongoing neural activity with TMS and resulting changes in perception or cognition, if any, would prove the causal relationship between neural activity and perception/cognition (Oshio et al. 2010).

TMS is a very important tool in the induction of short-term plasticity; a number of protocols have been proposed. Repetitive TMS (rTMS) at low frequencies (0.2–1 Hz) suppresses cortical excitability while rTMS at high frequencies (5 Hz or higher) increases cortical excitability. Theta burst stimulation (TBS) protocols deliver very short, high-frequency trains of stimulation at theta frequency (5 Hz). Intermittent TBS increases cortical excitability while continuous TBS decreases cortical excitability (Huang et al. 2005). Many studies have applied plasticity-inducing TMS protocols to therapy in Parkinson's disease (Fregni et al. 2005), dystonia (Murase et al. 2005), stroke (Kim et al. 2006), pain syndrome (Lefaucheur et al. 2001), and mood disorders (Herrmann and Ebmeier 2006). However, rTMS has the potential to cause seizures, even in healthy humans, although a single-pulse TMS to the brain is considered to be safe. Therefore, clinicians and researchers should adhere closely to safety guidelines (Rossi et al. 2009).

5.3.4.2 Transcranial Current Stimulation

Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation technique that delivers a weak direct current to the brain. It has long been known from animal studies that a weak direct current from intracerebral or epidural electrodes modulates cortical activity and may induce long-lasting changes in cortical excitability. tDCS typically delivers a weak direct current (1–2 mA) for 10–20 min through electrodes placed on the scalp. Brain regions under the anodal electrode show increased cortical excitability (excitatory effects) while those under the cathodal electrode show decreased cortical excitability (inhibitory effects). How tDCS induces change in cortical excitability is not completely understood; many factors may be involved, such as changes in the membrane potentials of neuronal populations and release of neurotransmitters (Tanaka et al. 2013b). Since its re-emergence (Nitsche and Paulus 2000), however, a number of studies have confirmed the capacity of tDCS to modulate cortical excitability and suggested its potential to induce behavioral changes (Kasahara et al. 2013; Tanaka et al. 2009). The potential to induce neuroplasticity means that clinically useful plasticity may by induced in patients with disorders such as stroke (Tanaka et al. 2011).

Other non-invasive electrical stimulation techniques – such as transcranial alternating current stimulation (tACS) and transcranial random noise stimulation (tRNS) – have been proposed as candidates to modulate motor, sensory, and cognitive processes (Guleyupoglu et al. 2013).

5.3.4.3 Deep Brain Stimulation (DBS)

As briefly mentioned earlier, DBS of the subthalamic nucleus, globus pallidus, or thalamus is a widely accepted technique for treating movement disorders (advanced Parkinson's, dystonia and tremor) and, more recently, psychiatric disorders such as depression.

5.4 Clinical Systems Neuroscience: Future

Traditionally, tools in clinical systems neuroscience have been used to clarify the pathophysiology, or to assist diagnosis, of disorders involving the nervous system. However, brain stimulation techniques have long been used as therapeutic tools to enhance functions of the nervous system. DBS has been successfully used as an functional therapy for movement disorders. Today there is a strong and continuous trend toward application of systems neuroscience tools to restore, supplement, or replace impaired functions in patients with dysfunctions of the nervous system. Such efforts include the development of BMI, robot-assisted rehabilitation, and neurofeedback. In the near future, these innovative methods will not only furnish

treatment options, but also provide completely new strategies, for the treatment of patients with nervous system dysfunctions. Another trend that is growing in popularity is neuro-IT. The next two sections focus on BMI and neurofeedback.

5.4.1 *Brain–*Machine Interface (BMI)

Outward BMIs, or brain–computer interfaces (BCIs), are emerging neuro-IT that decodes a behavioral decision from brain activity (effectively mind reading) and feeds the decoded information to control IT devices. A typical way of using BMI is to decode the motor intention of a patient and translate it into control signals to move a robotic hand (Hochberg et al. 2012) or a computer cursor (Wolpaw and McFarland 2004). Outward BMIs were originally developed as a neuroprosthesis for tetraplegic patients with spinal cord injury or amyotrophic lateral sclerosis, or hemiplegic patients with stroke. Inward BMIs were developed to replace lost sensation with neuro-IT. Inward BMIs include direct delivery of visual signals from engineered photosensors to the visual cortex.

ECoG may help in the development of high-performance BMI by taking advantage of the high signal-to-noise ratio (Yanagisawa et al. 2012). The invasiveness of the surgical procedure, long-term safety, and stability of implanted ECoG electrodes remain concerns for clinical application.

5.4.2 *Neurofeedback*

Neurofeedback is an emerging area of interest in clinical systems neuroscience. Using EEG or real-time fMRI, brain activity information is retrieved from the brain of a participant and shown to the participant online as sensory stimuli. The participant is then asked to concentrate on changing the sensory stimuli linked to the information about his/her own brain activity. There is evidence to show that this can be done. Interestingly, changes in brain activity may result in changes in behavior (Shibata et al. 2011). This suggests that neurofeedback has the potential to be utilized as a therapeutic tool (deCharms et al. 2005; Subramanian et al. 2011).

5.5 Concluding Remarks

Clinical systems neuroscience has come to the fore as a result of mutual interactions between neuroscience and clinical medicine, as distinct from unidirectional translation from basic research to clinical medicine. The author foresees even greater advances in clinical systems neuroscience in the coming decade. Such changes may

completely change how neuropsychiatric disorders are dealt with. Clinicians and researchers are recommended to join in this exciting activity.

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Part III
Risk Factors in Neurodegeneration

Chapter 6
Genetic Risk Factors for Neurodegenerative Diseases

Ken Inoue

Abstract The contribution of a single gene to each neurodegenerative disease is diverse in its effect size, depth, and mode of action. In those relatively rare neurodegenerative diseases that show a Mendelian pattern of inheritance, mutations in a major causative gene are solely responsible for the disease phenotype and underlying pathogenesis; whereas, the occurrence of more common neurodegenerative diseases likely requires the combined effect of alterations in many genetic factors and independent environmental factors. The pathological process of neuronal degeneration stems from deficits in neuronal or non-neuronal genes. Therefore, understanding the genetic landscape of neurodegeneration requires delineation of the general or unique effect of individual disease-related genes on the neurodegeneration process. Additionally, the interaction among genes should be determined. This chapter overviews, from distinct aspects, representative examples of genetic factors that have a major impact on the pathophysiology of neurodegenerative diseases.

Keywords Mendelian inheritance • Susceptibility • Alzheimer's disease • Amyotrophic lateral sclerosis • Frontotemporal dementia • Leukodystrophy • Astrocyte • Oligodendrocyte • Microglia

6.1 Introduction

Recent advances in the technology underlying comprehensive genetic and genomic analyses have enabled rapid and high-throughput studies that delineate numerous causative and susceptibility genes for neurodegenerative disorders. It has become apparent, from the many examples reported during the last two decades or so, that genetic factors play a major role in the pathological development of many neurodegenerative diseases. Moreover, we know that the effect size and depth of each genetic factor may vary among different diseases and genes. In a simple model

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K. Wada (ed.), *Neurodegenerative Disorders as Systemic Diseases*,
DOI 10.1007/978-4-431-54541-5_6

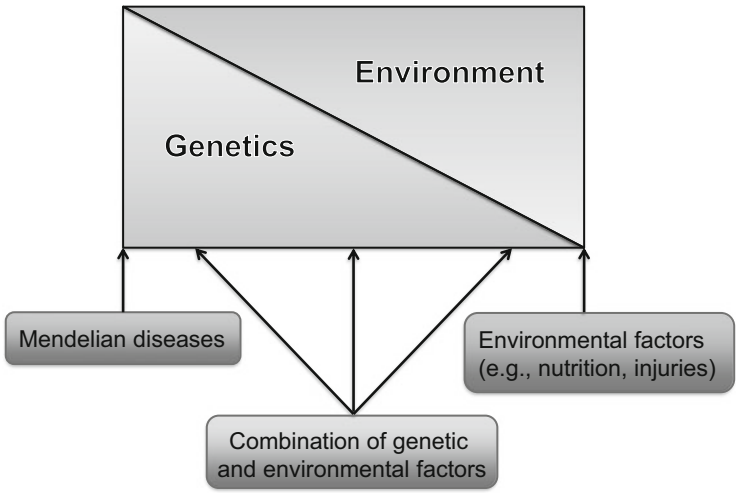

Fig. 6.1 The genetic and environmental factors that contribute to the occurrence of neurodegenerative diseases. (*Left*) Disease defined by a mutation in a single gene (as observed in monogenic Mendelian diseases). (*Right*) Some diseases are actually the result of environmental factors (such as injuries or nutrition). (*Middle*) The most common neurodegenerative diseases (in which multiple factors including susceptibility genes and environmental effects contribute to disease occurrence)

showing the contribution of genetic and environmental factors, Mendelian disorders are placed at the extreme end where an abnormality in a single gene is sufficient to cause a disease (Fig. 6.1). At the other end, some diseases are entirely dependent on environmental factors, such as infections and traumatic accidents. In reality, most neurodegenerative diseases are placed somewhere in the middle of these two extremes. Therefore, an understanding of both genetic and environmental factors is critical to delineating the pathogenesis of neurodegenerative diseases.

What is important yet difficult to understand is that genetic and environmental factors not only contribute to the pathogenesis independently, but also interact with each other to elicit their effect. Investigation and determination of such an interaction between genetic and environmental factors requires analysis of the disease as a systematic disorganization; techniques to uncover such dynamic interactions have not been well developed.

This chapter focuses on the genetic factors that primarily influence the pathology of neurodegenerative diseases at different levels and discusses the effect of environmental factors on the genetic basis of neurodegenerative diseases.

6.2 Neuronal Autonomous Genetic Factors

6.2.1 Genetic Factors That Directly Cause Neurodegenerative Disorders

Single genetic factors, mostly alterations in the coding exons of genes, can directly cause neurodegenerative diseases. In rare Mendelian disorders such single-gene defects are responsible for most of the disease mechanisms and phenotypes. This is the most simple and straightforward way of modeling the influence of genetic factors on neurodegeneration. A good example of such modeling is Huntington's disease (HD). HD is an autosomal-dominant neuropsychiatric disease characterized by progressive movement disorder, most commonly presenting as chorea, progressive cognitive decline leading to dementia, and various psychiatric symptoms that often precede diagnosis (Paulson and Albin 2011). The mutation causing HD is a CAG repeat expansion in the *HTT* gene (Group THsDCR 1993). This unique mutation is highly specific; no other type of mutation in *HTT* has been found to cause HD and all affected individuals with HD carry the CAG repeat expansion in *HTT*. It has been well recognized that the number of repeats is inversely correlated with age of onset (Andrew et al. 1993). Similarly, expanded CAG repeats commonly lead to a lengthy polyglutamine (polyQ) stretch in other autosomal-dominant neurodegenerative disorders including spinocerebellar ataxias, spinobulbar muscular atrophy, and dentatorubral–pallidoluysian atrophy (Zoghbi and Orr 2000). The strong correlation between the number of CAG repeats and age of onset has also been identified in these diseases, suggesting that the disease-associated polymorphic genotype also determines disease onset. Although the presence of a common pathology influencing the age of onset (and presumably disease progression) among these diseases has been expected, molecular mechanisms underlying these polyQ expansions appear to be quite complex, as reviewed elsewhere (La Spada et al. 2011). Nevertheless, CAG expansion involving multiple genes is a unique genetic factor that is specifically associated with neurodegeneration.

Sometimes, a single clinical phenotype can be caused by an alteration in many different genes. This condition is called "genetic heterogeneity." Charcot–Marie–Tooth (CMT) disease is one of the most common neurogenetic disorders, with a prevalence of 1 in 2500. CMT is characterized by bilateral distal wasting, weakness, and sensory loss that begins in the lower limbs and slowly progresses in a length-dependent manner. CMT is further divided into two forms, CMT1 (demyelinating neuropathy) and CMT2 (axonal neuropathy), based on electrophysiological findings. CMT can be dominant, recessive, or X-linked; more than 60 CMT-associated genes have been identified and the list is still growing (Timmerman et al. 2014). Mutations in each gene likely affect the diverse functions of Schwann cells (associated with CMT1) and neurons (associated with CMT2) in the peripheral nervous system. The most common CMT-causing mutation is the genomic duplication of 17p11.2 that harbors *PMP22*, which encodes a myelin membrane protein. Another myelin-associated gene, *MPZ*, is also commonly

mutated in patients with CMT1. Mutations in *GJB1,* encoding connexin32, affect the gap junctions of the myelin membrane. Genes involved in transcription and mRNA processing (*EGR2* and *CTDP1*), cytoskeletal structure (*PRX*, *IFN2*, *FGD4*, and *FBLN5*), endosomal sorting and cell signaling (*LITAF*, *SH3TC2*, *MTMR2*, *MTMR13*, *SBF1*, *FIG4*, *DNM2*, and *NDRG1*), and mitochondria (*GDAP1* and *HK1*) in Schwann cells can be affected in CMT1 (reviewed in Rossor et al. 2013). In CMT2, abnormalities that are even more diverse occur in the subcellular components of the neuronal axon and cell body, including defective nuclear envelope and mRNA processing, endoplasmic reticulum and Golgi apparatus, endosomal sorting and cell signaling, proteasome and protein aggregation, mitochondria, channels, axonal transport, and synaptic transmission (Rossor et al. 2013). These findings suggest that a single neurodegenerative phenotype can occur as a consequence of multi-layered pathological steps affecting tissues, cells, subcellular functional units, and genes, each of which can be responsible for the disease phenotype when mutated.

Single-gene alterations may only explain the common forms of neurodegenerative diseases in a small number of patients, while the vast majority of patients with the same disease may carry no such alterations. Dissecting the molecular pathology of such mutations not only explains the disease in rare cases, but also sheds light on the pathogenesis in most patients, because the rare genetic defect may affect the pathway followed by the common form of the disease. Alzheimer's disease serves as an example of such a case. Analyses of a small number of early-onset Alzheimer disease families showing an autosomal-dominant pattern of inheritance uncovered highly penetrant mutations in three genes: *APP*, *PSEN1*, and *PSEN2* (Goate et al. 1991; Sherrington et al. 1995; Rogaev et al. 1995; Levy-Lahad et al. 1995). *APP* encodes a single transmembrane protein from which amyloid β (Aβ) polypeptides are cleaved by β- and γ-secretases. Aβ polypeptides polymerize to form insoluble Aβ aggregates, the main component of senile plaque, which is the pathological hallmark in patients' brains. Most *APP* mutations are heterozygous missense alterations located in or near Aβ-coding exons (Goate et al. 1991). Rare whole-gene duplications (Rovelet-Lecrux et al. 2006) as well as small recessive deletions and missense alterations have also been reported (Di Fede et al. 2009). These *APP* mutations either change Aβ production, increase the $A\beta_{42}$ to $A\beta_{40}$ ratio (which differs in length according to cleavage sites; $A\beta_{42}$ is more toxic), or enhance fibril formation. Interestingly, one particular *APP* single-nucleotide polymorphism (SNP), which leads to a change in Ala673Thr, was reported to strongly protect carriers from Alzheimer's disease (1/OR = 5.29) (Jonsson et al. 2012). This SNP is located close to the cleavage site of BACE1 (β-site APP cleaving enzyme 1) and functionally inhibits BACE1 cleavage to produce Aβ. *PSEN1* and *PSEN2* are both critical components of γ-secretase, and mutations in either gene result in an increased ratio of $A\beta_{42}$ to $A\beta_{40}$ (De Strooper et al. 1998; Scheuner et al. 1996). Findings from these early-onset familial Alzheimer disease cases provided the solid genetic basis for the central role of Aβ in the pathogenesis of the common form of Alzheimer's disease.

6.2.2 *Genetic Factors That Affect Susceptibility to Neurodegenerative Disorders*

In most patients suffering from neurodegenerative diseases, genetic factors play a more complex role in the pathogenesis, onset, phenotype, and prognosis of the disease than in patients suffering from rare Mendelian forms of diseases (discussed in the previous section). Multiple genes appear to orchestrate together with environmental factors to formulate disease status. Recent advances in genome-wide association studies (GWAS) have been successfully delineating the susceptibility genes that contribute to each part of pathogenesis in the complex process of neurodegenerative diseases.

The best known example of such susceptibility genes with the largest effect size is the *APOE* gene in Alzheimer's disease (Corder et al. 1993). In fact, the Manhattan Plot (an overview of GWAS results encompassing the entire human genome) demonstrated a single incomparable high peak at the *APOE* locus, while other associated loci across the entire genome revealed only modest effects. The *APOE* gene harbors three polymorphic alleles, ε2, ε3, and ε4, of which the ε4 allele is a risk allele for Alzheimer's disease (Farrer et al. 1997). A meta-analysis in individuals of European descent showed that the risk of Alzheimer's disease is increased in people carrying one copy of the ε4 allele (ε2/ε4, odds ratio 2.6; ε3/ε4, odds ratio 3.2) or two copies (ε4/ε4, odds ratio 14.9), compared with those carrying an ε3/ε3 genotype (Farrer et al. 1997). This association is also observed in other populations with a weaker (African American ε4/ε4, odds ratio 5.7; Hispanic ε4/ε4, odds ratio 2.2) or a stronger (Japanese ε4/ε4, odds ratio 33.1) effect (Farrer et al. 1997). *APOE* genotypes strongly affect Aβ deposition as a result of haplotype-specific differences in metabolizing Aβ. Findings from multiple studies in humans and mice suggest that *APOE* ε4 increases the risk by initiating and accelerating the accumulation, aggregation, and deposition of Aβ in the brain (Liu et al. 2013). *APOE* ε4 is less efficient at clearing Aβ from the brain. *APOE* genotypes are also associated with mild cognitive impairment (MCI), which is now considered a pre–Alzheimer disease condition. Individuals with MCI who carry the ε4 genotype are at a higher risk of progression from MCI to Alzheimer-type dementia (Petersen et al. 1995) and suffer a faster cognitive decline than non-carriers (Cosentino et al. 2008). Thus, *APOE* serves as a major susceptibility gene for Alzheimer's disease with the ε4 allele increasing the risk by direct enhancement of Aβ pathogenesis.

By contrast, other susceptibility genes in at least nine loci (*CLU*, *CR1*, *PICALM*, *BIN1*, *EPHA1*, *ABCA7*, *MS4A* cluster, *CD33*, and *CD2AP*), identified and confirmed by multiple GWAS, have shown a much smaller effect size (reviewed in Bettens et al. 2013). Pathogenic mechanisms of variations in these nine susceptibility genes have not yet been established; however, these susceptibility genes may be related to specific functions including lipid processing (*CLU* and *ABCA7*), functioning of the complement system, inflammation, immune system (*CLU*, *CR1*, *ABCA7*, *CD33*, and *EPHA1*), and synaptic cell functions such as endocytosis (*PICALM*, *BIN1*, *CD33*, and *CD2AP*). However, the mechanisms by which these

gene variations affect the pathological process of Alzheimer's disease remains largely undetermined. Interestingly, common gene variants that have a predominant effect when mutated (i.e., *APP*, *PSEN1*, and *PSEN2*) are not risk factors for the complex forms of Alzheimer's disease (Harold et al. 2009; Lambert et al. 2009). This contrasts with the findings in Parkinson's disease, wherein common variants in the genes that are responsible for the Mendelian form of the disease (i.e., *MAPT*, *SNCA*, and *LRRK2*) also serve as risk alleles in the common form of the disease (Simon-Sanchez et al. 2009; Satake et al. 2009).

6.2.3 Genetics Factors That Contribute to Multiple Neurodegenerative Disorders

Genetic exploration and identification of the genes contributing to neurodegenerative disorders have begun to uncover the fact that clinically distinct neurodegenerative disorders share a common genetic basis to a significant degree. A single gene can contribute to the occurrence of different diseases. There are striking examples of this in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD).

ALS is a devastating degenerative disorder of motoneurons. It is characterized by the onset of focal weakness, typically in the limbs but sometimes in bulbar muscles. This progresses to paralysis of almost all skeletal muscles, leading to death from respiratory failure typically within 5 years (Sreedharan and Brown 2013). FTD is a distinct form of dementia characterized by progressive degeneration of the frontal or temporal lobe, or both (Loy et al. 2014). FTD is clinically characterized by deterioration in behavior and speech, but memory and visuospatial function are spared to a certain degree. ALS and FTD have been thought of as distinct clinical entities, although rare overlapping cases have been reported. The seminal molecular pathology discovery connecting ALS and FTD was the identification of TDP-43 as the major component of ubiquitinated neuronal cytoplasmic inclusions in both diseases (Neumann et al. 2006), which also led to the identification of mutations in the *TARDBP* gene (which encodes TDP-43) in autosomal-dominant ALS and FTD families (Sreedharan et al. 2008; Chio et al. 2010). More recently, a massive expansion of a hexanucleotide repeat in an intron of C9ORF72 linked to chromosome 9q21 was found to cause ALS and FTD (Renton et al. 2011; DeJesus-Hernandez et al. 2011). Subsequently, it became apparent that this locus was responsible for a major part of both familial ALS (~40 %) and FTD (~25 %) (Majounie et al. 2012). In addition, at least three other genes are known to link ALS and FTD. *FUS* encodes an RNA-binding protein that has functional homology with TDP-43. *FUS* mutations have been found in about 4 % of familial ALS cases without dementia (Kwiatkowski et al. 2009; Vance et al. 2009); however, in rare families, *FUS* mutations also cause FTD (Blair et al. 2010). Note that FTD exhibiting a *FUS*-positive histopathological subtype is commonly observed; however, most of these cases do not carry *FUS* mutations (Snowden et al. 2011).

Mutations in the *VCP* gene, which encodes valosin-containing protein, were initially found to cause inclusion body myopathy, an unusual clinical syndrome characterized by FTD, and Paget's disease of the bone (Watts et al. 2004); however, they were later found to be responsible for 1–2 % of familial ALS cases too (Johnson et al. 2010). Ubiquitin 2 regulates the proteasome degradation of ubiquitinated proteins. The gene encoding this protein, *UBQLN2,* is responsible for rare familial cases of ALS and ALS/FTD syndrome (Deng et al. 2011). Since *UBQLN2* pathology has been observed in patients with ALS who do not carry mutations in this gene, this protein may play an important role in the final common pathway associated with motor neuron degradation. These cumulative genetic findings have drastically changed our understanding of unexpectedly common molecular pathologies between ALS and FTD.

6.3 Non-neuronal Genetic Factors Affecting Neurons

In a number of neurodegenerative diseases, the disease genes primarily function in non-neuronal cells including astrocytes, oligodendrocytes, microglia, and vascular cells. Deficits in these non-neuronal genes also affect neurons by causing neurodegeneration. The following examples highlight the contribution of genetic factors affecting cells other than neurons.

6.3.1 Genetic Deficits in Astroglia That Cause Neurodegeneration

Astrocytes constitute the largest volume of cells in the central nervous system (CNS). Named for its star-like shape, astrocytes are distributed throughout the CNS in both the gray and white matter. Once considered little more than scaffolds for neurons, astrocytes play multiple roles by interacting with many types of cells and structural components in the CNS – including neurons, oligodendrocytes, other astrocytes, blood vessels, pial membranes, and the ependymal layer that lines ventricles – to maintain tissue homeostasis (Lanciotti et al. 2013).

In their interaction with neurons, astrocytes extend many fine processes to contact synapses and establish highly regulated bidirectional communication. By secreting trophic factors – such as brain-derived nerve growth factor (BDNF), glial cell–derived neurotrophic factor (GDNF), nerve growth factor (NGF), insulin-like growth factor (IGF), and thrombospondin – astrocytes contribute to the formation, maintenance, and remodeling of synapses. Astrocytes also modulate neuronal function by secreting cholesterol, apolipoprotein E, glutathione, and hydrogen sulfide. They express receptors to take up neurotransmitters – including glutamate, gamma-aminobutyric acid (GABA), norepinephrine, dopamine, serotonin,

acetylcholine, and glycine – to remove them from the synaptic cleft and maintain their low extracellular concentrations. The clearance of glutamate, mainly mediated by a high-affinity excitatory amino acid transporter 1 (EAAT1, also known as "GLT1"), is critical to protecting neurons from excitotoxicity (Rothstein et al. 1996). Dysfunction of EAAT1 and accumulation of excessive extracellular glutamate have been implicated in several neurodegenerative processes and associated diseases, such as epilepsy, stroke, ALS, HD, and Alzheimer's disease (Coulter and Eid 2012; Rothstein 2009; Faideau et al. 2010; Simpson et al. 2010). Astrocytes also supply neurons with energy substrates. Both glucose and lactate are transported to neurons to maintain the energy source for intense neuronal activity. Astrocytes also control the homeostasis of water and ion fluxes in the synaptic interstitial space through numerous ion channels and transporters.

Astrocytes also provide growth factors – such as platelet-derived growth factor alpha (PDGF-α), fibroblast growth factor 2 (FGF2), neurotrophin-3 (NT-3), IGF-1, and ciliary neurotrophic factor (CNTF) – which are necessary for the survival of oligodendrocytes (Lanciotti et al. 2013). Astrocytes are thought to regulate CNS myelination probably by secreting growth factors; elucidation of the underlying mechanism is important to understanding how astrocytes contribute to CNS repair and remyelination, which could be useful in the treatment of demyelinating diseases.

Astrocytes not only play essential roles to maintain healthy CNS homeostasis, they are also involved in many pathological processes. Specifically, genetic deficits in astrocyte-specific genes can cause neurodegenerative disorders, characterized as cystic leukodystrophies including Alexander disease (AxD), megalencephalic leukodystrophy with subcortical cysts (MLC), and vanishing white matter (VWM). These are all rare genetic diseases that commonly show the characteristic features of demyelinating leukodystrophy with progressive cystic or spongy (vacuolating) degeneration of myelin.

AxD is an autosomal-dominant disease caused by heterozygous mutations in the astrocyte-specific type III intermediate filament *GFAP* gene (Brenner et al. 2001). Clinical manifestations of AxD vary in severity and onset from more severe infantile and juvenile forms to milder adult onset forms (Messing et al. 2012). Patients with the infantile form show progressive features with seizures, bulbar dysfunction, psychomotor regression, and short lifespan. Pathologically, the brains of patients with severe AxD show loss of oligodendrocytes and myelin; cystic degeneration; neuronal loss most commonly in the hippocampus, striatum, and neocortex; and the presence of Rosenthal fibers in the cytoplasm of astrocytes (Messing et al. 2012). The exact mechanisms for neurodegeneration in AxD remain unknown, but both toxic gain-of-function and impairment of normal astrocyte supportive function have been implicated. Specifically, a marked downregulation of GLT-1 in astrocytes expressing mutant GFAP, and the vulnerability of hippocampal neurons to glutamate-induced cytotoxicity in co-cultures with astrocytes expressing mutant *GFAP* have been reported (Mignot et al. 2007). These findings may link *GFAP* mutations to glial glutamate transporter-1 (GLT-1) dysfunction and impairment of the neuron–astrocyte interaction, suggestive of a potential

mechanism underlying neuronal loss in AxD. Similarly, dysregulation of the homeostatic functions of astrocytes – including extracellular K^+ buffering by Kir4.1 and Na^+/K^+–ATPase activity – have been implicated in the demyelination of AxD (Hagemann et al. 2005).

6.3.2 *Genetic Deficits in Oligodendrocytes That Cause Neurodegeneration*

Mature oligodendrocytes extend cell processes that are modified into thin sheaths of plasma membrane wrapping around a portion of an axon in a spiral fashion (so-called "myelin"). Each oligodendrocyte interacts with as many as 50 different axons by extending multiple processes. Each axon is ensheathed by multiple continuous segments of myelin, separated by small areas of bare axons exposed to interstitial spaces called "nodes of Ranvier." Myelin functions as an insulator of electronic circuits in neuronal networks, where it enables rapid, efficient, and distant transmission without attenuation. In addition to these unique features of myelin as a structural component for highly efficient axonal conduction, oligodendrocytes communicate with axons through several signaling processes. Axonal signals that support myelin include neuregulins, neurotrophins, and the electrical activity of axons, while oligodendrocytes support axons through the function of some myelin membrane–bound proteins, such as *PLP1* and *CNP1*. Mice in which either *PLP1* or *CNP1* has been disrupted develop normal myelin, suggesting that these myelin proteins are dispensable for normal myelination. On the other hand, these mice develop broad axonal swelling, indicating that *PLP1* and *CNP1* support axons independent of myelin (Nave and Trapp 2008). Of these two myelin proteins only *PLP1* is directly associated with human disease.

Leukodystrophy comprises a group of rare genetic disorders that primarily target white matter in which myelinating oligodendrocytes and axons predominate. Pelizaeus–Merzbacher disease (PMD) is a hypomyelinating leukodystrophy characterized by deficit in CNS myelination (Inoue 2005). PMD is caused by mutations in *PLP1*, which encodes a major myelin membrane protein. PMD-causing *PLP1* mutations include point mutations that mostly result in amino acid substitutions, genomic duplications that lead to an additional copy of the gene, and null mutations caused by deletions or early-truncating mutations (Inoue 2005). In PMD the structure of the neuronal network is essentially intact. However, slowly progressive axonal degeneration may occur later in the disease probably because of the loss of axonal support by myelin and the resulting metabolic and electronic insufficiency in the axons. Activated microglia have also been found at the site of hypomyelinated axons; thus, microglia may additionally contribute to hypomyelination-associated axonopathy (Ip et al. 2006). In addition to these features (generally observed in all types of mutations), patients with null mutations show an apparent length-dependent progressive axonopathy

characterized by axonal swelling (Griffiths et al. 1998; Garbern et al. 2002). This is despite the fact that clinical severity and associated leukodystrophy observed by magnetic resonance imaging (MRI) in patients with null mutations are surprisingly milder than in patients with either point mutations or duplications. It is not completely understood why a milder condition with almost normal myelinating oligodendrocytes with no *PLP1* expression is more susceptible to axonal degeneration than severe conditions with bare axons and almost no mature oligodendrocytes caused by other types of mutations. Nevertheless, the expression of myelin-specific genes such as *PLP1* appears to play a role in preventing the degeneration of axons.

More than 30 leukodystrophies and their causative genes are known, and most, if not all, involve neurodegeneration (Kohlschutter and Eichler 2011), suggesting that a variety of genetic factors lead to this specific type of neurodegeneration. These diseases commonly show disruption of the myelin sheath in the CNS accompanied by axonal degeneration and inflammatory changes, together called "demyelination." The genetic bases of leukodystrophies are complex; genes involved in many different cells and functions have been implicated (Kohlschutter and Eichler 2011). X-linked adrenoleukodystrophy is caused by mutations in *ABCD1*, which encodes a member of the superfamily of ATP-binding cassette transporters (Steinberg et al. 1993). *ABCD1* is present on the peroxisomal membrane and is involved in the transport of very long chain fatty acids (VLCFA)–coenzyme A (CoA) synthetase. Defects in *ABCD1* result in accumulation of VLCFA, which is toxic to brain cells, especially microglia and oligodendrocytes, and thus leads to axonal degeneration. Mutations in a gene encoding the lysosomal enzyme, arylsulfatase A, cause metachromatic leukodystrophy, which is characterized by abnormal accumulation of cerebroside sulfate (the major component of myelin lipids), which leads to myelin breakdown and neuronal death. Further examples of neurodegenerative leukodystrophies are hypomyelination with atrophy of the basal ganglia and cerebellum (mutations in the β-tubulin4A gene), Canavan disease (mutations in the aspartoacylase gene), and Krabbe disease (mutations in the galactocerebrosidase gene).

6.3.3 *Genetic Deficits in Microglia That Cause Neurodegeneration*

Microglial activation and neuroinflammation are major components in neurodegeneration. Numerous genes and pathways are associated with the important role microglia play in neurodegeneration. However, the focus here is on a small number of microglia-specific genes, in which mutations primarily cause neurodegenerative disorders. Nasu–Hakola disease (NHD), also known as "polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy," is a rare autosomal-recessive neurodegenerative disease characterized by multifocal bone

cysts and progressive early-onset dementia. Two genes are known to be responsible for NHD – *TYROBP* and *TREM2* (Paloneva et al. 2000, 2002). Both these genes encode components of cell surface receptors that are expressed not only in blood immune system cells, but also in microglia in the CNS and bone osteoclasts. *TYROBP* and *TREM2* form a receptor-signaling complex that triggers the activation of immune responses in macrophages and dendritic cells (Lanier and Bakker 2000). Although the exact molecular pathogenesis is unknown, loss-of-function mutations in *TYROBP* and *TREM2* are predicted to cause dysfunction of microglia. The brains of patients with NHD show sudanophilic leukodystrophy characterized by loss of myelin and nerve fibers, and gliosis especially in the frontal and temporal lobes (Nasu et al. 1973). Note that these broad neurodegenerative changes involving oligodendrocytes, astrocytes, and neurons primarily result from the abnormal function of microglia. NHD is an extremely rare disease and the vast majority of patients are either Finnish or Japanese (Hakola 1972; Nasu et al. 1973). However, the NHD-associated microglial gene, *TREM2*, appears to have a broader impact on common neurodegenerative diseases. Heterozygous mutations in *TREM2* are associated with an increased risk of several neurodegenerative disorders including Alzheimer's disease, Parkinson's disease, ALS, and FTD (Guerreiro et al. 2013; Jonsson et al. 2013; Rayaprolu et al. 2013; Cady et al. 2014; Cuyvers et al. 2014; Borroni et al. 2014). Although the allele frequency of the variant *TREM2* is relatively low (~0.3 %), the effect size is remarkably high in Alzheimer's disease (odds ratio > 3, which is close to that of the *APOE4* allele). The exact mechanisms by which *TREM2* mutations increase susceptibility to different neurodegenerative diseases is still unknown, but a recent study suggested that reduced function of *TREM2* impairs phagocytosis, which may contribute to neurodegeneration (Kleinberger et al. 2014). These findings suggest that normal function of microglia is critical to preventing neurodegeneration in the brain.

6.4 Conclusions

This chapter overviewed the genetic factors that contribute to the development of neurodegenerative disorders from a wide variety of viewpoints. Neurodegenerative disorders can occur because of a mutation in a single gene or because of multiple contributions from alterations in some genes, or possibly many genes. In addition, environmental factors may also influence the development of neurodegenerative disorders through many different pathophysiological pathways. Such findings have of course been made possible by the rapid and dramatic technological advances in comprehensive genetic and genomic analyses, including genome-wide linkage analysis, GWAS, SNP array analysis, array comparative genomic hybridization (aCGH) analysis, whole-exome sequencing, and whole-genome sequencing. However, despite these advances, we have barely scratched the surface of the genetic contribution to pathogenesis of the most common neurodegenerative disorders. A prominent peak of the pathophysiological landscape of neurodegenerative disorders

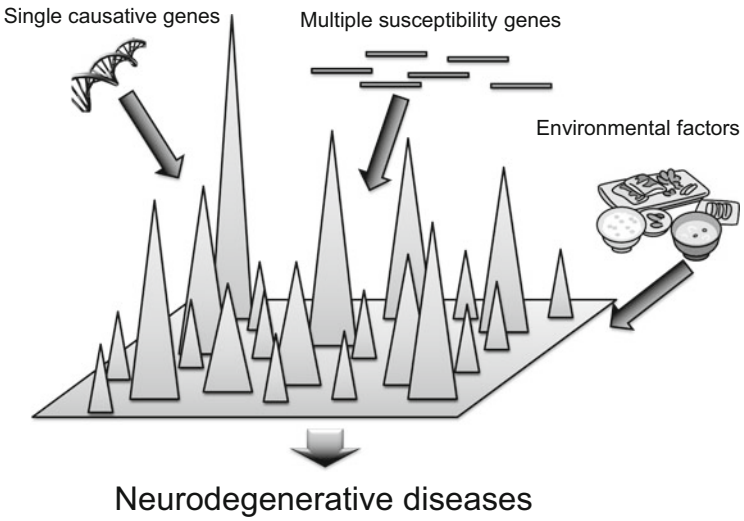

Fig. 6.2 The fundamental basis of genetic and environmental factors that contribute to the occurrence of neurodegenerative diseases

delineated by the discovery of underlying genetic and genomic bases can now be seen (Fig. 6.2). However, many small peaks representing genes with smaller effect size have yet to be identified. Furthermore, the risk factors stemming from interactions within these genetic factors or between genetic and environmental factors have not been fully elucidated. The fundamental basis of neurodegenerative disorders awaits clarification by future advances in high-throughput deep genomic exploration technology incorporating the multi-factorial interaction with environmental effects.

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Chapter 7
Intermediate Phenotype Approach for Neuropsychiatric Disorders

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Abstract Neuropsychiatric disorders are the result of complex interactions between multiple genetic variants with small effects, and environmental factors. The effects of susceptibility genes for neuropsychiatric disorders would be more penetrant at the level of neurologically intermediate phenotypes, such as cognitive impairments and reduced brain volumes, than at the level of a phenotypically heterogeneous neuropsychiatric symptom/behavior. The intermediate phenotype approach - a unique, powerful and standardized strategy – has recently been used to identify risk genes for neuropsychiatric disorders, as well as to characterize the neural systems affected by the genetic risk variants, in order to elucidate the biological mechanisms implicated in neuropsychiatric disorders. Intermediate phenotypes are defined as being heritable; quantitatively measurable; stable over time; related to the disorder and its symptoms in the general population; showing increased expression in unaffected relatives of probands; and co-segregating with the disorder in families. The intermediate phenotypes for neuropsychiatric

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K. Wada (ed.), *Neurodegenerative Disorders as Systemic Diseases*,
DOI 10.1007/978-4-431-54541-5_7

disorders are roughly classified as neurocognition, neuroimaging, neurophysiology, and others. Early studies used intermediate phenotypes to investigate the association between intermediate phenotypes and well-known functions of single-nucleotide polymorphisms (SNPs), such as catechol-O-methyltransferase (*COMT*). Genome-wide association studies (GWAS) of neuropsychiatric disorders have identified genes with unknown functions, such as *ZNF804A*. Another advantage of using intermediate phenotypes is that such an approach can investigate the unknown function of genes implicated in diseases. In this chapter, we discuss the concept of the intermediate phenotype approach, and discuss previous and recent studies in neuropsychiatric research, particularly schizophrenia. Finally, we discuss future developments of the intermediate phenotype approach.

Keywords Intermediate phenotype • Neuropsychiatric disorders • Schizophrenia • Genome-wide association study • Gene • SNP • Neurocognition • Neuroimaging • Neurophysiology

7.1 Concept of the Intermediate Phenotype

Neuropsychiatric disorders are phenotypically heterogeneous, with a complex genetic basis. Genetic factors play a major role in the pathogenesis of neuropsychiatric disorders and twin and family studies have emphasized the important role of genetic components in several neuropsychiatric disorders. For example, the estimated heritabilities of neuropsychiatric disorders are as follows: 90 % for autism spectrum disorder (Freitag 2007), 80–90 % for schizophrenia and bipolar disorder (Sullivan et al. 2003; McGuffin et al. 2003), 40 % for major depression (Sullivan et al. 2000), 30–50 % for anxiety disorders (Hettema et al. 2001) and 30–50 % for obsessive–compulsive disorder (van Grootheest et al. 2005). Researchers have attempted to find the genetic variants using linkage and candidate gene approaches, but the results of these genetic association studies have been largely inconsistent, although a few loci and some genetic variants were found to be consistent. Although rare mutations and copy number variants may account for some cases (Schreiner et al. 2013), other affected individuals are believed to acquire their risk for disorders through the inheritance of several common SNP-based variants, likely acting multiplicatively. A genome-wide association study (GWAS) approach, which examines hundreds of thousands of SNPs, could be a powerful, standard tool to identify common susceptibility variants for complex disorders, including neuropsychiatric disorders. GWAS of neuropsychiatric disorders, such as schizophrenia, bipolar disorder, and major depressive disorder, have been published (Schwab and Wildenauer 2013). For example, several large-scale GWAS on schizophrenia have successfully identified a few risk variants located in the *ZNF804A*, *NRGN* and *TCF4* genes, as well as in *MIR137* and a major histocompatibility complex (MHC) region (Stefansson et al. 2009; O'Donovan et al. 2008; Ripke et al. 2011). However, each risk-genetic variant for schizophrenia

only has small effects, with odds ratios which range from 1.1 to 1.2. However, although it is commonly accepted that the risks for developing neuropsychiatric disorders are mediated by the combined effects of hundreds of multiple genetic variants with small effects, rather than by a few genetic variants with large effects, previous GWAS of schizophrenia only explain a small aspect of the genetic architecture of these disorders. To solve this problem, and to minimize phenotypic and genetic heterogeneity, intermediate phenotypes rather than the diagnostic categorization are emphasized (Potkin et al. 2009). At the genetic level, collections of smaller numbers of SNPs may manifest as abnormalities in intermediate phenotypes. This approach has the potential to reduce clinical and genetic heterogeneity by examining intermediate phenotypes that reflect underlying genetic vulnerability better than the neuropsychiatric disorder itself.

Intermediate phenotypes are quantitative and biological traits, mediated between gene and clinical phenotype – such as syndrome and behavior – on a causal pathway leading to neuropsychiatric disorders, and might, therefore, be more directly related to the biological effects of susceptibility genes on the key pathway (Egan et al. 2001). Intermediate phenotypes can be used to convert a complex and phenotypically heterogeneous disorder with multifactorial genetic inheritance, to more homogeneous phenotypes with a simpler genetic component. The criteria of intermediate phenotypes propose that a phenotype should (i) be heritable, (ii) exhibit good psychometric properties, (iii) be stable over time, (iv) be related to the disorder and its symptoms in the general population, (v) show increased expression in the unaffected relatives of probands, and (vi) co-segregate with the disorder in families (Meyer-Lindenberg and Weinberger 2006). There are two concepts of an intermediate phenotype approach (Fig. 7.1). This strategy is usually used to discover the genetic variant related to disease (Fig. 7.1a), however, the concept has recently expanded. Although a GWAS approach to neuropsychiatric disorders has successfully resulted in the identification of several genetic variants implicated in the disorders, the biological functions of these identified genes are still poorly known. An intermediate phenotype approach is also useful when investigating the unknown function of genes implicated in diseases (Fig. 7.1b). This strategy can characterize how risk gene variants can modulate neural systems that are impaired in neuropsychiatric disorders. These two concepts have different hypotheses, but the methods to test these hypotheses are the same, and include association analyses of intermediate phenotype data and genetic variations. This is a more meaningful way to elucidate the biological mechanisms of risk genes. The intermediate phenotype approach is genetically and biologically less complex than the clinical syndrome/behavior approach, and genes showing association at the syndromal level will show greater effect sizes on variation in intermediate phenotypes. In this chapter, we review the intermediate phenotype approach for neuropsychiatric disorders with a focus on previous and recent studies.

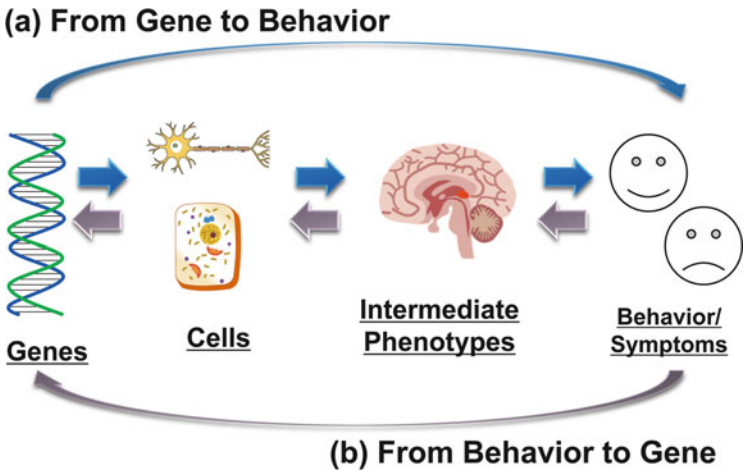

Fig. 7.1 Two concepts of intermediate phenotype approach. Susceptibility genes for neuropsychiatric disorders do not directly encode for the clinical syndrome/behaviors. The abnormal behaviors observed in neuropsychiatric disorders are produced by intermediate steps that occur between genes and syndrome/behaviors. Intermediate steps such as cell activity and neural circuits underlie the syndrome/behaviors of neuropsychiatric disorders. Intermediate phenotype is likely associated with a more basic and proximal etiological process rather than pathogenesis of disease itself. (**a**) From genes to behavior. One concept is that intermediate phenotype approach is a tool to discover risk genes for neuropsychiatric disorders. (**b**) From behavior to genes. The other concept of the approach is a strategy to characterize neural systems affected by risk gene variants for elucidating the neural systems impaired in neuropsychiatric disorders

7.2 Intermediate Phenotype for Neuropsychiatric Disorders (Neurocognition, Neuroimaging, and Neurophysiology)

Intermediate phenotypes in medicine other than neuropsychiatric disorders, include lipid levels and risk of coronary heart disease (Castelli et al. 1992), sodium homeostasis and risk of hypertension (O'Connor et al. 2000), and body mass index and risk of diabetes (Walters et al. 2010b). The paradigm of an intermediate phenotype for neuropsychiatric disorders first emerged in 2001 (Egan et al. 2001). This study reported that a functional variation in the catechol-O-methyltransferase *(COMT)* genotype (Val158Met) was reported to be associated with altered prefrontal activity during a working memory task. The COMT is a major mammalian enzyme involved in the metabolic degradation of catecholamines; this activity is higher in the Val158 protein than in the Met158 protein. The Val158 genotype was associated with lower executive performance, less efficient physiological response in the prefrontal cortex, and a risk of schizophrenia (Egan et al. 2001). Since then, hundreds of articles related to intermediate phenotypes have been published, and several candidates for intermediate phenotypes of neuropsychiatric disorders have been identified in these articles. The intermediate phenotypes for neuropsychiatric

disorders are roughly classified into: neurocognition, neuroimaging, neurophysiology, and others.

7.3 Previous and Recent Studies of Intermediate Phenotypes

We will focus on our previous and recent studies of schizophrenia and neurocognitive, neuroimaging, and neurophysiological intermediate phenotypes as examples, but this approach can also be extended to other neuropsychiatric disorders and neurodegenerative diseases.

7.3.1 Neurocognitive Intermediate Phenotypes

Neuropsychological studies of schizophrenia support the assumption that deficits in general cognitive ability, and more discrete aspects of neurocognition such as attention, memory and executive functions, are heritable, stable over the course of the illness, present in probands as well as in their healthy relatives and are differentially linked to the risk of schizophrenia (Burdick et al. 2009). Therefore, neurocognitive dysfunctions represent good potential candidates for intermediate phenotypes in studies of neuropsychiatric disorders, particularly schizophrenia. IQ, assessed by the Wechsler Adult Intelligence Scale (WAIS), perseverative errors and the number of categories achieved in Wisconsin Card Sorting Tests (WCST) to assess executive function, and the performance index (d') of Continuous Performance Tests (CPT) used to assess attentional function, are all prominently used as neurocognitive intermediate phenotypes for schizophrenia, because their effect sizes are large (Allen et al. 2009; Burdick et al. 2009).

Using a candidate gene approach, and based on the following criteria: (1) strength of evidence for association with schizophrenia based on sample size and number of replications; (2) linkage to a gene locus associated with schizophrenia; (3) biological plausibility based on evidence of altered function and expression in vivo or in vitro; (4) evidence of altered expression in schizophrenic brain, based on measures of mRNA or protein, or relative expression of isoforms or alleles (Preston and Weinberger 2005), several candidate genes have been highlighted: *COMT* (22q11); *DTNBP1* (6p22); *NRG1* (8p12-21); *RGS4* (1q21-22); *GRM3* (7q21-22); *DISC1* (1q42); and *G72*(*DAOA*) (13q32-34). Firstly, we present associations between these genes and neurocognitive intermediate phenotypes. As described above, the association between *COMT* and schizophrenia using intermediate phenotypes, has already been investigated in this field. The study reported that the *COMT* genotype was related to perseverative errors in the WCST (Egan et al. 2001). They found that the low-activity Met158 allele carrier of *COMT* had

a higher cognitive performance, and that the Val158 allele is significantly transmitted to schizophrenic offspring. These data suggested that the *COMT* Val158 allele impairs prefrontal cognition and physiology, and thereby slightly increases the risk of schizophrenia. Subsequent study found that high-activity Val158 allele homozygous had the poorest performance of working memory on n-back tasks (Goldberg et al. 2003). Siblings and patients with schizophrenia performed significantly worse than controls and the allelic effects on performance were found to be similar across groups. These findings were supported by other studies, but not all studies. In addition to the perseverative errors of WCST, the *COMT* polymorphism was associated with other neurocognitive intermediate phenotypes assessed by a Trail Making task, verbal fluency, verbal recall, IQ score and n-back task accuracy. However, a meta-analysis of reported associations between the *COMT* Val158Met and measures of memory and executive function, found no association between the *COMT* genotype and the majority of phenotypes, except for IQ score (Barnett et al. 2008).

The dystrobrevin binding protein 1 (*DTNBP1*) gene is linked to a risk for schizophrenia, and several studies, including ours have reported that several SNPs and haplotypes in the *DTNBP1* gene influence general cognitive ability and memory function, in both schizophrenic patients and healthy control subjects (Hashimoto et al. 2009, 2010a). A meta-analysis supports two SNPs (rs1018381 and rs2619522) in *DTNBP1* modestly influencing general cognitive ability (Zhang et al. 2010). Like these genes, several associations of other genes detected by candidate gene approaches with neurocognitive intermediate phenotypes, have been reported in this decade (Rose and Donohoe 2013): *NRG1* and verbal fluency and attention; *RGS4* and verbal fluency; *DISC1* and memory function; *G72*(*DAOA*) and episode memory, working memory, verbal fluency and attention. Meta-analyses of the reported associations will be performed in the future. We have reported association studies between other genes that are implicated in schizophrenia and neurocognitive intermediate phenotypes (Hashimoto et al. 2007, 2013; Ohi et al. 2013b): *PACAP* and memory performance, *KCNH2* and IQ, attention/vigilance and working memory and *AKT1* and attention.

The GWAS of schizophrenia have identified several risk variants located in the *ZNF804A*, *NRGN* and *TCF4* genes, *MIR137* and MHC region, as a widely-used benchmark for genome-wide significance ($p < 5.0 \times 10^{-8}$) (Stefansson et al. 2009; O'Donovan et al. 2008; Ripke et al. 2011). The number of variants detected by GWAS is rapidly increasing (Consortium 2011; Ripke et al. 2013). The biological functions of many of these genes are unknown. Therefore, an intermediate phenotype approach has been used as a tool to investigate the biological function of these genes. The SNP rs1344706 in the *ZNF804A* gene was associated with schizophrenia in the first GWAS (O'Donovan et al. 2008); the biological function of this gene was unknown at that time. Subsequently, although several studies, including ours, have been reported (Fernandes et al. 2014; Stefanis et al. 2013; Walters et al. 2010a; Balog et al. 2011; Hashimoto et al. 2010b; Voineskos et al. 2011; Chen et al. 2012), the results were inconsistent. The rs1344706 variant was associated with a range of cognitive phenotypes, attention, memory, working memory and executive functions

in some studies (Walters et al. 2010a; Balog et al. 2011; Hashimoto et al. 2010b; Voineskos et al. 2011; Chen et al. 2012), but other studies have reported no effect on the same phenotypes in other samples (Fernandes et al. 2014; Stefanis et al. 2013). Moreover, directions of reported association are also inconsistent. Some studies reported that the *ZNF804A* risk A allele was associated with better neurocognitive performance in patients with schizophrenia (Walters et al. 2010a; Chen et al. 2012), while others reported that the risk A allele was associated with worse performance (Balog et al. 2011; Hashimoto et al. 2010b; Voineskos et al. 2011; Chen et al. 2012). The rs12807809 located approximately 3 kb upstream of the *NRGN*, is a genetic variant detected by a second GWAS for schizophrenia (Stefansson et al. 2009). Neurogrannin (NRGN) plays an important role in the Ca^{2+}–CaM signaling pathway, including the post-synaptic activation of CaM-dependent protein kinase II (CaMKII) by CaM, which is associated with strengthened N-methyl-D-aspartate (NMDA) receptor signaling (Li et al. 1999). Although several studies have investigated the association between the SNP and neurocognitive phenotypes (Donohoe et al. 2011b; Krug et al. 2013; Ohi et al. 2013c; Walters et al. 2013), the associations were not found to be significant. We found an association between IQ and *NRGN* diplotype (a combination of haplotypes) but not with SNP in patients with schizophrenia (Ohi et al. 2013c). The *TCF4* rs9960767 was also detected by a second GWAS of schizophrenia. Based on evidence that *Tcf4* transgenic mice display profound deficits in sensorimotor gating and contextual and cued fear conditioning (Brzozka et al. 2010), and that severe dysfunction of *TCF4* causes Pitt–Hopkins syndrome with expression of mental retardation, microcephaly, facial dysmorphisms, and intermittent hyperventilation (Walters et al. 2010b), three studies have investigated influences of rs9960767 on neurocognitive intermediate phenotypes of schizophrenia (Lennertz et al. 2011b; Albanna et al. 2014; Lennertz et al. 2011a). Lennertz et al. reported an influence of the rs9960767 variant on verbal memory (Lennertz et al. 2011b). Contrary to the expectation that the schizophrenia-associated allele of the *TCF4* gene would lead to impaired verbal memory, they found that the carriers of the disease-associated allele showed better neurocognitive performance, in patients with schizophrenia. Lennertz et al. did not detect any significant association between rs9960767 and other comprehensive neuropsychological tests in their other study (Lennertz et al. 2011a). Albanna et al. reported the risk allele of the SNP was related to worse performance in Reasoning/Problem-Solving (Albanna et al. 2014). One marker in the MHC region, rs6904071, was associated with delayed episodic memory (Walters et al. 2013).

7.3.2 Neuroimaging-based Intermediate Phenotypes

Structural and functional neuroimaging has yielded intermediate phenotypes for neuropsychiatric disorders, because a number of MRI studies have revealed whole brain and region-specific differences between patients and controls. Similar to

neurocognitive intermediate phenotypes, many studies have showed evidence that neuroimaging data were suitable for intermediate phenotypes (Birnbaum and Weinberger 2013; Allen et al. 2009). As studies for functional neuroimaging have discussed elsewhere (Birnbaum and Weinberger 2013), we present only structural neuroimaging intermediate phenotypes. Voxel-based-Morphometry (VBM) and FreeSurfer techniques are mainly used to investigate gray matter abnormalities in patients with neuropsychiatric disorders compared with controls, while diffusion tensor imaging (DTI) technique is used to investigate white matter abnormalities.

As described above, we present in the order of genes detected by candidate gene and GWAS approaches. Many neuroimaging studies of *COMT* using structural magnetic resonance imaging (MRI) have reported significant associations between the Val158Met variant and brain structures (Ira et al. 2013; Ohnishi et al. 2006), although some studies have failed to replicate these findings (Barnes et al. 2009; Wang et al. 2013). A recent systematic review showed that smaller temporal and frontal brain areas were associated with the *COMT* Val allele in patients with schizophrenia, and their relatives (Ira et al. 2013). Several studies have found that the SNPs or/and schizophrenia-risk haplotypes in the *DTNBP1* were associated with different brain regions such as the anterior cingulate cortex, hippocampal, prefrontal, occipital, and total brain volumes, but one study failed to find this association (Donohoe et al. 2010; Dutt et al. 2009; Narr et al. 2009; Trost et al. 2013; Tognin et al. 2011). Several associations between other genes and brain structures have been reported (Rose and Donohoe 2013; Buckholtz et al. 2007), although the associated regions were inconsistent, and the associated subjects were different (only in patients, controls or both subjects); *RGS4* and decreased gray matter volumes in dorsolateral prefrontal cortex, thalamus and superior temporal gyrus, and white matter volumes in ventral prefrontal region; *NRG1* and total gray matter, total white matter, lateral ventricle, frontotemporal and hippocampal volumes, and white matter density in the interior capsule; *GRM3* and white matter integrity of cortico-cerebellar-thalamic-cortical circuit (Mounce et al. 2014); *DISC1* and several gray matter volumes in the prefrontal cortex, superior frontal gyrus, hippocampus, insular cortex, anterior cingulate cortex and supramarginal gyrus, and fractional anisotropy in prefrontal white matter volume (Hashimoto et al. 2006); *G72*(*DAOA*) and frontal volume and cortical thickness in middle temporal, inferior parietal, and lateral occipital cortices. We have also reported associations between other genes and neuroimaging-based intermediate phenotypes; *PACAP* and hippocampal volume; *VAV3* and superior and middle temporal gyri; *AKT1* and inferior parietal lobule (Ohi et al. 2013b; Hashimoto et al. 2007; Aleksic et al. 2013).

Compared with other intermediate phenotypes, many of the associations between genetic variants detected by a GWAS approach and neuroimaging-based intermediate phenotypes have been reported. Several studies have used neuroimaging-based intermediate phenotypes to examine whether the SNP rs1344706 in the *ZNF804A* detected by the first GWAS, impacts upon brain structure, including gray matter and white matter volumes. However, the reported associations were inconsistent in either regions or subjects, as follows: Lencz

et al. first reported that the risk allele homozygotes had larger total white matter volumes, and reduced gray matter volumes in several regions, including the angular gyrus, parahippocampal gyrus, posterior cingulate, and medial orbitofrontal gyrus/gyrus rectus, among healthy subjects when controlling for white matter volumes (Lencz et al. 2010). Voineskos et al. showed that individuals homozygous for the risk variant, demonstrated reduced cortical gray matter thickness in the superior temporal gyrus and the anterior and posterior cingulate cortices in healthy individuals (Voineskos et al. 2011). On the other hand, Donohoe et al. demonstrated that the risk allele when homozygous had relatively larger gray matter volumes, particularly hippocampal volumes, in patients with schizophrenia, but not in controls (Donohoe et al. 2011a). Wassink et al. showed this SNP influenced white matter volume in patients with schizophrenia and controls in opposite directions (Wassink et al. 2012). Wei et al. indicated that the risk allele carriers presented higher white matter density in the schizophrenia patients, and lower white matter density in healthy controls (Wei et al. 2012). There were some associations between the risk allele and lower fractional anisotropy in several regions (Kuswanto et al. 2012; Ikuta et al. 2013). On the other hand, there were also reports of no effect of the SNP on white matter integrity (Sprooten et al. 2012; Wei et al. 2013; Fernandes et al. 2014; Voineskos et al. 2011), on gray matter volumes (Wassink et al. 2012), on cortical thickness (Bergmann et al. 2013) or on any brain volumes (Cousijn et al. 2012).

The rs12807809 SNP around *NRGN* is also associated with several brain regions. Ohi et al. first reported the association of rs12807809 with the gray matter volume of the anterior cingulate cortex in patients with schizophrenia, but not in controls (Ohi et al. 2012). In addition, it was reported that the risk T-allele homozygotes had a thinner left entorhinal cortex in patients with schizophrenia (Thong et al. 2013). Two other studies showed an association between this SNP of *NRGN* and cortical thickness, but there are also opposite directions of the association (Rose et al. 2012; Walton et al. 2013). The *MIR137* risk genotype was associated with reduced white matter integrity throughout the brain, as well as smaller hippocampi and larger lateral ventricles (Lett et al. 2013) in patients with schizophrenia. However, a study showed no association between this SNP and white matter microstructure (Kelly et al. 2014). One marker in the MHC region, rs6904071, was associated with hippocampal volume (Walters et al. 2013). A second stage GWAS in schizophrenia was conducted using the first stage GWAS to increase the sample size. This second stage study found additional risk genes, such as the *CNNM2*, *PCGEM1*, *TRIM26*, *CSMD1*, *MMP16*, *NT5C2* and *CCDC68* (Consortium 2011). The risk variant rs7914558 in the *CNNM2* gene was associated with the volume of the bilateral inferior frontal gyri, and no significant association between other risk genes and gray matter morphology was observed (Ohi et al. 2013a). However, another research group showed increased gray matter volume in the temporal pole and the anterior cingulate cortex, in subjects with a risk allele in the *CNNM2* (Rose et al. 2013a). The same group also reported no association between the risk SNP in *CSMD1* and gray matter volume (Rose et al. 2013b).

7.3.3 Neurophysiological Intermediate Phenotypes

Neurophysiological examinations have the advantages of being easy to undertake and of using simple non-language-related stimulation, regardless of age, sex, race and language, as well as several neurophysiological deficits in schizophrenia which frequently occur before the onset of the psychotic symptoms has been reported. These neurophysiological candidate intermediate phenotypes in schizophrenia, include abnormalities in prepulse inhibition (PPI), smooth pursuit eye movements, auditory P50 event-related potential (ERP), P300, mismatch negativity (MMN) and prefrontal activation using near-infrared spectroscopy (NIRS) (Turetsky et al. 2007). Evidence suggests that many of these neurophysiological deficits are distinct from each other. They are stable, mostly independent of symptom state and medications, and are also observed in healthy relatives. These candidates have relatively high heritabilities (Greenwood et al. 2007; Sakakibara et al. 2014; Hall et al. 2006).

As described above, we present in the order of candidate gene approach-based genes and GWAS-based genes. Compared with neurocognitive and neuroimaging-based intermediate phenotypes, the number of reports using neurophysiological intermediate phenotypes is small. The *COMT* Met158 carriers affect elevated PPI levels in healthy subjects (Roussos et al. 2008) as well as in schizophrenia patients (Quednow et al. 2010), increased prefrontal activation during the verbal fluency task in patients with schizophrenia (Takizawa et al. 2009), and lower P50 deficits (Lu et al. 2007). On the other hand, the results using eye movements were inconsistent; *COMT* Met158 allele has an alleviating effect on eye movement disturbances in patients (Rybakowski et al. 2002) and in controls (Thaker et al. 2004), while the Met158 carrier is associated with worse performance on the antisaccade task both in patients (Thaker et al. 2004), and in controls (Haraldsson et al. 2010). Reported associations between other genes detected by candidate gene approaches and neurophysiological phenotypes are as follows (Rose and Donohoe 2013); *NRG1* and P300 latency; *RGS4* and eye movements (Kattoulas et al. 2012); *GRM3* and MMN (Kawakubo et al. 2011); *DISC1* and P300 latency (Blackwood et al. 2001). We have also reported some associations between other genes and neurophysiological intermediate phenotypes; *RELA* and PPI; *SIGMAR1* and prefrontal activation; (Ohi et al. 2011; Hashimoto et al. 2011). There were a few studies investigating association of GWAS-based genes and neurophysiological intermediate phenotypes: *ZNF804A* and P300 (O'Donoghue et al. 2014; Del Re et al. 2014); *TCF4* and PPI (Quednow et al. 2011) and P3 amplitude (Hall et al. 2014); *MIR137* and P300 (Decoster et al. 2012).

7.3.4 Other Intermediate Phenotypes

As the concept of the intermediate phenotype expands, new candidate intermediate phenotypes are arising, such as gene expression in post-mortem brain and lymphoblast, personality traits using Temperament and Character Inventory (TCI), Neuroticism-Extraversion-Openness Personality Inventory (NEO-PI), and Schizotypal Personality Questionnaire (SPQ). However, as the conditions for intermediate phenotypes, such as heritability, stability over time, or showing increased expression in the unaffected relatives of probands, may be attributed to chance, the results obtained using these intermediate phenotype approaches should be interpreted carefully.

7.4 Future Development of the Intermediate Phenotype Approach

Overall, the numerous effects of the risk genetic variants in genes identified by both candidate gene and GWAS approaches on several intermediated phenotypes have been reported, but the reported associations have not been consistent. This inconsistency may be due to a small number of subjects in the association studies between genes and intermediate phenotypes, when compared with those in genetic association studies between genes and diseases. In addition, this inconsistency may also be due to differences in the methods of measurement (a different type of test) and the tested subjects (only patients, controls or both). Therefore, further replications of these studies, using larger sample sizes and a common methodology, and subsequent meta-analysis of reported associations, are necessary to lead to reliable conclusions.

To control confounding factors (e.g. medications and duration of illness), intermediate phenotypes studies were conducted using only healthy subjects. However, using only healthy subjects may overlook more characteristic associations observed in patients. In our opinion, studies using both patients and controls are important to investigate associations between genes and intermediate phenotypes. In addition, studies using unaffected relatives of patients with neuropsychiatric disorders, may also be a valid approach because they carry risk genes for the disorder without the confounding factors relating to the state of the disorder.

Following a genome-wide linkage study of intermediate phenotypes; e.g. eye movement (3p14), spatial processing (2p25 and 16q23), PPI (5p15), verbal memory (8q24), attention (10q26) and facial memory (10q26 and 12p12) (Greenwood et al. 2013), several GWAS using intermediate phenotypes as a phenotype have recently been published. These studies sought new genetic variations to explain the risk for neuropsychiatric disorders, as well as their underlying mechanisms of neurocognition, neuroimaging, and neurophysiology. In particular, GWAS on neuroimaging-based intermediate phenotypes have frequently been undertaken. In

the early stages of GWAS on brain morphology, the Alzheimer's Disease Neuroimaging Initiative (ADNI) carried out a GWAS of brain structures in fewer than 1000 subjects, including healthy controls and individuals with both mild cognitive impairment or Alzheimer's disease. This research group used several brain phenotypes and methods of analysis (Shen et al. 2010; Stein et al. 2010a, b). Although extensive efforts using structural phenotypes and different strategies were made, these studies failed to identify new genetic variants with any genome-wide significance. Within the last few years, several large-scale collaborative consortiums, such as the Enhancing NeuroImaging Genetics through Meta-Analysis (ENIGMA) Consortium, have been established to overcome the problem of sample size (Thompson et al. 2014; Psaty et al. 2009; Schumann et al. 2010). The ENIGMA Consortium is a collaborative network of researchers working together on a range of large-scale studies that integrate data from 70 institutions worldwide. The ENIGMA studies have analyzed neuroimaging data from more than 12,000 subjects. ENIGMA's first project was a GWAS to identify common genomic variants associated with hippocampal or intracranial volume (Stein et al. 2012). The intergenic variant rs7294919, located at 12q24.22, was associated with hippocampal volume, and rs10784502, located within *HMGA2* at 12q14.3, was associated with intracranial volume. Researchers continue to explore genetic associations with subcortical volumes (ENIGMA2) and white matter microstructure (ENIGMA-DTI). They are also focused on understanding the impact of schizophrenia, bipolar illness, major depression and attention deficit/hyperactivity disorder (ADHD) on the brain. On the other hand, Hass et al. performed a GWAS of hippocampal volume using 328 subjects, including both healthy controls and schizophrenic patients, but no SNP was found to reach genome-wide significance (Hass et al. 2013). This failure may have been due to the small sample size employed.

Although several GWAS which investigated neurocognitive phenotypes impaired in schizophrenia, such as general cognitive ability, executive function, processing speed, and verbal fluency, have been conducted in other cohorts that did not include patients with schizophrenia (Davies et al. 2011; Cox et al. 2014; Luciano et al. 2011; Lencz et al. 2014; Benyamin et al. 2014), these studies reported that no single variant was found to be associated with any of the neurocognitive phenotypes at the level of genome-wide significance. To increase sample size and to identify any genetic variants related to neurocognitive intermediate phenotypes, the Cognitive Genomics consorTium (COGENT) - an international consortium of nine teams of researchers across seven countries - was founded, mirroring the ENIGMA neuroimaging consortium. On the other hand, there are few GWAS focusing on neurophysiological phenotypes in patients with schizophrenia or other neuropsychiatric disorders. GWAS on P300 did not find any genome-wide significant variant in approximately 1000 unrelated individuals drawn from a study of alcohol dependence (Zlojutro et al. 2010), while GWAS on MMN found a nominal genome-wide significant variant on chromosome 4q32.1 ($p = 5.14E\text{-}8$) in a small number of dyslexic children (Roeske et al. 2011). These failures may be due to the small effect size of each genetic variant on the phenotypes. To detect a genome-wide significant variant using GWAS of intermediate phenotypes, a larger

sample size would be required because the level of significance in GWAS is very strict (<5.0E-8). Researchers in the field should, therefore, form a large consortium, such as ENIGMA and COGENT, to increase sample sizes and thereby detect the signals. This also highlights the importance of large-scale studies of intermediate phenotypes – both within and between categories – of neuropsychiatric disorders, such as the Consortium on the Genetics of Schizophrenia (COGS) and the Bipolar Schizophrenia Network on Intermediate Phenotypes (B-SNIP). An advantage of these studies is that they address the question of whether risk variants related to intermediate phenotypes are specific to schizophrenia or generalized to neuropsychiatric disorders across various conditions, including schizophrenia or bipolar disorder.

Polygenic risk score analysis has recently been developed to summarize the genetic risk components of neuropsychiatric disorders. It uses thousands of common alleles with very small effects (Purcell et al. 2009). A polygenic risk score for a particular disease can be calculated for each individual in a sample from published genetic association data, by summing the known effect size of each individual SNP multiplied by the number of reference alleles present for that SNP in a particular individual. A substantial proportion of the heritability of schizophrenia is explained by a polygenic component consisting of many common SNPs of extremely small effect. The polygenic schizophrenia risk scores (PSS) also contribute to the risk of bipolar disorder, but not to that of several non-psychiatric diseases (Purcell et al. 2009). By applying polygenic SNP scores derived from a large-scale cognitive GWAS meta-analysis to case-controlled cohorts, COGENT has examined whether alleles related to reduced neurocognitive function could also serve to increase the risk of schizophrenia (Lencz et al. 2014). Patients with schizophrenia had lower cognitive polygenic scores derived from a large-scale cognitive GWAS meta-analysis compared with controls, and the PSS were associated with lower general cognitive ability, thus indicating a genetic overlap between schizophrenia and general cognitive ability. In addition, an association analysis between the PSS and brain volume has also been reported (Terwisscha van Scheltinga et al. 2013). The PSS, which was calculated using the Psychiatric Genetics Consortium case control data, was associated with total brain and white matter volumes, but not gray matter volume, explaining approximately 5 % of the variance in the total brain and white matter volumes. This report is the first association study of the polygenic score and brain structure. However, it remains unclear whether PSS affects variation in specific gray matter volume. We investigated the effect of PSS on specific gray matter volume, using VBM and VBM-based region of interest (ROI) methods. Both analyses revealed that PSS was significantly negatively correlated with the local gray matter volume in the left superior temporal gyrus and the calculated total left superior temporal gyrus volume in the patients with schizophrenia, whereas there was no effect of the score on the region in the controls. Higher polygenic scores were associated with a smaller left superior temporal gyrus volume. The polygenic score explained approximately 3.2 % of the variance in the total left superior temporal gyrus in the patients with schizophrenia. These findings suggest

that genetic variants are associated with the reduced superior temporal gyrus volume observed in patients with schizophrenia.

GWAS of neuropsychiatric disorders, along with intermediate phenotypes, have identified several risk variants. If sample size were increased, then many genetic variants with a small effect size would be discovered from the large volume of data. Of these variants, however, most SNPs would be located in non-coding regions. How these SNPs might influence disorders and phenotypes is unknown. These SNPs may only be the flag which harbors signals related to neuropsychiatric disorders and intermediate phenotypes. Expression Quantitative Trait Loci (eQTL) based on correlations between genetic variants and RNA transcript expression levels, are the genomic regions regulating RNA transcript expression levels. The eQTL provide a possible mechanism by which these variants may influence disorders and phenotypes. It has been shown that SNPs associated with common diseases identified by GWAS, are enriched with expression-affecting SNPs (eSNPs) (Fu et al. 2012) and regulatory regions of the genome (Powell et al. 2013), suggesting that the functional mechanism by which many GWAS variants affect disease susceptibility, is through gene regulation. The majority of eQTL that have been identified are located within the cis-region (± 1 MB) of the transcription start site (TSS). Cis-eQTL tend to have larger effect sizes compared to trans-eQTL (Powell et al. 2013) and are more likely to reflect a direct functional relationship between the SNP and the measured expression levels. To understand the mechanisms of genetic susceptibility for neuropsychiatric diseases, we require knowledge of the genetic control of regulatory variation in a different types of tissue, such as lymphoblast from whole blood, and brain cells from the post-mortem brain. Future advances in the intermediate phenotype approach will contribute to elucidating the pathology of neuropsychiatric disorders.

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Part IV
Future Directions for Therapy

Chapter 8
Significance of Mechanism-Oriented Research Toward Neuronal Protection Therapy Against Neurodegenerative Disorders ~ ZNRF1 E3 Ubiquitin Ligase as a Critical Mediator for Wallerian Degeneration and Neuronal Apoptosis

Shuji Wakatsuki and Toshiyuki Araki

Abstract Therapeutic strategies for neurological disorders are now spreading in many directions. Previously, the removal of regeneration-inhibitory environments in the brain, together with the introduction of regeneration-promotive characteristics of the peripheral nerves has been explored. More recently, the effect of transplantation of stem cell-derived cells on traumatic injury and neurodegenerative disorders has been examined. Among various approaches aimed at a recovery from neurodegeneration, neuronal protection based on the understanding of degeneration mechanisms is an attractive alternative, especially for neurodegenerative disorders.

Axon degeneration is a hallmark of many neurological disorders, including neuropathies and neurodegenerative diseases. Previous studies have shown that subcellular signaling which promotes axonal degeneration is independent from the typical cell death signal. Whereas, axonal protection mechanism, as shown in a naturally occurring mutant strain *wallerian degeneration slow* (*wlds*) mice, can save both axons and cell bodies from some types of insults, but the existence of common regulatory mechanism(s) between Wallerian degeneration and neuronal apoptosis remains to be fully elucidated. In this chapter, we introduce a general overview of both well-established and newly discovered pathways that control the progression of Wallerian degeneration, and we also describe how E3 ubiquitin ligase zinc and ring finger 1 (ZNRF1) functions as a critical mediator for neurodegenerative pathways, Wallerian degeneration and neuronal apoptosis, by translating oxidative stress into subcellular signaling in neurons. Our results presented here, suggest that the pathophysiological significance of ZNRF1-mediated signaling in

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K. Wada (ed.), *Neurodegenerative Disorders as Systemic Diseases*,
DOI 10.1007/978-4-431-54541-5_8

the regulation of both Wallerian degeneration and neuronal apoptosis is a potential therapeutic avenue against neurodegenerative disorders.

Keywords Traumatic injury • Peripheral nervous system • Axon • Apoptosis • Caspase • Kinases • Oxidative stress • Nicotinamide adenine dinucleotide • Neuroprotective • Neurodegenerative diseases

8.1 Therapeutic Strategies for Neurological Diseases

One of the features which distinguishes the central nervous system (CNS) from the peripheral nervous system (PNS) is its capability for regeneration. Damaged axons in the PNS can regenerate and regain full functional capability, but it can take a long time for this to be completed. On the other hand, regeneration of neurons and their processes in the CNS, once lost by disorders or injury, is severely limited (Brosius Lutz and Barres 2014). There have been two main hypotheses to explain the difficulty of regeneration in the CNS. One is that a substance or environment which inhibits regeneration is present in the CNS but not in the PNS and the other is that a substance or environment which promotes regeneration is present in the PNS but not in the CNS (Aguayo et al. 1981; Yiu and He 2006; Chen et al. 2007). Decades of research efforts have shown that both of these hypotheses are true, at least in part, and the molecular background to the inhibition of regeneration in the CNS, and the regeneration-promotive environment in the PNS have gradually been revealed. There has also been an accumulation of examples showing that limited CNS regeneration can be achieved using animal models of spinal cord injury, by combining substitution of factors/reagents which can promote regeneration, and the removal/neutralization of factors which inhibit regeneration. These types of therapeutic approach toward neurological disorders are discussed in other chapters in this book.

In neurodegenerative diseases, particular types of CNS neurons (dopaminergic neurons in the mid-brain, for instance) degenerate and die. Drug treatments currently available for such diseases mostly aim to increase the production/release of neuroactive substances required for neuronal functionality (such as L-DOPA treatment for Parkinson's disease, and acetylcholine therapy for Alzheimer's disease) (Brichta et al. 2013; Schliebs and Arendt 2011). On the other hand, approaches that aim to maintain or increase the number of healthy neurons have also been explored with the aim being the fundamental/complete recovery from neurodegenerative disorders. One such approach is the transplantation of stem cell-derived neurons/glial cells (Sandoe and Eggan 2013). By development of reprogramming technology to generate stem cells (induced pluripotent stem cell or iPS cells) from somatic cells, this approach has gained attention because iPS cell technology significantly lowers the hurdle of developing novel transplantation therapy using stem cell-derived differentiated cells (Ross and Akimov 2014).

Despite earlier hopes and expectations, transplantation-based therapies for neurological disorders have many limitations. Firstly, fiber connection between transplanted neurons and host neurons is mostly non-physiological. For instance, dopaminergic neuron transplantation in Parkinson's disease, which is regarded as being closest to a clinical application among all of the transplantation therapies in the field of regenerative medicine, is most often designed so that stem cell derived transplants are injected into striatum, which is the physiological projection area for substantia nigra neurons which are known to degenerate in the disease (Nishimura and Takahashi 2013). In this case, a neuronal graft is ectopically introduced and therefore the neuronal network in substantia nigra is not reconstructed after transplantation. However, to improve motor function in the host, an increase of dopamine, released in the striatum is observed after transplantation, which seems to be sufficient. In the case of transplantation of stem cell-derived cells to the injured spinal cord, which is another hopeful field for the application of regenerative medicine, transplanted cells are not expected to replace neurons lost by injury or diseases (Tsuji et al. 2011). The grafts may form local connections to surrounding host neurons, but they do not usually connect to distant physiological targets. Instead, the expected roles of the grafts include the generation of a novel neuronal network in the host environment, which may be trained so as to be utilized by host. These examples suggest that currently planned transplantation therapies could be applied to certain neurological disorders only, and could result in limited recovery effects. Therefore, transplantation therapy may not be the "silver bullet" able to retain healthy neurons in the fight against neurodegenerative disorders.

Another alternative approach to maintaining neuronal health, is to protect neurons against insults that causes degeneration based on an understanding of the mechanism of neuronal degeneration in various disorders. This approach may appear to give only a palliative effect when compared to other possible therapeutic alternatives, and it is certainly not applicable to physical injuries that damage neurons instantaneously. However, neuronal protection therapy could still be important for most neurodegenerative disorders. In such diseases, neuronal functions are still preserved at a certain level at disease onset, and it takes years (sometimes tens of years) for progression to occur. Therefore, if the neuronal protection therapy is introduced at the onset of the disease and it results in the preservation of neuronal functions even if only to extend the period of time before neuronal function is lost, that still means a period of several years where function is preserved for patients. Since most neurodegenerative disorders develop in middle-aged and old-aged people, neuronal protection therapy could give a life-time preservation of neurological functions. For these reasons, we think it is relevant to explore the possibility of establishing neuronal preservation therapy by understanding the mechanism of neurodegeneration.

Neuronal cells are morphologically characterized by their long processes, i.e., axons and dendrites. In pathogenesis of most neurodegenerative disorders, the degeneration of axons precedes the death of cell bodies (Neukomm and Freeman 2014). Axonal degeneration is therefore often described as an early hallmark of neuronal degeneration in many neurodegenerative disorders. While axonal

degeneration and neuronal cell death are inseparable processes in the pathogenesis of neurodegenerative disorders, there is evidence that the mechanism for prototypical neuronal apoptosis does not include the progression of axonal degeneration. Another important observation regarding axonal degeneration is that the decay is not due to a lack of support from the cell body, but is from a series of enzymatically controlled mechanisms for active destruction. Typical axonal degeneration which is observed after an axon is cut, is called Wallerian degeneration (Waller 1851). Wallerian degeneration is delayed for at least a week in a naturally occurring mutant strain *wallerian degeneration slow* (*wlds*) mice, which express the Wallerian degeneration slow (WldS) protein and exhibits a gain-of-function phenotype of axonal protection by the expression of its active component nicotinamide mononucleotide adenylyltransferase 1 protein (NMNAT1) in axons, as described in detail later (Araki et al. 2004; Sasaki et al. 2009b; Babetto et al. 2010). Remarkably, the expression of WldS or NMNAT1 in axons can delay axonal degeneration in many neurological disorder models as well (Coleman and Freeman 2010; Wang et al. 2012; Conforti et al. 2014). Sterile α- and armadillo-motif-containing protein 1 (SARM1) was identified as a pro-degenerative protein required for axonal degeneration, and the depletion of this protein is sufficient to protect axons from degeneration in both primary cultures of mammalian neurons, and in fruit flies (Osterloh et al. 2012). These observations suggest that axonal degeneration is regulated by an active, self-destructive mechanism(s). Thus we became interested in the mechanism of axonal degeneration as a potentially important target for neuronal protection therapy of neurodegenerative disorders.

Previous studies have shown that subcellular signaling that promotes axonal degeneration is independent from the typical cell death signal (Finn et al. 2000; Raff et al. 2002; Whitmore et al. 2003). However,,the axonal protection mechanism can save both axons and cell bodies against some types of insults. For instance, *wlds* mice, which are characterized by significantly delayed Wallerian degeneration, also show protection against neuronal cell death observed in some disease models (Table 8.1) (Coleman and Freeman 2010; Wang et al. 2012; Conforti et al. 2014), suggesting that some types of disease-associated neuronal insults elicits signaling which promotes both axonal degeneration and neuronal cell death. However, the existence of common regulatory mechanism(s) remains unclear. Here, we introduce a general overview of some well-established and newly discovered pathways that control the progression of Wallerian degeneration, and also describe how an E3 ubiquitin ligase ZNRF1 functions as a critical mediator for Wallerian degeneration and neuronal apoptosis, by translating oxidative stress into subcellular signaling in neurons. Our results presented here suggest that the pathophysiological significance of ZNRF1-mediated signaling in the regulation of both Wallerian degeneration and neuronal apoptosis is a potential therapeutic avenue against neurodegenerative disorders.

Table 8.1 Effect of *wlds* mutation on axonal degeneration and neuronal apoptosis

Insult	Effect	Axonal degeneration	Neuronal apoptosis	References
6OHDA	Some axons preserved for ~11 days	+	–	Sajadi et al. (2004)
MPTP	Enhances survival, prevents nigrostriatal axon degeneration, and attenuates neurotransmitter loss	+	–	Hasbani and O'Malley (2006)
Taxol	Resistant to paclitaxel neuropathy by behavioral, electrophysiological, and pathological measures.	+	ND	Wang et al. (2002)
Diabetes	Alleviated abnormal sensory responses, nerve conduction, retina dysfunction and reduction of surviving retinal ganglion cells	+	+	Zhu et al. (2011)
P0 null	Reduces motoneuron loss and axonal loss at ~3 months	+	+	Samsam et al. (2003)
PMP22 null	Reduces axonal loss and clinical impairments without altering demyelination	+	ND	Meyer zu Horste et al. (2011)
SOD1 mutant transgene (G37R, G85R, and G93A)	Modest exention in lifespan (G93A)	–	–	Vande Velde et al. (2004), Fischer et al. (2005), and Rose et al. (2008)
pmn	Attenuates symptoms, extends life span, prevents axonnal degeneration, rescues motoneuron number and size, and delays retrograde transport deficits	+	+	Ferri et al. (2003)
gad	Reduction of pathology at 4 month	+	ND	Mi et al. (2005)
sma	No protective effect	–	–	Rose et al. (2008) and Kariya et al. (2009)

6OHDA 6-hydroxydopamine, *MPTP* 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, *P0* protein zezo, *PMP22* peripheral myelin protein 22, *SOD1* superoxide dismutase 1, *pmn* peripheral motor neuropathy, *gad* dracile axonal dystrophy, *sma* Spinal muscular atrophy, *ND* not described

8.2 Wallerian Degeneration

Axonal degeneration is observed in a wide variety of pathological conditions. A classic example of axonal degeneration is Wallerian degeneration, which occurs after an axon is cut (Waller 1851). Wallerian degeneration is observed as a major

component in many neurodegenerative diseases, such as Parkinson's Disease and Alzheimer's Disease (Fig. 8.1a) (Coleman 2005; Saxena and Caroni 2007; Wang et al. 2012). An active and evolutionarily conserved mechanism drives Wallerian degeneration (Fang and Bonini 2012). Wallerian degeneration can be investigated in both the peripheral and central nervous system (PNS and CNS) using injury-induced axonal degeneration models in vivo and in vitro (Coleman and Freeman 2010; Wang et al. 2012). Acutely severed axons exhibit the profiles of microtubule disassembly, blebbing of the distal axon, followed by axonal fragmentation; similar processes of Wallerian degeneration have been observed in several organisms including fruit flies and nematodes (Coleman and Freeman 2010). Some chemically induced acute injuries such as the local destruction of microtubule structure, with the microtubule disrupting anti-cancer agent vinblastine, induce axonal degeneration closely resembling Wallerian degeneration, and should, therefore, be considered as equivalent to physical lesions (Fig. 8.1a). Thus, understanding the mechanisms of axonal degeneration in simple traumatic injuries allows us to model how axons are lost in more complex neurodegenerative conditions.

8.3 Key Molecules Involved in the Regulation of Wallerian Degeneration Process

A much clearer understanding of the cellular and molecular basis of axonal degeneration during development, and in some neurological conditions, has been elucidated in the past decade (Conforti et al. 2014; Neukomm and Freeman 2014). Next we introduce a general overview of some well-established and some newly discovered pathways that control the progression of Wallerian degeneration (Fig. 8.1b).

8.3.1 NMNAT Proteins

The interest in the mechanisms underlying Wallerian degeneration has grown dramatically since the discovery of the *wlds* mice (Table 8.1) (Coleman and Freeman 2010; Fang and Bonini 2012; Wang et al. 2012). The *wlds* mutation comprises an 85-kb tandem triplication, and subsequent cloning of the mutation has revealed the presence of a genomic triplication, generating a unique fusion protein called WldS protein, consisting of the first 70 amino acid residues of the ubiquitin-chain elongation factor E4b fused to the complete sequence of NMNAT1 and a brief linker sequence (Coleman et al. 1998; Conforti et al. 2000; Mack et al. 2001). We and others have previously shown that overexpression of NMNAT enzymatic activity, which mediates NAD synthesis, is essential for the axonal protection phenotype observed in *wlds* mice (Araki et al. 2004; Sasaki

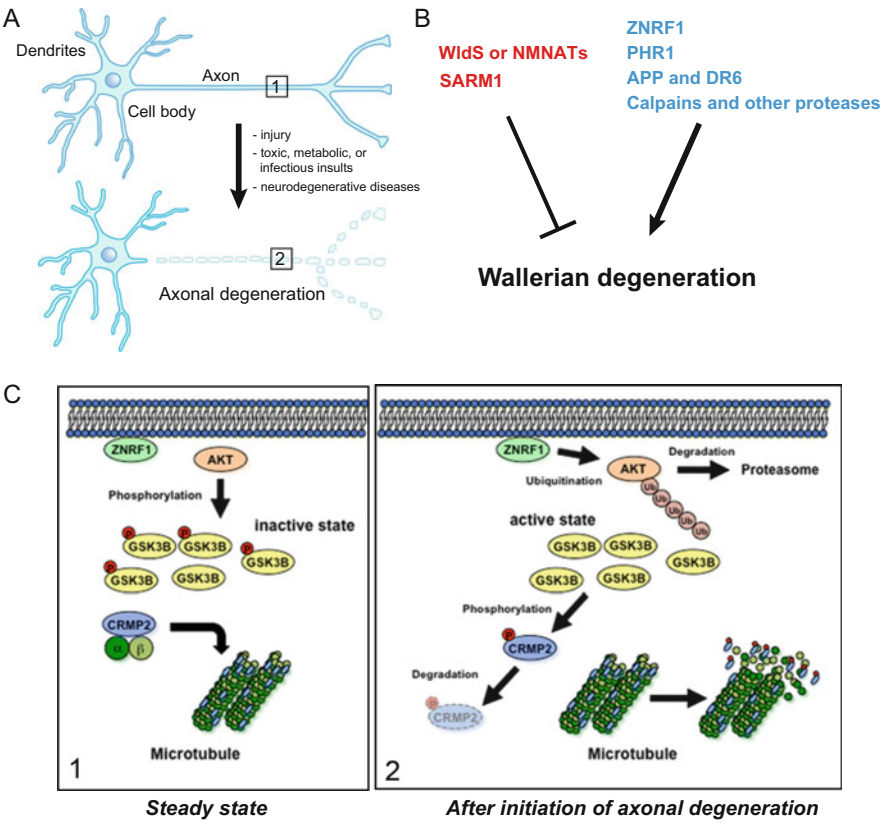

Fig. 8.1 Molecular pathways regulating Wallerian degeneration. (**a**) When an axon is cut, the isolated distal portion of axon rapidly undergoes Wallerian degeneration. Similar degeneration is also observed in the nerves in many neurological disorders including neurodegenerative diseases. (**b**) Molecules that mediate axonal degeneration are the E3 ubiquitin ligase ZNRF1, PHR1, or the Ca^{2+}-activated cysteine protease Calpains. Loss of these molecules can protect axons from degeneration. By contrast, *wlds* and NMNATs can inhibit the progression of axonal degeneration (see text for details). (**c**) The ZNRF1–AKT–GSK3B–CRMP2 pathway controls cytoskeletal integrity during axonal degeneration. In the steady state axon (*boxed* area 1 in **a**, *left panel*), AKT phosphorylates GSK3B and thereby inactivates it. CRMP2 is not phosphorylated in the steady state axons and continues to maintain the microtubule architecture. Upon initiation of axonal degeneration (*boxed* area 2 in **a**, *right panel*), ZNRF1 targets AKT to the UPS-mediated degradation, and thereby activates GSK3B. Activated GSK3B phosphorylates CRMP2 to destabilize microtubule assembly in the axons. Inhibition of this pathway results in axonal protection against degeneration

et al. 2009a; Babetto et al. 2010). In addition to NMNAT1, both NMNAT2 (located mainly in Golgi apparatus) and NMNAT3 (located mainly in mitochondria) have also been identified in mammals (Berger et al. 2005). Neuronal expression of the WldS protein and NMNAT3 but not NMNAT1 protein in vivo, protects axons from Wallerian degeneration, and suggests that mitochondrial expression of NMNAT

activity may play a role in the mechanism of axonal protection observed in *wlds* mice (Yahata et al. 2009). Thus, the subcellular localization of the WldS protein is thought to be one of the important determinants for its role in axonal protection.

Expression of WldS, or other NMNATs, markedly delays axonal degeneration triggered by a variety of physical or toxic insults (Table 8.1) (Coleman and Freeman 2010; Wang et al. 2012; Conforti et al. 2014). These observations suggest that NMNAT enzymatic activity is required for axonal protection, but the correlation between neuronal NAD levels and axonal survival is lacking so far. For instance, high concentrations of exogenous NAD are reported to delay axonal degeneration in the in vitro Wallerian degeneration model (Araki et al. 2004; Wang et al. 2005; Sasaki et al. 2006), but increasing NAD levels in mice by removing the NAD consuming enzyme poly-ADP ribose polymerase 1, which catalyzes the NAD-dependent addition of ADP-ribose polymers to a variety of proteins, gives no protective effects on axonal degeneration (Sasaki et al. 2009b). Furthermore, pharmacological inhibition of nicotinamide phosphoribosyltransferase, which lowers intracellular NAD level, provides only moderate effects against axonal degeneration, and does not affect NMNAT-mediated axonal protection either (Sasaki et al. 2009b; Shen et al. 2013). Thus, the exact mechanism by which the WldS protein or NMNAT proteins suppress axonal degeneration has not been fully elucidated.

8.3.2 SARM1

Drosophila sterile α- and armadillo-motif-containing protein (dSARM) has been found through the extensive screening of ethyl methanesulfonate-treated fruit flies, to provide strong axonal protection (Osterloh et al. 2012). Deletion of the SARM1 protein in mice is sufficient to protect axons from degeneration for days after peripheral nerve transection, to an extent similar to that observed in the *wlds* axons (Osterloh et al. 2012). SARM1 is the fifth Toll/IL-1 receptor (TIR) domain-containing adaptor protein identified to regulate Toll-like receptor downstream signaling, and has therefore been studied with respect to innate immunity, and has been shown to control the expression of several inflammatory cytokines (Lin et al. 2014). Unlike the other TIR domain-containing adaptor proteins, SARM1 is predominantly expressed in neurons in the brain and promotes neuronal death after oxygen-glucose deprivation (Kim et al. 2007; Chen et al. 2011; Lin et al. 2014). These observations suggest that SARM1 regulates the innate immune responses of the CNS through regulating cytokine expression by neurons. By contrast, *Sarm1/tir-1* has been shown to regulate the left–right asymmetric expression of odorant receptor genes in olfactory neurons in nematoda (Chuang and Bargmann 2005). In mammals, SARM1 has been also shown to play important roles in controlling neuronal morphology, via the interaction with a transmembrane heparan sulfate proteoglycan syndecan-2 to induce the mitogen activated protein kinase, kinase 4–c-Jun amino-terminal kinase (JNK), pathway. Further studies are

needed to elucidate a possible mechanism(s) for the SARM1-mediated axonal protection effect.

8.3.3 Calpains and Other Proteases

Protease activation – such as in calpains – has also been strongly implicated in axonal degeneration (George et al. 1995; Ikegami et al. 2004; Gerdts et al. 2011). For example, inhibition of calpains delays axonal degeneration induced by vinblastine or by NGF withdrawal (Gerdts et al. 2011). Calpains are Ca^{2+}-activated cysteine proteases linked to a variety of physiological processes, and their activity is critically controlled, not only by intracellular Ca^{2+}, but also by the endogenous inhibitor calpastatin (Goll et al. 2003; Liu et al. 2004). Endogenous calpastatin has been proposed to play pro-survival roles in adult neurons under degenerative conditions, including in models of brain ischemia (Bano et al. 2005; Takano et al. 2005; Rao et al. 2008; Vosler et al. 2011; D'Orsi et al. 2012) and MPTP/6-hydroxydopamine (6OHDA)-induced PD models (Crocker et al. 2003; Grant et al. 2009). Recently, exogenous transgenic expression of calpastatin in mice provided definitive evidence of calpain involvement in sciatic and optic nerve degeneration after transection (Yang et al. 2013). Calpastatin depletion was observed in degenerating axons, while maintaining calpastatin in transected axons inhibited degeneration in vitro, and in the optic nerve in vivo. Calpastatin depletion also occurred in a caspase-dependent manner in trophic factor-deprived sensory axons in vitro, and was required for this model of developmental degeneration. These findings suggest that inhibition of calpain activity by calpastatin is one of the critical steps for the progression of axonal degeneration and calpain activation can be implicated in neurodegenerative disorders (Liu et al. 2004; Araujo and Carvalho 2005; Camins et al. 2006).

8.3.4 N-APP and DR6

Signaling components, including the Fas death receptor (CD95), tumor necrosis factor (TNF) receptors, and the p75 receptor involved in developmental axonal destruction, are known to be observed in the brain of neurodegenerative disorders (Haase et al. 2008). However, as these signaling pathways are also upregulated in response to neuronal injury, it is uncertain whether these components contribute to primary causative mechanisms or if they reflect secondary processes such as neuroinflammation. Nikolaev et al. reported a physiological function for the amyloid precursor protein (APP)-processing product N-APP in developmental axonal pruning and neuronal apoptosis, in which N-APP binds directly to a death receptor to trigger the culling of axons and neurons during development, and possibly in disease-associated neurodegeneration as well (Nikolaev et al. 2009). Using in vitro

axonal degeneration and genetic mouse models, they determined that death receptor 6 (DR6) is required for these two self-destructive processes both in vivo and in trophic factor-deprived neuronal cultures, and they found that DR6-dependent axonal pruning is mediated by caspase 6, and neuronal apoptosis by caspase 3.

8.3.5 UPS and E3 Ligases

One of the first mechanistic insights into the subcellular signaling of axonal degeneration was the demonstration of the involvement of ubiquitin-proteasomal protein degradation system (UPS) in the progression of axonal degeneration. Zhai et al. showed that the application of a pharmacological inhibitor for UPS can cause delayed axonal degeneration of transected axons both in vitro and in the optic nerve in vivo (Zhai et al. 2003). However, the exact E3 ubiquitin ligase required for the progression of axonal degeneration in mammals, has long been unclear. We and others identified the E3 ubiquitin ligases and ZNRF1 and PAM-Highwire-Rpm-1 (PHR1) as the critical regulators for axonal degeneration, respectively.

8.3.5.1 ZNRF1

We previously identified ZNRF1 protein as an E3 ligase that is widely and constitutively expressed in neurons during development, as well as in adulthood (Araki and Milbrandt 2003). Interestingly, overexpression of a dominant-negative form of ZNRF1 can prevent Wallerian degeneration. These observations raise the possibility that ZNRF1 is an E3 ligase involved in the regulation of Wallerian degeneration. During the functional analysis of the UPS in axons during degeneration, we noticed that the expression level of protein kinase B (AKT) and its enzymatic activity, showed significant decreases during degeneration, and that UPS is involved in the downregulation of AKT. From these findings, we hypothesize that ZNRF1-mediated protein degradation is involved in the post-translational regulation of AKT expression. We therefore examined the possibility that ZNRF1 mediates AKT degradation in degenerating axons, and found that ZNRF1 promotes Wallerian degeneration by causing AKT to degrade via the UPS (Fig. 8.1c) (Wakatsuki et al. 2011). AKT phosphorylates glycogen synthase kinase 3B (GSK3B) and thereby inactivates it in axons. AKT overexpression significantly delays axonal degeneration. Overexpression of the active form of GSK3B induces collapsin response mediator protein 2 (CRMP2) phosphorylation at 514th Threonine (T514), which is required for the microtubule reorganization observed in the degenerating axon. The inhibition of GSK3B and the overexpression of non-phosphorylated CRMP2, both protected axons from Wallerian degeneration. These observations indicated the molecular identity of the ZNRF1-mediated regulation of kinase signaling that controls cytoskeletal integrity during Wallerian degeneration.

8.3.5.2 PHR1

Deoxyribodipyrimidine photo-lyase (PHR1) is an evolutionarily conserved family of large E3 ubiquitin ligases that are central regulators of axonal biology. Mutations of PHR1 orthologs from worms to mice lead to dramatic defects in the development of synapses and axons (Schaefer et al. 2000; Bloom et al. 2007; Lewcock et al. 2007). In invertebrates, PHR1 proteins accelerate the regenerative response following nerve injury, and delay synapse loss in genetic models of cytoskeletal instability (Xiong et al. 2010; Nix et al. 2011). In mice, loss of PHR1 results in the prolonged survival of severed axons, both in the PNS and CNS, as well as the preservation of motor and sensory nerve terminals (Babetto et al. 2013). Interestingly, PHR1 promotes self-destruction through control of the expression levels of NMNAT2 in injured axons (Xiong et al. 2012). These observations suggest that pharmacological inhibition of PHR1 function may be an attractive therapeutic candidate for ameliorating axonal loss in neurodegenerative diseases.

8.4 ZNRF1 Activation Induces Neuronal Apoptosis

As described previously, CRMP2 degradation, induced by its phosphorylation at T514 (CRMP2 pT514), leads to microtubule destabilization, and thereby promotes axonal degeneration. Therefore, CRMP2 pT514 can be an indicator for the activation of ZNRF1-mediated signaling in neurons. CRMP2 pT514 is often observed in neurons in animal models, and in patients with brain ischemia or neurodegenerative diseases (Ryan and Pimplikar 2005; Cole et al. 2007; Hou et al. 2009; Williamson et al. 2011). To show that ZNRF1-mediated signaling is activated in neurons under oxidative stress, we chose to examine a focal cerebral ischemia model. Focal ischemia is known to cause different types of cell death; neurons in the ischemic core undergo necrotic cell death, whereas neurons in the ischemic penumbra surrounding the ischemic core, mostly show delayed neuronal apoptosis, and oxidative stress is strongly implicated in the latter (Ueda and Fujita 2004; Broughton et al. 2009). Using a middle coronary artery occlusion (MCAo) model in adult male mice, we found increased CRMP2 pT514 immunoreactivity in neurons in the ischemic penumbra but not in the infarct core (data not shown). These results raised the possibility that ZNRF1-mediated AKT degradation is involved in the regulation of oxidative stress induced neuronal apoptosis. To show that the ZNRF1–AKT–GSK3B–CRMP2 pathway can be involved in the regulation of oxidative stress-induced pathology in the nervous system, we examined the significance of ZNRF1–AKT–GSK3B–CRMP2 pathway activation in oxidative stress-induced pathology, and found that the up-regulation of ZNRF1 activity by oxidative stress is sufficient to trigger neuronal degeneration and the prevention of phosphorylation-induced ZNRF1 activation protects neurons from both axonal degeneration and apoptosis (Wakatsuki et al., unpublished results).

Next, we discuss our recent findings, which show that ZNRF1 activation induces neuronal apoptosis.

8.4.1 ZNRF1 Is Activated by Oxidative Stress in Neurons

Application of 6OHDA has frequently been used as an inducer of oxidative stress in cultured neurons, as well as in animal tissues (Grunblatt et al. 2000; Blandini and Armentero 2012). To examine whether ZNRF1 is involved in oxidative stress-induced neuronal apoptosis, we assessed the expression levels of AKT in 6OHDA-treated primary cultured cortical neurons overexpressing wild-type ZNRF1, a dominant-negative form of ZNRF1 C184A, or under RNAi-mediated ZNRF1 down-regulation (Fig. 8.2). We found that AKT degradation is induced in 6OHDA-treated neurons. Importantly, AKT degradation was prevented by the overexpression of ZNRF1 C184A to an extent similar to the treatment of the proteasome inhibitor MG132, but was not affected by the overexpression of wild-type ZNRF1 (Fig. 8.2a, b). In neurons expressing shRNA for ZNRF1, we also observed that AKT expression levels are (relatively) maintained compared to the control (data not shown). These results suggest that ubiquitin ligase activity of ZNRF1 is induced by oxidative stress in neurons.

8.4.2 ZNRF1 Is Activated by Its Phosphorylation at Y103

As described previously, the ZNRF1 protein is widely and constitutively expressed in neurons during development as well as in adulthood (Araki and Milbrandt 2003). This suggests that, whereas we previously showed that ZNRF1–AKT–GSK3B–CRMP2 is a major signaling pathway in the promotion of Wallerian degeneration, ZNRF1 expression is not sufficient to induce Wallerian degeneration (Wakatsuki et al. 2011). It has been reported that some E3 ligases are not constitutively active, but are subject to regulation by their post-translational modifications including phosphorylation (Zhong et al. 2005; Gallagher et al. 2006). We therefore examined the possibility that ZNRF1 activity is regulated by its phosphorylation. Interestingly, ZNRF1 was phosphorylated at tyrosine residue(s) in SHSY5Y neuroblastoma treated with 6OHDA. Employing a web-based program that predicts potential tyrosine phosphorylation site(s) of ZNRF1 and the cognate kinases (NetPhos and NetPhosK, respectively), we found that Y103 can be phosphorylated by receptor tyrosine kinases including epidermal growth factor receptor tyrosine kinase (EGFR). Using antiserum against phosphorylated ZNRF1 at Y103 (ZNRF1 pY103), we found that ZNRF1 pY103 is induced by 6OHDA treatment in cultured cortical neurons in both dose- and time- dependent fashion (data not shown) and is also observed in in vivo settings, including a mouse model of 6OHDA-induced brain lesion (data not shown) and the MCAo model (Fig. 8.2d). To show that

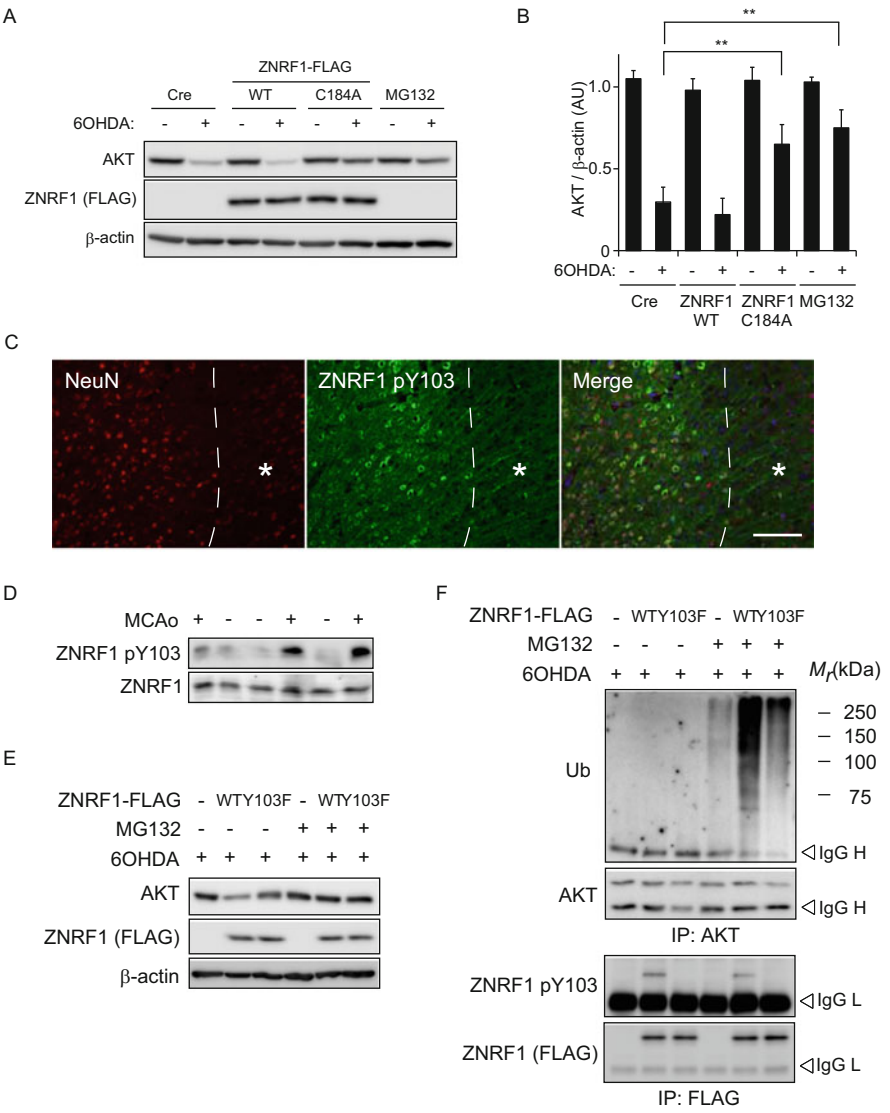

Fig. 8.2 Phosphorylation of ZNRF1 at Y103 in response to oxidative stress correlates with the up-regulation of its ubiquitin ligase activity toward AKT protein. (**a**, **b**) ZNRF1 expression promoted UPS-mediated AKT degradation in 6OHDA-treated neurons. Cultured cortical neurons were infected with adenovirus vector expressing myc-tagged wild-type ZNRF1 or dominant-negative ZNRF1 C184A mutant for 48 h and maintained with or without 20 μM 6OHDA for 3 h to induce neurotoxicity. After culture for 16 h in the presence or absence of proteasome inhibitor MG132, cell lysates were prepared from each culture and analysed by immunoblot analysis using antibodies against AKT or myc-tag (for ZNRF1). Cre only-expressing neurons or MG132-treated neurons served as negative and positive controls, respectively. β-actin served as the loading control. Representative immunoblot (**a**) and quantified expression levels for AKT normalized to β-actin are shown in **b** in comparison with control culture (Cre, +6OHDA) (mean ± SEM, five independent experiments). The asterisks indicate significant difference ($P < 0.001$) from control. (**c**) Phosphorylation of ZNRF1 at Y103 in neurons in the MCAo animal model. Representative

ZNRF1 pY103 is linked to its ubiquitin ligase activity, we examined AKT ubiquitination in 6OHDA-treated cultured cortical neurons overexpressing wild-type ZNRF1 or ZNRF1 Y103F mutant. We found that the expression level of AKT was significantly decreased in neurons expressing wild-type ZNRF1, but not in the ZNRF1 Y103F mutant (Fig. 8.2e, f). AKT ubiquitination, on the other hand, was increased in wild-type ZNRF1-expressing cells, but not in cells expressing the ZNRF1 Y103F mutant. These results suggest that ZNRF1 pY103 results in activation of the ubiquitin ligase activity. Importantly, EGFR inhibition by compound 56 - a potent inhibitor of EGFR - significantly inhibited ZNRF1 phosphorylation and AKT ubiquitination (data not shown). These results indicate that ZNRF1 is specifically phosphorylated at Y103 in neurons in response to oxidative stress, and that the oxidative stress-induced ZNRF1 pY103 is mediated by EGFR.

8.4.3 Inhibition of ZNRF1 Activation Protects Primary Cultured Neurons from Oxidative Stress-Induced Apoptosis

To examine whether activation of ZNRF1 by phosphorylation at Y103 in response to oxidative stress in neurons leads to apoptosis, we assessed the expression of cleaved caspase 3, a marker for prototypical apoptosis pathway activation, in 6OHDA-treated cultured cortical neurons overexpressing ZNRF1C184A or ZNRF1 Y103F. We found that 6OHDA-induced activation of the apoptosis signaling pathway was blocked by expression of these mutant forms of ZNRF1, suggesting that ZNRF1 activation in neurons by oxidative stress resulted in increased apoptosis signaling. To further analyze subcellular signaling downstream of ZNRF1, we examined the expression of cleaved caspase 3 in 6OHDA-treated

Fig. 8.2 (continued) photomicrographs for immunostaining of a neuron-specific marker NeuN or ZNRF1 pY103 on coronal sections after 4 h of MCAo. The dashed line indicates a border between ischemic penumbra and infarct core. Scale bar = 50 μm. Note that ZNRF1 pY103 immunoreactivity is observed in the penumbral neurons in sharp contrast with the absence of its immunoreactivity in the infarct core (asterisk). (**d**) Cell lysates were prepared from tissue sections of the ipsilateral (+) or the contralateral (−) striatum in MCAo animal model after 4 h of occlusion, and subjected to immunoblot analysis using antibodies against ZNRF1 pY103 or ZNRF1. (**e**, **f**) Wild-type (WT) or phosphorylation-resistant form of ZNRF1 Y103F mutant (Y103F) expressing cultured cortical neurons were treated with 20 μM 6OHDA for 3 h and maintained in the presence or absence of MG132 for 16 h. Cre only-expressing neurons served as a negative control (labeled as "-"). (**e**) Protein expression in 5 % input of cell lysates was confirmed by immunoblot analysis using antibodies against AKT or FLAG-tag (for ZNRF1) in a bottom panel. β-actin served as the loading control. (**f**) Cell lysates were subject to immunoprecipitation using antibodies against AKT, and the resultant immunoprecipitates were analyzed by immunoblot using antibodies against ubiquitin or AKT, respectively (*top*). With regard to ZNRF1-FLAG WT or Y103F, the immunoprecipitates using anti-FLAG antibody were analyzed by immunoblot using antibodies against FLAG-tag (for ZNRF1) or ZNRF1 pY103 (*middle*)

cultured cortical neurons overexpressing myristoylated form of AKT (myrAKT; a constitutively active form of AKT) or kinase-dead form of GSK3B (GSK3B K85M) (Fig. 8.3a, b). We found that expression of myrAKT and GSK3B K85M both prevented 6OHDA-induced apoptosis. These results suggested that AKT–GSK3B kinase cascade is involved in ZNRF1 activation-induced neuronal apoptosis signaling. Similar results were obtained in multiple different types of neurons, such as SHSY5Y neuroblastoma or the ventral mesencephalic neurons cultures, which is known to be rich in dopaminergic neurons (data not shown). These results suggest that oxidative-stress induced activation of ZNRF1 in neurons, results in apoptosis via ZNRF1-dependent proteasomal degradation of AKT, and resultant activation of GSK3B.

Next, we extended our previous identification of the role of ZNRF1, and showed that oxidative stress-induced activation of ZNRF1 turns on the downstream kinase cascade of AKT-GSK3B. Since our previous results showed that ZNRF1-induced degradation of AKT in proteasome constitutes the major signaling pathway in promoting Wallerian degeneration, we decided to examine whether the oxidative stress-induced activation of ZNRF1 plays a role in promoting Wallerian degeneration as well. To this end, we generated the in vitro Wallerian degeneration model using the same primary cultured dorsal root ganglion (DRG) neurons as we used in our previous work, and examined ZNRF1 Y103 in neurites before and after the induction of Wallerian degeneration (Fig. 8.3c, d). We found that ZNRF1 pY103 is highly induced by initiation of Wallerian degeneration. These data suggest that oxidative stress-induced phosphorylation of ZNRF1, turns on subcellular signaling to promote neuronal apoptosis, as well as Wallerian degeneration.

8.4.4 Inhibition of ZNRF1 Activation Protects Dopaminergic Neurons from Oxidative Stress-Induced Cell Death In Vivo

To assess whether the oxidative-stress induced activation of ZNRF1 in neurons also results in apoptosis in vivo, we examined 6OHDA-induced apoptosis in transgenic mice overexpressing ZNRF1 C184A. For this purpose we generated two independent transgenic (Tg) mice lines (lines 474 and 492) bearing ZNRF1 C184A-IRES-GFP cDNA, whose expression can be induced by Cre-mediated excision of loxP-flanked cassette. Using these Tg lines, we performed an intrastriatal injection of adenoviruses expressing Cre recombinase, and induced the transgene expression in the ipsilateral striatum, as confirmed by immunoblot or fluorescence microscopy analysis (Fig. 8.4a). Intrastriatal injections of 6OHDA produce degeneration of dopamine neurons in the substantia nigra (SN) and damage of their nerve endings in the striatum (Grunblatt et al. 2000; Cheng et al. 2011; Blandini and Armentero 2012). After injection of 6OHDA within ipsilateral striatum, the viability of neurons was determined by quantifying a marker for dopaminergic neurons,

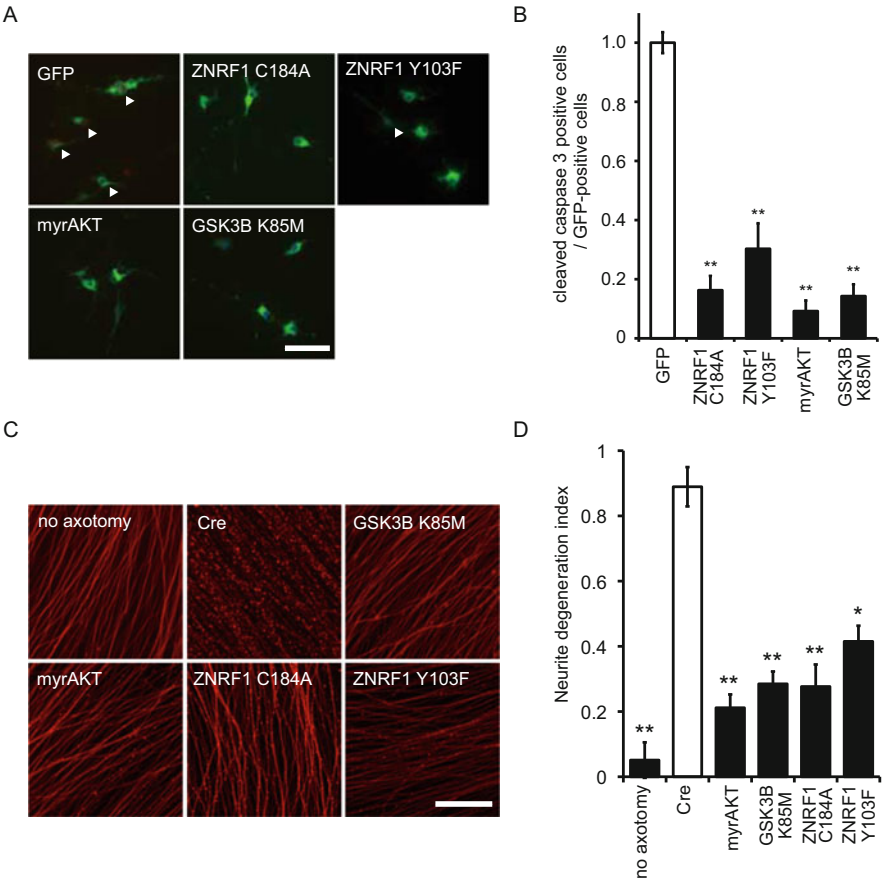

Fig. 8.3 Phosphorylation of ZNRF1 at Y103 is involved in the progression of Wallerian degeneration and oxidative stress-induced neuronal apoptosis. (**a**, **b**) Oxidative stress-induced neuronal apoptosis is not observed when ZNRF1 phosphorylation at Y103 is inhibited in cultured primary cortical neurons. Cleaved caspase 3 immunofluorescence was assessed to determine the effect of AKT, GSK3B, ZNRF1 or their mutant expression. Representative photomicrographs for cleaved caspase 3 immunostaining are shown in (**a**). Open arrowheads indicate cleaved caspase 3-positive cells. (**b**) The ratio of the cleaved caspase 3-positive cell number to total number of GFP-positive cells for each condition are shown (mean ± SEM, five independent experiments). The asterisks indicate significant difference ($P < 0.001$) from control (open bar, labeled as "GFP"). (**c**, **d**) Neurite protection effect by ZNRF1–AKT–GSK3B pathway molecules was assessed by *in vitro* Wallerian degeneration model using neurites from cultured DRG explant neurons overexpressing indicated proteins via adenoviral vector infection. Representative photomicrographs of degenerating neurites from neurons expressing indicated genes using adenoviral vectors at 24 h after initiation of Wallerian degeneration are shown in **c**. Scale bar = 25 μm. Neurite degeneration index values calculated for each condition at 24 h are shown in **d** (five independent experiments). The asterisks indicate significant difference ($*P < 0.05$ and $**P < 0.01$) from Cre-only expression control (labeled as "Cre")

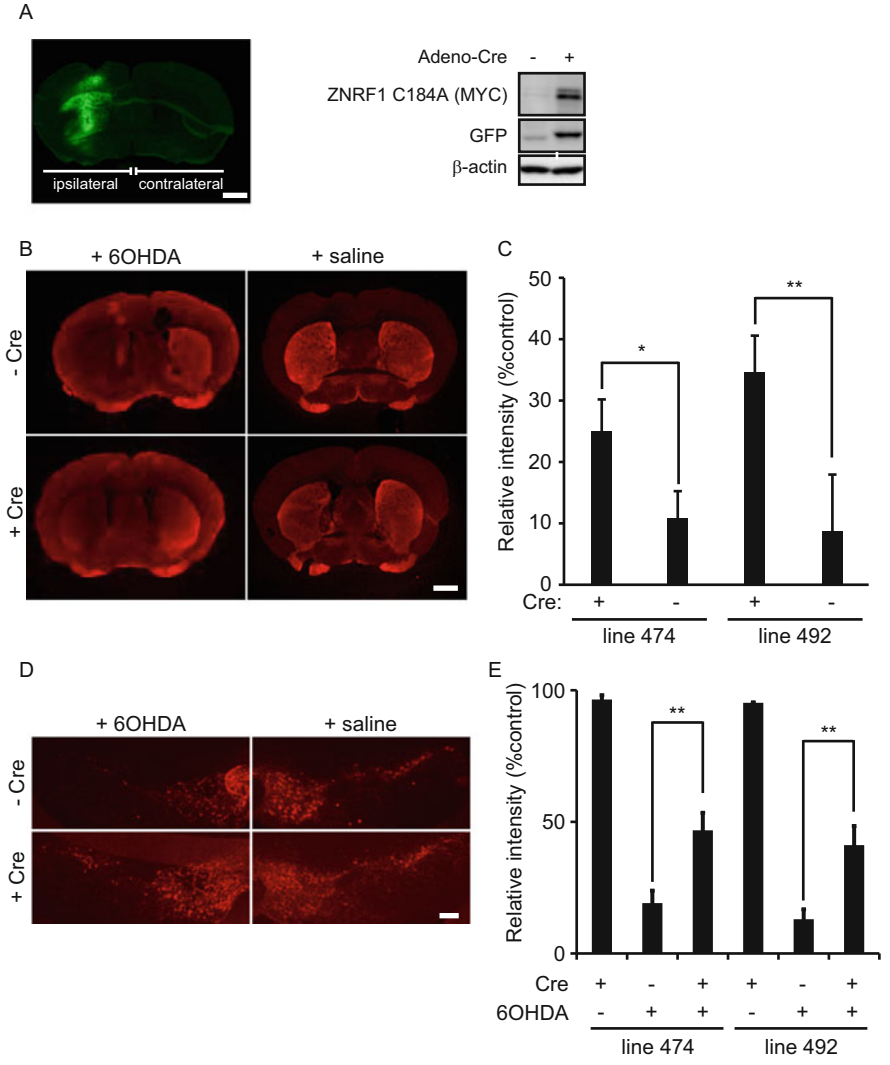

Fig. 8.4 Inhibition of ZNRF1 ubiquitin ligase activity preserves dopaminergic neurons in an *in vivo* 6OHDA-lesioned model. Unilateral intrastriatal injection of adenoviral vector for expression of Cre recombinase was performed to induce the expression of ZNRF1 C184A in the two independent Tg mice lines (lines 474 and 492) bearing ZNRF1 C184A-IRES-GFP cDNA, whose expression can be induced by Cre-mediated excision of loxP-flanked cassette. Cell lysates were prepared from tissue sections of the ipsilateral (+) or the contralateral (−) striatum 5 d after adenovirus injection and transgene expression levels were confirmed by immunostaining using anti-GFP antibody and immunoblot analysis using antibodies against myc-tag or GFP. Representative photomicrographs or immunoblots are shown in (**a**). β-actin served as a loading control. (**b**–**e**) Five days after induction of transgene expression, neuroprotective effect of ZNRF1 C184A expression was assessed by TH immunoreactivity at 7 d after 6OHDA injection in striatum (**b**, **c**), and SN (**d**, **e**). (**b**) Representative photomicrographs for immunostaining of TH on coronal sections

tyrosine hydroxylase (TH) immunoreactivity, and was then compared to the uninfected control. We found by quantification of TH-positive intensities within the striatum (Fig. 8.4b, c) and the SN (Fig. 8.4d, e) in the two independent Tg mice lines, that the expression of ZNRF1 C184A preserves dopaminergic neurons in a 6OHDA-lesioned model. These observations strongly suggest that inhibition of ZNRF1 ubiquitin ligase activity protects neurons against oxidative stress-induced apoptosis and Wallerian degeneration in vivo. Collectively, our present data indicate that the up-regulation of ZNRF1 activity by oxidative stress is sufficient to trigger neuronal degeneration, and prevention of phosphorylation-induced ZNRF1 activation protects neurons from both Wallerian degeneration and apoptosis.

8.5 Concluding Remarks and Future Directions

We have shown that ZNRF1 ubiquitin ligase functions as a critical mediator for the neurodegenerative pathways, Wallerian degeneration and neuronal apoptosis, by translating oxidative stress into subcellular signaling in neurons. ZNRF1 is likely phosphorylated at Y103 by EGFR in response to oxidative stress in neurons, although it is also possible that tyrosine phosphatase(s) involved in ZNRF1 de-phosphorylation is inhibited in response to oxidative stress, since protein tyrosine phosphatase activity is often negatively regulated by oxidation (Tonks 2006). Previously, we showed in Schwann cells that ZNRF1 ubiquitinates another substrate glutamine synthetase in injured nerves. The increased oxidative stress in de-differentiated Schwann cells of injured nerves, oxidizes proteins including glutamine synthetase (Saitoh and Araki 2010). The proteins destabilized by oxidization are subject to proteasomal degradation, suggesting that oxidative stress may also serve as an initiation signal for ZNRF1-dependent degradation. In Schwann cells, ZNRF1 expression is transcriptionally induced by the Schwann cell phenotypic change into its de-differentiated state to degrade glutamine synthetase. Here we found another mechanism for the regulation of ZNRF1 ubiquitin ligase activity by phosphorylation.

Enzymatic activity of some E3 ligases is subject to regulation by protein phosphorylation. For instance, an E3 ligase Itch protein alters its catalytic activity through its conformational change by JNK-mediated phosphorylation, to induce disruption of an intramolecular inhibitory interaction (Gallagher et al. 2006). Also,

Fig. 8.4 (continued) are shown. Scale bar = 500 μm. (**c**) Relative immunofluorescent intensities in ipsilateral to contralateral striatum for each condition in the two independent Tg lines are shown (mean ± SEM, three independent experiments). The asterisk indicates significant difference ($*P < 0.05$, $**P < 0.01$) from the no infection control. (**d**) Representative photomicrographs of immunostaining of TH on SN are shown. Scale bar = 250 μm. (**e**) Relative immunofluorescent intensities in ipsilateral to contralateral SN for each condition in the two independent Tg lines are shown (mean ± SEM, three independent experiments). The asterisks indicate significant difference ($**P < 0.01$) from the no infection control

phosphorylation of ZNRF2, another member of ZNRF family proteins, influences its interaction with the substrate, 14-3-3 protein and its association with intracellular membranes (Hoxhaj et al. 2012). In this context, phosphorylation of ZNRF2 at Ser 19, which is located outside the zing finger region responsible for its substrate binding, releases ZNRF2 from the plasma membrane to the cytosol, resulting in decreased catalytic activity. In contrast, phosphorylation of ZNRF1 at Y103, which also occurs outside the zinc finger domain, leads to the up-regulation of its catalytic activity. Thus far, we have found that this phosphorylation does not affect its ability to associate with AKT (data not shown), but the detailed mechanism of regulation of ZNRF1 activity remains unclear. Further studies are needed to determine whether phosphorylation of ZNRF1 at Y103 affects its intracellular localization and/or other mechanism(s) to modify the activity of ZNRF1.

Injury-induced axonal degeneration, and axonal elimination during development, are similar processes in the sense that the axons once connected to their targets are destroyed in both situations, however, the subcellular signaling behind each mechanism is not identical. For instance, overexpression of the X-linked inhibitor of apoptosis (XIAP) protein, which regulates caspase activity in degenerating axons, provides protection against axonal degeneration induced by trophic factor withdrawal, but the XIAP/caspase regulatory loop is dispensable for Wallerian degeneration (Unsain et al. 2013). On the other hand, expression of the Wlds protein is ineffective in delaying developmental axonal elimination (Hoopfer et al. 2006). Previously, we showed that inhibition of ZNRF1-mediated AKT degradation could give protection to trophic factor deprivation-induced neurite degeneration (Wakatsuki et al. 2011). These results suggest that subcellular signaling elicited by activation of ZNRF1 may be involved in the regulation of axonal degeneration, not only in neurodegenerative conditions, but also in normal neural development. Regulation of ZNRF1 activity may play a role in the pathogenesis of some neurodegenerative disorders, as well as in the neural circuit formation during development.

Can targeting axonal degeneration provide therapeutic benefits for neurodegenerative diseases? In *wlds* mice, WldS protein significantly delayed axonal degeneration in the PNS of disease models such as progressive motor neuronopathy (*pmn*) mice (Ferri et al. 2003) and myelin protein zero (*P0*) null mutants, which is a model of Charcot-Marie-Tooth disease (Table 8.1) (Meyer zu Horste et al. 2011). In the CNS, WldS protein also protects against both genetic and toxic insults, as well as transient global cerebral ischemia (Gillingwater et al. 2004). Thus, expression of WldS protein can protect axons from degeneration, not only in response to trauma, but also in metabolic, inflammatory, and hereditary neuropathies, as well as in neurodegenerative diseases (Coleman and Freeman 2010; Wang et al. 2012; Conforti et al. 2014). Interestingly, we showed that inhibition of ZNRF1 activation prevents neuronal apoptosis along with Wallerian degeneration by using an in vivo model of oxidative stress-induced neurodegeneration. This is in sharp contrast to the neuronal protection phenotype of *wlds* mice against 6OHDA-induced neurotoxicity, in which delayed Wallerian degeneration only, was observed (Cheng and Burke 2010). While the results obtained from *wlds* mice suggested that the

subcellular signaling promoting Wallerian degeneration is independent from neuronal cell death, our findings demonstrated that prevention of ZNRF1 activation may be a better strategy for protection, and that ZNRF1 activation may be a general self-destructive, pathologically relevant program of inducing neurodegeneration.

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Chapter 9
Drug Development for Neurodegenerative Diseases

Yoshitaka Nagai and Eiko N. Minakawa

Abstract Neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and the polyglutamine diseases, have been defined as a group of intractable disorders, which are characterized by the progressive degeneration of neurons in various regions of the brain, resulting in neurological and psychiatric symptoms. Molecular genetics and biological studies have revealed that most neurodegenerative diseases are caused by protein misfolding and aggregation, and hence they are considered to belong to the so-called protein misfolding diseases. Moreover, recent emerging evidence has suggested that the misfolded protein aggregates formed in these diseases have similar intrinsic characteristics, i.e., they are propagated by prion-like infectious mechanisms. Therefore, various therapeutic strategies targeting protein misfolding and aggregation are being extensively explored. Here we introduce emerging disease-modifying therapeutic approaches against neurodegenerative diseases, particularly those targeting the misfolding and aggregation of toxic proteins. The development of anti-misfolding and anti-aggregation agents that are commonly effective against a wide range of neurodegenerative diseases is eagerly anticipated in the near future.

Keywords Neurodegenerative diseases • Protein misfolding diseases • Alzheimer's disease • Parkinson's disease • Amyotrophic lateral sclerosis • Polyglutamine diseases • Protein misfolding • Aggregation • Amyloid • Disease-modifying therapy

9.1 Introduction

Neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), and spinocerebellar ataxias (SCAs), have been defined as a group of devastating intractable disorders characterized by the progressive and selective degeneration of neurons in

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K. Wada (ed.), *Neurodegenerative Disorders as Systemic Diseases*,
DOI 10.1007/978-4-431-54541-5_9

distinct regions of the central nervous system (CNS) in each disease. These diseases exhibit a variety of progressive neurological and psychiatric symptoms depending on the affected regions in each disease, such as cognitive and motor impairment, which typically appear in middle age. Since the pathogenesis of these diseases had remained poorly understood until recently, no effective therapies to prevent or delay these diseases have been established to date except for some symptomatic therapies. Pathological and biochemical studies have revealed a key clue in the pathogenesis of these diseases i.e., the depositions of proteinaceous aggregates/inclusions in patients' brains, such as senile plaques (composed of amyloid-β; Aβ) and neurofibrillary tangles (tau) in AD, Lewy bodies (α-synuclein; α-Syn) in PD, and ubiquitinated inclusions (TAR DNA-binding protein 43; TDP-43) in ALS, although it had been controversial until recently whether these aggregates are the cause or the result of these diseases. However, identification of causative genetic mutations in rare familial forms of neurodegenerative diseases and their subsequent molecular biological analyses have dramatically extended our understanding of their pathomechanisms. It is noteworthy that most such mutations in the disease-causative genes, such as Aβ precursor protein (APP), presenilin-1 (PS1), presenilin-2 (PS2), α-Syn, tau, superoxide dismutase 1 (SOD1), TDP-43, huntingtin, ataxins, etc., commonly render the mutant proteins prone to misfold and aggregate, or result in the overproduction of aggregation-prone proteins, which eventually leads to the accumulation of these aggregated proteins as inclusion bodies in the brain (Fig. 9.1) (Ross and Poirier 2005; Nagai and Popiel 2008). Protein inclusions composed of

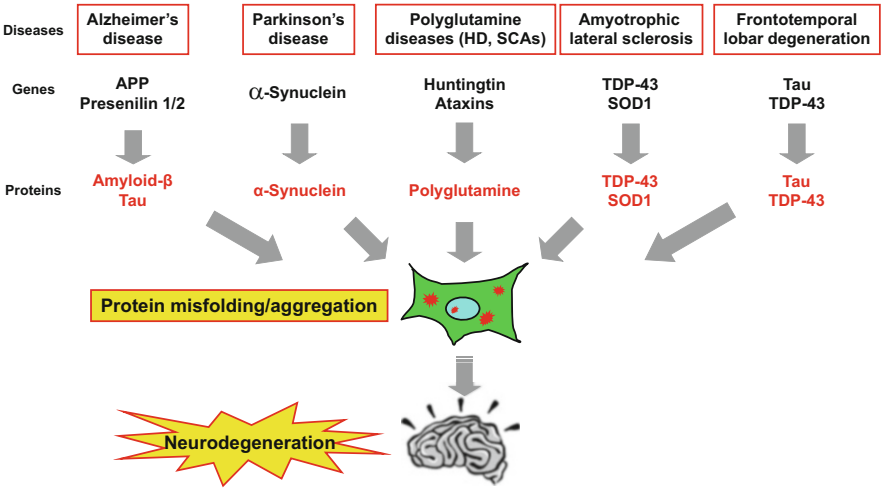

Fig. 9.1 Abnormal protein misfolding and aggregation as the common molecular pathogenesis of neurodegenerative diseases. Most of the genetic mutations responsible for the inherited neurodegenerative diseases commonly render the mutant proteins prone to misfold and aggregate, or result in the overproduction of aggregation-prone proteins, leading to their accumulation as inclusion bodies in the brain, eventually resulting in neurodegeneration. These facts strongly indicate that abnormal protein misfolding and aggregation cause neurodegeneration through common pathogenic mechanisms, and hence they are called protein misfolding diseases

these mutant proteins in the inherited neurodegenerative diseases are quite similar in general to those composed of the corresponding wild-type proteins observed in the sporadic diseases, suggesting that common pathomechanisms underlie both inherited and sporadic diseases.

One of the striking features shared by these protein aggregates is that they have common β-sheet-rich amyloid fibrillar structures, despite their distinct primary amino acid sequences. Accumulating evidence now suggests that rather than the insoluble mature amyloid fibrils, soluble pre-fibrillar intermediates of these proteins, such as β-sheet-rich misfolded monomers, oligomers, or protofibrils, are more toxic (Haass and Selkoe 2007). Furthermore, various experimental animal models expressing these mutant proteins have been established, using mice, flies, and worms, and have been proven to induce progressive neurodegeneration accompanied with protein aggregates/inclusions in the brain, faithfully recapitulating pathological features of the human diseases. These facts, taken together, strongly indicate that abnormal protein misfolding and aggregation cause neurodegeneration by common pathogenic mechanisms in these neurodegenerative diseases, and hence they are called conformational diseases or protein misfolding diseases (Fig. 9.1) (Carrell and Lomas 1997; Dobson 2003).

Although the detailed mechanisms by which these misfolded protein oligomers/aggregates cause neurodegeneration have remained unclear, the dysfunction of various cellular and neuronal processes has been proposed as being responsible for neurodegeneration, including mitochondrial dysfunction, endoplasmic reticulum stress, oxidative stress, inefficient protein degradation, transcriptional dysregulation, intracellular and axonal transport impairment, synaptic dysfunction, excitotoxicity, Ca^{2+} dysregulation, membrane damage, and the activation of caspases. Accordingly, various disease-modifying therapeutic approaches targeting each of these affected processes are currently being investigated. Among these therapeutic targets, protein misfolding and aggregation are considered to be the most ideal therapeutic targets since they are the earliest events in the pathogenic cascade, and hence inhibition of misfolding/aggregation is expected to widely suppress a broad range of downstream pathogenic changes. In this chapter, various emerging disease-modifying therapeutic approaches for neurodegenerative diseases, particularly those targeting the misfolding and aggregation of toxic proteins are introduced and reviewed. Problems that need to be solved during the development of molecular-targeted disease-modifying therapies are discussed, as are the future prospects of research in this area.

9.2 Alzheimer's Disease

Alzheimer's disease (AD) is the most common cause of degenerative dementia, which is pathologically characterized by senile plaques composed of Aβ and neurofibrillary tangles (NFTs) composed of tau. The prevalence of AD is estimated

to be more than 30 million people worldwide (5 million people in the United States), and the prevalence increases with age, being 5% of people over 65-years-old and about 30% of people over 85-years-old (Hebert et al. 2013). The estimated annual health-care cost was approximately $200 billion in the United States in 2012, and hence AD is a serious threat to human beings and countermeasures are urgently anticipated.

Since the discovery of mutations in either the APP, PS1, or PS2 gene in familial AD (FAD), most of which overproduce aggregation-prone Aβ42, the "amyloid hypothesis" considering the deposition of Aβ in the brain as the primary cause of AD pathogenesis, has been widely accepted (Hardy and Selkoe 2002; Hardy and Higgins 1992). Accordingly, a large amount of effort put into developing potential therapies for AD has focused on targeting the Aβ deposition. Other therapeutic approaches have also been investigated, for example those targeting tau, which is a major component of the NFTs that are believed to form as a downstream event of Aβ deposition. Although these therapeutic candidates still need to be validated in clinical trials, various promising approaches to combat this devastating disorder are in development as shown below (Table 9.1) (Ghezzi et al. 2013; Nygaard 2013).

Table 9.1 Potential disease-modifying therapies in clinical development for Alzheimer's disease

Drug	Stage	Outcome	Findings
γ-Secretase inhibitors			
Semagacestat	Phase III	Terminated	Worsening, skin cancers and infections
Avagacestat	Phase II	Terminated	Worsening
β-Secretase inhibitors			
LY2886721	Phase II/III	Terminated	Live toxicity
MK-8931	Phase II/III	Ongoing	
Active immunization			
AN1792	Phase II	Terminated	Meningoencephalitis
CAD106	Phase II	Ongoing	
Passive immunization			
Bapineuzumab	Phase III	Completed	Low efficacy
Solanezumab	Phase III	Completed	Low efficacy
Gantenerumab	Phase III	Ongoing	
Crenezumab	Phase II	Ongoing	
Aβ aggregation inhibitors			
Tramiprosate	Phase III	Completed	Low efficacy
PBT2	Phase II	Completed	Low efficacy
Scyllo-inositol	Phase II	Completed	Low efficacy
Tau aggregation inhibitors			
Methylene blue	Phase II	Completed	

9.2.1 *Reduction of Amyloid-β Production: γ- and β-Secretase Inhibitors*

Aβ is produced by the sequential cleavage of its precursor APP by two enzymes, namely β-secretase and γ-secretase (Fig. 9.2). β-secretase first cleaves APP at the extracellular cleavage site, and then γ-secretase cleaves the resultant C-terminal APP fragment within the transmembrane domain to generate several Aβ species of 36-43 amino acids. Among the Aβ peptides, the 40-amino-acid peptide (Aβ40) is the most abundant species, whereas the 42-amino-acid peptide (Aβ42) is less abundant but has a higher aggregation propensity. APP is also known to be cleaved by a third enzyme, α-secretase, within the Aβ sequence, resulting in no production of Aβ. Therefore, β-secretase and γ-secretase are considered as rational therapeutic targets to reduce Aβ production (Bignante et al. 2013).

9.2.1.1 γ-Secretase Inhibitors

γ-Secretase is a protease complex consisting of nicastrin, Aph-1, and Pen-2 in addition to presenilin, whose mutations cause FAD (Francis et al. 2002). Since γ-secretase is involved in the intramembranous cleavage of not only APP, but also other essential substrate proteins, such as Notch, ErbB4, and N-cadherin, simple inhibition of its enzymatic activity would possibly cause detrimental effects. Indeed, PS1 knockout mice were shown to result in embryonic lethality, with a deformed skeleton and impaired neurogenesis, owing to a defect of the Notch signaling pathway (Shen et al. 1997).

The first γ-secretase inhibitor that was developed and extensively evaluated in clinical studies was Semagacestat (LY450139), which was shown to decrease the

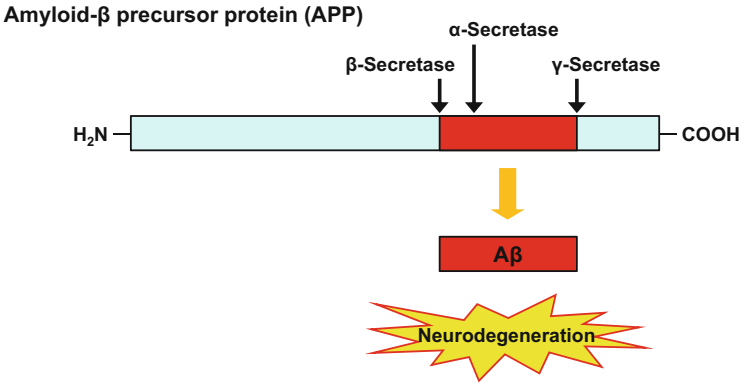

Fig. 9.2 Production of the amyloid-β peptide by cleavage from Aβ precursor protein. Aβ is produced by the sequential cleavage of APP by β-secretase, followed by γ-secretase. APP is also known to be cleaved within the Aβ sequence by α-secretase, resulting in no production of Aβ

Aβ production in the CNS and Aβ concentrations in plasma. Large Phase III trials assessing the clinical efficacy of Semagacestat in AD patients were terminated before their completion due to the worsening of cognitive function as well as other adverse effects, such as skin cancers and infections (Doody et al. 2013). These undesired events were thought to result from the inhibition of Notch signaling. Therefore, γ-secretase inhibitors that specifically inhibit the APP cleavage and spare the Notch cleavage, or the so-called γ-secretase modulators that only shift the γ-secretase cleavage position to produce the less aggregation-prone Aβ40 without affecting the Notch cleavage, have been extensively investigated. Although Avagacestat (BMS-708163), a γ-secretase inhibitor with a minimal effect on Notch signaling, showed a good safety and tolerability profile, and reduced the cerebrospinal fluid (CSF) concentrations of Aβ in a Phase I trial, a Phase II trial in AD patients again showed a trend toward clinical worsening and was thus terminated (Coric et al. 2012). The investigation of other Notch-sparing γ-secretase inhibitors, such as Begacestat (GSI-953) and CHF-5074, are still ongoing (http://www.clinicaltrial.gov).

9.2.1.2 β-Secretase Inhibitors

β-Secretase, also referred to as β-site APP cleaving enzyme 1 (BACE1), is an aspartyl protease, which initiates Aβ production by cleaving the extracellular domain of APP, in competition with α-secretase, before the intramembranous cleavage of the C-terminal fragments (Vassar et al. 1999). Although β-secretase also has other substrates, such as neuregulin 1, BACE1 knockout mice were initially reported to be viable and to show normal development and behavior with no overt phenotype, in contrast to PS1 knockout mice (Luo et al. 2001). Most importantly, BACE1 deficiency in APP transgenic mice has been shown to result in a dramatic reduction of cerebral Aβ levels and a rescue from Aβ-dependent memory deficits (Ohno et al. 2004), indicating BACE1 inhibition as a promising therapeutic strategy for AD without serious adverse effects.

After the successive failures of γ-secretase inhibitors in clinical trials, β-secretase inhibitors became the next focus of attention, as they were expected to have fewer off-target effects. LY2886721 is an orally available BACE1 inhibitor, which was shown to reduce cerebral Aβ concentrations in preclinical animal models. However, a Phase II trial of LY2886721 in patients with mild cognitive impairment or mild AD was recently terminated due to abnormalities in liver biochemical tests, although its administration resulted in dose-dependent decreases in both plasma and CSF Aβ40 concentrations. MK-8931 is a potent β-secretase inhibitor, which was shown to reduce Aβ levels in the CSF and brains of rodents and primates. Following the Phase I trial demonstrating the general safety of MK-8931 and its significant effect on decreasing CSF Aβ levels, two large Phase II/III randomized control trials (RCTs) are currently ongoing for mild-to-moderate AD patients.

9.2.2 *Removal of Amyloid-β: Active and Passive Immunization*

Various immunological therapeutic approaches against Aβ, including both active and passive immunization, have been actively investigated (Lannfelt et al. 2014). Antibodies against Aβ are thought to enter the brain to some extent, and to remove Aβ from preexisting amyloid plaques via microglial activation and subsequent phagocytosis (Bard et al. 2000). Aβ immunotherapy is also expected to act in the peripheral circulation to reduce Aβ levels, leading to the excretion of monomeric Aβ from the brain according to the equilibrium of Aβ between the peripheral blood and CNS, which is called the "peripheral sink hypothesis" (DeMattos et al. 2001). Aβ antibodies are also expected to directly interfere with the aggregation and further accumulation of Aβ in the CNS. The active immunization approach offers many advantages over passive immunization, in particular, a sustained antibody response by a limited number of vaccinations, and its lower cost. Its potential disadvantage is the variability in the antibody response among patients, including the occurrence of unexpected responses. On the other hand, the potential advantages of passive immunization are the reproducible and reliable delivery of therapeutic antibodies to the patient, and their rapid clearance. This also results in the disadvantage of the requirement for repeated antibody infusions over extended periods.

9.2.2.1 Active Immunization

The potential of Aβ immunotherapy for AD was first demonstrated by Schenk et al. in 1999. They vaccinated APP transgenic mice with Aβ itself, and found a significant reduction of cerebral Aβ plaques, and an amelioration of cognitive impairment (Schenk et al. 1999). However, the first Phase II clinical trial of active immunization using the AN1792 vaccine in AD patients was terminated because 18 of the 298 patients developed meningoencephalitis after 2–3 injections (Gilman et al. 2005). Long-term follow-up through clinical and postmortem neuropathological examinations performed on some of the patients, demonstrated a reduction of brain Aβ plaques in the immunized patients, but no significant differences in cognitive function between antibody responders and the placebo group (Holmes et al. 2008). These results clearly indicate the promising potential of Aβ immunotherapy to remove Aβ plaques, but also suggest that plaque removal may not prevent the progressive cognitive decline in AD.

Recently, a new vaccine called CAD106 was developed, which presents the N-terminal Aβ1-6 peptide epitope to drive a B-cell response and to avoid the T-cell activation that is responsible for meningoencephalitis. CAD106 was shown to successfully reduce Aβ deposition in APP transgenic mice. A Phase I clinical trial of CAD106 found it to be safe and well tolerated, with indications of an

antibody response to Aβ, a reduction in serum Aβ levels, and no signs of meningoencephalitis (Winblad et al. 2012).

9.2.2.2 Passive Immunization

Another solution to avoid undesired T cell-induced inflammation that can cause side effects, is to use passive immunization with humanized antibodies. The humanized monoclonal anti-Aβ antibody bapineuzumab, which binds both soluble and insoluble fibrillar Aβ, was shown to reduce the amyloid burden in APP transgenic mice (Bard et al. 2000). This was the first antibody to be tested in clinical trials, and was shown to be generally safe and well tolerated in Phase I/II studies (Wyeth, Madison, NJ, USA, and Élan Corporation, Dublin, Ireland). Recently, two large multicenter, randomized, double-blind, and placebo-controlled Phase III trials were conducted to assess the clinical efficacy of bapineuzumab in mild-to-moderate AD patients. In these trials, the intravenous administration of bapineuzumab resulted in reduced Aβ loads in the brain, detected by positron emission tomography using Pittsburgh compound B (PIB-PET) and reduced phosphorylated tau levels in the CSF of AD patients carrying the ApoE4 allele. However, this treatment failed to improve clinical outcomes, such as cognitive function, and also failed to meet the primary clinical endpoints in these patients (Salloway et al. 2014).

Solanezumab (LY2062430; Eli Lilly) is a newer humanized monoclonal antibody, which binds to an epitope in the central region of Aβ and preferentially targets soluble Aβ oligomers. Solanezumab treatment was shown to increase plasma Aβ concentrations, and to reverse memory deficits in APP transgenic mice (Dodart et al. 2002). In a Phase II trial, the safety and tolerability of solanezumab were shown, together with a dose-dependent increase in plasma and CSF Aβ levels. Two subsequent large Phase III clinical trials of solanezumab in patients with mild-to-moderate AD, however, failed to slow the cognitive decline, although CSF Aβ levels were increased after the treatment (Doody et al. 2014). Pooled data from both trials in mild AD patients revealed a significant slowing of cognitive decline after 78 weeks of treatment, indicating that treatment should be started earlier in the disease process for the maximum beneficial outcome. Eli Lilly and Co. has started a third Phase III clinical trial of solanezumab in patients with mild AD, in which they are also analyzing amyloid depositions by PET.

Gantenerumab (R1450; Hoffman-LaRoche) is a novel human anti-Aβ antibody that has been optimized for high affinity and preferential binding to a conformational epitope expressed on Aβ fibrils using phage display technology (Bohrmann et al. 2012). Gantenerumab treatment significantly reduced the brain Aβ plaques, and prevented new plaque formation without affecting plasma Aβ levels in APP/PS2 transgenic mice. A large Phase III clinical trial of gantenerumab is currently underway.

Crenezumab (MABT5102A; Genentech Inc.) is a novel human anti-Aβ antibody with an IgG4 backbone rather than an IgG1 backbone, which is expected to have a milder effect on microglia and should thus avoid side effects, such as

microhemorrhages and vasogenic edema. Genentech and the Banner Alzheimer's Institute, together with the National Institutes of Health (NIH) have started a prevention trial of Crenezumab in the large Colombian AD family with a PS1 mutation.

9.2.3 Amyloid-β Aggregation Inhibitors

According to the "amyloid hypothesis", the aggregation and subsequent deposition of Aβ peptides as amyloid plaques in the brain are thought to trigger the pathogenic cascade in AD (Hardy and Selkoe 2002; Hardy and Higgins 1992). Conformational changes and aggregation of Aβ are facilitated by its interaction with various biomolecules such as other proteins, lipids, and metals. Therefore, various therapeutic approaches to prevent Aβ aggregation, either by inhibiting its self-assembly, or its interaction with other biomolecules have been investigated as described below (Dasilva et al. 2010).

Soto and colleagues first developed the idea of using Aβ aggregation inhibitors as potential therapies for AD. The core amyloidogenic sequences of Aβ required for its self-assembly are reported to be the hydrophobic residues 16-20 (KLVFF) and residues 25-35. They designed Aβ-based peptide inhibitors, the so-called "β-sheet breaker peptides", by introducing a single proline substitution in the former core amyloidogenic region to abolish the β-sheet propensity of Aβ (Soto et al. 1996). These β-sheet breaker peptides were indeed shown to inhibit Aβ fibril formation and to disassemble preformed Aβ fibrils in vitro, and furthermore, were shown to reduce amyloid deposition, neuronal loss, and accompanying brain inflammation in Aβ-injected amyloidosis rats, and APP transgenic mice (Soto et al. 1998; Permanne et al. 2002). These studies clearly indicated that Aβ aggregation is one of the causes of neurodegeneration in the pathogenesis of AD, and that the inhibition of Aβ aggregation leads to the suppression of downstream pathogenic events.

Tramiprosate (3-amino-1-propanesulfonic acid/Alzhemed; Neurochem Inc.) is a glycosaminoglycan (GAG) mimetic that interferes with the binding of GAGs with the Aβ peptide, thereby preventing Aβ fibril formation. Tramiprosate treatment was reported to reduce the amyloid plaque load, as well as soluble and insoluble Aβ levels in the brain of APP transgenic mice (Gervais et al. 2007). Although tramiprosate was shown in a Phase II study to be safe and well tolerated, to cross the blood-brain barrier (BBB), and to dose-dependently reduce CSF Aβ levels, a large Phase III clinical trial in mild-to-moderate AD patients failed to meet the predefined primary endpoints. However, post-hoc analyses revealed its positive and significant effects on secondary endpoints, including the attenuation of hippocampal volume loss, as well as a trend toward the slowing of cognitive decline (Aisen et al. 2011), suggesting the potential of tramiprosate as a disease-modifying drug for AD.

PBT2, an 8-hydroxy quinolone derivative, works as a chelator of copper, zinc and iron, and thereby disrupts the aberrant interactions of these metals with Aβ.

Treatment of APP/PS1 transgenic mice with PBT2 resulted in a decrease in the brain Aβ levels and cognitive improvements (Adlard et al. 2008). In a Phase IIa clinical trial in early AD patients, the cognitive efficacy of PBT2 was limited to only two measures of executive function, although a dose-dependent and significant reduction in CSF Aβ42 levels was observed (Lannfelt et al. 2008).

Scyllo-inositol (cyclohexanehexol/AZD-103), a derivative of the natural glycolipid phosphatidylinositols, has been shown to interfere with Aβ aggregation by competing with phosphatidylinositol lipids for binding to Aβ, which is known to facilitate Aβ fibril formation. McLaurin and colleagues showed that oral administration of scyllo-inositol reduces brain Aβ levels and amyloid plaques, and improves cognitive deficits in APP transgenic mice (McLaurin et al. 2006). These therapeutic effects were still observed even when mice were treated after the onset of AD-like symptoms. However, a Phase II clinical trial of scyllo-inositol resulted in no evidence of clinical benefit, and only a decrease in the CSF Aβ levels was observed (Salloway et al. 2011). Therefore, the clinical efficacy of scyllo-inositol awaits validation in a larger Phase III study.

Epigallocatechin-3-gallate (EGCG) is a major polyphenolic component of green tea. Although much attention was paid to its anti-oxidant activity against Aβ-induced toxicity in earlier studies, EGCG was subsequently shown to bind directly to Aβ, thereby inhibiting its fibrillization, and instead, redirecting Aβ to assemble into non-toxic large spherical aggregates in vitro (Ehrnhoefer et al. 2008). EGCG also targets other aggregation-prone proteins, such as mutant huntingtin with a polyglutamine expansion, tau, α-Syn, prion, transthyretin (TTR), and islet amyloid polypeptide (IAPP) to inhibit their amyloid fibrillization. Administration of EGCG to APP transgenic mice was reported to reduce Aβ levels and amyloid plaque load, as well as cognitive impairment, indicating its therapeutic potential in vivo (Rezai-Zadeh et al. 2005).

Curcumin is a major constituent of the dietary spice turmeric, which has been reported to exert anti-oxidant and anti-inflammatory properties (Monroy et al. 2013). Curcumin was found to inhibit Aβ fibril formation and also to destabilize preformed Aβ fibrils in vitro (Ono et al. 2004). It was shown to bind directly to the fibrillar conformation of Aβ, and to also inhibit the aggregation of other amyloidogenic proteins including Aβ, α-Syn, prion, TTR and IAPP. In vivo studies showed that peripherally-injected curcumin crosses the BBB and labels amyloid plaques in APP transgenic mice, and its administration reduced amyloid plaque burden (Yang et al. 2005). In a Phase II clinical trial in mild-to-moderate AD patients, oral administration of curcumin was shown to be generally safe and well tolerated, but it failed to show any clinical or biomarker efficacy (Ringman et al. 2012), and the plasma curcumin levels were found to be very low. The major obstacle to the clinical application of curcumin is its poor bioavailability, mainly due to its insolubility, poor absorption, and rapid metabolism (Anand et al. 2007). Therefore, derivatives of curcumin with improved bioavailability and delivery, are highly anticipated due their role as potential therapeutic agents against these incurable diseases in the near future.

9.2.4 Degradation of Amyloid-β

Since the aggregation and subsequent deposition of Aβ in the brain are regarded as the primary culprits in the pathogenesis of AD (Hardy and Selkoe 2002), the degradation of Aβ, and not only its production, is thought to be one of the more attractive therapeutic targets for AD. In particular, a recent report showing that the clearance of Aβ, rather than its production, is altered in AD patients, has highlighted the importance of Aβ degradation (Mawuenyega et al. 2010). Although there are many enzymes capable of cleaving Aβ in vitro, only a few have been confirmed to be involved in degrading Aβ in vivo.

Neprilysin, a zinc-dependent metalloprotease, is the most extensively studied Aβ-degrading enzyme in the brain (Iwata et al. 2000). Genetic disruption of the neprilysin gene in mice confirmed its importance in the degradation of endogenous Aβ, as well as exogenously administered Aβ (Iwata et al. 2001). On the other hand, expression of neprilysin in APP transgenic mice, either by crossing them with neprilysin transgenic mice or by viral vector-mediated gene transfer, has been shown to significantly reduce brain Aβ levels and amyloid plaques, and to improve their life span (Leissring et al. 2003; Marr et al. 2003), indicating its therapeutic potential. It is noteworthy that the effect of environmental enrichment on reducing Aβ load in APP mice is closely correlated with the upregulation of neprilysin levels (Lazarov et al. 2005).

Insulin-degrading enzyme (IDE), a thiol metalloendopeptidase that degrades small peptides such as insulin and glucagon, is another protease that plays an important role in degrading Aβ in the brain (Qiu et al. 1998). Indeed, IDE knockout mice exhibited increased levels of endogenous Aβ in the brain, and developed hyperinsulinemia and glucose intolerance, which have also been proven to be risk factors for AD (Farris et al. 2003). The overexpression of IDE as well as neprilysin, has been shown to reduce the Aβ load and associated brain changes in APP transgenic mice (Leissring et al. 2003). Therefore, the discovery of these Aβ-degrading enzymes has revealed novel therapeutic opportunities for AD through the upregulation of their activities.

9.2.5 Tau Aggregation Inhibitors

From the failures of most Aβ-targeted disease-modifying therapies in demonstrating clinical efficacy for AD patients as described above, two major concerns have emerged. First, the time point of treatment initiation might be too late in the course of the disease. In most preclinical studies, therapeutic interventions were started before disease onset in genetically-engineered animals destined to develop the disease. On the other hand, AD-related pathological changes are considered to begin many years before the clinical onset of symptoms (Price and Morris 1999; Bateman et al. 2012), which might prevent the translation of preclinical results

obtained from animal models, to clinical efficacy in human AD patients. Another concern is the validity of the therapeutic target. Notably, although Aβ immunotherapies have succeeded in the prevention and removal of amyloid deposition in AD patients, they did not lead to an improvement of cognitive decline. While both amyloid plaques and NFTs are the characteristic pathological features of AD, NFT pathology is known to be more closely correlated with the severity of clinical symptoms of AD than amyloid plaques (Arriagada et al. 1992). Furthermore, the reduction of endogenous tau levels was reported to ameliorate cognitive deficits without affecting the Aβ pathology in APP transgenic mice (Roberson et al. 2007). These facts challenge the current amyloid cascade hypothesis for AD pathogenesis. In addition, the discovery of tau mutations in frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17), another form of degenerative dementia, highlighted the significant role of tau in the pathogenesis, which is solely sufficient to cause neurodegeneration in the absence of amyloid pathology (Hutton et al. 1998; Spillantini et al. 1998). Therefore, an alternative therapeutic target, namely tau, the key component of NFTs, is currently being considered and intensively investigated (Giacobini and Gold 2013).

Tau is a member of the microtubule-associated protein family that is involved in the assembly and stabilization of microtubules. It is predominantly expressed in neurons, particularly in their axons, to maintain neuronal morphology and axonal transport. Six tau isoforms are produced via alternative mRNA splicing, which mainly differ in their number of three or four microtubule binding repeats, and all are expressed in the adult human brain. In the AD brain, tau is abnormally hyperphosphorylated and aggregated into NFTs. Hyperphosphorylation of tau is known to reduce the binding ability of tau to microtubules, similar to its FTDP-linked mutations (Hong et al. 1998). However, whereas the hyperphosphorylation of tau is reported to promote its self-assembly into NFTs (Alonso et al. 2001), tau itself can assemble into β-sheet-rich filaments through its microtubule binding repeat. Among the various aggregated species, tau oligomers are believed to be toxic, leading to synaptic dysfunction (Lasagna-Reeves et al. 2011). Thus, therapeutic approaches to inhibit tau aggregation are currently being investigated.

The first tau aggregation inhibitor reported was the phenothiazine methylene blue (MB), which blocks tau-tau interactions through the microtubule-binding repeat domain (Wischik et al. 1996). Administration of MB was shown to reduce the soluble tau levels, but not pre-existing NFTs, resulting in an improvement of cognitive deficits in tau transgenic mice (O'Leary et al. 2010). A Phase II clinical trial of MB in mild-to-moderate AD patients was conducted and reported to have beneficial effects (Schirmer et al. 2011). Detailed reports on this Phase II study are anticipated, and Phase III trials with a new form of this compound are now underway (Wischik et al. 2014).

9.2.6 Tau Immunotherapy

Recent studies have indicated that tau aggregates can be secreted from neurons, and extracellular tau aggregates contribute to the neuron-to-neuron propagation of tau pathology via a prion-like transmission mechanism (Clavaguera et al. 2009; Frost et al. 2009). Therefore, therapeutic approaches to remove extracellular tau aggregates by immunotherapy are under investigation.

The first active immunization study using recombinant human tau with no phosphorylation resulted in the induction of tauopathy-like pathologies and encephalomyelitis with neurological deficits in mice, indicating the potential risk of using unphosphorylated tau to elicit an autoimmune response (Rosenmann et al. 2006). However, subsequent active immunization studies using phosphorylated tau have succeeded in reducing tau pathology and improving cognitive deficits in mutant tau transgenic mice (Asuni et al. 2007). Passive immunization studies using either phosphorylated tau antibodies or a conformation-dependent tau antibody, have also demonstrated significant improvements in motor deficits and tau pathology in mutant tau transgenic mice (Boutajangout et al. 2011; Chai et al. 2011). Taken together, these results indicate the potential of tau immunotherapy as a therapeutic approach for AD and related tauopathies.

9.2.7 Tau Phosphorylation Inhibitors

The hyperphosphorylation of tau is believed to be one of the most critical steps in tau-mediated neurodegeneration, although it is still controversial as to whether phosphorylation of tau triggers its aggregation, or is just a consequence of its deposition. Therefore, kinases and phosphatases involved in tau phosphorylation and dephosphorylation, respectively, are regarded as therapeutic targets for AD and other tauopathies. Although several kinases, such as glycogen synthase kinase 3 β (GSK-3β), cyclin dependent kinase 5 (CDK5), casein kinase 1 (CK1), and mitogen activated protein kinases (MAPKs) have been reported to phosphorylate tau in vitro, GSK-3β is believed to be the most promising candidate tau kinase because it is activated by Aβ and is essential for Aβ-induced neurotoxicity, thus linking Aβ to tau phosphorylation.

Administration of the GSK-3 inhibitor lithium chloride has been shown to reduce the levels of tau phosphorylation and insoluble aggregation, as well as the degree of axonal degeneration in mutant tau transgenic mice (Noble et al. 2005). Accordingly, small short-term clinical trials of lithium chloride have been conducted in mild-to-moderate AD patients, resulting in no beneficial effect on cognitive impairment, but a significant decrease in CSF phosphorylated tau levels (Forlenza et al. 2011; Hampel et al. 2009). Tideglusib (NP-12), a novel specific GSK-3 inhibitor, was tested in double-transgenic mice co-expressing human mutant APP and tau, resulting in decreased tau phosphorylation and Aβ deposition,

as well as the prevention of memory deficits (Sereno et al. 2009). Currently, phase II clinical trials of tideglusib in patients with AD and progressive supranuclear palsy (PSP), one of the tauopathies, are ongoing.

Another approach to inhibit tau hyperphosphorylation is to activate the protein phosphatases that dephosphorylate tau. Protein phosphatase 2A (PP2A) was reported to be the main phosphatase involved in tau phosphorylation in the brain (Goedert et al. 1992; Gong et al. 2000). Sodium selenate was shown to increase PP2A activity, to prevent tau hyperphosphorylation and neurodegeneration, and to improve motor performance in mouse models of tauopathy (van Eersel et al. 2010).

9.3 Parkinson's Disease

Parkinson's disease is the second most common neurodegenerative disorder after AD, and is the most common movement disorder that causes progressive motor symptoms such as rest tremor, rigidity, bradykinesia, gait disturbance and postural instability (de Lau et al. Lancet Neurol 2006). It has recently been recognized that patients also exhibit various non-motor symptoms including olfactory deficit, depression, pain, sleep disturbance and autonomic dysfunction (Chaudhuri et al. Lancet Neurol 2009). The pathological hallmark of PD is the selective loss of dopaminergic neurons in the substantia nigra pars compacta, accompanied by Lewy bodies and Lewy neurites, both of which are distinctive intraneuronal inclusions (Lees et al. Lancet 2009). The main component of these inclusions is the misfolded and aggregated form of α-Syn, which is at least partially post-translationally modified by phosphorylation. Treatments currently available for PD are limited to symptomatic therapies such as dopamine replacement, which only partially improves symptoms, and no effective disease-modifying therapy has been developed to date.

Since the discovery of both missense and multiplication mutations in the α-Syn gene as causes of familial PD and dementia with Lewy bodies (DLB), and the identification of single-nucleotide polymorphisms (SNPs) in the α-Syn gene as a risk factor of sporadic PD, α-Syn has been suggested to play a critical role in the pathogenesis of PD and related synucleinopathies. Indeed, experimental over-expression of α-Syn was shown to mimic several aspects of PD in transgenic animals, such as motor dysfunction, α-Syn aggregation/accumulation, and neuro-degeneration. Importantly, familial PD-linked mutations in α-Syn were shown to accelerate its aggregation as well as neurotoxicity. Furthermore, aggregated α-Syn has recently been shown to be secreted from cells, and is believed to be transmitted from neuron to neuron via prion-like mechanisms, thus contributing to the progressive spread of pathology (Jucker and Walker 2013). Therefore, similar to the approaches against AD, various therapeutic approaches targeting protein misfolding and aggregation of α-Syn have been investigated against PD and the other synucleinopathies, although most of them are still at preclinical experimental stages.

In 2001, Masliah and colleagues first demonstrated that β-synuclein (β-Syn) acts as an inhibitor of α-Syn aggregation, and that overexpressed β-Syn ameliorated motor deficits and neurodegeneration in α-Syn transgenic mice (Hashimoto et al. 2001), although β-Syn itself was later found to be linked to DLB, and could possibly induce neurotoxicity. El-Agnaf and colleagues designed a synthetic peptide derived from the hydrophobic non-Aβ component domain of α-Syn, which has been shown to prevent α-Syn aggregation and toxicity (El-Agnaf et al. 2004).

Various small chemical compounds, including baicalein, EGCG, curcumin, rifampicin, etc., have been discovered to show inhibitory activities on α-Syn aggregation in vitro, although most of them still remain to be evaluated for their therapeutic effects in vivo (Ono et al. 2008; Ono and Yamada 2006). Among these, the flavinoid compound baicalein was shown not only to inhibit the fibrillation of α-Syn, but also to disaggregate its preformed fibrils (Zhu et al. 2004). EGCG was reported to modify the fibrillogenesis pathway of α-Syn, as in Aβ (Ehrnhoefer et al. 2008). More recently, EGCG was found to delay the climbing dysfunction and to reduce oxidative stress in a PD *Drosophila* model expressing α-Syn (Siddique et al. 2014a). Curcumin was also shown to delay the decrease in activity, to reduce oxidative stress, and to extend the life span in α-Syn expressing PD flies (Siddique et al. 2014b), suggesting that multiple mechanisms may be involved in its therapeutic effects (Monroy et al. 2013). The macrocyclic antibiotic rifampicin is an inhibitor of α-Syn aggregation (Li et al. 2004) that is currently being developed for clinical evaluation. Rifampicin treatment was reported to suppress α-Syn aggregation and its associated neurodegeneration in a mouse model of multiple system atrophy (MSA), a synucleinopathy, which expresses α-Syn in oligodendrocytes (Ubhi et al. 2008). However, a clinical trial of rifampicin in MSA patients was recently terminated because of no significant clinical efficacy (Low et al. 2014).

As with immunological therapeutic approaches for AD, various immunotherapies targeting α-Syn have been extensively investigated. The first study was reported by Masliah and colleagues, in which vaccination of α-Syn transgenic mice with recombinant human α-Syn resulted in the production of high affinity antibodies accompanied with a decreased accumulation of aggregated α-Syn and reduced neurodegeneration (Masliah et al. 2005). Passive immunization with exogenously administered human α-Syn antibodies has been shown to prevent the neuron-to-astroglia transmission of α-Syn, and to ameliorate neurodegeneration and behavioral deficits in α-Syn expressing mice (Bae et al. 2012). Among the various immunotherapies targeting α-Syn, active immunization by vaccination with AFFITOPE® PD01 (Affiris, Vienna, Austria) is currently being evaluated in a Phase I clinical study for PD patients. This antigen consists of a peptide carrier conjugate, with aluminium hydroxide as the immunological adjuvant, and targets aggregated α-Syn but not β-Syn. AFFITOPE® PD01 has been shown to reduce the level of α-Syn deposits in the brain, to decrease neuronal loss, and to improve the behavioral deficit in the Morris Water Maze test in two independent PD mouse models (Schneeberger et al. 2012).

Phosphorylation of α-Syn has been implicated to be linked to the pathology of PD, because α-Syn is hyperphosphorylated, particularly at serine 129 (Ser129), in

Lewy bodies of PD patients and animal models overexpressing α-Syn (Braithwaite et al. 2012). Although several functional consequences of α-Syn phosphorylation have been reported from in vitro studies, the dephosphorylation of α-Syn at Ser129 in α-Syn transgenic mice by the upregulation of phosphoprotein phosphatase 2A (PP2A), was associated with reduced α-Syn aggregation, improved dendritic arborization and neuronal activity, and amelioration of motor deficits (Lee et al. 2011).

Although our understanding of the pathogenesis of PD has greatly increased in the past 20 years, no agents have yet been proved to alter its disease course to stop or slow its clinical progression. Further research toward the development of disease-modifying therapies for PD is anticipated.

9.4 Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is the most common adult form of motor neuron disease, affecting the upper and lower motor neurons in the brain and spinal cord. Patients develop progressive muscle weakness, typically leading to death, mainly due to respiratory failure within three to five years from disease onset. Similar to AD and PD, a key pathological characteristic of ALS is the presence of cytoplasmic inclusions/aggregates in degenerating motor neurons. The predominant form of aggregates are ubiquitinated and classified as Lewy body-like inclusions, while ubiquitin-negative eosinophilic inclusions called Bunina bodies, are also found.

Although *SOD1* was the first gene identified as a causative gene for familial ALS (FALS), mutations in the *SOD1* gene account for only 20% of FALS patients, and the inclusions found in the vast majority of sporadic and FALS patients are SOD1-negative. Nevertheless, most SOD1 mutants were found to be prone to misfold and aggregate, which is associated with neurotoxicity in cellular and animal models, and to be accumulated in SOD1-linked FALS patients, suggesting that these diseases can also be categorized as protein misfolding diseases (Shaw and Valentine 2007). Lansbury and colleagues performed *in silico* screening for small molecules that would stabilize the SOD1 dimer. They successfully identified 15 chemical compounds that inhibit the aggregation of FALS-linked SOD1 mutants in vitro, as potential lead compounds against FALS (Ray et al. 2005).

A significant breakthrough was achieved in 2006, when TDP-43 was identified as a major component of the ubiquitinated inclusions in the majority of patients with sporadic ALS, and tau-negative frontotemporal lobar degeneration (FTLD) (Neumann et al. 2006). Subsequently, mutations in the *TDP-43* gene were identified as genetic causes of FALS (Sreedharan et al. 2008). Importantly, TDP-43 has a glutamine/asparagine-rich domain with a strong propensity to aggregate at the C-terminus, where most FALS-linked mutations are located, and indeed, mutations in TDP-43 were shown to accelerate its aggregation (Guo et al. 2011; Johnson et al. 2009). These facts strongly indicate that aggregation of TDP-43 is the initial

event in the pathogenic cascade of ALS and FTLD, which are collectively called the TDP-43 proteinopathies.

Research toward counteracting TDP-43 aggregation using similar approaches to those used in AD and PD has been initiated in the past several years, and their therapeutic effects still remain to be evaluated in in vivo models. Methylene blue (MB) and dimebon have been reported to reduce the formation of TDP-43 aggregates in cell culture (Yamashita et al. 2009). However, the administration of MB has failed to show any protective effects in two transgenic mouse models of ALS expressing either mutant SOD1 (G93A) or mutant TDP-43 (G348C) (Audet et al. 2012). Further intensive research toward understanding the molecular basis of ALS will hopefully lead to the establishment of effective therapeutic strategies to overcome this devastating disease in the near future.

9.5 Polyglutamine Diseases

The polyglutamine (polyQ) diseases are a group of inherited neurodegenerative diseases, and are caused in common by an expansion mutation of the glutamine-encoding CAG repeat (>35–40 repeats) in the various disease-causative genes. So far nine diseases, including Huntington's disease (HD), spinocerebellar ataxia (SCA) type 1, 2, 3, 6, 7 and 17, dentatorubral pallidoluysian atrophy (DRPLA), and spinobulbar muscular atrophy (SBMA), have been found to belong to this group (Table 9.2) (Bauer and Nukina 2009; Nagai and Popiel 2008; Orr and Zoghbi 2007). It is important to note that the disease-causative proteins have neither sequence homology nor any functional similarities, except for the polyQ stretch itself, the size of which tightly correlates with disease severity. Furthermore, these diseases (except for SBMA) are inherited in an autosomal dominant manner. These unique genetic characteristics strongly indicate that the polyQ diseases are caused by gain of toxic function mechanisms triggered by the expanded polyQ stretch itself (Nagai and Popiel 2008). Indeed, expression of the expanded polyQ peptide alone, or a polyQ stretch fused with non-native proteins, has been proven to cause neurodegeneration in experimental animal models.

In the common molecular pathogenesis of the polyQ diseases, expansions of the polyQ stretch were shown to induce misfolding of the host proteins, leading to the formation of insoluble β-sheet-rich aggregates/inclusion bodies in affected neurons, eventually resulting in neurodegeneration. Although it still remains controversial as to whether insoluble inclusion bodies are neurotoxic, protective, or just resultant by-products, soluble β-sheet-rich misfolded monomers and oligomers have been demonstrated to trigger cytotoxicity (Nagai et al. 2007; Schaffar et al. 2004; Takahashi et al. 2008). Therefore, the misfolding and aggregation of expanded polyQ proteins are considered to be ideal therapeutic targets, as they are the most upstream events in the pathogenic cascade, and hence inhibition of misfolding/aggregation is expected to widely suppress various downstream pathogenic events

Table 9.2 The polyglutamine diseases

Diseases	Gene	CAG repeat		Affected regions
		Normal	Disease	
Spinobulbar muscular atrophy (SBMA)/Kennedy's disease	androgen receptor	9–36	38–65	Brainstem, spinal cord
Huntington's disease (HD)	huntingtin	6–35	36–180	Caudate nucleus, putamen, cerebral cortex
Spinocerebellar ataxia type 1 (SCA1)	ataxin-1	6–39	39–83	Cerebellar cortex, dentate nucleus, brainstem, cerebral cortex
Spinocerebellar ataxia type 2 (SCA2)	ataxin-2	14–32	32–200	Cerebellar cortex, brainstem, cerebral cortex, peripheral nerves
Spinocerebellar ataxia type 3 (SCA3)/Machado-Joseph disease	ataxin-3	12–41	55–84	Dentate nucleus, basal ganglia, brainstem, spinal cord
Spinocerebellar ataxia type 6 (SCA6)	α1A calcium channel	4–19	20–33	Cerebellar cortex
Spinocerebellar ataxia type 7 (SCA7)	ataxin-7	4–35	37–306	Cerebellum, brainstem, retina
Spinocerebellar ataxia type 17 (SCA17)	TATA-binding protein	25–44	46–63	Cerebellum, cerebral cortex, basal ganglia
Dentatorubral-pallidoluysian atrophy (DRPLA)	atrophin-1	6–36	49–88	Dentate and red nuclei, cerebellum, cerebral cortex, brainstem

(Herbst and Wanker 2006; Michalik and Van Broeckhoven 2003; Nagai and Popiel 2008).

9.5.1 Intrabodies

Intracellular antibodies, or so-called intrabodies that recognize the expanded polyQ protein have been applied to inhibit its aggregation. The first reported intrabody, C4, was identified from a single-chain Fv phage-display library by Messer and colleagues in 2001, which recognizes the N-terminal region adjacent to the polyQ stretch of huntingtin (Htt). Indeed, they showed that expression of C4 in cell culture potently inhibits the formation of polyQ-expanded mutant Htt inclusions (Lecerf et al. 2001). Furthermore, they successfully demonstrated that the expression of C4 suppresses neurodegeneration in a *Drosophila* model of HD (Wolfgang et al. 2005), and its viral vector-mediated expression delays Htt aggregation in the brain of HD model mice (Snyder-Keller et al. 2010).

Other intrabodies include V_L 12.3, which recognizes the N-terminal region of Htt (Colby et al. 2004), and MW7 and Happ1, both of which recognize the proline-

rich region (PRR) of Htt (Khoshnan et al. 2002; Southwell et al. 2008). V_L 12.3 was shown to suppress Htt aggregation and cytotoxicity in yeast, although it failed to show any beneficial effects on HD mouse models upon viral vector-mediated expression (Southwell et al. 2009). On the other hand, Happ1 was shown to promote the turnover of mutant Htt, and Happ1 expression successfully suppressed Htt aggregation, and improved motor and cognitive deficits in HD mouse models (Southwell et al. 2008, 2009). Viral vector-mediated expression of another intrabody EM48, which is thought to recognize a C-terminal epitope of human Htt exon1, was also shown to suppress Htt neuropil aggregation and to ameliorate neurological symptoms in a mouse model of HD (Wang et al. 2008).

The application of intrabodies appears to be an attractive approach with regard to their high binding affinity to the disease-causative proteins. However, most of these intrabodies cannot be applied to polyQ diseases other than HD, and may possibly exert unfavorable effects on the normal Htt protein, because intrabodies that specifically recognize the expanded polyQ stretch itself have not been identified so far. The most critical issue is their delivery into the human brain, as they currently require to be expressed via gene delivery due to their large size.

9.5.2 Peptides

Peptides have potential as therapeutic molecules since they are of relatively low molecular weight, and are therefore expected to be delivered to the target organ by their administration (Takeuchi et al. 2014). Indeed, various peptides-based therapeutic approaches have been applied to inhibit protein aggregation, and have been reported to exert therapeutic effects in cell culture and animal models of other neurodegenerative diseases, such as AD and PD (El-Agnaf et al. 2004; Soto et al. 1998).

Based on the hypothesis that peptides with selective binding affinity to the expanded polyQ stretch are expected to interfere with the conformational changes and aggregation of polyQ proteins, we screened combinatorial peptide phage display libraries. We successfully identified polyQ binding peptide 1 (QBP1: SNWKWWPGIFD), which selectively binds to the abnormally expanded polyQ stretch with a dissociation constant of 5.7 μM (Nagai et al. 2000; Okamoto et al. 2009; Popiel et al. 2013), and showed that QBP1 indeed inhibits the toxic β-sheet conformational transition and aggregation of the expanded polyQ protein in vitro (Nagai et al. 2007, 2000). We also found that expression of QBP1 significantly suppresses polyQ oligomer formation and polyQ-induced cytotoxicity in cell culture (Nagai et al. 2000; Takahashi et al. 2008, 2007), and suppresses polyQ-induced neurodegeneration in *Drosophila* models of the polyQ diseases (Nagai et al. 2003). We further demonstrated that QBP1 conjugated with protein transduction domains (PTD-QBP1) is efficiently delivered into cultured cells, and its oral administration successfully suppresses polyQ-induced premature death in *Drosophila*, indicating the potential of PTD-QBP1 as a therapeutic molecule (Popiel

et al. 2007). We further investigated the therapeutic potential of PTD-QBP1 on a polyQ diseases mouse model; however, repeated intraperitoneal injection of PTD-QBP1 resulted in only a modest improvement of body weight loss, with no improvement in the motor phenotypes, nor suppression of inclusions in the brains, probably due to its low BBB permeability (Popiel et al. 2009). Therefore, one of the future directions is obviously to design low molecular weight chemical analogs of QBP1 with high BBB permeability (Popiel et al. 2013; Tomita et al. 2009).

Kazantsev *et al*. designed a bivalent peptide consisting of two short polyQ stretches connected by an α-helix spacer, and showed that this suppressor peptide delays polyQ inclusion formation and neurodegeneration in cell culture and fly models (Kazantsev et al. 2002). However, its binding selectivity to the expanded polyQ stretch was not well characterized, and short polyQ stretches are reported to be recruited into expanded polyQ protein aggregates, and possibly to accelerate the aggregation and cytotoxicity of the expanded polyQ protein under certain conditions (Slepko et al. 2006). On the other hand, Chen *et al*. also employed a combinatorial screening approach to identify potential polyQ aggregation inhibitors from a library consisting of peptoids (polymers of N-substituted glycines), which have advantages in their stability, cell permeability, and structural diversity (Chen et al. 2011). They selected the peptoid HQP09, and found that it effectively inhibits polyQ aggregation in vitro. Interestingly, HQP09 did not show any competition for binding to the polyQ stretch with QBP1, possibly suggesting that these two inhibitors recognize the expanded polyQ stretch in a different manner. Importantly, they performed a structure-activity relationship study to determine the pharmacophore of HQP09, and developed the minimal derivative peptoid HQP09_9 (4-mer, MW = 585). Whereas subcutaneous injection of HQP09_9 failed to exert therapeutic effects on a mouse model of HD, probably due to its poor BBB permeability, intracerebroventricular injection of HQP09 resulted in reduced Htt inclusions and improved motor behavioral outcomes of HD mice.

9.5.3 Small Chemical Compounds

As described above, there has been abundant experimental evidence that suppressing misfolding and aggregation of the expanded polyQ protein would lead to therapeutic effects on animal models of the polyQ diseases. Therefore, in developing clinically applicable pharmacological therapies for the polyQ diseases, searching for small chemical compounds that inhibit polyQ protein aggregation, has been extensively conducted (Herbst and Wanker 2006).

The first report on small chemical compounds with inhibitory activities for polyQ aggregation was published by Wanker and colleagues, in which the amyloid-binding azo-dye Congo red and some other chemicals, were shown to inhibit aggregation of the polyQ-expanded Htt protein in vitro (Heiser et al. 2000). However, since Congo red does not efficiently cross the BBB (Frid et al. 2007), its therapeutic effects on polyQ disease mice, and clinical prospects for human patients

remain controversial (Sanchez et al. 2003; Wood et al. 2007). Wanker's group further established an automated high-throughput screening system, and identified approximately 300 polyQ aggregate inhibitors from a large-scale chemical compound library (~184,000 compounds provided by Merck) (Heiser et al. 2002). Among these hit chemical compounds, they showed that some benzothiazoles including PGL-135, efficiently inhibit Htt aggregation both in vitro and in cell culture. However, a subsequent study could not confirm the therapeutic effects of benzothiazoles in a HD mouse model (Hockly et al. 2006). They also screened a natural substance library (~5,000 compounds), and identified EGCG, which inhibits Htt aggregation in vitro, and suppresses polyQ-induced neurodegeneration in an HD *Drosophila* model (Ehrnhoefer et al. 2006). They also subsequently showed the inhibitory activity of EGCG on aggregation of α-Syn and Aβ (Ehrnhoefer et al. 2008). More recently, EGCG has also been shown to be effective in a *Caenorhabditis elegans* model of SCA3, suggesting its potential as a therapeutic candidate for amyloid-related neurodegenerative diseases (Bonanomi et al. 2014). Nukina and colleagues also performed in vitro screening of approximately 200 non-toxic chemical compounds, and identified trehalose, a disaccharide, as a polyQ aggregate inhibitor. They further demonstrated that oral administration of trehalose delays the onset of neurological symptoms in a polyQ disease mouse model (Tanaka et al. 2004). Considering that trehalose is safe for humans and is known to act as a chemical chaperone, which stabilizes protein native conformations in general, it would be a promising therapeutic candidate not only for the polyQ diseases, but also for other neurodegenerative diseases.

Several studies have examined aggregation inhibitors reported for other neurodegenerative proteins, such as Aβ, tau, prion, and α-Syn, for their activity on polyQ proteins. Using a zebrafish model of the polyQ diseases, *N*'-benzylidene-benzohydrazide derivatives, known as anti-prion compounds, were shown to inhibit polyQ protein aggregation as well (Schiffer et al. 2007). Methylene blue (MB), a tau aggregation inhibitor, has also been shown to inhibit mutant Htt aggregation, and to suppress neurological phenotypes in HD fly and mouse models (Sontag et al. 2012), although MB suppressed only Htt aggregation, and not its toxicity in a HD zebrafish model (van Bebber et al. 2010). Curcumin, an aggregation inhibitors for Aβ, α-Syn, and prion, has also been shown to inhibit polyQ aggregation in yeast (Verma et al. 2012), although it failed to show therapeutic effects on a knock-in mouse model of HD (Hickey et al. 2012).

Cellular model-based screening for polyQ aggregation inhibitors has also been conducted. Diamond and colleagues applied the fluorescence resonance energy transfer (FRET) technique to quantify inclusions of fluorescently-tagged polyQ proteins in cultured cells, and performed screening for its inhibitors. They successfully identified the Rho-activated protein kinase (ROCK) inhibitor Y-27632 from approximately 2,800 biologically active chemical compounds, and showed that Y-27632 suppresses neurodegeneration in a polyQ disease *Drosophila* model (Pollitt et al. 2003). They further demonstrated that the administration of Y-27632, as well as the clinically approved ROCK inhibitor HA-1077 (Fasudil) successfully improves motor dysfunction and retinal degeneration in an HD mouse

model (Li et al. 2009, 2013), suggesting that the ROCK signaling pathway could be a promising therapeutic target for the polyQ diseases. Using a yeast model, Kazantsev and colleagues screened 16,000 chemical compounds for their ability to suppress polyQ-expanded Htt aggregation and growth impairment, and identified four hit compounds. Among various structural derivatives of these hits, they identified C2-8, a sulfobenzoic acid derivative, which inhibits Htt inclusions in cell and brain slice culture models and suppresses neurodegeneration in a *Drosophila* model of HD (Zhang et al. 2005). It is noteworthy that C2-8 has been shown to penetrate the BBB by both oral and intraperitoneal administration, and to suppress Htt inclusions in the brain of HD mice, although its therapeutic efficacy on the neurological symptoms remains controversial (Chopra et al. 2007; Wang et al. 2013). Using luciferase-based protein aggregate reporters, the Johns Hopkins Clinical Compound Library (~1,500 FDA-approved drugs) was screened, and leflunomide, a dihydroorotate dehydrogenase inhibitor, and its active metabolite teriflunomide were identified as polyQ aggregation inhibitors, although their therapeutic effects in vivo have not yet been evaluated (Fuentealba et al. 2012). In addition to our efforts toward developing QBP1-based small chemical analogs as described above, we have also screened chemical compound libraries (~46,000 chemical compounds) using a simple polyQ aggregation turbidimetric assay (Nagai et al. 2000), and identified a number of novel polyQ aggregate inhibitor chemical compounds (unpublished). We are currently evaluating their therapeutic efficacy in vivo using fly and mouse models, expecting the identification of potential therapeutic candidates for the further clinical evaluation in polyQ disease patients in the near future.

9.6 Future Perspectives

As we introduced above, although protein misfolding and aggregation have been revealed as key steps in the common pathogenesis of neurodegenerative diseases, clinically approved disease-modifying therapies targeting these processes have not been established until now. Moreover, most clinical trials for AD, such as those of γ-secretase inhibitors and immunotherapies for Aβ, have failed to demonstrate clinical efficacy (Doody et al. 2013, 2014; Salloway et al. 2014). What can we learn from these failures of anti-amyloid approaches for AD? One of the problems is the discrepancy between animal models and human patients. Most animal models used in preclinical studies for AD are transgenic mice overexpressing either APP, PS1, tau, or their combinations. These mice are far from mimicking the genetic conditions, not only of most human sporadic AD patients, but also of FAD patients. In this regard, it is notable that novel knock-in mice harboring pathogenic mutations in the endogenous mouse APP gene have recently been established, which show Aβ pathology as well as memory impairment in an age-dependent manner (Saito et al. 2014).

Another obvious problem is the disease stages of AD at which the interventions are started. The Dominantly Inherited Alzheimer Network (DIAN) study revealed that the earliest biochemical changes can be detected as early as 25 years before expected symptom onset, in carriers with an FAD-linked PS1 mutation (Bateman et al. 2012). CSF Aβ42 levels in FAD carriers, although initially higher than those in non-carriers, gradually decline from 25 years before expected onset. Subsequently, Aβ deposition in the brain, and hippocampal atrophy, could be detected by PIB-PET and MRI, and CSF tau levels were increased from 15 years before expected onset. By 10 years before expected onset, decreased brain metabolism and memory impairment were observed. Global cognitive impairment was detected 5 years before expected onset. Finally, participants typically met diagnostic criteria for dementia at an average of 3 years after their estimated age at onset. Although these biological disease cascades in FAD may not directly apply to those in late-onset sporadic AD, these findings suggest that the current time point in making a diagnosis of AD and starting interventions, may be too late to prevent the disease progression. It is important to note that most preclinical trials are designed as preventive studies using transgenic mouse models that are destined to develop the disease.

In addition, there has been a lot of discussion so far on the molecular target for AD therapy, namely, Aβ vs tau (Giacobini and Gold 2013). Some people believe that Aβ-targeted therapies have failed because the true neurotoxic target that causes memory and cognitive impairment in AD is tau. Nevertheless, even if tau-targeted disease-modifying therapies are developed in the near future, the above two issues, i.e., the discrepancy between animal models and human patients, and the appropriate time to start intervention, need to be considered for the successful translation of preclinical studies to clinical efficacy in human patients.

It is noteworthy that the results of a Phase III clinical trial of leuprorelin, a luteinizing hormone-releasing hormone agonist, in patients with SBMA, one of the polyQ diseases, also highlights that early intervention is critical for its clinical efficacy (Katsuno et al. 2010). Subgroup analysis indicated a significant improvement in swallowing function by leuprorelin treatment only in SBMA patients with a shorter duration after onset (<10 years), whereas it failed to show clinical efficacy when all the patients enrolled were analyzed as one group. In contrast to sporadic diseases, in which the detection of the earliest changes and prediction of disease onset at the presymptomatic stage are extremely difficult, familial diseases can be diagnosed by genetic testing. In addition to the DIAN study on FAD-mutation carriers, there are also two ongoing multicenter studies on presymptomatic HD carriers, namely, PREDICT-HD (Paulsen et al. 2006, 2014) and TRACK-HD (Tabrizi et al. 2009, 2013), aimed at identifying biological disease cascades as potential biomarkers. Therefore, one future direction is to design clinical trials for evaluating preventive clinical efficacy on presymptomatic carriers with inherited neurodegenerative diseases.

Acknowledgments We thank Dr. H. Akiko Popiel for critical reading of the manuscript, and lab members for their helpful discussions. This work was supported in part by Grants-in-Aid for

Scientific Research on Innovative Areas (Synapse and Neurocircuit Pathology to Y.N.) and Strategic Research Program for Brain Sciences (Integrated research on neuropsychiatric disorders to Y.N.) from the Ministry of Education, Culture, Sports, Science, and Technology, Japan; by Grants-in-Aid for Scientific Research (B) and Challenging Exploratory Research to Y.N., and for Young Scientists (B) to E.N.M. from the Japan Society for the Promotion of Science, Japan; by Health Labour Sciences Research Grants for Research on Development of New Drugs and Research on Measures for Intractable Diseases to Y.N. from the Ministry of Health, Labour and Welfare, Japan; and by a grant from Core Research for Evolutional Science and Technology (CREST) of the Japan Science and Technology Agency to Y.N.

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Chapter 10
Physical Therapy and Rehabilitation in Patients with Degenerative Cerebellar Diseases: Current Evidence and Future Direction

Ichiro Miyai

Abstract After brain damage, use-dependent plasticity of the spared neural network plays a crucial role in improving neural deficits and promoting motor learning and relearning using the impaired limbs. In degenerative cerebellar diseases, it is to be elucidated whether a similar mechanism works or not, since pathological processes are basically progressive. The fundamental question regarding the efficacy of neurorehabilitation in cerebellar degenerative diseases, is whether it is beneficial in terms of both the short- and long-term effect. To answer this question, two important issues need to be considered. The first is whether impaired motor learning due to cerebellar dysfunction is compensated for by repeated practice, since the cerebellum plays a crucial role in motor learning. The second issue is how long functional gains can be sustained provided that intensive rehabilitation results in significant gains. Recent studies have shown that intensive rehabilitation focusing on balance and mobility improves motor function for a period of up to 1 year in patients with degenerative cerebellar diseases. To obtain meaningful long-term gains, a combination of intermittent intensive short-term rehabilitation and home-based practice and support may be a practical way of managing patients. Future studies may elucidate a potential role of neuromodulation coupled with rehabilitative intervention to enhance the gains and to minimize functional decline.

Keywords Ataxia • Degenerative cerebellar diseases • Rehabilitation • Use-dependent plasticity • Motor learning • Neuromodulation

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K. Wada (ed.), *Neurodegenerative Disorders as Systemic Diseases*,
DOI 10.1007/978-4-431-54541-5_10

10.1 Introduction

Accumulated evidence shows that early and intensive rehabilitation improves the functional outcome of mono-phasic brain insult, i.e. stroke. Task-specific intervention is most likely to benefit disabled patients. The functional gains are sustained, provided that patients are able to maintain their daily activities involving the impaired limbs. Thus intensive rehabilitation immediately after brain damage, followed by less intensive intervention that is compensated for by promoting patients' activities of daily life (ADL) is the principal model for managing disabled people after acute brain diseases. On the other hand, longitudinal decline in impairment and disability is inevitable in patients with degenerative cerebellar diseases. In this situation, environmental setting and the timely provision of solutions to the emerging problems of daily life, as well as intervention to improve patients' impairment and disability are essential. Although a comprehensive approach is meaningful for patients and caregivers, recent advances in neurorehabilitation will give further insight into the scientific approach to patients with degenerative neurological disease.

10.2 Neural Mechanisms of Functional Improvement in Degenerative Cerebellar Diseases

In terms of neural mechanisms underlying functional recovery after a single episode of brain damage, use-dependent plasticity of the spared neural network plays a crucial role in improving neural deficits and promoting motor learning and relearning, using the impaired limbs. Altered motor mapping in the primary motor cortex, such as enlargement of hand representation, is associated with the motor recovery of hand function after brain damage in animals and humans (Chollet et al. 1991; Weiller et al. 1992, 1993; Nudo et al. 1996; Ward et al. 2003). Such reorganization of neural networks depends on use-dependent plasticity, and this finding is the rationale for neurorehabilitation after brain damage.

There are fewer studies regarding the neural mechanisms of functional improvement after cerebellar lesion, especially of mechanisms underlying the improvement of gait and balance. Cortical activation during gait and balance control can be evaluated by using functional near-infrared spectroscopy (fNIRS) that records cortical changes in hemoglobin oxygenation, coupled with increased blood flow induced by neural activation (Miyai et al. 2001). During sustained treadmill walking at a constant speed, prefrontal and medial sensorimotor activations tend to decline, probably due to the shift of the locomotor control center from the supraspinal to the spinal central pattern generator (Suzuki et al. 2004; Miyai et al. 2006). In patients with cerebellar stroke, however, recruitment of the prefrontal cortex was sustained in patients, suggesting a compensatory role of prefrontal areas in connection with the cerebellum, for calibration of ataxic gait (Mihara et al. 2007,

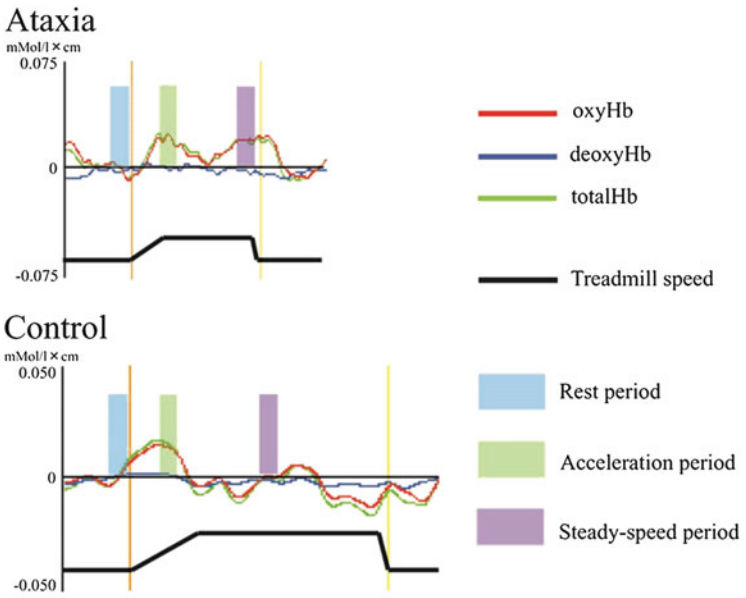

Fig. 10.1 Sustained increase of oxygenated hemoglobin (oxyHb) signals during treadmill walking in a patients with cerebellar ataxia. While oxyHb signals in the prefrontal cortex gradually decreased during treadmill walking at a steady speed in a healthy subject, a patient with cerebellar ataxia showed sustained increase of oxyHb signals (Adapted from Mihara et al. 2007)

Fig. 10.1). This suggests that cerebellar damage might also induce reorganization of neural networks.

Neural mechanisms for balance control can be assessed by using an event-related fNIRS. A time-line analysis revealed that prefrontal activation was induced to adapt to postural perturbation delivered by combined brisk forward and backward movement on a platform. Activation in the right posterior parietal cortex and supplementary motor area (SMA) was enhanced when auditory warning signals were provided before the delivery of perturbation (Mihara et al. 2008). In a cross-sectional study of balance recovery after hemiplegic stroke, postural perturbation-related cortical activation was observed in the prefrontal cortical areas in both hemispheres, as well as the premotor and parietal cortex in the unaffected hemisphere. There was a significant correlation between balance ability as assessed by Berg Balance Scale, and activation in the SMA and prefrontal cortex (Mihara et al. 2012a). In the subsequent longitudinal observation, postural perturbation-related cortical activation in the SMA of the affected and unaffected hemispheres was significantly increased after intensive rehabilitation (Fujimoto et al. 2014). The increment of the postural-perturbation-related oxygenated hemoglobin signals in the SMA of the unaffected hemisphere, significantly correlated with the gain in balance function as measured by the Berg Balance Scale (Fig. 10.2). These findings support that idea that the SMA plays an important role in postural balance control, and suggest that the SMA is a crucial area for balance recovery after a hemiplegic

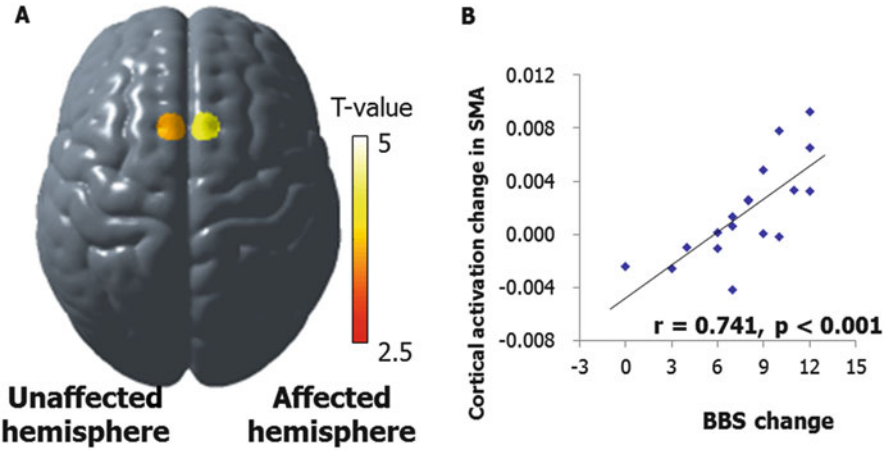

Fig. 10.2 Cortical changes underlying balance recovery in patients with hemiplegic stroke. Increase in SMA activities (**a**): BA 6, MNI coordinate $x/y/z = -9.0/6.6/76$. 0in the unaffected hemisphere, 7.8/7.0/75.9 in the affected hemisphere) of the supplementary motor area (SMA) showed significant correlation with improvement of balance score as measured by Berg Balance Scale after intensive rehabilitation for 41 days in average (**b**). Longitudinal data are from 20 hemiplegic patients with first-ever subcortical stroke (mean ± SD age: 62.2 ± 12.6, 11 males) (Adapted from Fujimoto et al. 2014)

stroke. Although these refer to mechanisms underlying balance recovery after a hemiplegic stroke, similar observations are made in patients with degenerative cerebellar disease (data unpublished).

In degenerative cerebellar diseases, it is to be elucidated whether similar mechanisms works or not, since pathological processes are basically progressive. It is possible that certain plastic changes contributing to functional improvement after rehabilitation cannot be taken over in the next phase of the disease. Regarding this issue, previous functional neuroimaging studies have suggested that cerebellar dysfunction might be compensated for by neural networks other than the cerebellum, including frontoparietal cortical areas, especially those involved in the cerebellar-cortical loops. In patients with multiple system atrophy, hand movement induced less bilateral cerebellar activation and greater left superior parietal activation than in healthy subjects. They also had less bilateral cerebellar and greater supplementary motor and left superior parietal activation than patients with Parkinson disease (Payoux et al. 2010). It is also of interest whether such functional modulation leads to structural reorganization. A recent study using voxel-based morphometry (VMA) implied that balance training improved balance performance in patients with degenerative cerebellar diseases, along with gray matter increase in the non-affected cerebral cortex as well as the less affected cerebellar cortex within the cerebellar-cortical loops. The most compelling change in gray matter volume was seen in the dorsal premotor cortex that is shown to project to the primary motor cortex and cerebellar motor areas, including lobules VI and Crus I and VIII (Burciu et al. 2013). It is to be elucidated whether such changes reflect a recovery from

degeneration or intact processes of cerebellar plasticity in the remaining healthy tissue. In terms of the impact of rehabilitative intervention on damaged neural networks, further investigations will be required to discover whether enhanced physical activities induce delay pathological process in the affected area. In animal models of spinocerebellar ataxia type 1 (SCA1) caused by expansion of a translated CAG repeat in Ataxin-1, exercise induced up-regulation of epidermal growth factor as well as expanded life span, although the impact on motor function was unclear (Fryer et al. 2011).

10.3 Specific Issues in Considering Neurorehabilitation to Cerebellar Degenerative Diseases

The fundamental question regarding the efficacy of neurorehabilitation in cerebellar degenerative diseases is whether it is truly beneficial in terms of both short- and long-term effects. In considering the dose-dependent effect of task-specific training in stroke patients (Kwakkel et al. 1999, 2004), it is assumed that such patients have preserved their motor learning ability and that the disease process is basically non-progressive. Thus two important issues should be considered to answer this question. The first is whether impaired motor learning due to cerebellar dysfunction is compensated for by repeated practice, i.e. intensive task-oriented rehabilitation, since the cerebellum plays a crucial role in motor learning (Ito 2001).

Motor learning is essential to learn to use chopsticks, play a musical instrument, ride a bicycle, and swim. The main component of motor learning is the improved performance realized by repeating a task-oriented practice. In an experimental setting, motor learning includes both adaptation learning and motor sequence learning. Adaptation learning is a form of motor learning based on sensory information, such as skill acquisition for using a computer mouse, controlling a robot arm under a modified force field, and prism adaptation. The transformation process of sensory information to motor command is captured as an internal model. Internal models include both forward and inverse models (Wolpert and Kawato 1998; Wolpert and Ghahramani 2000). Forward internal models can predict subsequent sensory consequences from efference copies of issued motor commands. Inverse internal models calculate the necessary feedforward motor commands from the desired trajectory information (Fig. 10.3, Wolpert and Kawato 1998; Kawato 1999; Kitazawa 2013). In the cerebellum, feedback-error learning contributes to the formation of internal models. Climbing fibers from the inferior olivary nucleus send error signals to the cerebellar cortex, and internal models are then acquired based on synaptic plasticity of the Purkinje cells, i.e. long-term depression (Ito and Kano 1982).

On the other hand, motor sequence learning, the knowledge of the sequence of a series of movements is acquired by repeating those serial movements. This form of motor learning can be regarded as the formation of an internal model for the motor

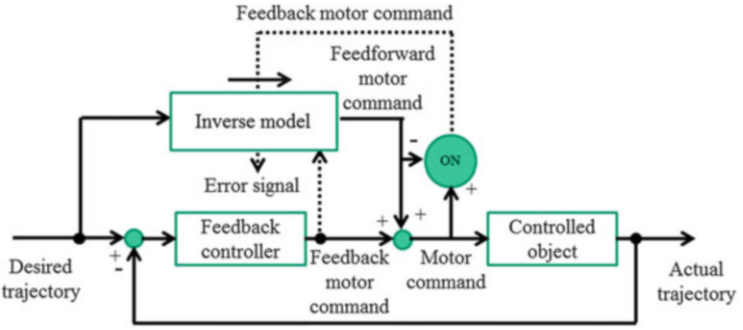

Fig. 10.3 Cerebellar feedback-error-learning. Motor learning continues until motor commands completely equals feedforward motor command without needs for feedback motor command (Adapted from Wolpert and Kawato 1998; Kawato 1999; Kitazawa 2013. *ON* olivary nucleus)

sequence to move smoothly by predicting the next movement. In a rehabilitation setting, the repeated practice of ADL including transfer from bed to wheelchair and toileting, is aimed to complete these behavioral series safely and automatically. Thus in terms of motor learning, the cerebellum play a crucial role for supervised learning based on errors, while the basal ganglia are engaged in reinforcement learning based on reward prediction (Doya 1999; Schultz et al. 1993). Acquired motor skills are consolidated and retained in the cerebellar-cortical loops and striatal-cortical loops. For retention, it is assumed that retention of adaptation learning and motor sequence learning, mainly depend more on the cerebellum and related cortical regions, and the striatum and related cortical regions, respectively (Doyon and Benali 2005; Doyon 2008).

How well can patients with cerebellar damage learn motor skills? An adaptation learning task consisting of throwing balls at a target wearing a prism, revealed that prism adaptation was impaired or absent in patients with olivo-cerebellar lesions, except lesions in the dentate-thalamic projection (Martin et al. 1996). In an experiment using eye-blink conditioning, patients with degenerative cerebellar diseases showed inappropriately-timed conditioned responses with respect to the conditioned stimulus (Topka et al. 1993). Hatakenaka et al. (2012) investigated the relationship between motor learning and gains in ADL after intensive rehabilitation in patients with infratentorial stroke. Fast motor learning was assessed by the repetition of 30 s pursuit rotor (PR) tasks. Both patients and controls learned the PR skill, although the gains in PR performance were significantly lower in patients. In terms of brain activities, cerebellar patients showed sustained prefrontal activation, while healthy subjects showed reduced prefrontal activation coupled with increased activation in the supplementary motor area after repeated PR tasks. These findings suggest that feedback control of the PR task shifted to feedforward control with task repetitions in healthy subjects, while cerebellar patients continued to rely on feedback information. Furthermore, reduced learning significantly correlated with smaller ADL gains assessed by the Functional Independence Measure (FIM, Keith et al. 1987). Thus impaired motor sequence learning by the PR task was

correlated with reduced gains after intensive rehabilitation for ataxic stroke patients. To relearn motor sequences required to perform ADLs, such as transfer, toileting, and dressing independently, preserved motor learning may influence learning efficacy. This indicates that augmented practice may be an optimal strategy to compensate for the impaired motor learning in patients with cerebellar damage. Regarding this issue it is reasonable to test whether intensive rehabilitation induces clinically significant gains in degenerative cerebellar diseases.

The second issue is how long functional gains can be sustained, provided that intensive rehabilitation results in significant gains. To answer this question, longitudinal assessment after focused rehabilitative intervention, including comparison of data for natural decline, will be necessary. The results from a European cohort (Jacobi et al. 2011) indicated that the annual increase of the Scale for the Assessment and Rating of Ataxia (SARA, Schmitz-Hubsch et al. 2006; Yabe et al. 2008) score was greatest in SCA1 (2.18 ± 0.17, mean $\pm$ SE) followed by SCA3 (1.61 ± 0.12) and SCA2 (1.40 ± 0.11). SARA progression in SCA6 was slowest and nonlinear (first year: 0.35 ± 0.34, second year: 1.44 ± 0.34). In a Japanese cohort of patients with SCA 6, there was a 3.1 ± 4.3 point change of the International Corporative Ataxia Rating Scale (Trouillas et al. 1997) for 4 years in a retrospective study, and the change of SARA scores was 1.5 ± 2.0 per year in a prospective follow-up (Tsuji et al. 2008; Nakajima et al. 2009). Thus no significant difference in the functional status between assessment at the baseline and 1-year follow-up might be interpreted as an improvement. However, to elucidate if repeated rehabilitative intervention may affect the natural history of degenerative diseases, longitudinal evaluation over further years may be necessary. Individual variables, including gender, age at onset, duration of disease and genetic factors such as length of CAG repeat, also influence long-term outcomes (Klockgether et al. 1998; Jacobi et al. 2011; Schmitz-Hubsch et al. 2008), and these factors need to be adjusted.

10.4 Effect of Intensive Rehabilitation in Degenerative Cerebellar Diseases

There have been two studies testing the effect of intensive rehabilitation on cerebellar ataxia, gait and balance in patients with degenerative cerebellar diseases (Ilg et al. 2009, 2010; Miyai et al. 2012). These studies have shown that the immediate effect of intensive rehabilitation focusing on balance and mobility function is warranted (Table10.1). Ilg et al. (2009) provided 1 h of coordinative training for 3 days per weeks, for 4 weeks, in 10 patients with cerebellar degeneration, 6 with degeneration of afferent pathways. The rehabilitation program included static balance e.g. standing on one leg, dynamic balance e.g. sidesteps, climbing stairs, whole body movements to train the trunk-limb coordination, steps to prevent falling and falling strategies in order to prevent trauma, and movements

Table 10.1 Results of intensive rehabilitation in patients with degenerative cerebellar diseases

	Ilg et al. (2009, 2010)	Miyai et al. (2012)
Number of Pt	16	42
Type of disease	SCA6(2),SCA2(1),ADCA(1), IDCA(6)/FA(3), SANDO(2),SN(1)	SCA 6(20), ADCA(6),IDCA (16)
Age ± SD (Range)	61.4 ± 11.2 (44 ~ 79)	62.5 ± 8.0 (40 ~ 82)
Gender	8 M, 8 F	22 M, 20 F
Duration of disease	12.9 ± 7.8 (3 ~ 25 years)	9.8 ± 6.2(7 month ~ 30 years)
Baseline SARA	15.8 ± 4.3 (11 ~ 24)	11.3 ± 3.8 (5 ~ 21.5)
Control	No	Crossover for short-term effect
Intervention	1 h × 3 per week × 4 weeks	2 h × 5 + 1 h × 2 per week × 4 weeks
Post training	Home training protocols	Unmonitored self-training
Outcome measures	SARA, gait speed, balance, BBS, GAS	SARA, FIM, gait speed, cadence, FAC, falls
Assessment point	4 weeks pre, baseline, post, 8 weeks, 1 year	Baseline, post 0, 4, 12, 24 weeks
Main results	SARA & gait improved 8 weeks & 1 year post rehabilitation only in patients with cerebellar ataxia not afferent ataxia	SARA & gait improved 12 weeks & 24 weeks, respectively

SCA6 spinocerebellar ataxia type 6, *SCA2* spinocerebellar ataxia type 2, *SCA31* spinocerebellar ataxia type 31, *CCA* cortical cerebellar atrophy, *FA* Friedreich's ataxia, *SANDO* sensory ataxic neuropathy with dysarthria and ophthalmoparesis caused by mutations in the polymerase gamma gene, *SN* sensory neuropathy, *SARA* scale for the assessment and rating of ataxia

to treat or prevent contracture especially movements of the shoulders and spine. At the end of the session, patients received an individual written training schedule to enable them to perform the exercises by themselves at home for 1 h each day. Significant improvements in gait performance, including velocity, lateral sway, and intralimb coordination and reduction of ataxia symptoms including regulation of balance in static and dynamic balance tasks, were observed in patients with cerebellar degeneration. These improvement were sustained at a follow-up assessment 8 weeks after the intervention. Of note, these interventions were not effective in patients with afferent ataxia. Subsequent follow-up study revealed that these gains in gait and ataxia were significant at 1 year (Ilg et al. 2010).

In a study by Miyai et al. (2012), patients with degenerative cerebellar disease received 4-week inpatient rehabilitation, including 1-h physical therapy and 1-h occupational therapy per day on weekdays, and 1 h of either physical or occupational therapy on weekends (Cerebellar Ataxia Rehabilitation: CAR trial). Forty-two patients with spinocerebellar degeneration were randomly assigned to the immediate group or the delayed-entry control group. The short-term outcomes of the immediate and control group were then compared. Long-term evaluation of

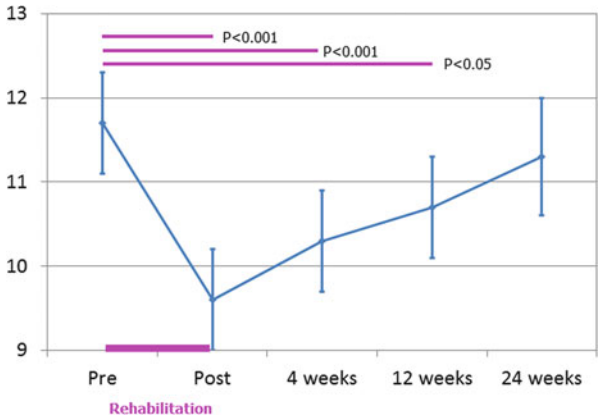

Fig. 10.4 Longitudinal changes of SARA after intensive rehabilitation ($n = 41$). Although functional status tended to decline to the baseline level within 24 weeks, gain in SARA score was significant up to 12 weeks (Wilcoxon signed-rank test)

both groups took place at 4, 12, and 24 weeks after the intervention. Each therapy focused on the improvement of ataxia and ADL. Physical therapy emphasized improving postural balance and gait, and the program included general conditioning, range-of-motion exercise for trunk and limbs, muscle strengthening, static and dynamic balance exercises with standing, kneeling, sitting, and quadruped standing, mobilizing the spine in both prone and supine positions, walking indoors and outdoors, and climbing up and down stairs. The occupational therapy program emphasized improving ADL and the program comprised relaxation, hygiene, dressing, writing, eating, toileting, bathing, balance exercises, reaching, coordinative tasks of the upper limbs and trunk, and dual motor tasks such as handling objects while standing and walking. After discharge, patients were instructed to keep to a similar level of activity in their daily lives as they did before. Patients receiving home-based rehabilitation at a baseline level, usually 20–40 min of physical therapy per week, were advised to continue the program after discharge at the same frequency as they did before admission. The immediate group showed significantly greater functional gains in ataxia, gait speed and ADL than the control group. The improvement of truncal ataxia was more prominent than that of limb ataxia. These gains in ataxia and gait were sustained at 12 weeks and 24 weeks, respectively (Figs. 10.4 and 10.5). At least one measure was better than at the baseline, at 24 weeks in 22 patients. Patients with sustained improvement had less severe ataxia (lower SARA score) than those without sustained improvement (8.9 ± 0.7 vs. 12.7 ± 0.9, $p < 0.05$). The receiver operator characteristic curve (ROC) analysis showed that the baseline SARA score was moderately predictive of the sustained improvement at 24 weeks.

Although these studies show the significant effect of focused rehabilitation in patients with degenerative cerebellar disease (Table 10.1), several unresolved questions remain, including dose and duration of intensive rehabilitation, identification of the specific components of intervention that work, strategies that maintain functional gains after intensive rehabilitation, strategies for patients with severe ataxia, strategies for patients presenting with additional pyramidal/extrapyramidal signs, i.e. multiple system atrophy, and strategies for enhancing outcome by the

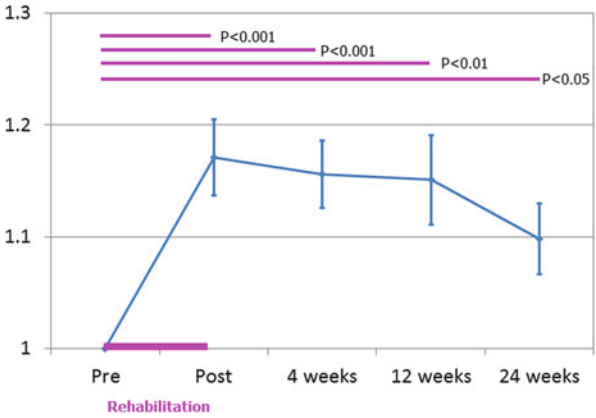

Fig. 10.5 Longitudinal changes of normalized gait speed after intensive rehabilitation ($n = 40$). Gait speed was the most sensitive to changes. There was a 17 % increase in gait speed immediately after the intervention and still 10 % increase at 24 weeks after the intervention

combination of rehabilitation and pharmacological neuro-modulation/brain stimulation (Ilg et al. 2014). In terms of the minimum dose required to obtain meaningful gains, it seems to be at least 3 h per week for 4 weeks. It is unclear whether greater amounts of intervention will result in further improvement since the functional outcome of balance and gait after intervention of 14 h per week for 4 weeks seems to be comparable with the outcome after 3 h per week for 4 weeks. This then raises the possibility that home-based intensive rehabilitation could be a feasible intervention for patients with degenerative cerebellar diseases. Other issues will be discussed in the following sections.

10.5 Specific Components of Intervention

As described in the previous section, the rehabilitative approach to degenerative cerebellar diseases is comprehensive, and includes practice for improving balance, gait and ADL. This raises the question of whether there is a specific intervention that can improve cerebellar ataxia. A similar problem emerged with strategies of motor rehabilitation in stroke patients. Several meta-analyses from small randomized controlled trials, have shown that strategies focused on high-intensity and repetitive task-specific practice, are likely to promote motor recovery after stroke (Langhorne et al. 2009). During the last decade, there has been an accumulation of several well-designed large-scale randomized control trials. In particular, the EXCITE (Extremity Constraint Induced Therapy Evaluation) trial (Wolf et al. 2006) is the first large multi-center randomized controlled trial for the efficacy of constraint movement induced therapy (CIMT), designed according to CONSORT (Consolidated Standards of Reporting Trials) (Altman et al. 2001; Schulz et al. 2010). In this study, 222 participants who had suffered a stroke within the previous 3–9 months, were assigned to receive either CIMT) or usual and customary care (ranging from no treatment after concluding formal rehabilitation, to pharmacologic or physiotherapeutic interventions). The CIMT group, where

patients wore a restraining mitt on the less-affected hand, while engaging in repetitive task practice and behavioral shaping with the hemiplegic hand, showed greater gains than the usual care group in both timed tasks of the affected upper extremity, and in the measure of daily use of the affected upper extremity. These improvements in arm motor function persisted for at least 1 year.

In the EXCITE trial, an augmented period of intervention for the affected upper extremity might be the essential determinant of efficacy. Indeed, a recent multicenter randomized controlled trial, revealed that intensive robot-assisted therapy resulted in greater gains in motor paresis of the affected upper extremity than usual care, but dose-matched intervention by a therapist induced comparable outcomes (Lo et al. 2010). This is also the case with gait practice for stroke patients. Duncan et al. (2011) investigated the effect of body-weight supported treadmill training and home gait exercise by therapists in 408 stroke patients. No significant differences in improvement were found between early (2 months) locomotor training and home exercise, or between late (6 months) locomotor training and home exercise, including walking speed, motor recovery, balance, functional status, and quality of life. Of note, the delay in initiating the late locomotor training did not affect the outcome at 1 year, suggesting a longer time window for the locomotor intervention compared with the upper extremity intervention.

Thus potential strategies detected by small randomized controlled trials, eventually failed to show a superiority in outcome when well-designed large-scale studies were performed. It is likely that the dose-component of intervention dominates other specific strategies at this point. In patients with cerebellar ataxia, weight loading to the upper and lower limb, balance training on the foam platform, and stepwise increase of walking speed on the treadmill have been tried in clinical settings. Although these have not been tested even by non-randomized controlled trials, it is doubtful whether further randomized controlled trials will show any difference in outcome. Lessons from these clinical trials for stroke patients suggest that at least the amount and frequency of experimental intervention should be matched with controls in future studies in order to detect the specific factors contributing to the improvement of ataxia.

10.6 Intervention to Minimize Long-Term Decline

Previous studies have claimed that short-term intensive rehabilitation induces a long-term improvement of cerebellar ataxia and gait of up to 1 year in patients with degenerative cerebellar diseases. The gains might be meaningful when compared with the natural decline of the diseases. This suggests that patients have sufficient motor learning ability to benefit from intensive practice. Follow-up data over 3 years in 4 cases in the CAR trial, revealed that gains in SARA after repeated boost rehabilitation were almost comparable with those obtained after the initial intervention (Ilg et al. 2014). An initial 4-week intensive intervention resulted in a 2.5 point improvement of SARA on average, and after an average period of 26 weeks SARA worsened by 7.6 points and the re-boost therapy resulted in a 2.0

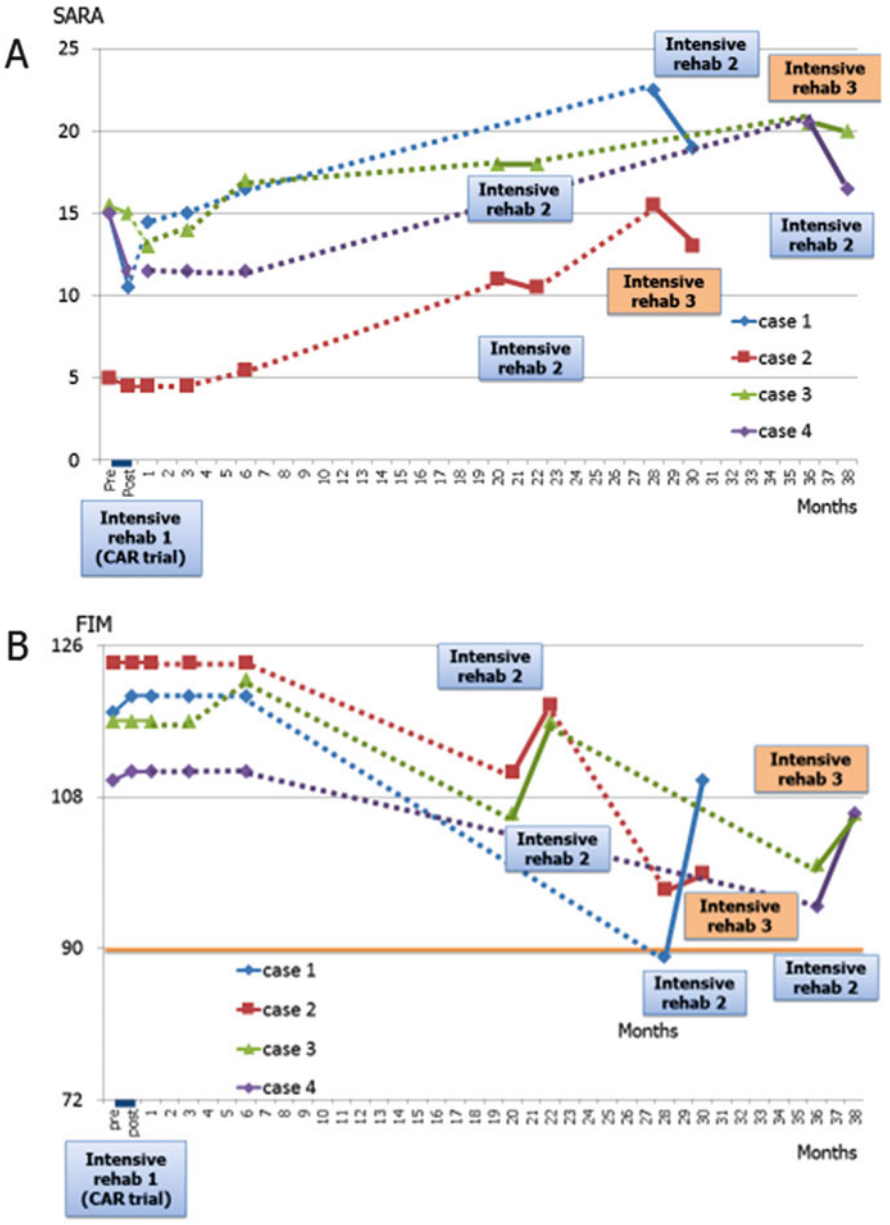

Fig. 10.6 Effect of boost rehabilitation in patients with degenerative cerebellar diseases. All 4 patients participated in CAR trial (Miyai et al 2012) and underwent 4-week intensive rehabilitation. Case 2 and 3 had re-boost intensive rehabilitation twice and case 1 and 4 had re-boost rehabilitation once. Solid lines represent intervention periods of intensive rehabilitation. *Dotted lines* represent follow-up periods with home-based rehabilitation of 40–80 min per week. (**a**) Effect on SARA score. First intervention results in 2.5 point improvement of SARA on average and after average of 26 weeks SARA worsened by 7.6 points and the re-boost therapy resulted in 2.0 point improvement. This suggests that the effect of re-boost rehabilitation on SARA was comparable with the first intensive rehabilitation. (**b**) Effect on FIM score. First intervention

point improvement. All patients received low-intensity home therapy between the intensive interventions. However, home therapy of up to 80 min per week, failed to maintain the gains (Fig. 10.6a). On the other hand, the effect on ADL, as assessed by FIM, showed a different trend. Firstly, intervention resulted in a 0.8 point improvement of FIM on average, because all patients were independent at this point and there was a ceiling effect on improvement of FIM. After an average of 26 weeks, FIM worsened considerably by 17.8 points, and the re-boost therapy resulted in a 12.8 point improvement. This suggests that the effect of re-boost rehabilitation on FIM was meaningful when cerebellar ataxia was severe enough to influence patients' ADL. Again low-intensity home therapy failed to maintain their ADL (Fig. 10.6b). These observations suggest that the target or goal of rehabilitative intervention should change from improving the impairment of gait and balance to relearning motor strategies in order to achieve independent – or less dependent – ADLs by compensation, once the impairment itself overwhelms the level of patients' independent mobility.

Thus for the retention of learned motor skills, it is important to maintain and promote daily activities. For this purpose, monitoring activities and providing a solution to patients and their caregivers if a certain activity becomes difficult for them, will be a practical way forward. Taub et al. introduced the "transfer package", a set of techniques to facilitate the transfer of therapeutic gains obtained from CIMT in the therapeutic setting, to the life situation (Taub et al. 2013). Use of the transfer package, regardless of the type of training received, promoted daily use of the affected upper extremity. This suggests that motivating patients to keep to their activities along with some advice and encouragement, may work in community-based rehabilitation. Similar observations were reported in gait training. In a multicenter randomized controlled trial (Dobkin et al. 2010), post-acute inpatients were assigned to feedback groups of either self-selected fast walking speed immediately after a single, daily 10-m walk, or to no reinforcement of speed after the walk, performed within the context of routine physical therapy. The walking speed at discharge in the experimental group was approximately 30 % greater than that of the control group. This suggests that feedback of knowledge of performance will be another key in maintaining activities in the community, either via reward-based learning or through motivation to enhance ADL. Internet video support to empower home-exercise (http://scd-msa.net/rehabilitation/) might be a potential option, provided that interactive communication is guaranteed.

Fig. 10.6 (continued) results in 0.8 point improvement of FIM on average because all patients were independent at this point and there was a ceiling effect on improvement of FIM. After average of 26 weeks FIM considerably worsened by 17.8 points and the re-boost therapy resulted in 12.8 point improvement. This suggests that the effect of re-boost rehabilitation on FIM was meaningful when cerebellar ataxia influenced patients' ADL. FIM score greater than 90 means that a patient's ADL is at least supervision level

10.7 Evolution of New Technologies

As addressed in Sect. 10.2, previous neuroimaging studies have suggested that cerebellar dysfunction might be compensated for by neural networks other than the cerebellum. In patients with multiple system atrophy, hand movement induced greater recruitment of frontal mesial areas and superior parietal cortices (Payoux et al. 2010) Sustained recruitment of the prefrontal areas was associated with execution of pursuit rotor tasks (Hatakenaka et al. 2012) and treadmill walking (Mihara et al. 2007) in patients with cerebellar stroke. After balance training, gray matter volume increased in both the dorsal premotor cortex and cerebellar motor areas (Burciu et al. 2013). These observations only describe the relationship between motor performance and brain activities. To investigate causality of such recruitment of motor related area in improving functional outcome, it is essential to test whether neuromodulation of these areas augments functional gains.

Currently available methods for neuromodulation include repetitive transcranial magnetic stimulation (TMS), transcranial direct current stimulation (tDCS), and neuro-pharmacological modulation using selective serotonin reuptake inhibitors and other enhancers of monoamine neurotransmission. In terms of brain stimulation, the role of TMS in promoting cerebellar plasticity, the exact positioning of electrode stimulation, and the duration of the after effects of tDCS remain unclear (Grimaldi et al. 2014). Although a pilot study has suggested some short-term benefit of rTMS (Shiga et al. 2002), the long-term neural consequences of non-invasive cerebellar modulation are unclear.

Neuropharmacological modulation, especially modulation of mono-amine neurotransmission using amphetamine (Walker-Batson et al. 1995), levodopa (Scheidtmann et al. 2001), and serotonin reuptake inhibitors (Miyai and Reding 1998; Chollet et al. 2011) has been tried in order to promote functional recovery after a stroke. In degenerative cerebellar diseases, there have been few randomized controlled trials. In Japan, the thyrotropin releasing hormone tartrate, has been approved for improving ataxic symptoms (Sobue et al. 1983). In future studies, it seems to be essential that such neuro-modulatory interventions are coupled with physical therapy and other exercises (Feeney et al. 1982).

Functional neuroimaging and morphological studies have suggested the relationship between improvement of ataxia and balance, and recruitment of motor related areas (Mihara et al. 2008, 2012a; Burciu et al. 2013; Fujimoto et al. 2014). To investigate the causality of such a relationship, it is essential to test if enhanced activation of the target area induces functional improvement. Recent technical progress in fMRI has enabled real-time feedback of blood oxygenation level-dependent (BOLD) signals. One can control the regional signals according to the feedback information (deCharms 2008; Subramanian et al. 2011). We have also developed a real-time neurofeedback system using fNIRS. In healthy subjects, feedback of oxygenated hemoglobin signals in the premotor area, enhanced its activation when compared with feedback from sham signals (Mihara et al. 2012a, b). A randomized controlled trial revealed that the premotor enhancement by real-

time fNIRS neurofeedback promoted motor recovery of paretic hand in stroke patients (Mihara et al. 2013). A goal for future study is to discover whether neuromodulation of motor related areas, such as the premotor cortex and SMA, could lead to improved control of balance and gait in degenerative cerebellar diseases.

10.8 Model of Rehabilitative Intervention for Degenerative Cerebellar Diseases

For a more sophisticated and comprehensive development of rehabilitative intervention, neural and non-neural mechanisms underlying the efficacy of intervention should be elucidated. Non-neural mechanisms include cardiopulmonary fitness, strengthening of muscle, and improvement of disuse syndrome. The effect of fitness on locomotor outcome has been repeatedly reported in patients with stroke (Langhorne et al. 2009). Thus rehabilitative intervention should be provided on the premise that basic tolerance is preserved by fitness and nutritional control. Since the diseases are gradually progressive, it is also important to control the patients' environment to ensure daily activities are safe, according to their ADL status, and to ensure the timely provision of solutions to any emerging problems of daily life.

Neural mechanisms include the motor learning of compensatory strategies for balance and gait, the recruitment of other areas in the cerebellum that are relatively intact, and the recruitment of the cerebral cortex and cortico-basal ganglia network. To maximize these inherent mechanisms against brain damage, intermittent intensive rehabilitation, coupled with some home-based support, such as a "transfer package" including the education of patients and their caregivers, is a practical

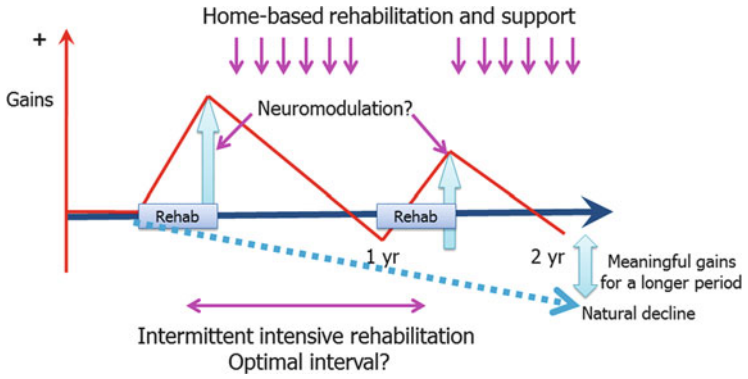

Fig. 10.7 Model of rehabilitative intervention for degenerative cerebellar diseases. This figure describe potential intervention for long-term meaningful gains for patients with cerebellar degenerative diseases based on the current evidence as discussed in this chapter. Combination of intermittent intensive short-term rehabilitation and home-based rehabilitation and support may minimize natural decline of the diseases. Possible role of neuromodulation to enhance the effect of intensive rehabilitation is to be elucidated. See text for details

intervention designed to minimize longitudinal decline. Further studies regarding the optimal interval of intensive rehabilitation, and optimal ways of delivering home-based support are needed. In reality, however, resources for rehabilitation and care are limited. Thus behavioral enforcement and empowerment in daily life may lead to increased total activity. Finally, emerging neuromodulation techniques may have an additional effect on the comprehensive intervention methods discussed (Fig. 10.7).

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Chapter 11
Home- and Community-based Medical Care for Neurodegenerative Diseases: ALS as an Illustration

Takamura Nagasaka and Yoshihisa Takiyama

Abstract Neurodegenerative diseases generally have a slow, progressive course involving a great deal of suffering for patients. We need to be patient and tolerant when facing such a disease, and to this end we should develop an efficient system of multidisciplinary care, both physical and psychological. For home-health based care, a multidisciplinary approach to planning treatment is required, involving a team that includes a number of doctors and other healthcare professionals who are experts in different fields. For neurodegenerative diseases, the primary disciplines involved are medicine, nursing, physical and rehabilitation medicine, caregiving, and welfare etc.

The condition of amyotrophic lateral sclerosis (ALS) brings difficulties that can cause worry or emotional stress. A patient and their family members have to endure the stresses and strains of daily life with the disease. For a patient to enjoy a worthwhile and relatively stable life, we need to provide multidisciplinary care.

Keywords Neurodegenerative disease • Motor neuron disease • Amyotrophic lateral sclerosis • Multidisciplinary management • Home care • Palliative care • End of life

11.1 Introduction

The aim of this chapter is to describe the clinical management of home- and community-based care for amyotrophic lateral sclerosis (ALS) as a representative major neurodegenerative disease. ALS is one of the most intractable diseases, and patients have to consider how to spend the final part of their life. In this regard, home-based care has important implications for a patient and their family members, because patients depend upon the help and assistance of caregivers.

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K. Wada (ed.), *Neurodegenerative Disorders as Systemic Diseases*,
DOI 10.1007/978-4-431-54541-5_11

To overcome any difficulties and to make the limited remaining life of a patient as worthwhile as possible, participants in a patient's care should be encouraged to learn both the meaning of, and the practical issues for, medical treatment and the management of care. Perspectives and understanding of matters surrounding ALS as a prototype of intimate care might be valuable for the home- and community-based care of other neurodegenerative disorders.

11.2 General Aspects

11.2.1 Neurodegenerative Diseases and Total Care

Neurodegenerative diseases are age-dependent intractable diseases caused by degeneration of the central nervous system (CNS). Slow progressive deterioration of their condition due to the absence of a complete cure is inevitable for patients with neurodegenerative diseases. An effect of an aging of population is that the number of people diagnosed as having a neurodegenerative disease has been increasing. Neurodegenerative diseases share certain common features with each other, including histopathology, clinical course, and molecular mechanisms of pathogenesis. The clinical and pathological manifestations of neurodegenerative diseases affect "specific subsets of neurons", arise without a clear explanation and can be either inherited or acquired, "progress relentlessly", are age-related, increase in frequency with advancing age, and are accompanied by microscopic signs of four stages of disorder: neuronal pathology, neuronal cell death, disappearance of neuronal cell bodies, and glial proliferation (Przedborski et al. 2003).

In the terminal stage of the disease, many patients have special management needs regarding their nutrition, respiration, body motion, and communication, amongst other things. Patients also have to endure long-term recuperation from medical care. In this situation, home care is an ideal method for treatment of the disease.

Among several neurodegenerative disorders, ALS is regarded by many as an excellent model for palliative care intervention in neurology. Several factors are involved in managing home-based medical treatment and care of the disease. After overcoming issues in the management and care of ALS, we would then be able to tackle other fatal, progressive neurologic diseases, such as multiple system atrophy, Huntington's chorea, and late-stage Parkinson's disease (Mitsumoto and Rabkin 2007).

For example, international consensus guidelines would assist in the development of a framework for active palliative care engagement in ALS and other neurodegenerative diseases (Bede et al. 2011).

11.2.2 ALS as an Intractable Disease

11.2.2.1 "Time Marches on Inexorably"

ALS is a representative intractable disease, progressing relentlessly and showing no mercy to patients. Since Charcot described ALS, the disease diagnosis has been difficult, but diagnostic criteria have been proposed and evaluated (Brooks 1994; Miller et al. 1999; Brooks et al. 2000; de Carvalho et al. 2008; Costa et al. 2012). As there is no treatment, the disease takes entire possession of a patient's body and the majority of ALS patients die from respiratory failure. Recently, several findings have demonstrated that ALS can have multiple causes including different gene mutations, which indicates that multiple molecular mechanisms must cause ALS, i.e., the ALS syndrome (Ravits et al. 2013). However, there has, as yet, been no real breakthrough in the medical treatment for ALS, and it is still necessary to provide special care for patients. There is considerable evidence that a multidisciplinary approach that includes palliative care, improves both the survival and quality of life of patients (QOL) with ALS (van den Berg et al. 2005; Traynor et al. 2003; Chiò et al. 2004). For such an approach, mutual trust between a patient and a physician is clearly important in the comprehensive treatment of ALS, particularly in the initial stage of therapeutic relations, and this period should be paid particular attention (Borasio et al. 1998). A recommended strategy for the care of patients with ALS has been summarized in a review (Radunović et al. 2007). The summary comprised eight parts: system control, ankle-foot orthosis/ wheelchairs/ home modifications, Riluzole, assisted ventilation, nutritional support, percutaneous endoscopic gastrostomy (PEG) or radiologically inserted gastronomy (RIG), palliative care, advanced directives, and four stages of progression of the disease: coming to terms with diagnosis and disability, coping with substantially impaired function, the end-of-life stage, and the after death stage. The European Federation of Neurological Societies (EFNS) task force on the management of ALS recommends that a palliative care approach should be adopted from the time of diagnosis (Andersen et al. 2005).

11.2.2.2 Breaking the News

To ensure good communication between a patient and their family, and therapeutic and care members throughout the whole course of the disease, the initial explanation, or "breaking the news", is one of the most important elements, and there is no standard procedure. To inform someone of a diagnosis of ALS is extremely difficult and a difficult task for a doctor, because informing the patient that they have the disease is essentially informing them that they are dying. However, this should be encouraged, because it is the first step in palliative care and ensuring successful day-to-day communication and clinical practice among patients, caregivers and members of medical support teams. To reduce the stress on the patient,

information on ALS should be provided in a stepwise manner and at a pace comfortable to the patient, using honest and empathic words to engender as positive a mood as possible, and should be delivered in the presence of the patient's family, not in an out-patient booth (Johnston et al. 1996; Borasio et al. 1998). Notification should begin with asking what the patient/family know about the condition, following an explanation on how to approach the diagnosis. Points that should be covered are as follows: identification of positive prognostic indicators, an explanation that support is available, that the patient and family are not alone, and that interventions can help to maintain independence, QOL, and dignity, through a multidisciplinary approach, a network centered upon the patient, and a social program. Informants should allow plenty of time for questions and for reflection. In some cases, long-term survival of more than 10 years, for up to 10 % of patients is possible, due either to the different phenotypes of the disease (Hu et al. 1998; Katz et al. 1999; Sasaki and Iwata 1999; Le Forestier et al. 2001; Vucic and Kiernan 2007) or to successful care including ventilatory support and a positive outlook. Accordingly, a physician should also provide hope. Comprehensive guidelines for communicating the diagnosis have been presented for the management of ALS (Andersen et al. 2005; Miller et al. 2009a, b). After a diagnosis of ALS have been made, the autonomy and independence of the patient should be respected when care is planned, and the care plan should be made on the basis of accepted guidelines or a standard plan, and should be centered on the patient's community.

In our experience, particularly in Japan, only the patient's family were notified of a diagnosis of ALS in the past if the patient had a sensitive personality and pessimistic outlook.

However, this approach failed because the patient could not receive adequate treatment and care, and spent their remaining time unprofitably. Nowadays, the patient is informed of the diagnosis.

11.2.3 Familial ALS

In ALS, 10 % of cases have been reported to be familial in nature (Chiò et al. 2012c), and 5.1 % in a reported review and meta-analysis (Byrne et al. 2011), and 16 % in Ireland with overlap between ALS, frontotemporal dementia (FTD), and neuropsychiatric disease (Byrne et al. 2013). The list of ALS-related genes is rapidly increasing with the introduction of new technologies such as exome sequencing and ALS DNA banks throughout the world. About two-thirds of familial cases are caused by four genes: *C9ORF72*, *SOD1*, *TDP-43* and *FUS*, and at least 21 other less common genes and loci have been detected (Andersen 2006; Van Es et al. 2010; Chiò et al. 2012c; Majounie et al. 2012; Millecamps et al. 2012; Ogaki et al. 2012; Tsai et al. 2012). Familial ALS (FALS) patients are always younger than in sporadic cases. Relatively young patients experience more severe feelings of hopelessness than older patients do. For FALS patients, caregivers are often the parents, children or young adults. Several

complications including autonomic nervous system impairment, and sensory disturbance etc. are characteristic features of FALS. These situations and symptoms are usually not conducive to living at home. Accordingly, intensive intervention by home care staff, based on a multidisciplinary care approach is much needed.

11.2.4 ALS with Cognitive Impairment and FTD

ALS is increasingly being recognized as a complex multisystem disorder with prominent nonmotor manifestations, including a broad range of neuropsychological and behavioral deficits (Zago et al. 2011). It was reported that 28 % of patients were cognitively impaired (Gordon et al. 2010). Currently, there is limited recognition of the impact of cognitive impairment on disease management in ALS. The presence of cognitive and behavioral impairment in ALS has serious implications for both the patient and caregivers, especially for home-based care. A report revealed that the presence of neurobehavioral dysfunction – or reduced acceptance/understanding of ALS at diagnosis – is a strong predictor of a poor outcome, even if a ventilator and/or nutrition support is introduced (Chiò et al. 2012a). ALS patients with cognitive impairment are at higher risk of falls, injuries, and choking episodes. They also show poorer compliance with walking aids, feeding tubes, and noninvasive ventilation (NIV). Cognitive impairment also affects the ability of this patient group to make important financial and legal decisions, and the ability to competently engage in end-of-life decisions. A report demonstrated that executive impairment at the initial visit was associated with significantly higher rates of attrition due to disability or death, and faster rates of motor functional decline, particularly in bulbar function (Gordon et al. 2010; Elamin et al. 2013).

In patients with ALS without dementia, executive dysfunction, but not impairment in other cognitive domains, is an important negative prognostic indicator (Elamin et al. 2011).

There is a significant clinicopathological and genetic overlap between ALS and FTD (Phukan et al. 2007). The complications of FTD are a major problem for home-based care. Delusion is particularly common in patients who develop FTD/ALS (Lillo et al. 2010) and survival is significantly shorter in FTD/ALS. A report demonstrated that patients with simultaneous onset of cognitive and motor symptoms had shorter survival times than those with discrete onset for FTD/ALS (Hu et al. 2009). With these issues, domiciliary medical care and treatment are difficult to maintain.

The treatment of cognitive impairment in ALS is difficult as there are no specific drugs. Off-label use of medications for dementia includes donepezil, rivastigmine, galantamine, and memantine. Serotonin selective reuptake inhibitors (SSRIs) are commonly used for behavioral control in patients exhibiting aggression, agitation, disinhibition, and depression. Delirium or nocturnal agitation can be treated with low-dose olanzapine, risperidone, or quetiapine.

Wicks and Frost (2008) pointed out that despite a desire for more information about cognitive symptoms from patients and their carers, healthcare professionals

continue to primarily address only the physical consequences of the disease. So we should take notice of this.

11.2.5 Recent Treatment of ALS

Although a useful curative treatment for ALS has not yet been developed, the attitude toward care has gradually changed. In "symptomatic care of patients with ALS" published in 1975 (Smith and Norris 1975), QOL was acknowledged as an integral aspect of patient care in the 1980s, while "motor neuron disease: towards better care" was published in 1985 (Norris et al. 1985). In the 1990s, ongoing clinical research was being performed in the areas of speech, swallowing, nutrition, exercise, respiratory care, emotional well-being, QOL, caregiver burden, and health assessment tools such as the ALS functional rating scale (ALSFRS-R), Norris Limb and Bulbar Scale, and the Appel Functional Rating Scale (Oliveira and Pereira 2009).

Despite several clinical trials of various agents for ALS, anti-glutamate Riluzole, approved by the US Food and Drug Administration, the Japanese Ministry of Welfare and Health, and elsewhere, has only been used as a disease-modifying drug, although the effect is slight (Bensimon et al. 1994; Miller et al. 2012). Recently, several treatment modalities were launched. A trial of ALS patients involving the administration of hepatocyte growth factor (HGF) in cerebrospinal fluid (CSF) is ongoing in Japan. Sreedharan and Brwon have reviewed small molecules in trial that include NP001 (modifies activation of macrophages), CK2017357 (activates troponin to enhance muscle contractility), ozanezumab (blocks inibition of axonal outgrowth), arimoclomol (tested only in SOD1-mediated ALS; improves cellular stress responses), mexiletine (reduces excessive neuronal firing), and rasagiline (neuroptotective properties). Trials of stem cell therapy are also ongoing. A study of autologous, marrow-derived mesenchymal stem cells prepared ex vivo using a proprietary protocol, and testing of fetal human stem cells administered via direct intraspinal injection to individuals who are receiving a full immunosupression protocol, as used in organ transplantation, have been performed (Sreedharan and Brown 2013). There have also been trials involving modified antisense oligonucleotides used to silence SOD1-mediated ALS, after confirmation in transgenic ALS rats (Miller et al. 2013). It is notable that not only patients with ALS, but also their families, are looking forward to the development of a ground-breaking treatment.

11.3 Fundamental Issues for Home-based Medical Care

11.3.1 Impairment in ALS

Familiarity with the impairments caused by ALS is essential in caring for the patient, especially in a home-based care setting. The commonest symptoms are weakness, wasting, fasciculations, cramps, spasticity, communication difficulties, dyspnea, chronic hypoventilation, excessive watery saliva, persistent secretions, dysphagia, emotional lability, depression, anxiety, insomnia, fatigue, constipation, and pain. In terms of motor dysfunction, muscle weakness of the four extremities and the trunk, due to lower motor neuron impairment, leads to restricted activity. Spastic paraplegia due to upper motor neuron impairment brings leg and joint pain, and also restricts activity. Patients with lower limb onset and flail arm/flail leg syndrome have a good prognosis.

It has been proposed that a poorer prognosis is apparent with the presence of several features including a short interval between the first symptom and the diagnosis, presence of dementia, bulbar or respiratory onset disease, an older age of onset, marked weight loss, presence of pure upper or pure lower motor syndromes, malnutrition/ hypermetabolism, rapidly progressive decline in ALSFRS, beneficial vascular risk profile, increased homocysteine, vital capacity <50 % of normal, and sniff nasal inspiratory pressure <40 cm H_2O (Hardiman 2013). In home-based care, we should be more conscious of these issues to enable provision of adequate care and treatment.

11.3.2 Communication Disturbance

Difficulty in communication is one of the major impairments in patients with ALS.

For ALS patients undergoing home-based care, maintaining communication with others is one of the most important issues for maintaining a good QOL. From this point of view, appropriate timing of referral for augmentative and alternative communication (AAC) assessment and intervention, continues to be an important clinical decision-making issue (Beukelman et al. 2011). Encouragement of communication using several types of equipment has begun to be performed. Microprocessor units are available. Professor Stephen Hawking has well illustrated how to communicate with computer communicating systems. Dysarthria progressing to mutism occurs in ALS in all cases. It is no exaggeration to say that QOL may be influenced by communication impairment in patients under home-based care. Therefore, a patient with dysarthria and their interlocutor must optimize the opportunities for better communication. A study on predictive factors of communication impairment in ALS patients under tracheostomy with a ventilator has shown faster progression (Nakayama et al. 2013) while prosthetic treatments can be helpful for improving articulation. A character board is useful, a patient

being able to indicate a character on the board, following reading by the operator. Communication options for ALS patients have been expanded with the technological advancement of so-called augmentative and alternative communication (AAC) devices (Beukelman et al. 2007, 2011). A systematic review of AAC devices revealed four critical variables, i.e., language representation, output, motor access, and the microprocessor unit. For language representation, devices are being designed that integrate natural language processing and prediction algorithms for word, utterance and conversational level units allowing natural speech. Voice banking is often considered as an early treatment option involving customized communication devices in the future. Most devices offer a variety of access methods including keyboards, touch screens, and touch sensors etc. For advanced stage patients with ALS with only minimal movement of the head and eyes, access interface strategies have been proposed including eye or head tracking (Fager et al. 2011; Spataro et al. 2014). These devices are useful for not only patients, but also for caregivers (Hwang et al. 2014). One disadvantage of these devices is that the operation of a computer can be difficult for older people. Despite the systemic motor deficit progress in ALS, skill learning always remains intact, even up to the very final stages (Silvoni et al. 2013). Although AAC devices are useful for mutual communication, it has been shown how recipient animation of an authored written contribution is an important element of the interaction, particularly regarding how the recipient reveals their stance or reaction to what has been written (Bloch and Clarke 2013). Using these devices, patients in our hospital wrote blog entries on a daily basis, which resulted in social communication and its attendant pleasure.

When ocular movement is intact, an eye-tracking computer system is a valuable device for patients with ALS, although the progress of oculomotor impairment may limit its functional use (Spataro et al. 2014). Useful technological advances include brain-computer interfaces (BCIs) and thought translation devices, although they are not yet widely available. BCIs could be used in advanced stages of the disease, since patients do not require the preserved ocular-motor ability necessary for eye-tracking applications. Visual or auditory P300-based BCIs might also be effective in aiding communication (Cipresso et al. 2012; McCane et al. 2014). Although communication impairment might be alleviated using these devices, it should be noted that debate on the related specific ethical issues still exists (Phillips 2006; Nijboer et al. 2013).

11.3.3 Autonomic Nervous System Disorders in ALS

As a pure motor disorder, traditional thinking about the physical and neurological findings in ALS patients excluded autonomic nervous system disorders, sensory disturbance, and decubitus ulcers. Several recent findings have modified this thinking, especially concerning hereditary ALS. In sporadic ALS patients, increasing muscle sympathetic activity has been reported (Shindo et al. 2004, 2011). The data suggest that an autonomic nervous system disorder might potentially exist,

especially in long-term patients. We sometimes experience decubitus ulcers, constipation, diarrhea, and urinary retention in patients with long-time survival as a result of ventilation. A study showed that urinary incontinence was increased in patients with ALS aged ≥60 years with the predominant presentation of urge incontinence. A high prevalence of constipation without stool incontinence was also noted (Nübling et al. 2014). Caring for ALS patients with autonomic nervous system disorders at home is a much greater challenge.

11.3.4 Management of Progression of ALS

ALS is a progressive condition that significantly reduces life expectancy, and death occurs mostly in a predictable manner. Therefore, in order for ALS patients to live at home, systematic management of the progression of the disease, from the earliest stages onwards, is essential. The QOL of the patient and caregiver can be improved through the symptomatic treatment of patients. Because many patients change their preferences for life-sustaining therapy as the disease progresses, especially at an advanced stage, a patient should have a chance to reconsider their own ideas and to make decisions as to cardiopulmonary resuscitation, and treatment of respiratory and nutrition problems. As a result, ventilation or percutaneous gastrostomy could be introduced (Silverstein et al. 1991). When a patient determine whether to receive these intervention therapy or not, it is important to ask the caregiver's opinion (Chiò et al. 2005).

A report by the quality standards subcommittee of the American Academy of Neurology (AAN) recommended that multidisciplinary clinic referral should be considered in the management of patients with ALS to optimize health care delivery and to prolong survival (Level B), and may also be considered to enhance QOL (Level C). In the treatment of refractory sialorrhea, botulinum toxin B should be considered (Level B), and low-dose radiation therapy for the salivary glands may be considered also (Level C). In the treatment of pseudobulbar affect, dextromethorphan and quinidine should be considered if approved by the US Food and Drug Administration (Level B). For patients who develop fatigue while taking Riluzole, withholding the drug may be considered (Level C). Because many patients with ALS exhibit cognitive impairment, which in some cases meets the criteria for dementia, screening for cognitive and behavioral impairments should be considered in patients with ALS (Level B). Other existing management strategies all lack strong supporting evidence (Miller et al. 2009b). The report also recommended that Riluzole should be offered to slow disease progression (Level A). PEG should be considered to stabilize weight and to prolong survival in patients with ALS (Level B). NIV should be considered to treat respiratory insufficiency in order to increase survival (Level B) and to slow the decline of forced vital capacity (Level B). NIV may also be considered in order to improve QOL (Level C). Early initiation of NIV may increase compliance (Level C), and insufflation/exsufflation may be considered to help clear secretions (Level C) (Miller et al. 2009a). Disease progression can be monitored using a variety of clinical scales. The Revised ALSFRS-R is

currently the most widely used measurement tool, and a decrease in score is a predictor of reduced survival (Traynor et al. 2003). For home-based care, dysphagia and respiratory insufficiency are major problems. Practical measures for the management of ALS patients are discussed below.

11.3.4.1 Cramping and Spasticity

Cramps are a common complaint in ALS. They can be treated with massaging and physiotherapy, and with phenytoin, carbamazepine, benzodiazepines such as clonazepam, diazepam, etc. and kampo (Japanese herbal medicines). Spasticity means increased tonus and is caused by upper motor neuron degeneration, and may cause pain and lead to gait disturbance due to the impaired coordination of limbs that results through stiffness. Muscle relaxants including baclofen and tizanidine may be effective. Spasticity can also be treated with physiotherapy to increase or maintain the range of motion and to avoid contractures. However, a reduction in the spasticity of the lower limbs may reduce mobility due to the development of muscular weakness through lower motor neuron impairment. Therefore, careful assessment by an experienced physiotherapist is essential.

11.3.4.2 Pseudobulbar Affect

Pseudobulbar affect, pathological crying or laughing, is a consequence of corticobulbar degeneration and occurs in up to 50 % of patients. It is important to take notice of the symptoms as it results in the loss of communication between patients and caregivers due to emotional overexpression or through difficulty in speaking. It has traditionally been treated with amitriptyline or SSRIs. Dextromethorphan combined with quinidine may be beneficial, although this treatment may be limited by side effects (Brooks et al. 2004; Miller et al. 2009b).

11.3.4.3 Pain

ALS patients always complain of discomfort due to the immobility of their bodies and the effects of gravity. About half of ALS patients report pain relating to QOL (Pagnini et al. 2012b). A report demonstrated that 23 % of patients complain of shoulder pain at some time during the course of their illness (Ho et al. 2011). To reduce body pain, a frequent change of position is needed. Regular physiotherapy is also useful for reducing and preventing the symptoms (see also *Rehabilitation*). Treatment should begin with simple analgesics such as paracetamol (acetamynophen) and Non-steroidal anti-inflammatory drugs (NSAIDs), followed by weak opioids such as tramadol, and then strong opioids such as morphine. External medicines such as compresses or liniments can easily be used.

11.3.4.4 Respiratory Insufficiency

Most ALS patients die from respiratory failure involving pneumonia (Gelanis 2001), and in the late stage of the disease, develop dyspnea, a feeling of choking, thoracic heaviness, restlessness, and anxiety. Despite the inevitable weakness of the respiratory and oropharyngeal muscles, choking as the cause of death is rare in ALS (O'Brien et al. 1992; Mandler et al. 2001). The onset of the disease rarely involves respiratory impairment (de Carvalho et al. 1996; Shoesmith et al. 2007), and the respiratory-onset disease has the worst prognosis (Chiò et al. 2009). Nowadays, noninvasive positive pressure ventilation (NPPV) has become the standard treatment for patients with advanced respiratory insufficiency (Tripodoro and De Vito 2008). Although the presence of respiratory muscle weakness is an independent predictor of QOL, evaluation of respiratory function is a very important issue in ALS care and management (Lechtzin et al. 2007), and even more so in multidisciplinary home-based care. Because of the slow progression of respiratory muscle weakness, symptoms of respiratory insufficiency may not be detectable, either subjectively or objectively. Respiratory failure should be anticipated when the diagnosis has been established (Bradley et al. 2002). Early signs of respiratory insufficiency may include morning headaches, daytime somnolence, and fatigue with or without disturbed sleep, or orthopnea, dyspnea, poor concentration, and nocturia. Therefore, assessment of respiratory insufficiency is important and should include interviews, physical examinations, pulmonary function tests, overnight or diurnal pulse oximetry, and arterial blood gas measurements, especially in the early morning. Reversible causes of shortness of breath such as infections, pulmonary emboli, or bronchospasms should also be considered when assessing dyspnea.

During home visits, accurate estimation of respiratory function is necessary. Forced vital capacity (FVC) is the most widely used method in the assessment of respiratory insufficiency in ALS, but limitations include the insensitivity to significant changes in respiratory function partly down to difficulties in performing the test due to muscle weakness in the face and limbs. The erect-supine FVC difference, the supine FVC being significantly lower than the erect FVC, is significantly greater in patients complaining of dyspnea, orthopnea, and daytime fatigue (Varrato et al. 2001). SNIP, which correlates well with diaphragm strength and nocturnal hypoxemia reflecting changes in respiratory muscle strength, is useful in the accurate measurement of respiratory function, particularly in patients with bulbar involvement, since a face mask is not required (Morgan et al. 2005). Abnormal arterial blood gas findings develop at a late stage, and hypercapnia leads to a very poor prognosis. For home-based care, transcutaneous carbon dioxide/oxygen sensors (pulseoxymetry, etc.) can be useful instead of regular arterial blood measurements. When a patient with ALS suffers from acute ventilation with acute respiratory failure, they will almost always need permanent ventilation (Bradley et al. 2002). CO_2 narcosis is one of the emergency situations in ALS patients not using tracheostomy positive pressure ventilation (TPPV). Once CO_2 retention has occurred, the diminished respiratory drive leads to carbon dioxide retention, which

causes headaches, dyspnea, somnolence, and ultimately narcosis. The routine use of supplemental oxygen is not recommended in ALS other than when using TPPV, as it may promote or enhance the narcosis greatly. Yearly influenza vaccinations and polyvalent pneumococcal vaccine are recommended for patients at home. In patients with TPPV, decreased internal pressure of the oral cavity may lead to tinnitus.

11.3.4.5 Non-Invasive Positive Pressure Ventilation (NIPPV)

The most significant advance and the strategy favored in ALS care, has been the domiciliary provision of NIPPV for treating respiratory insufficiency (Rafiq et al. 2012). NIPPV is useful for extending the patient's survival and decreasing respiratory problems, so NIPPV should be introduced early on. It has been shown that bilevel NIPPV improves the prognosis of ALS patients with significant respiratory insufficiency and also improves QOL without increasing the stress and burden on the caregiver (de Carvalho et al. 1996; Aboussouan and Lewis 1999; Kleopa et al. 1999; Bourke et al. 2006; Mustfa et al. 2006; Baxter et al. 2013). However, for young male patients and patients attending specialists, NIPPV tends to be introduced early on (Chiò et al. 2012b). Clinical guidelines for the use of NIPPV in the management of ALS have been presented to professionals and co-workers by the National Institute of Health and Clinical Excellence (NICE) in the UK. The guidelines comprise an assessment pathway for identifying and assessing respiratory insufficiency (NICE clinical guidelines). The criteria for initiating NIPPV suggested at a meeting of the European ALS/MND consortium sponsored by the European Neuromuscular Centre (ENC) are as follows: at least one of the symptoms related to respiratory muscle weakness: dyspnea, orthopnea, disturbed sleep, morning headaches, poor concentration, anorexia, excessive daytime sleepiness (ESS >9), evidence of muscle weakness, a FVC $=<$ 80 % or SNIP $=<$ 40 cm H_2O, and evidence of either significant nocturnal desaturation on overnight oximetry or a morning ear lobe gas level of pC02 $>=$ 6.5 kPa (Leigh et al. 2003). The other criteria recommended are a vital capacity of less than 50 % of that predicted and a SNIP value of less than 40 cm H_2O. A diagnostic indication is when symptoms of respiratory insufficiency are associated with nocturnal hypoxemia and an elevated early morning blood CO_2 level (Miller et al. 2009a). Pulse oximetry should be performed on NIPPV, and patients should be reviewed at regular intervals by a respiratory physician in order to ensure that the pressure settings and attachment time are optimized (Pinto et al. 2003, 2010). A study showed that home telemonitoring, a modern tool for NIPPV, reduces healthcare utilization (Pinto et al. 2010). A report revealed that NIPPV extends survival, particularly in those who are compliant with $>=$ 5 h of NIPPV each day and those without severe bulbar dysfunction (Bourke et al. 2006). However, frequent nocturnal patient-ventilator asynchrony in patients consistently using nocturnal NIPPV was reported (Atkeson et al. 2011). Common initial complaints with NIPPV, such as leaks, abdominal bloating, and facial discomfort, can be easily

managed. Some patients have difficulty tolerating NIPPV with the presence of bulbar symptoms, including increased secretions or cognitive impairment, or an inability to manually adjust the mask. Of those who can tolerate NIPPV while hospitalized, a significant proportion do not persevere following discharge. When a patient with respiratory insufficiency is unable to use NIPPV, a discussion about tracheostomy and invasive ventilation, complete with information on the relative advantages and disadvantages from the point of view of palliative care, should take place as early as possible (Albert et al. 2009). For dyspnea and despite NIPPV, a combination of opiates and benzodiazepines is used. Opioids are both safe and effective for dyspnea (See paragraph, palliative care).

11.3.4.6 TPPV

Invasive mechanical ventilation, involving the use of a tracheostomy or endotracheal tube, may be considered in patients who cannot tolerate NIPPV or who suffer from acute respiratory failure. A report stated that most decisions about TPPV tend to be taken in emergency situations in the absence of advance directives, and in young positively-motivated patients with predominantly bulbar symptoms in whom NIPPV fails (Heritier Barras et al. 2013). While the use of a tracheostomy tube may extend life, there are many ethical issues to be considered. In terms of the withdrawal of mechanical ventilation, current thinking is differs by country due to different legal ethics etc. In the more likely event that patients and carers decline TPPV, a patient and family should be provided with assurances that palliative care strategies can control symptoms in the terminal phase of the disease. Unlike in cases of NIPPV, patients using TPPV should be aware of the risk of losing all methods of conventional communication, including eye movements, and that they may reach a locked-in state. In the absence of advanced technology such as BCIs, the thinking of these patients who have become incapable of communicating may be difficult to assess. It is therefore desirable that the patient's wishes and thinking are made known prior to the loss of communication, and that TPPV is not introduced. It also should be taken into account that the routine use of TPPV at home may be prohibitively expensive and the patient may be forced to use a social program or system for home care out of necessity.

On the other hand, in Switzerland and France, current practice tends to discourage the use of TPPV due to the fear of a locked-in state, the high burden placed on caregivers, and the unmasking of cognitive disorders occurring as the disease progresses (Heritier Barras et al. 2013).

11.3.4.7 Sialorrhea and Bronchial Secretions

Sialorrhea (excessive salivation) is associated with dysphagia, and the impairment of smooth salivary secretion. Sialorrhea can be treated with oral medications such as amitriptyline, atropine, and trihexyphenidyl hydrochloride etc. (Cooper-Knock

et al. 2011), cautious injection of botulinum toxin into the salivary glands (Gilio et al. 2010) and (Winterholler et al. 2001), or salivary gland irradiation (Neppelberg et al. 2007). However, sialorrhea can be difficult to manage with medication in patients used to employing portable suction equipment or a continuous suction machine.

Neuromuscular respiratory weakness leads to ineffective coughing and retained airway secretions, predisposing patients to recurrent chest infections. Bronchial secretions can be treated with mucolytics, nebulized beta-adrenergic antagonists, or anticholinergics. As with chronic bronchitis, treatment with antibiotics for several weeks may be effective. Physiotherapy, manually assisted coughing techniques, and modified postural drainage can also be helpful. It was reported that expiratory aid by regular use of mechanical cough-assisting and insufflation-exsufflation devices, may facilitate airway clearance in patients at an advanced stage (Lahrmann et al. 2003; Sancho et al. 2004). The combination of NIPPV and cough-assisting machine techniques, decreases pulmonary complications, morbidity, and hospital admissions (Simonds 2006). In the past in Japan, in order to prevent sputum storage and athelectasis, high volume ventilation may have been chosen.

11.3.4.8 Weight Loss and Nutritional Support

Weight loss and malnutrition are common symptoms in ALS patients. These symptoms can result from dysphagia, wasting of muscle and fat, difficulties in eating due to weakness of the limbs and trunk, and hypermetabolism, particularly in those with compromised respiration. Dysphagia should be evaluated using clinical scales and fiber-optic examinations. The data indicate appropriate nutritional administration; modification of food and fluid form / consistency, postural advice for eating and digestion, and parenteral feeding by nasal tube or gastrostomy. Placement of a gastrostomy tube is recommended at an earlier stage for those who have symptomatic dysphagia or significant weight loss, and respiratory insufficiency. The practice is particularly recommended for a patient who is receiving home-based care, or is in a nursing home. The data suggest that gastrostomy tube placement using the percutaneous endoscopic method (PEG) or surgery under local anesthesia can both be accomplished safely in ALS patients with erect or supine % FVC measurement values of less than 50 % than predicted using NIPPV, oxygen, oxymetry monitoring, and conscious sedation (Gregory et al. 2002). Advantages include improved nutrition, although the survival effect is likely to be marginal. Radiologically inserted gastrostomy (RIG) is preferred over PEG in patients with severe bulbar symptoms and/or respiratory complications in order to avoid aspiration and respiratory emergencies (Greenwood 2013). If there is evidence of respiratory insufficiency, NIPPV should be introduced before gastrostomy. If the patients wish to be fitted with a tracheostomy tube, simultaneous laryngectomy is useful, as it enables patients to eat for a longer period.

Hypermetabolism, physical activity, and mechanical ventilation may be sources of energy expenditure (Genton et al. 2011). A study demonstrated that ALS patients

are hypermetabolic by an average of 10 % with no association with a reduction in respiratory function, smoking, hyperthyroidism, spasticity and fasciculation intensities, or infection (Desport et al. 2001). Defects in energy metabolism including weight loss, hypermetabolism, and hyperlipidemia in ALS patients correlate with the duration of survival (Dupuis et al. 2011; Shimizu et al. 2012). It was shown that high calorie diets increased survival in a mouse model (Dupuis et al. 2004; Mattson et al. 2007), while mild obesity was associated with greater survival in patients with ALS (Gallo et al. 2013; O'Reilly et al. 2013). Evidence has been reported that hypercaloric enteral nutrition is safe and tolerated in patients with ALS (Wills et al. 2014). A study demonstrated that NIPPV can reduce energy expenditure in ALS patients by alleviating the ventilatory burden imposed on inspiratory neck muscles to compensate for diaphragm weakness (Georges et al. 2014). In addition to energy expenditure, the volume of water taken in is a significant matter because a surplus water intake leads to heart failure or edema, and a shortage to either dehydration or coarse sputum. Due to their everyday importance, control of energy expenditure and water are important for maintaining a basic physical condition in home-based care.

11.3.5 Rehabilitation

It is unclear whether exercise is necessary or not in patients with ALS (de Almeida et al. 2012). A report showed that a program of regular moderate physical exercise has a positive but short-lived effect on disability in ALS patients (Drory et al. 2001). In the Netherlands, the protocol for rehabilitative management in ALS was used in 89 % of all treated ALS patients, and organized rehabilitation medicine was effective in the symptomatic and palliative treatment of ALS patients (van den Berg et al. 2003). In our prefecture, a rehabilitation hospital puts effort into the rehabilitation of ALS patients. An investigation revealed that aerobic exercise therapy and cognitive behavioral therapy are both effective in improving functioning and QOL in ALS (van Groenestijn et al. 2011). A report suggested that exercise may be beneficial in ALS patients once NIPPV is used to control peripheral and muscle oxygenation (Pinto et al. 1999). In home care, from the perspective of total health care including mental care, ordinary exercise is recommended as early as possible.

11.3.6 Mental Care

ALS is a fatal disease and the patient needs to be prepared to accept death. The QOL of patients is often influenced by psychological factors including reactive depression due to the ALS. Therefore, good mental care is very important for maintaining QOL. Several studies revealed no increasing prevalence of syndromal depressive

disorders even in patients at a late-stage of the disease (Rabkin et al. 2005). It has been confirmed that ALS patients with psychological distress have a significantly greater risk of death (McDonald et al. 1994). A report concluded that the desire to hasten dying in the end-stage of the disease is simply a feature of depression (Albert et al. 2005). Research indicated that ALS patients can still have a high QOL despite progression of the disease, and that the prevalence of depressive disorders remains far below that which one would expect (Norris et al. 2010). Fatigue, depression, and excessive somnolence are pronounced in ALS and lead to a poorer QOL (Lou et al. 2003). Although fatigue and depression are overlapping conditions, it has been reported that fatigue is more prevalent and persistent than depression, and is associated with disease severity (McElhiney et al. 2009).

Counseling for patients, and also for caregivers, is useful in helping to manage the psychological aspects of the disease. Both SSRIs and tricyclic antidepressants can be used for a depressive state or depression. Amitriptyline is often the preferred choice if sialorrhea, insomnia, or pseudobulbar effect coexist. Anxiety can be treated with benzodiazepines.

A study demonstrated that ALS patients have a poor quality of sleep in association with a decreased ALSFRS-R score, deep depression and an increase of Epworth Sleepiness Scale (ESS) score (Lo Coco et al. 2011). Meanwhile, sleep problems might arise from dyspnea, anxiety, depression, difficulty in changing position, suction of sputum or from the use of a ventilator. Identification of the cause of the sleep disturbance is crucial prior to beginning home care because it is influenced by several physiological problems. When sleep disturbance due to respiratory insufficiency appears NIPPV may be appropriate, but a study showed that nocturnal NIPPV improves oxygenation but has no benefit for sleep (Katzberg et al. 2013). A report suggested that the treatment of sleep problems might be useful in alleviating fatigue in patients with nocturnal symptoms such as nocturia and muscle cramps (Lo Coco and La Bella 2012). One should be aware of sleep problems in home care. Although sleep medication is beneficial, it should be avoided if there is worsening respiratory failure and bulbar palsy caused by increasing muscular weakness or decreasing consciousness.

11.3.7 QOL

Quality of life is determined by the pleasure and satisfaction an individual draws from living. Health-related quality of life is determined by the influence that an individual's health has on their day-to-day living. A fine balance between appropriate information for home care and a patient's acceptance of the situation leads to better quality decision-making. The QOL of patients is affected by the disparity between a patient's choices and their clinician's view of the situation based on evidence, knowledge, and experience.

Assessment of QOL is important in maintaining a good quality of care (Neudert et al. 2001b).

To measure the QOL of individuals with intractable diseases, many scales have been developed (Heffernan and Jenkinson 2005), and several ALS-specific health-related QOL scales have been proposed such as amyotrophic lateral sclerosis assessment questionnaires (ALSAQ-40 and ALSAQ-5). (Jenkinson et al. 1999; Jenkinson and Fitzpatrick 2001). It has been reported that QOL is well maintained in ALS, despite decreases in physical function, and this may be attributed to a psychological response shift (Hardiman et al. 2004). On the other hand, health-related QOL decreases in patients with ALS commensurate with their physical decline, and some patients experience high levels of psychological distress. The QOL in patients with ALS is determined by several factors including psychological and existential factors, relationships, and other support factors, religion and spirituality, in addition to patient strength and level of physical function (Van den Berg et al. 2005). In Japan, a report pointed out that the general QOL of community-dwelling patients with intractable neurological diseases assessed by means of a Short Form (36) Health Survey (SF-36), and that of their caregivers by means of Short Form (8) Health Survey (SF-8) was significantly lower than the national standard values. Low correlation between patients' QOL and caregivers' QOL was also reported (Miyashita et al. 2011).

11.3.8 Palliative Therapy

Because of its severe clinical characteristics, ALS represents a paradigm for palliative care in neurodegenerative diseases. Almost all patients wish to live in their own home rather than in a hospital or in the homes of others. Living at home allows a patient to live as full a life as possible, right up to the end stage. Home care has many benefits. There is considerable evidence that palliative care intervention improves QOL in both patients and carers (Bede et al. 2011). It was indicated in a recent 10-year literature review of family caregiving in ALS, that caregiver burden and QOL studies have dominated the research field, and that palliative care interventions should be focused on in the future (Aoun et al. 2013). Accordingly, the aim of palliative care is to maximize the QOL of patients and families by relieving symptoms, providing emotional and psychological support, removing obstacles to a peaceful death, and supporting the family in bereavement (Borasio et al. 2001b).

Palliative care should be based in the local community and given from the time of diagnosis, and advance directives regarding the end of life should be discussed early on and revisited regularly (Johnston et al. 1996; Bradley et al. 2001; Borasio et al. 2001a). Advanced directives for ALS with respiratory failure and the use of mechanical ventilation have been proposed (Benditt et al. 2001). From the early period of home care management, it was thought that palliative care offers a more peaceful death for ALS sufferers (Oliver 1995). Therefore, assurances should be made that palliative care strategies can control symptoms in the terminal phase of the illness.

In the past, one of the prototypes of palliative care was provided in hospices, where the family is the unit of care, and particular attention is paid to the prompt and effective relief of distressing symptoms, in conjunction with psychosocial and spiritual support (O'Brien et al. 1992). A flexible model of care with evidence-based palliative interventions at specific trigger points in various clinical domains has been proposed.

One of the important attitudes regarding palliative drugs is that the drugs should always be for the control of symptoms and not for the shortening of life (Oliver 1997). For symptomatic treatment of dyspnea, anxiolytics, such as benzodiazepines, are widely used in combination with opioids to reduce anxiety and distress. Moreover, opioid and other narcotic medications are effective in treating pain and discomfort long before the last stages of the illness, including restlessness and confusion due to hypercapnia (Oliver 1998). It has been established that the doses of morphine used in ALS are significantly lower than those used in cancer-related pain syndromes. Opiates are effective in the management of pain, nocturnal discomfort, and dyspnea long before the terminal phase of ALS. A report demonstrated that patients died peacefully at home from carbon dioxide narcosis with palliative medications (Kühnlein et al. 2008).

11.3.8.1 End of Life Issues

A report of a pre-2000 survey revealed that two-thirds of patients were living in hospices and would probably die in their preferred location, receiving morphine. Therefore, it should be considered that information about hospice enrollment is made in a timely manner (Casarett et al. 2004). The specialist palliative care provided by hospice institutions can help patients during the terminal stages of ALS (Oliver and Webb 2000). Meanwhile, a survey revealed that around half of the patients died at home, and that most ALS patients died peacefully, and no patient choked to death in either Germany or the UK (Neudert et al. 2001a). As hospices are not common in Japan, patients always die in hospital, although patients spend all their time until near the end of life at home and wish to die there. In terms of the end of life at home, doctors should take the initiative in preparing patients for the acceptance of death. In the terminal phase of the disease, an appropriate supporting protocol of care and treatment is important for maintaining the quality of dying and death. Steady consistent care appropriate for ALS patients from the earliest stage is also necessary for a satisfactory end to life. A study demonstrated that almost 90 % of patients had advance directives and their decisions were honored by healthcare professionals, and most caregivers mentioned that the patient remained calm in the face of death after due preparation (Ganzini et al. 2002). It was demonstrated that advance directives were in place for 88.9 % of 1014 patients with ALS in America and Canada, and were followed in 96.8 % of cases, and that most patients died peacefully (Mandler et al. 2001). In this regard, clinical evidence and standards regarding how to provide optimal end-of-life care specifically in ALS are needed (Mitsumoto et al. 2005). At the terminal stage, the carer burden is excessive, and

may exacerbate patient distress and the desire for hastening death (Whitehead et al. 2012), so physicians should consider this along with psychiatric complications (Stutzki et al. 2014).

Recent studies have focused on euthanasia and physician-assisted suicide (PAS) (Reagan et al. 2003). In most countries, it is currently illegal for physicians to grant euthanasia and PAS including continuous deep sedation (CDS) with the object of death. In Sweden, where euthanasia or PAS is illegal, patients with ALS are at very high risk of suicide, and the relative risk is even higher during the earlier stages of the disease (Fang et al. 2008). In the Netherlands, actions assisting the ending of the life of patients are allowed, as long as the actions are consistent with the requirements of due care laid down in the Euthanasia Act of 2002 (Maessen et al. 2009). Several studies on terminally ill patients suggested that high and increasing rates of euthanasia or PAS could be the result of inadequate palliative care, unrecognized depression, patients feeling they were a burden on others, hopelessness, the financial burden, or lessening of QOL (Ganzini et al. 2002; Emanuel and Battin 1998; Bascom and Tolle 2002). A report demonstrated that fear of suffocation and dependency is the most frequent reason for euthanasia in ALS patients (Maessen et al. 2010). One in five patients with ALS died as a result of euthanasia or PAS in the Netherlands, although euthanasia or PAS was still illegal, but there was no punishment for the action in 2002 (Veldink et al. 2002). The report also showed that disability before death was significantly more severe in patients who died as a result of euthanasia, than among those who died in other ways. Instead of these active measures, facilitation of CO_2 retention with oxygen inhalation could lead to a quiet death through narcosis. The preference of a patient for cardiopulmonary resuscitation and ventilator care may change over the course of the disease, so directives should be reviewed with any participating spouse carers (Chiò et al. 2005).

11.3.8.2 After Separation

A patient's death has a lasting impact on surviving family members (Martin and Turnbull 2001). Some family members, especially caregivers, feel exhausted, and others may have concerns regarding ALS and wish to discuss them. A survey demonstrated that grief and bereavement support should be an integral part of ALS care (Hebert et al. 2005). Through the use of well-chosen words, a bereaved family can be helped to come to terms with the death, and the bond between the physician and the late patient's family strengthened (Bedell et al. 2001).

In our prefecture, relatives of family members affected by ALS can join a voluntary association of patients, families and people with valuable experience of ALS. They come for relief and in order to better understand the meaning of their loss through communication and activities with members of the association.

11.4 Practical Issues

11.4.1 Caregiver Distress

A diagnosis of ALS impacts on family members, and the roles of the patient and other family members within a family may change. It may be that a family loses a breadwinner and a housekeeper simultaneously. Instead of strengthening the bonds between family members, these changes can have a major destabilizing effect on family relationships. Although the perspectives of ALS patients and caregivers are very similar to the above mentioned therapeutic approaches, the needs and goals of patients and their caregivers are different (Trail et al. 2003). Many studies have shown that the self-reported QOL of carers may be lower than that of the patients. Caregivers have a great influence on the quality of care and the patient's QOL as the disease progresses, therefore consideration of the caregiver burden from both physical and emotional perspectives is a key factor in both initial and continuing home-based care (Pagnini et al. 2012a; Trail et al. 2003). A burden on the carer also occurs in relation to patient respiratory issues (Pagnini et al. 2012c). Sleeplessness due to supporting a patient on a ventilator at night can have a major impact on a carer's body, possibly leading to exhaustion. Attention should therefore be paid to the psychological condition of caregivers, and adequate mental support including counseling should be provided. At every stage of the illness, especially in the late stage or with ventilation, caregivers should take regular respite from their work, in order to make caring for a patient at home over a long period time less of a physical burden. To give some respite to caregivers, the patient might enter a hospital, hospice or group home for a short time on a routine basis. We have been trying to construct a network in which a patient is able to enter a hospital for a short period, thereby enabling caregivers to get sufficient rest.

A study showed that suffering associated with ALS is no more severe than that associated with Parkinson's disease and related disorders in the view of caregivers, and both groups appear to have unmet palliative care needs at the end of life (Goy et al. 2008). To this end, learning about home-based care of ALS is useful for patients suffering from other neurodegenerative disorders and their caregivers.

11.4.2 Differences in Home Care Among Countries or Districts

There is a national trend toward moving end-of-life care from hospitals to community-based settings, such as homes, hospices, nursing homes, group homes, or assisted living quarters (Tilden et al. 2004). In Japan, some families or patients do not health care professionals to entering their homes according to past tradition.

An interesting study comparing data from the Limousin Referral Centre, France, and a Uruguayan population revealed that the survival of ALS patients in Uruguay is 9 months shorter than that in France, and concluded that this was due to the heterogeneity of medical care and the absence of ALS referral centers in Uruguay (Gil et al. 2009). The study emphasized the importance of comprehensive care for ALS patients. A survey demonstrated that ALS clinics were absent in all countries in South America, and that there was a lack of appropriate controls with insufficient epidemiologic data (Cronin et al. 2007). In order to better understand and scientifically document approaches to ALS and its treatment in underdeveloped regions of South America, and in order to explore the interactions and synergies of research, clinical care, and patient advocacy, several approaches were adopted in order to educate both physicians and patients with ALS in Ecuador (Bucheli et al. 2013). In Switzerland and France, current practice tends to discourage the use of TPPV in ALS because of the fear of locked-in syndrome, the high burden placed on caregivers, and the unmasking of cognitive disorders (Heritier Barras et al. 2013). Although it depends on the region or country, a patient's religion can help them to come to terms with their impending death. Religiousness and spiritual factors helped to maintain QOL for ALS patients (Simmons et al. 2000; Walsh et al. 2003), and influenced patients' decisions in their choice of certain types of medical support system aimed at prolonging their life (Murphy et al. 2000). Caregivers with a strong religious belief also exhibit high self-reported QOL scores (Calvo et al. 2011).

11.4.3 Multidisciplinary Care

The treatment of ALS is a complex matter for patients, caregivers and professionals because of the severe disability involved, and the need to manage medical, psychological and social problems (Mitsumoto and Del Bene 2000). Owing to the variation in complaints, conditions, disease course, and rate of progression of the disease from person to person in ALS, there are no simple management protocols or critical pathways. To overcome the condition, multidisciplinary teams of professionals specialized in ALS care should be formed to improve the standards of care and QOL of patients and caregivers. It has been shown that patients receiving multidisciplinary care had a better QOL (Traynor et al. 2003; Chiò et al. 2006; van den Berg et al. 2005). Multidisciplinary care for ALS patients prolongs their survival, particularly in patients with bulbar dysfunction (Traynor et al. 2003), and improves the mental QOL of patients with ALS (van den Berg et al. 2005). When symptoms are addressed and treated in the early stages of the disease, management in a specialized setting is also more cost effective, e.g., patients who attend specialist clinics have fewer and shorter hospital admissions (Chiò et al. 2006). Accordingly, optimal management of the disease requires the expertise of a large multidisciplinary team with experience of home-based care of ALS. While such teams tend to be based in larger centers, most patients are cared for in

the community. A management strategy for a close liaison of hospital-based multidisciplinary care with community-based intervention in a “hub and spoke” or “radial system” model with close cooperation, is recommended. Such a system can be constructed in a community, using a hospital as the hub. Besides taking part in multidisciplinary care, family doctors can act as practitioners.

The multidisciplinary team should be composed of a neurologist, a family doctor, a respiratory physician, a palliative care physician, a psychology counselor, and allied professionals including physiotherapists, occupational therapists, speech and language therapists, nutrition experts, and medical social service personnel. Dentists may also participate in the team.

Evidence-based guidelines for clinical management recommended by the EFNS (Andersen et al. 2005), and the AAN (Miller et al. 2009b) emphasize the importance of multidisciplinary care for ALS patients. A study showed that the costs of multidisciplinary ALS care were practically identical to the costs of general care (van der Steen et al. 2009). Our hospital functions at the center of our prefecture and has links to several communities providing home-based care centered upon patients. Moreover, teams for home-based care under the association of patients with ALS act concertedly to aid both communication and aspiration care.

11.4.4 Home Care Network

In this chapter, the term ‘network’ has two definitions: a ‘home care network’ means a network centered on the patient, and a ‘social network’ means a network with both public and social connections. A prime object of home care for patients is slowing down the deterioration of a patient’s Activities of Daily Living (ADL) and QOL. It is also important that caregivers avoid sacrificing too much of their own lives. To help deal with these issues, a home care network is very important. The establishment of a home care network requires a good deal of effort and for good quality home care management, communication among all those involved, namely, medical staff, patients and their families, home doctors, nurses, physical therapists, helpers and hospitals, is very important. Care managers mainly plan care schedules for ALS patients at home and a stable home care network strengthens the bond between not only related persons, but also the general public or community. Medical staff help patients, families, and caregivers with the medical, emotional, and financial challenges of coping with ALS, particularly during the final stages of the disease. Social workers provide vital support such as assistance in dealing with several public systems, obtaining financial aid, preparing living wills, and finding support groups for patients and caregivers. Home nurses are available, not only to provide medical care, but also to teach caregivers about tasks such as maintaining respirators, feeding, and moving patients to avoid painful skin problems and contractures. Moreover, home nurses work in conjunction with physicians to ensure proper medication, pain control, and to provide other care affecting the QOL of patients who wish to remain at home. Attending physicians and co-workers can also

counsel patients and caregivers about end-of-life issues. Interaction between patients, caregivers, and community workers has been investigated, and it was concluded that support networks varied in size and composition, and carer age was identified as a discriminator of the availability and consistency of support (Ray and Street 2005). Community workers should endeavor to provide a consistent level of care and services. Care conferences attended by a patient, doctors, homecare nurses, home helpers, a care manager and also hospital nurses, should be held at times of change to the care plan including at discharge. In our prefecture, for patients using a ventilator at home, a ventilator agency provides several services including maintenance of ventilators and suction machines, and supports a variety of situations throughout the day. We can obtain information from agency staff about situations concerning ventilators or ventilation, in order that we can better understand the respiratory status of a patient, and relay the information on our medical and home care. Recently, the Ministry of Health, Labour and Welfare in Japan made the groundbreaking decision that suction by means of tracheostomy tube by a home helper, a long-held wish of persons taking part in home care, would be allowed in addition to use of such by family members and professionals. The decision has improved home-based care regardless of the disease type.

In our survey on the situation of home care in our prefecture by a research team of the Ministry of Health, Labor and Welfare in Japan, we found that some small networks centered upon ALS patients already existed. The combination of several small networks is helpful in establishing a social network (Fig. 11.1). Therefore the fundamental structure for building a social network already exists. In 1996 we proposed a small home care network for a patient with TPPV, with family members, visiting nurses, home helpers and university students as volunteers. Tracheostomy tube exchange has been performed in our hospital and at home by a family doctor alternately, every 2 weeks.

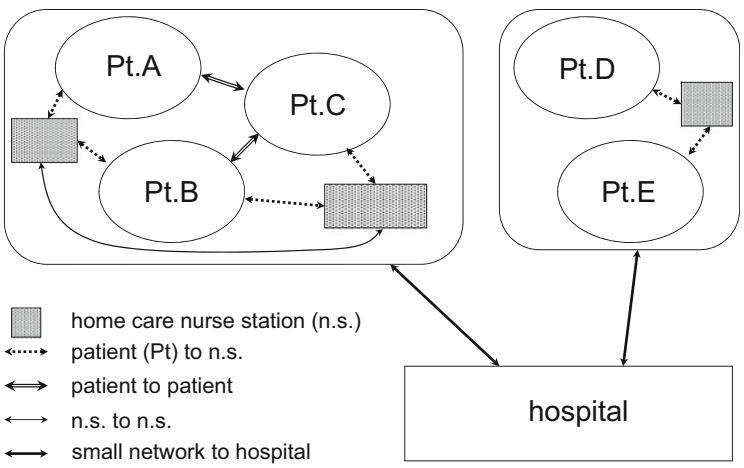

Fig. 11.1 Conjunction of small networks centered upon patients

11.4.5 Social Program or System

For continuing home-based care, consideration of the economic costs as well as the human costs associated with caregiving is especially important. In order for patients to spend time at home, utilization of a social program is crucial because it covers care costs. Patients with ALS think that coordination is an important factor (Mitsumoto et al. 2006). There are several programs or systems currently available in Japan, such as a system for rare diseases specified by the Japanese government as being worrisome, having no effective treatment and of unknown causes, a nursing care insurance system, and physical disability certificates. Under these organizations, care managers belonging to a nursing care insurance system draw up plans for home care. With these programs, the cost of house modifications is covered to enable patients to spend their time at home in comfort and safety. It was reported that case management appears to have no benefit for patients with ALS or their caregivers within the context of multidisciplinary ALS care teams (Creemers et al. 2014). However, e.g., in Japan, multidisciplinary teams have been poorly constructed, so case management is important in community-based care. Moreover, one method by which we can unify quality of care among small networks is by the development of a critical path for care.

11.4.6 Measures to Deal with Natural Disasters

A manual for dealing with natural disasters should be prepared for home care for patients using a ventilator. In the event of a natural disaster, it is essential that a ventilator keeps working (Nakajima 2009). Reports from the large earthquake in the Tohoku area of Japan in March 2011, suggested the requirement of guidelines for dealing with a natural disaster, and the medical transfer of patients with ventilators and means of communication (Aoki 2013). For rehabilitation, we propose an organization that provides the support of local medical services, constructs a community program for delivery of medical information, and includes educational training to produce specialized medical practitioners etc. (Aoki 2012). A list of patients living with ventilators and their individual care plans designed for disasters needs to be prepared so that patients can be transported to hospital at an appropriate time, when electricity supply and visiting nurse care systems are damaged. Satellite telephones are very useful for communicating with such patients and medical teams when normal telephone communication is limited. For patients using a ventilator at home, an emergency call to the ambulance service can be crucial.

11.4.7 Association of Patients and Their Family Members

To continue home care for patients, the association of patients and their family members is very helpful, both mentally and physically. The purpose of the association is to promote mutual help among patients, families and medical care staff in order to enable a full and satisfactory life through patient home care. Fifteen years ago, an association of patients with ALS and their family members was suggested by an ALS patient in our prefecture. The patient proposed a system of home-based care for patients with TPPV in Japan (Yamaguchi et al. 2001) after the association had been formed. In those days, nobody with TPPV lived in their own house. Initially, we called a meeting with home nursing station staff, public health nurses, and administrative officers of the health welfare section invited to attend. Now the association has matured and it has many members, and it collaborates with other associations for patients with other neurodegenerative disorders, e.g., Parkinson's disease. Several events hosted by the association including general meetings, exchange meetings, concerts etc. have been held on a regular basis, and we have been participating in meetings held by the association. This works to strengthen the relationship between patients and medical staff, including doctors.

PatientsLikeMe is an online community built to aid information exchange between patients. The site provides customized disease-specific outcome and visualization tools to help patients understand and share information about their conditions (Frost and Massagli 2008). Although ALS patients and caregivers often use the internet to obtain information about clinical practice and care, we should endeavor to help them get a better understanding by helping them to interpret the information they find (Chiò et al. 2008). In this regard, the activity of an association based on a community is very important.

11.4.8 Social Participation

Some ALS patients are encouraged to take part in social and community activities, especially in the early stages of the disease. ALS patients should consider social participation as a means of maintaining a positive approach to life. In later stages of the disease, a few patients are able to continue social activities with their ventilators. If someone wishes to go abroad, ALS patients can take a trip by air with their own ventilators. Wheelchairs allow ALS patients to broaden their horizons, this is especially the case for patients using a ventilator. A report detailed the most desirable wheelchair features, and found that motorized wheelchairs offer patients a greater sense of independence, and an improved sense of well-being (Trail et al. 2001).

11.5 Trials for Home- and Community-based Care

11.5.1 A Questionnaire Survey

We investigated the conditions of in-home care patients with ALS who live in our prefecture, under the research team of the Ministry of Health, Labour and Welfare in Japan, over a period of 7 years. During the first year, we undertook a questionnaire survey for ALS patients, family caregivers, hospitals, home nursing stations, visiting nurses, and public health nurses, about the formation of an in-home care system, interest in a community network for home-based care, problems experienced during in-home care, and requests for starting up a care network. We found that a limited network centered upon a patient already existed, and it was shown that patients hoped to have home care for as long a period as possible. The following year, we investigated the formation and function of small networks for patients by visiting them. It was found that there are many differences between networks, and it was realized that a unified care approach is good for patients and for establishing a larger social network. To this end, we created a guidebook to home care. Two years after publication of the guidebook, we undertook a questionnaire survey covering the same areas as the first survey. The main results were that mutual communication had been facilitated among small networks and the quality of care had become standardized. The analysis of results was shared with patients, families and care staff. We also assessed the methods of communication in ALS patients with moderate to severe motor weakness, and associated in-home care provision, by use of a questionnaire and inspection visits over the last 2 years. We emphasized the need for a variety of communication methods, and highlighted the incomplete utilization of social resources including various official support.

Following on from these surveys, it should be possible to construct an individual support system for at-home patients with ALS. We had aimed to combine individual systems by sharing information and techniques for home care. The recognition of an individual's role and responsibility in participating in their home care would lead to a better QOL among patients and their families, especially caregivers.

11.5.2 Guidebook for Home Care in ALS

To provide unified home care services, the building of a network is one of the best methods. Although it is not easy to build a large, fully-working network system, a guidebook that serves to unify the provision of home care in small networks, is relatively easy to create. There is a need for a range of support services to be made available, from which carers can select those most appropriate to them. However, some support services are not always available for carers, and accordingly there is a need for carers to have better access to training in physical care (O'Brien

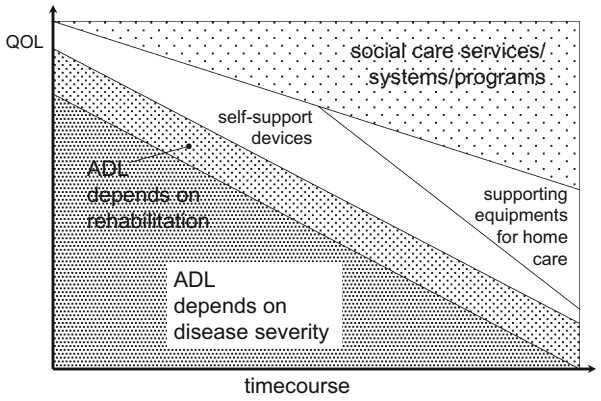

Fig. 11.2 Maintenance of the QOL in AL. *QOL* quality of life, *ADL* activities of daily living

et al. 2012). Guidelines for the multidisciplinary care of home-ventilated ALS patients have been proposed (Eng 2006).

Based on the results of analysis of the questionnaire survey mentioned above, we published a guidebook for home care on behalf of ALS patients and caregivers at home. The book was written with the primary objective of maintaining a good QOL (Fig. 11.2). The book consists of seven chapters, namely About ALS, Impairments and rehabilitation, Basic methods of care, Preparation for home care (from hospital), Utilization of social resources for home care, Illustration of a case, and Some information on home care tools. We designed it to be easy to understand through the inclusion of many photographs and illustrations. The guidebook was sent to patients, hospitals, clinics, home care nurse stations, and health care centers in our prefecture. The guidebook was introduced at a meeting of the research team of the Ministry of Health, Labour and Welfare, and an affiliated patients association (JALSA; Japanese Amyotrophic Lateral Sclerosis Association). We recently presented the guidebook (written in Japanese) to the public, via the homepage of our department (URL:http://www.med.yamanashi.ac.jp/clinical/neurol/home.html).

11.5.3 Seminars on Neurodegenerative Diseases for Medical Participants: Doctors and Co-workers

We have been holding a seminars on neurodegenerative diseases for medical staff and public directors twice a year since 2009. Seminars by neurology specialists, and presentations of several subjects regarding intractable diseases, including case presentations, have been held alternately. The titles of past seminars are: Home-based care support for patients with intractable diseases, Medical practice of spinocerebellar degeneration, Medical practice of dystonia, Clinical history and future applications of muscular dystrophy, and Approach of a care network

supporting patients with intractable diseases in the Neurology Department of Kyushu University – a look back at the progress made over 16 years.

The aims of this program are: linkage between medical staff and public bureaus, the sharing of new information about concepts and treatment of diseases, and home care support for patients through the creation of patient-centered networks. Although our prefecture (Yamanashi), is relatively small, it should in theory be relatively easy to create and build networks. However, despite this, there are difficulties experienced when building networks due to a shortage of staff and a low population density. As a result, medical staff are required to move over a relatively large area. Through these seminars, it is possible to share information about many aspects of intractable diseases with people occupying various roles in the caregiving process. The seminars are also helpful as exchange meetings for home care networks.

We recently presented our experience on the positive outcomes of NIPPV and palliative care with morphine in ALS patients (the data were also presented at a meeting of the Japanese Medical Network for Seriously Intractable Disease). The presentation was significant because home care staff usually learn about practical issues without any comprehensive data. So the information provides home care staff relief, confidence and knowledge of practical methods.

11.5.4 Future Perspectives

In Japan, several nationwide clinical and genetic surveys of patients with neurodegenerative diseases, such as those by the Japan Spastic Paraplegia Research Consortium (JASPAC) for hereditary spastic paraplegia (Takiyama et al. 2010; Shimazaki et al. 2012) and the Japan Multiple System Atrophy Research Consortium (JAMSAC) for multiple system atrophy (Ichikawa et al. 2011), have been performed. In 2006, a longitudinal multicenter study of Japanese ALS patients using a telephone survey system, by the Japanese Consortium for Amyotrophic Lateral Sclerosis research (JaCALS), was started (Atsuta et al. 2011). This multicenter study is useful in obtaining large amounts of clinical data, and can contribute to elucidation of disease pathogeneses and the development of treatments.

We will probably be able to provide home medical care to patients, and to obtain physical information on patients using an IT network system in the near future. Such an IT network is operated by staff including hospital staff, co-workers, patients and caregivers. In our university hospital, a system for sharing a patient's laboratory data, vital signs and other information among doctors belonging to our university hospital, home doctors and visiting nurses, has been in operation for a while. This system is mainly used for diabetes mellitus and ophthalmic diseases. We had a chance to try out this system for an ALS patient at home with a ventilator. In the future, home- or community-based care may well move in this direction and can be developed and improved through the use of an IT system.

11.6 Conclusion

At present, home-based or community-based care is aimed at patients with neurodegenerative diseases worldwide. Several keyphrases including multidisciplinary care, palliative care, measures to deal with natural disasters, home care networks, and measures for communication are all discussed as issues needing careful management. Mental health support for family caregivers, as well as for patients, is essential for continuing home-based care. Care for patients with ALS has become a prototype aimed at enabling patients and carers to move toward home-based or community-based care in other neurodegenerative diseases. From the perspective of care, we should aim to learn more about holistic medicine including physical, psychological, palliative, preventive, social, and regional aspects, and nursing for home-based care. We should continue to focus on patients and families at home, and encourage the development of home care networks.

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Part V
Health Promotion: Prevention and Quality of Life

Chapter 12
Information Environment and Brain Function: A New Concept of the Environment for the Brain

Manabu Honda

Abstract The brain is an organ which receives and processes information from the environment, and controls actions on the environment. The processing of information in the brain is mediated by chemical reactions, and therefore, "material" and "information" are practically equivalent inside the brain. It is interesting to note that the safety of the environment and related health impacts, has previously been evaluated solely using the material and energy scales mentioned above. However, given the role of the brain in higher animals, there is a growing concern that measurements of the environment solely based on material and energy may be inadequate. In this chapter, the author introduces the framework of "information environment science", first proposed by Tsutomu Oohashi in 1989, with the intention of promoting a more comprehensive understanding of the environment by integrating a new third scale of information with the existing two scales of material and energy. The author then discusses the concept of essential information, which can be thought of as an "essential nutrient" for the brain, and discusses studies in sound environment information as a possible candidate for essential information. It is hoped that such studies will lead to a broader acknowledgment and understanding of the information environment as a new environmental concept in brain research.

Keywords Material • Energy • Information • Environment • Essential information • Sound • Midbrain • Thalamus • Hypothalamus • Tropical rain forest

12.1 Introduction

It has been known for a long time that various environmental factors are involved in the onset and clinical course of many psychiatric and neurological disorders. At a macroscopic level, damage to the environment considerably reduces the sustainability and regenerative capacity of the global ecosystem, thereby affecting the

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K. Wada (ed.), *Neurodegenerative Disorders as Systemic Diseases*,
DOI 10.1007/978-4-431-54541-5_12

entire spectrum of living organisms dwelling on the planet, over an extended period of time. Therefore, the damage inflicted on our global environment poses serious problems, not only to humans, but also to the entire ecosystem. In more temporally and spatially limited areas than those pertaining to such global ecosystem problems, environmental damage can also seriously impact on human health. For example, in Japan, a variety of toxic substances polluting the environment have caused health hazards and had harmful effects on society. Examples include four major pollution-related diseases, i.e., Minamata disease, Niigata Minamata disease, Yokkaichi asthma, and Itai-Itai disease. The major symptoms of these diseases appertain to toxic effects on the central (CNS) or peripheral nervous systems (PNS). In this chapter, a disease or disorder caused primarily by an environmental factor is referred to as an "environmental disease." To counteract such environmental diseases, it is essential to identify and remove the toxin(s) responsible, so as to restore the environment to a state that is no longer harmful to human health, while at the same time providing treatment for humans who have contracted the disease.

However, identification of the causative factors of many environmental diseases is often both extremely difficult and time-consuming. Minamata disease, a toxic disease of the CNS caused by methyl mercury, first appeared in 1956 in Minamata City, Kumamoto Prefecture, Japan (*see* the web page by National Institute of Minamata Disease). In 1959, a research group from Kumamoto University suggested that an organic mercury compound may be the cause of Minamata disease, and wastewater from a local factory was strongly suspected to be the origin of the mercury. Despite such suspicions, however, the discharge from the suspected source was not stopped until 1968, when the causative agent was officially announced as being methyl mercury. Yet it took 12 years, owing to the lack of clear proof and various political, administrative, and economic factors, to take any decisive action. Meanwhile, Minamata disease continued to claim an increasing number of victims, and is now regarded as an unprecedented tragedy in the history of environmental illness in Japan.

Several scientific and sociological reasons can be posited for the apparent difficulty in identifying the cause(s) of an environmental disease. Here, the author proposes that a major reason is that in the absence of any overt health damage, there is no actual recognition that there is a problem. Of course, there exist cases of "criminal contamination" in which individuals or other entities were known to have intentionally contaminated the environment, or allowed the environment to remain contaminated in the full knowledge of a particular substance's toxicity. However, an even more serious problems are cases in which the cause is left unaddressed since any risk has long gone unrecognized. In particular, we should pay attention to the fact that a number of environmental problems may surface based on multifactorial, complex incidents in "disciplinary niches" where existing scientific disciplines have not yet officially recognized. Confronting a new environmental issue may require large-scale initiatives, which will perhaps encourage a fresh societal attitude toward the emergent problem. Positing this point of view, the author thinks that it is meaningful to create a new "framework" enabling the detection and assessment of previously unrecognized risk factors in the environment, that possess

the potential to cause (as yet unnoticed) damage to health. Needless to say, in order to solve the problems of environmental diseases, it is not sufficient to merely point out the potential risks, and a causal relationship has to be proved. Nonetheless, if there are no means by which to bring a particular potential problem to the attention of the scientific community, the need for proof of a causal relationship will remain unrecognized, and no preventive measures will be implemented.

Based on these considerations, in the initial part of this chapter, the author focuses on the brain as an organ which receives and processes information from the environment and controls actions on the environment, and introduces the framework of "information environment science," with the aim of discussing the nature of health in the context of the relationship between the brain and the environment. From the perspective of brain function, we then differentiate between safety as recognized in the material/energy environment, and safety as recognized in the information environment, and move on to discuss the concept of essential information, which manifests itself from discrepancies highlighted in the comparison between the two environments as discussed, and which can be regarded as an "essential nutrient" for the brain. Finally, the author introduces our studies in sound environment information aiming to determine a possible candidate for essential information. It is hoped that such studies will lead to a broader acknowledgment of the information environment as a new environmental concept in brain research, and that others will begin to focus attention on potential problems in the field and subject them to rigorous scientific investigation.

12.2 Environment for Brain Function

Multiple types of environmental damage are a threat to human health. In the environmental pollution incidents described above, as well as in other pollution-related problems (e.g., acid rain, air pollution, and asbestos contamination), damage to the environment by harmful substances results in damage to health, i.e., environmental diseases. Such kinds of health hazards can be viewed as the problems occuring in the material environment. By contrast, an increase in the sun's high-energy ultraviolet rays reaching the earth's surface as a result of holes in the ozone layer is believed to bring about an increased incidence of cancer in humans. Serious damage to health can also be caused by the widespread release of radioisotopes with a long half-life, and such a phenomenon has stubbornly persisted in Japan, since the March 11, 2011 nuclear accident in Fukushima Prefecture. In radioisotope-related health issues, it is the cytotoxicity of the high-energy radiation released by radioisotopes, rather than the toxicity of chemical substances, that is most damaging. Therefore, these effects can be viewed as health hazards associated with the energy environment, given that it is high-energy electromagnetic waves that are ultimately responsible for damage to human health, although the causative agents therein are actually chemical substances such as chlorofluorocarbons and radioisotopes. Global

warming can be viewed as a complex problem in which the material and energy environments are closely interconnected.

It is notable that the safety of the environment and related health impacts has previously been evaluated solely using the material and energy scales mentioned above. Such scales may suffice when, among the diverse environmental effects on living organisms, the physical aspect is the focus of attention. However, given the role of the brain in higher animals (i.e., the brain's functions of acquiring and processing information and regulating interaction with the environment), there have been growing concerns, beginning around 1980, that measurements of the environment exclusively based on material and energy may be inadequate. This concern was triggered by the so-called Tsukuba syndrome, a pathological phenomenon characterized by the frequency of suicides among researchers at Tsukuba Science City in Japan.

Tsukuba Science City is an 'artificial' city seen as a symbol of Japan's efforts to return to the forefront of the international community as an economic superpower, and was designed and constructed to represent the view of an ideal modern city. Research facilities, containing numerous laboratories boasting the most advanced, state-of-the-art equipment, were located in the orderly and clean surroundings of Science City, and were viewed as representing the pinnacle of artificial beauty. However, in Science City, there was also an unexpected occurrence of abnormally frequent suicides among its researchers. The incidence of suicide at that time was more than twice that of the national average. Moreover, suicides occurred without any clear reason and often appeared to be impulsive. This phenomenon of frequent suicide was not seen among people who had been living in traditional villages in the vicinity of Tsukuba before the construction of Science City. Instead, it only occurred among researchers who were supposedly enjoying life in this ideal environment.

The unfortunate situation in Tsukuba Science City, a research base for elite Japanese professionals including those specializing in environmental health, threatened the city's very existence as authorities puzzled over how to approach this pathology, which gradually came to be referred to as Tsukuba disease. Given that the occurrence of this phenomenon was localized to the immediate environment of Tsukuba Science City, the problem could be considered as an environmental issue, only resolvable by improving Science City's environs, just as environmental factors were recognized as factors causing Minamata disease. However, it was extremely difficult to isolate the origin of this phenomenon from the perspective of the material and energy environments, which were at that time the only available measures by which to assess the situation within the existing framework of environmental science.

With the emerging awareness of such problems serving as a starting point, Tsutomu Oohashi (environmental science) and Susumu Oda (psychiatric medicine) from The University of Tsukuba, Yoichiro Murakami (philosophy of science) from The University of Tokyo, and other advocates, pointed out the limitations of the conventional two-dimensional environmental outlook based merely on material and

energy, and they proposed the necessity of including information as an additional dimension of inquiry.

12.3 Equivalence of Material and Information in the Brain

Here I re-examine what material and information really mean to the brain. In the brain, when information is transmitted among a large number of interconnected neurons, the transmission efficiency is varied, with pieces of information converging or diverging in order to achieve complex information processing. Various mechanisms for the transmission of information exist, but in a typical synaptic transmission, information travels through an axon as an action potential and is converted at the synapse to stimulate the release of a chemical substance called a neurotransmitter into the synaptic cleft. The released neurotransmitter then binds to a receptor on the surface of the receiving neuron, causing a slight change in cellular membrane potential. A number of such minute potential changes are integrated and the receiving neuron may then generate an action potential. If it does, information is transmitted to the next neuron. Furthermore, the action potential, which transmits information as electrical impulses, transmits changes in membrane potential caused by changes in the ion permeability of the cell membrane. This is a totally different phenomenon from that of electric current, which involves the movement of electrons in a conductor. Thus, it becomes clear that the processing of information in the brain is mediated by chemical reactions, and that information inputs to the brain via the five senses ultimately elicit a reaction identical to the reaction that occurs upon administration of a specific extraneous chemical substance to a specific site in the brain. Therefore, "substance", or "material", and "information" are essentially equivalent inside the brain.

Moreover, an abnormality in brain function which can be caused by administering a certain substance to animals can, alternatively, be triggered by information. In drug development, for example, experimenters must in some way induce the disease under study in their animal models for them to be used in basic and preclinical studies. In studies of depression, the former practice was to create depression-like symptoms in animal models by administering reserpine. After repeatedly being exposed to the sound of a buzzer while receiving reserpine, the experimental animals eventually started showing symptoms of depression when simply hearing the sound of the buzzer, even in the absence of reserpine. However, it was still difficult to verify the net effect of the experimental drugs since reserpine itself demonstrated complex interactions, along with those of the drugs tested. For this reason, techniques have now been developed to induce symptoms of depression in animal models without need for use of any chemical substance. Two methods used are (1) the desperation, or "forced-swimming," model, wherein animals are placed in a cylindrical water tank with no surface on which to put their paws, and (2) the "forced-running" model in which a motor is attached to a mouse-friendly running wheel, inducing a state of depression in the mouse by forcing it to run

inside the wheel. These methods are both thought to achieve the equivalent of a particular chemical substance and a particular information input in terms of creating psychiatric disease-like symptoms.

On the other hand, the offspring of primates, such as monkeys, which are herd-forming animals, have been known to present with symptoms similar to autism and schizophrenia if they are isolated from their family early in their lives, and raised without the opportunity to communicate with their parents and offspring. Similar symptoms can be induced by administering certain chemical substances, such as meta-amphetamine. In addition, substance disorders in humans, such as decreased secretion of oxytocin, vasopressin, and other neuropeptides that are closely related to social development, have also been known to emerge in individuals neglected in childhood by parents, who severely limited their children's opportunities to communicate.

Furthermore, results from some former sensory deprivation experiments conducted by researchers, are fertile ground for considering the meaning of information inputs in the brain (Zubek 1969). In these experiments, audiovisual information was blocked through the use of blindfolds, earplugs, or other means; even somatosensory information was blocked by placing the subject in a fluid with a temperature and specific gravity identical to those of the human body. It has been reported that, when deprived almost totally of sensory information inputs from the five cardinal senses to the brain, as in these experiments, healthy young subjects began experiencing hallucinations and delusions within just a few minutes, and developed derangement within several tens of minutes.

The human brain is often compared to a computer. In this metaphor, one tends to consider that the baseline brain is in a state of idling with no sensory information input, and that it only starts functioning when information is entered. Such a tacit understanding is reflected in many psychological experiments, in which the baseline controls are subjects in a resting state who are not receiving any stimulation, nor making any movement. However, the results from the aforementioned sensory deprivation experiments suggest that the human brain continually processes sensory information from the five senses, regardless of whether such activity can be subjectively recognized, and it is not likely to continue functioning normally if these information inputs are largely blocked. Such characteristics of the processing of information in the brain, are further evidence that the body is in fact continuously undergoing chemical reactions referred to as basal metabolism, even when the body is at rest. The body would no longer function properly and various health problems may emerge if any of a group of chemical substances called essential nutrients were depleted.

12.4 Framework of Information Environment Science

On the basis of the various physiological and pathological findings concerning the relationship between the brain and information, the framework of "information environment science" was constructed by Tsutomu Oohashi in 1989, with the intention of gaining a more comprehensive understanding of the environment by integrating a new third scale of information into the already existing two scales of material and energy that were already being used to understand the environment (Oohashi 1989).

The key to information environment science, is whether or not the concept of "information" can be used appropriately in natural science terms. When viewed as a scientific conceptual tool, even though information always exists in association with physical phenomena in which some material and energy are involved, information can be manipulated as a code, once it is isolated from those specific physical phenomena, and then abstracted. This manipulation represents the so-called basic characteristics of information, and a majority of information science activities deal with information in such a framework. When the association with material and energy is cut off in the course of abstraction, physical restraints are removed and the degree of freedom in conceptual manipulation is greatly increased. However, the effectiveness of information environment science, in which real problems occurring between living organisms and their environment are the subjects of interest, is significantly limited. In information environment science, a fundamental tenet of "linking an information phenomenon to a life material phenomenon" is thereby proposed. In other words, information (i.e., genetic information) is the foundation of the basic functions of life, such as self-replication and propagation, and, at the same time, all biological phenomena currently existing on the earth can ultimately be reduced to chemical reactions. Therefore, in information environment science, the scope of application of the "information" concept is initially restricted to areas of interaction between living organisms and the environment. On that basis, information is defined as a scientific concept relating to temporal and spatial patterns that potentially elicit some sort of chemical reaction in life. Thus any information phenomena correspond to some sort of chemical reaction.

Defining information for life in this manner, allows an information phenomenon to hold dual meaning, as it can simultaneously mean the material phenomenon of a chemical process. Thus an integrated approach from either side to the issues regarding life-environment interaction can open a path toward a level of effectiveness and reliability comparable to that of material science. Therefore, in information environment science, molecular biology should be considered as a field of material science that is complementary to information science, with the intention being to better understand the environment by linking the two components organically. This conceptual breakthrough allows us to assess the "value of information" in conjunction with the "value for life." Furthermore, social and cultural problems with varying evaluations and judgments can be linked to an assessment of them as biological processes, e.g., whether the problem is of significance to life-related

Table 12.1 Comparison of safety issues regarding the environment

	Material/energy aspect	Information aspect
Things that should not be present for the maintenance of health	A permissible range is identified using objective parameters (e.g., dioxine < 4 pg/kg/day)	Examination has started but remains at an exploratory level (e.g., noise pollution, subsonic vibration)
Things that are essential for the maintenance of health	Essential nutrients exist and a permissible range is objectively identified (e.g., Vitamin B12 > 2.4 μg/day)	"Essential information" has yet to be examined
Human adaptability to the environment	It is widely recognized that limitation of adaptability does exist	It seems to be implicitly understood that humans can adapt to almost any type of information

This table is originally constructed based on the description in Oohashi (2003)

issues, such as the maintenance and propagation of life, and the stabilization of the ecosystem. As a result, it becomes possible to exert a more wide-reaching influence on society.

Within the framework of information environment science, the brain is viewed as an organ in which information phenomena and physical phenomena are integrated in a fundamental and straightforward manner, and the brain sciences are thus called on to provide a new perspective on environmental issues by bridging the gap between information science and molecular biology.

Through the use of information environment science, it is easy to see the discrepancy between attitudes toward the safety of the environment taken from the material/energy standpoint, and those taken from the information standpoint (Table 12.1) (Oohashi 2003). Environmental safety problems concerning material/energy are at least widely recognized, although any countermeasures taken are often insufficient. Furthermore, strong social enforcement and consensus-building arise once the problem becomes apparent. In contrast, the idea of a relationship between environmental safety problems and information has yet to gain clear recognition, let alone inform effective countermeasures to the problems.

Firstly, I will discuss concepts around "things that should not be present for the maintenance of health" in the environment. In several areas of material and energy, accepted margins of safety are comprehensively defined with objective numerical values, such as 4 pg/kg/day or less for a tolerable daily intake (TDI) of dioxine, known as "environmental hormones". Such references also include some scientifically or socially difficult-to-derive unified objective values, such as permissible radiation exposure levels, but it can be said that the importance of establishing such reference values is at least universally recognized. On the other hand, studies on some types of harmful information, such as noise and low-frequency vibration, have been initiated, but remain at an exploratory level. Noise regulations are formulated on the basis of the one-dimensional index of sound volume/pressure and are not concerned with the content of the sound itself. However, environmental sounds within tropical rainforests, for example, often exceed 60 dB and can even

approach 70 dB. According to current recommendations, such a level of sound would not be suitable for residential areas, yet, unlike urban mechanical noises, it would not be perceived as uncomfortable even at such a high volume. Moreover, although the flashing of a light at a constant interval is known to cause convulsions due to photosensitivity (e.g., the Pokemon and Babel incidents), the detailed stimulation parameters of flashing light have yet to be investigated.

Next, let us look at "things that are essential for the maintenance of health" in the environment. In the material and energy areas, the presence of factors that are essential for continuous survival in a healthy state, such as essential nutrients, is widely recognized, and efforts have been made to describe them as comprehensively as possible using objective indices. However, in the information environment field, even the idea of which types of information need to be present in the environment for the maintenance of health does not appear to have ever been pursued, even though the human brain stops working properly within a short period of time when the majority of information inputs to the brain are blocked, as described earlier. It is clear that such studies on the type of information indispensable to the maintenance of health are nonexistent.

In addition, there also exists a significant discrepancy in our recognition of the extent of human adaptability to the environment, when we compare the material and energy environment with the information environment. In particular, with respect to the material and energy environment, it is well known that there are limits to human adaptability and that health can be damaged, or survival become impossible, if those limits are exceeded. In contrast, in the case of the information environment, the prevailing concept (albeit only implicitly recognized) appears to be that it is possible to adapt to any sensory information input to the brain with some patience, no matter what the nature of the information is. In other words, the idea that "there is a limit to the adaptable range," which is the common understanding in the material and energy fields, does not appear to be generally accepted here. However, the abovementioned sensory deprivation experiments suggest that the brain is incapable of adapting when deprived of information input below a hypothetical threshold.

Once viewed in this manner, it is clear that safety, security, and health measures in the information environment are far from sufficient when compared to those in the material and energy environment, in terms of any scientific study, public interest, or ethical support. To improve this situation, it would seem reasonable to place a higher priority on studies of the "essential information for life," (Oohashi 2003) in order to raise its currently low profile. Therefore, in the next section, some examples of our approach to such problems are introduced.

12.5 Approach to Essential Information for Humans

It is very difficult to comprehensively specify and index the entire range of substances essential to human survival (i.e., essential nutrients). Nonetheless, even without a full understanding of these essential nutrients, modern humans have continued to survive throughout the course of evolutionary history to the present day, by consuming natural foods. In other words, all the nutrients necessary to maintain human survival are likely to be found in natural food consumed on a daily basis, without which the species *Homo sapiens* would have gone extinct at some point during evolutionary history.

The same rationale may be applicable to information. We have postulated that all the pieces of information necessary to support the survival of the human race are comprehensively included in the natural environment, in which the modern human genes have evolved. Therefore, if we can identify significant differences between information found in the environment that has historically nurtured human genes, and information contained in specific present-day environments, then the identification of the causes of such differences and the determination of their effect on the brain, which serves as the initiating point for reception and processing of such information, may be able to provide us with effective strategies to clarify what that essential information actually is.

As a consequence, our research group headed by Tsutomu Oohashi compared the natural environment of tropical rainforests, in which human genes have evolved along the primate lineage, against an artificial urban environment, in which environmental diseases, collectively referred to as "diseases of civilization", were prevalent. We first focused on sound information, which can readily be recorded, analyzed, and reproduced electronically.

Figure 12.1 shows the power spectra of various sounds that we recorded in the environment (Nishina and Oohashi 2007). In these graphs, the horizontal and vertical axes indicate frequency and power respectively, and power spectra sampled every 10 milliseconds are displayed in an array form, in which time flows from front to back. In general, a frequency of 20 kHz or above is considered inaudible to the human ear. In looking at the environmental sounds of a city as shown in the upper part of Fig. 12.1, the inside of an apartment room, for example, provides very good soundproofing measures that create a quiet, almost silent space with a relatively low level of sound pressure. Even with a TV on, only sound within the audible range was seen to increase. These conditions are essentially the same as conditions outdoors, with the result that urban noises are mostly concentrated in the audible range, and only rarely contain components of 20 kHz or above.

The lower part of Fig. 12.1 shows natural environmental sounds recorded in three places: a grove surrounding a residence in Tsukuba, a garden in Bali, and a typical rainforest. As can be seen at a glance, such places are rich in inaudible high-frequency sounds of 20 kHz and above. It has become apparent to us, with increasing confidence, that the main source of such high-frequency air vibration components is attributable to insects. In Fig. 12.2, median frequencies of the sounds

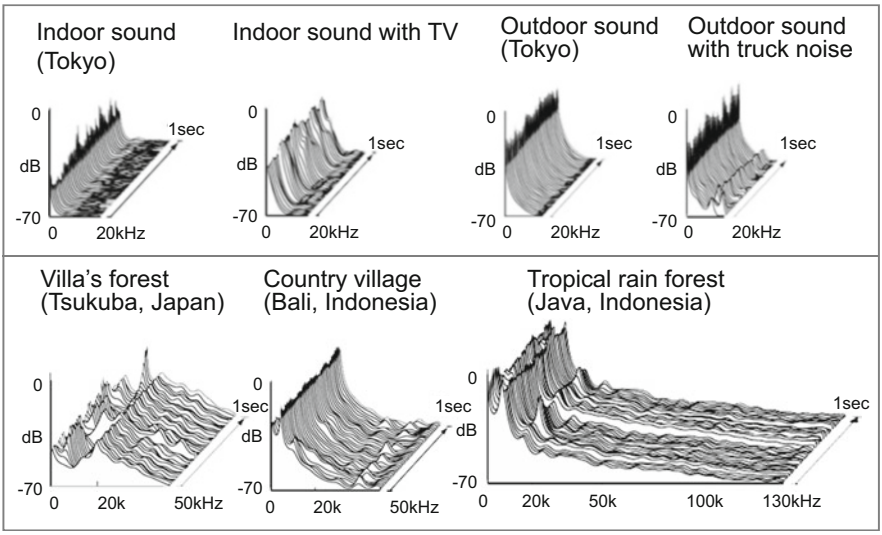

Fig. 12.1 Power spectra of various environmental sounds

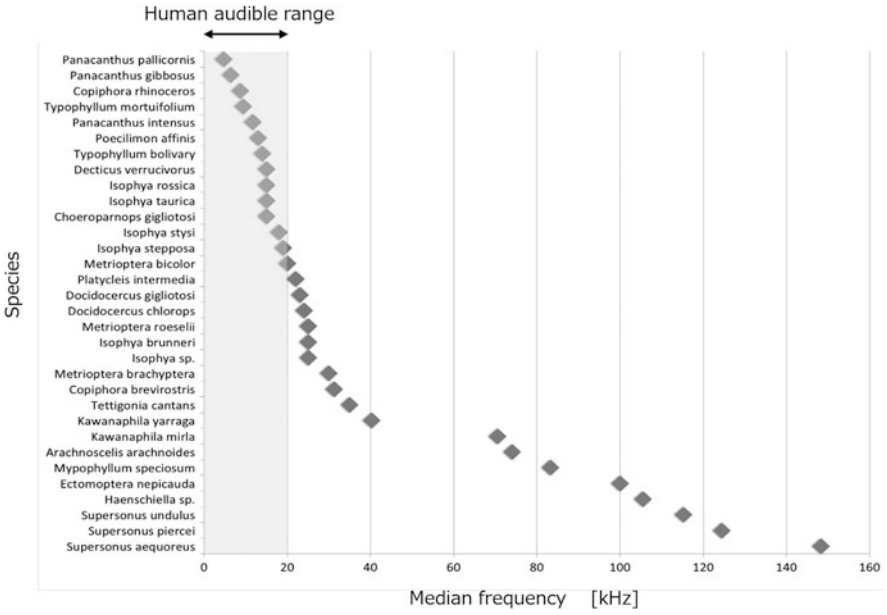

Fig. 12.2 Sound frequency of various insects (This figure is originally constructed based on the information provided by Sarria et al. 2014)

made by various insects are plotted by species (Sarria et al. 2014). Although the bandwidth of the sound of each insect is relatively narrow, the overall diversity of insect species is likely to be a driving force for the formation of the broad-band high-frequency component. It is evident from such plots that there is a considerable difference between native natural environmental sounds, and artificial urban environmental sounds in air vibrations containing ≥20 kHz frequency components that are inaudible to the human ear (at least at the signal level).

We would not expect to see problems occurring if the high-frequency sounds which are missing from the urban environment, but found abundantly in the natural environment of rainforests (where human genes evolved) have no effect on the human body. However, if they were to play a role that is essential for health, although such a role is currently difficult to appreciate, the environment of a modern city may be regarded as being at a near-malnutritional state of information, and one in which essential information is totally absent. To clarify this point, it is critical to determine what effect the inaudible high-frequency components have on the human body, particularly on the brain, which receives and processes sensory information.

We therefore measured cerebral blood flow under various sound presentation conditions by means of positron emission tomography (PET) experiments (Oohashi et al. 2000), wherein water ($H_2{}^{15}O$), having a very short half-life of 2 min, was injected into the body and the intracerebral distribution of the injected isotope imaged by PET. The results showed an activity enhancement with a very strong peak in the deep-lying brain structure when the subject heard a sound containing an abundance of high-frequency components, compared to that observed when the subject heard a sound produced by removing the high-frequency components from an identical sound, leaving only the audible sound component. Specifically, statistically significant activity increases were observed in the midbrain of the brainstem (Fig. 12.3, top) and in the thalamus (Fig. 12.3, bottom), which is part of the diencephalon. As shown in the graph on the right in Fig. 12.3, activity levels inside those parts of the brain were, instead, reduced when the subject heard only audible sound (HCS) compared to activity observed when the subject heard background noise (Baseline), which does not present any sound stimulus, revealing a difference from the response to a common auditory stimulus. Furthermore, those parts of the brain were not activated when an isolated inaudible high-frequency sound was introduced (LCS). These results indicate that responses in the midbrain and thalamus observed only when the high-frequency sound was presented together with the audible sound, are not just simple responses to high-frequency air vibrations, but are derived from certain interactions with the audible sound.

Activated areas detected by means of a strict statistical test, like the one used above, represent peaks of brain activity, but they do not give a complete depiction of the breadth of brain activity. Thus, we conducted a principal component analysis (PCA) to analyze the whole network in the brain responding to the presence or absence of high-frequency components (Honda et al. 2013). The results obtained indicated that the entire midbrain and diencephalon, including the hypothalamus, act as an integrated unit, whereas the midbrain and the thalamus individually form

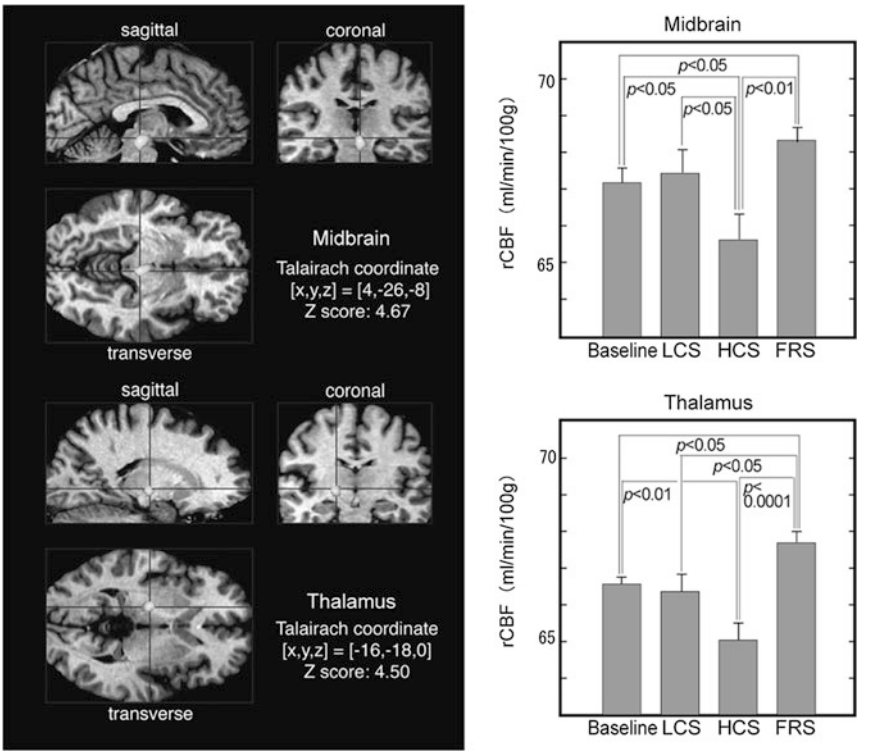

Fig. 12.3 Brain regions activated by sounds containing inaudible high-frequency components (Adopted from Oohashi et al. 2000). FRS:full-ragne sound, HCS: high-cut sound, LCS: low-cut sound

peaks as detected in the aforementioned analysis, and distant sites including the anterior cingulate gyrus and the medial prefrontal cortex are coordinated and act as a neural network (Fig. 12.4).

The midbrain serves as the base of a neural network called the diffuse modulatory system, which projects from the midbrain to various other areas inside the brain. In particular, monoaminergic nerves, which utilize dopamine as a neurotransmitter, project from the midbrain into sites in the frontal lobe identified by this analysis, such as the anterior cingulate gyrus and the medial prefrontal cortex, and which are known to function as the neural circuit of the reward system, producing sensations of pleasure. On the other hand, the midbrain/diencephalon maintains homeostasis as the highest center of the autonomic nervous system and the endocrine system, and it also affects the immune system. It has been shown that immunological functions such as natural killer cell activity are enhanced, and stress hormones such as adrenaline and cortisol are reduced, when a sound containing an abundance of high-frequency components is presented. We termed a series of such effects of inaudible high-frequency sounds, hypersonic effects.

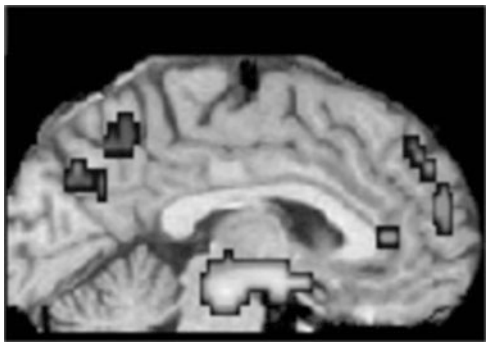

Fig. 12.4 Activated brain network depicted by principal component analysis

There are many nuclei, a cluster of densely packed neurons, in the deep part of the brain. These nuclei, which are activated through hypersonic effects, play an important role in the maintenance of a healthy life. Functional impairments of the midbrain/diencephalon containing such important nuclei in significant numbers, are currently attracting attention among researchers due their direct or indirect links with various modern-day illnesses. Such illnesses are rapidly increasing in tandem with the processes of urbanization and civilization. For example, the functional suppression of the monoaminergic nervous system projecting from the midbrain to the frontal lobe, is closely associated with depression – a major cause of the sharp increase in suicide observed in recent years. Moreover, the functional suppression of the nucleus basalis of Meynert, which lies anterior to the diencephalon, and the acetylcholine nerve system, which is the diffuse modulatory system projecting from there to the cerebral cortex, is closely related to symptoms of Alzheimer's disease. Furthermore, stress-induced dysfunction of the hypothalamus not only causes abnormalities in the endocrine system, leading to hypertension, diabetes mellitus, and various other lifestyle-related diseases, but it also disturbs the balance of the parts of the immune system that combat the development of cancers.

From findings made in the fields of acoustics, information science, and brain imaging among others, we believe that air vibration components exceeding the upper limit of the human audible range, which are abundant in the natural environmental sounds of a rainforest (and which may very well be the environment in which human genes and the brain evolved), but which are markedly absent from the artificial environmental sounds of modern cities, might serve as essential information for humans via functions within the deep-lying brain structure, such as the midbrain and diencephalon.

12.6 Concept of Information Medicine Generated by Information Environment Science

As explained at the beginning of this chapter, in order to eliminate the pathology of environmental diseases caused by mismatches between the environment and life-related phenomena, therapeutic techniques must be directed not only toward affected humans, but also toward the causative environment itself. This new concept of the environment for the brain, builds upon the foundation of information environment science as described above, and via empirical data obtained through scientific investigations conducted from this perspective, has improved the information environment by complementing it with essential information, leading to the creation of the concept of "information medicine", an innovative non-pharmacological approach that can aid in both the treatment and prevention of a variety of psychiatric and neurological disorders. Because information medicine involves environmental approaches to solve the root cause of the pathology in question by correcting the causative environmental factor, it can complement conventional therapies for an affected individual, such as drug-based treatments, rather than compete against them. In particular, with respect to the sound information we have studied, various advanced media technologies are currently available, and they may well represent a relatively easily achievable target. Therefore, in the last part of the chapter, I briefly introduce the concept of information medicine based on the hypersonic effect.

As described above, high-frequency components exceeding the upper limit of the audible range of humans, are abundant in the sounds of tropical rainforests, where the genome of the human race evolved. Experiments, including those using PET, have revealed that an area deep inside the human brain is activated when exposed to such environmental sounds, compared to the brain's baseline condition in which only background noise is present. However, caution should be exercised as to whether it is indeed true that "the deep brain activity is enhanced when hearing a sound containing high-frequency components". The reason for this is that human beings evolved in rainforests filled with high-frequency components for more than 20 Ma, right from the days of the ancestors of great apes. In fact, it is only a few thousand years since a very small number of humans, with genes and a brain evolved to suit such an information environment, began to move into an urban setting, in which almost no high-frequency components exist. In other words, an important issue that is often overlooked in modern brain science relates to the "standard state" or "baseline" of the human brain.

In laboratories, background noise without an added sound stimulus is usually used as a baseline, and the activity is generally considered "increased" when sounds containing high frequencies are heard as opposed to "decreased" when sounds deficient in high frequencies are heard, when compared to the activity in the said baseline state. However, from an evolutionary biological perspective as described above, the baseline may be the state surrounded by a high-frequency, rich environmental sound such as the rainforest, which in experimental terms is the "state where

a sound containing high-frequency components is presented (equal to the full-range sound (FRS) condition in Fig. 12.3)", rather than the background noise state where no sound stimulus is presented. If the data from the PET experiments are re-analyzed with the rainforest baseline, the result can be interpreted as indicating that the actual state of the brain of humans living in modern cities is a state in which the activity of the deep brain is chronically reduced when compared to the baseline for the human race, because the sound from the environment is lost, or, even if the sound remains, inaudible high-frequency components are lost as urbanization and civilization advance.

Such a chronic imbalance of deep brain activity resulting from the deviation of the information environment from its original state programmed in our genes and brains, may cause both mental and physical health problems. It may subsequently be possible to optimize deep brain activity by supplementing the urban environmental information in the everyday environments of modern humans, with the missing high-frequency components, through the use of cutting-edge media technologies, and to thereby contribute to the prophylaxis and therapy of various pathological conditions. Using this working hypothesis, we are currently accumulating evidence through basic researches as well as clinical researches with the aim of developing information medicine as a treatment for depression and dementia.

It is strongly hoped that these approaches to brain science, based on the novel concept of the environment for the brain, will eventually lead to treatment and prevention of various environmentally-induced psychiatric and neurological disorders, as well as helping to maintain good mental health.

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Chapter 13
Social Implementation of Neurodegenerative Disease Research and Neuroethics

Tamami Fukushi

Abstract Twenty-first century research and development into neuroscience has brought about new methodologies that can be used to investigate the biological basis of neurodegenerative diseases and to provide new insights into understanding neurodegenerative diseases. There is a recognition that functional and structural networks across the neural system and other biological systems in the human body may affect the occurrence and ingravescence of neurodegenerative diseases. There is also suggestion that diseases which were once considered to be different or independent may have closer relationship. Thus, it can confidently be said that neurodegenerative diseases are caused not only by the direct deficit of brain and nerves, but also by the effect on the neural system by deficits in other functional systems in the human body. This idea provides researchers with a new biological approach to neurodegenerative diseases. Various clinical studies will be conducted for subjects with neurodegenerative diseases in the near future on this basis. From the perspective of bioethics research, this chapter discusses current issues and future views regarding the research and development of treatment technology for neurodegenerative diseases by focusing on protection of the subject.

Keywords Neurodegenerative disease • Neuroethics • Neuroscience • Neurology • Psychiatry • Brain stimulation

13.1 Introduction

13.1.1 Progress of Neurodegenerative Disease Research

Advances in neuroscience have led in recent years to changes in the environment surrounding neurodegenerative diseases. In particular, disease research into the development of treatment technology has moved on from basic biology using animal models to clinical medicine directly targeting the human brain.

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K. Wada (ed.), *Neurodegenerative Disorders as Systemic Diseases*,
DOI 10.1007/978-4-431-54541-5_13

For example, the visualization technologies for pathology and diagnosis have advanced. The introduction of induced pluripotent stem cell (iPS) technology has enabled reproduction of more detailed pathogenic mechanisms and pathologies at the cellular level. As a result, molecular biological understanding of neurodegenerative diseases has progressed. The approach has expanded from one that only covers neurons or part of the brain to systemic one in which the structural and functional links between organs and the brain are targeted. In addition, researcher interest has now expanded to the interaction between the material and social environments surrounding the brain and body.

The extent to which scientific understanding of neurodegenerative diseases has advanced has already been written about by many researchers. The focus of this chapter is the process by which scientific understanding of neurodegenerative diseases can be implemented socially. This process may include conducting clinical studies with patients and therapeutic intervention. Such intervention is expected to reveal which changes in the brain function of patients impact personality or cognitive ability. The results of such studies *per se* may provide a new research target. Taken together, it is important to impart accurate knowledge about clinical research and neurodegenerative diseases to society as well as to progress patient treatment by insuring social participation and human rights.

13.1.2 Possibility of Neuroethics for Appropriate Conduct of Neurodegenerative Disease Research

Neuroethics comprehensively treats the various issues and challenges associated with implementing neurodegenerative disease studies on behalf of society such as the social, legal, ethical, and policy implications related to advances in neuroscience. Neuroethics was established at the international conference *Neuroethics: Mapping the Field* held in San Francisco in 2002. Conference topics included neuroscience research into the ethics of human research into the understanding of morality. Neuroethics can also be explained as the process in which neuroscience studies with human subjects is practiced, which includes academic analysis aimed at resolving ethical issues in society. It is a discipline that fuses neuroscience with humanities and social sciences (Marcus 2002).

The origin of the word “neuroethics” is fully explained by Fukushi and Sakura (2008a, b). Cranford advocated the word “neuroethicist” as a metaphor for the role played by a neurologist in end-of-life care (Cranford 1989). Cranford’s paper emphasizes the responsibility and role played by the neurologist in an institutional ethical committee, with regard to patient care after diagnosis of neurological disorder. By contrast, Pontius (1973) used the word “neuroethics” to criticise excessive intervention in the study of development of nerve function in infants and to point up the importance of considering the long-term effect of neuroscience research on subjects’ quality of life. These two articles suggested the conceptual

schema of neuroethics as originating from multiple views of neuroscience and humanity studies including bioethics, medical ethics, philosophy of mind, and research ethics (Fins 2008).

Indeed, the factors covered by neuroethics extend well beyond concerns for neuroscience research in infant development and end-of-life care. For example, there is the problem of the application criteria used for deep-brain stimulation in the treatment of Alzheimer's disease and mental illness (New Energy and Industrial Technology Development Organization 2008, 2009). There is the problem of the psychotropic drugs used for the enhancement of cognitive ability rather than disease treatment. There is the problem of judicial decisions based on brain function measurement technologies including brain imaging. Moreover, there is the problem of the ethical process and interpretation of research results concerning human morality, religious beliefs, and a sense of discrimination which are so close to human privacy and personal secrecy (Illes 2006).

In Japan researchers have tended to use the term "nerve" as part of the translation of "neuroethic." However, Osamu Sakura, who has played a leading role in introducing this academic area to Japan, pointed out that the Japanese translation of neuroethics should reflect the extensiveness of neuroscience and the problems posed by the involvement of unprofessional stakeholders (Fukushi et al. 2006: 2). He also recommended using the term "brain" as part of the Japanese translation.

13.2 Protection of Subjects in Neurodegenerative Disease Research

13.2.1 Suggestion from Neuroethics

Neurodegenerative disease study is expected to provide treatment technologies for patients with impaired brain function. However, since it is still in the embryonic stage, the study itself is not ready for medical service to be provided to the patient.

Ando et al. (2009) split differences in the ethical positioning of subjects between psychological research and medical research. In the case of medical research, the very existence of the patient led to the social demand for doctors and medical care. As a result medical professionals and medical research were born. The simple fact that most of the people engaged in medical research are patients is the cornerstone of the contractual relationship between doctors who provide the treatment and patients who receive it. This relationship led to the second contractual relationship in which experimental cooperation was established between subjects (patients) and researchers (doctors). Such a relationship is considered a win–win relationship in which benefits accrue to both parties by the development of new medical technologies and their application to the patient. In addition, to progress the experiment, the procedures and theories of medical ethics and bioethics are applied to the conclusion of contract.

The author now turns to how researcher's position subjects in psychological research. Unlike medical research, the demand for psychological research is not brought on by others, it stems rather from the interests of psychologists themselves. Hence, psychological research hinges on the existence of psychologists. In other words, the contractual relationship between subjects and researchers is established using a different format from that of medical research. The goodwill of subjects is necessary since no clear practical benefits can be provided to them as a result of psychological experiments. Researchers involved in psychological research must still show as much respect to subjects as in the case of medical research, albeit in different ways. When requesting experimental cooperation, respect and acknowledgement of subjects should be kept firmly in mind.

13.2.2 Lessons from Neuroethical Discussion for the Protection of Subjects

The above discussion means that psychological research can be considered curiosity-driven research. Moreover, if medical research is at a very early stage, the ethical positioning of subjects can also be considered curiosity-driven research, or very close to it. This means that two ethical positions – medical research and curiosity-driven research – may be applied to subjects in the early stages of neurodegenerative disease research. The balance between these two ethical positions may change depending on the developmental stage, characteristics of the target disease, and whether society understands the target disease and accepts the social participation of patients. The author now introduces several examples of ethical practice designed to protect subjects, which may trigger neuroethical discussion on how best to do so in neurodegenerative disease research.

13.2.2.1 Procedures for Notification and Follow-up in Case of Incidental Findings of Subjects Who Run Potential Risks of Neurological Disease or Even Death

This issue has been debated by many stakeholders (Wolf 2011; Seki et al. 2010). In the United States, despite official guidelines not yet being established, the federal government requests funded researchers to identify procedures for adoption in research protocols regarding incidental findings (National Institutes of Health 2010; Illes et al. 2008). It is much the same in Japan, where no unified governmental guidelines have yet been established. However, the Japan Neuroscience Society highlights the necessity for proper handling of incidental findings in their revised guidelines (Japan Neuroscience Society 2009). There is also a management protocol in Japan that is project specific. For example, in the case of the Japan Children's Study Group, the researchers found incidental findings in 40 of the 110 participants

in a structural MRI study conducted in a pediatric cohort in Japan (Seki et al. 2010). They categorized these cases into four classes based on referral emergency. The parents of two participants were recommended the "routine referral" category for their child and the parents of one participant the "urgent referral" category. This led to establishment of a management protocol to deal with incidental findings of MRI research using children for non-clinical purposes. In this protocol a wide range of stakeholders are involved not only in evaluating incidental findings but also how best to handle them further, dependent of course on whether participants want to be informed of incidental findings.

13.2.2.2 Protection of Patients with Brain Stimulation Procedures Using Magnetic and Electronic Apparatus

Ethical issues concerning brain stimulation for the treatment of neurodegenerative diseases need to be resolved urgently. In addition to brain stimulation being approved for neurological disease patients with motor deficit, it is now being actively tested for patients with psychiatric disease or Alzheimer's disease (Fukushi 2012; Takagi 2012). This issue was first discussed by neurosurgeons, psychiatrists, and neuroethicists in the early 2000s elsewhere in the world, while the argument in Japan only began in recent years.

Since the safety standards surrounding brain stimulation may change with advances in the scientific understanding of the human brain, it likely will take a long time for consensus to be reached regarding its use. Accordingly, there is always the possibility that adverse events may occur, despite experiments being performed in compliance with current safety standards, (Fukushi and Sakura 2008a). Knoch et al. (2006) demonstrated that repetitive transcranial magnetic stimulation (rTMS) of the right prefrontal would lower the threshold for acceptance of an unfair offer in the Ultimatum Game for healthy subjects. Their rTMS stimulation parameter was based on the safety criteria of the International Federation of Clinical Neurophysiology at the time (Hallet et al. 1999). Leslie Sergeant Jones, a neuroscientist at UCLA and a member of the Institutional Review Board (IRB), claimed in Jones (2007) that the study of Knoch et al. was based on the interpretation of "minimal risk" (45 CFR section 46.102). The International Federation of Clinical Neurophysiology revised the safety standard for TMS as a series of academic papers in 2009 (see Rossi et al. 2009).

The New Energy and Industrial Technology Development Organization undertook an international survey regarding ethical and safety guidelines for deep-brain stimulation (DBS) use in patients with psychiatric disease (2008). The principal investigator of this project – Takagi – described three points that need to be considered for the protection of subjects (New Energy and Industrial Technology Development Organization 2008; Takagi 2012):

1. *Team activity, in other words, collaboration with neurologists, neuropsychologists, psychiatrists, neurosurgeons, nurses, and bioethicists would be effective to share the responsibility as well as to prevent the "curiosity-driven" trials of DBS.*
2. *Multiapproval and monitoring systems, in which a combination of the US Food and Drug Administration (FDA)-like regulation, an institutional review board (IRB), a "data monitoring committee," and a "conflict of interest board," would work efficiently to monitor the safety, efficacy, and conflict of interest between the clinician and medical industries.*
3. *Consideration of patient selection based on multicriteria for patient screening and suitable informed consent with accurate information about the risk and benefit of DBS, which is not a perfect solution of one's disorder, would be required.*

Clinical application of DBS to psychiatric patients in Japan has yet to be approved. For this to happen, all the various stakeholders would need to fully investigate the social implications of this technology.

13.2.2.3 Ethical Considerations for Subjects Who Have Reduced or No Consent Ability

It is difficult to judge whether subjects with cognitive impairment have consent ability, especially since this judgment may affect the ethical procedures that have to be followed to protect the subject. It may also affect assessment of the efficacy of investigational drugs/devices in the development of treatment technology. This is an appropriate point for the author to introduce international guidelines regarding ethical procedures designed for clinical research subjects with reduced or no consent abilities, and then to discuss issues that warrant further ethical procedures.

This chapter looks at two sets of international guidelines. The international guidelines issued by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) represent the first set. Ethical arrangements for subjects in their guidelines (ICH 1996) are as follows:

Extract from the ICH E6 R1 4.8. Informed Consent of Trial Subjects

4.8.12 When a clinical trial (therapeutic or non-therapeutic) includes subjects who can only be enrolled in the trial with the consent of the subject's legally acceptable representative (e.g., minors, or patients with severe dementia), the subject should be informed about the trial to the extent compatible with the subject's understanding and, if capable, the subject should sign and personally date the written informed consent.

4.8.13 Except as described in 4.8.14, a non-therapeutic trial (i.e., a trial in which there is no anticipated direct clinical benefit to the subject), should be conducted

in subjects who personally give consent and who sign and date the written informed consent form.

4.8.14 Non-therapeutic trials may be conducted in subjects with consent of a legally acceptable representative provided the following conditions are fulfilled:
(a) The objectives of the trial cannot be met by means of a trial in subjects who can give informed consent personally.
(b) The foreseeable risks to the subjects are low.
(c) The negative impact on the subject's well-being is minimized and low.
(d) The trial is not prohibited by law.
(e) The approval/favourable opinion of the IRB/IEC is expressly sought on the inclusion of such subjects, and the written approval/ favourable opinion covers this aspect.

Guideline for Good Clinical Practice
Such trials, unless an exception is justified, should be conducted in patients having a disease or condition for which the investigational product is intended. Subjects in these trials should be particularly closely monitored and should be withdrawn if they appear to be unduly distressed.

4.8.15 In emergency situations, when prior consent of the subject is not possible, the consent of the subject's legally acceptable representative, if present, should be requested. When prior consent of the subject is not possible, and the subject's legally acceptable representative is not available, enrolment of the subject should require measures described in the protocol and/or elsewhere, with documented approval/favourable opinion by the IRB/IEC, to protect the rights, safety and well-being of the subject and to ensure compliance with applicable regulatory requirements. The subject or the subject's legally acceptable representative should be informed about the trial as soon as possible and consent to continue and other consent as appropriate (see 4.8.10) should be requested.

The second set of international guidelines was established in collaboration with the World Health Organization by the Council for International Organizations of Medical Sciences (CIOMS) to protect subjects lacking consent ability. Ethical arrangements for subjects in their guidelines (CIOMS 2002) are as follows:

Extract from the International Ethical Guidelines for Biomedical Research Involving Human Subjects
Guideline 15: Research involving individuals who by reason of mental or behavioural disorders are not capable of giving adequately informed consent
Before undertaking research involving individuals who by reason of mental or behavioural disorders are not capable of giving adequately informed consent, the investigator must ensure that:

- *such persons will not be subjects of research that might equally well be carried out on persons whose capacity to give adequately informed consent is not impaired;*

- *the purpose of the research is to obtain knowledge relevant to the particular health needs of persons with mental or behavioural disorders;*
- *the consent of each subject has been obtained to the extent of that person's capabilities, and a prospective subject's refusal to participate in research is always respected, unless, in exceptional circumstances, there is no reasonable medical alternative and local law permits overriding the objection; and,*
- *in cases where prospective subjects lack capacity to consent, permission is obtained from a responsible family member or a legally authorized representative in accordance with applicable law.*

The ICH and CIOMS guidelines recognize the need for experiments with human subjects as part and parcel of the research and development (R&D) of treatment technology for neurodegenerative diseases including mental illness. They also consider the possibility that such R&D might involve subjects who do not have enough capacity for consent. If the research protocol indicates the use of such subjects, researchers are required to clearly elucidate the reasons the given study has no suitable alternatives and cannot be achieved without the cooperation of such subjects.

The international guidelines described above define how subjects with reduced or no consent ability can be protected. However, they do not indicate the criteria needed to judge the consent ability capacity of a given subject. Moreover, the procedure for surrogate consent is subject to the laws of the country where the experiment is to be conducted. These facts indicate the difficulty – both scientifically and ethically – of defining and evaluating the capability of consent ability.

The criteria to define consent ability have been juristically and scientifically discussed in the United States for several decades. Although the U.S. federal government could indicate the pros and cons of such a judgment based on empirical analysis of consent capability, it still has not come up with a unified standard (Making Health Care Decisions 1982). Despite a tentative plan drawn up by scientists and bioethicists, it is still difficult to conclude an international agreement (Grisso and Appelbaum 1998).

The consent ability of patients can be categorized into four groups based on reports published in the United States and discussions arising from such reports concerning a number of abilities such as "the ability to communicate his/her own decision," "the ability to understand information regarding medical practice," "the ability to assess the current status and results of therapeutic practice properly," and "the ability to process information in a reasonable way." Evaluation techniques and measurement technologies for the scientific interpretation of each of these abilities are in urgent need of development. Once developed, information on consent ability common to various stakeholders – including psychiatrists, neurologists, clinical psychologists, cognitive psychologists, lawyers, and neuroethicists – can be collated for more productive discussion. At the time of writing neuroscience has not established objective indices regarding consent ability nor has it reached a consensus on how these indices should be built. Even though objective indices will be established in the future, it will not be easy to apply them in the real world.

Moreover, after their establishment, consensus will still need to be built regarding the procedures employed to judge consent ability – a difficult task since the various stakeholders will almost certainly have their own ethical views on how to judge it appropriately.

13.3 Conclusion

This chapter discussed the relationship between researchers and subjects regarding the implementation of neurodegenerative disease research in ways that are socially acceptable. The chapter further emphasized issues surrounding the consent ability of subjects participating in the clinical research of neurodegenerative diseases. The chapter should be of use to researchers who are already aware of these points, but might not have enough time to discuss them explicitly in their daily activities nor the opportunity to examine their own research in such a context.

The Japanese government requires researchers to promote public engagement with their research on a regular basis, especially those researchers in receipt of research grants. This is the reason researchers constantly explore means of communicating their research results to society in the most appropriate way. Neurodegenerative disease research, in particular, has a serious impact on society as a result of patient participation, because research outcomes may modulate the biological basis of social behavior not only of the patient but also of the people and social environments surrounding the patient. Every neurodegenerative disease researcher needs to recognize the significance of his/her own research activity and take responsibility for it. In addition, researchers' ethical views and morals should strictly adhere to research practice through ethical guidelines, safety criteria, and procedures specifically designed to protect patients and insure research integrity.

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