

Chapter 1

Imaging Neurodegeneration: What Can Magnetic Resonance Spectroscopy Contribute?

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Abstract With increased prevalence of neurodegenerative diseases with age and an aging society, neuroimaging for diagnosis, prognosis, and therapy monitoring in these diseases has become more important than ever. There is particularly a great need for robust biomarkers and surrogate markers of cerebral pathology that can facilitate development of effective treatments in these conditions. Many radionuclide and MRI modalities are currently used in clinical research, with some already accepted among diagnostic criteria for neurodegenerative diseases. Others are being evaluated for their potential to monitor the pathogenic events during neurodegeneration at multiple levels from the global network level down to the subcellular and molecular levels. This chapter places magnetic resonance spectroscopy (MRS) within the context of other imaging modalities for evaluating neurodegeneration and summarizes its unique role in simultaneously assessing multiple relevant pathophysiological events, including neuronal loss/dysfunction, gliosis, demyelination, impaired energetics, increased membrane turnover, demyelination, synaptic dysfunction, and oxidative stress. Finally, the steps that still need to be taken to facilitate wider utility of advanced MRS methodology are outlined.

Keywords Structural MRI • Diffusion MRI • Resting state fMRI • Positron emission tomography • Magnetization transfer MRI • Manganese-enhanced MRI • Susceptibility-weighted imaging • *N*-Acetylaspartate • Choline • Creatine • *Myo*-inositol • Lactate • Glutamate • Glutamine • GABA • Glutathione • Ascorbate

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Imaging Cerebral Pathology in Degenerative Brain Diseases

Neurological diseases affect as many as one billion people worldwide. Of these, neurodegenerative diseases cause progressive neuronal degeneration and death and are a major cause of disability and human suffering. With increased prevalence of these diseases with age and an aging society in developed countries (between 2000 and 2030, the number of individuals 65 years of age and older will double [1]), neuroimaging for diagnosis, prognosis, and therapy monitoring in these diseases has become more important than ever.

This volume on *MRS of Degenerative Brain Diseases* focuses on chronic, progressive neurodegenerative diseases, including both preclinical (animal model) and clinical applications. Thus, acute insults that cause neuronal damage and degeneration (as in stroke and traumatic brain injury) are not covered in the volume. Chronic neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), and spinocerebellar ataxia (SCA), represent a complex family of neurological disorders in which vulnerable neuron populations are progressively lost. Current treatments for these conditions are supportive as disease-modifying treatments have been elusive. A major impediment in therapy development has been the lack of robust biomarkers and surrogate markers of cerebral pathology [2–5]. Management currently relies on structural MRI and clinical measures, which are also the primary outcome measures in clinical trials. Assessing whether therapies impact the progression of neurodegeneration is particularly challenging because the slow progression and phenotypic variability in these diseases necessitate long clinical trials with large sample sizes. Clinical outcome measures typically used in these trials have many limitations: they do not distinguish between disease-modifying and purely symptomatic drug effects, they are of no use in the earliest, preclinical stage (when neuroprotective agents are likely to be most effective), and they usually have poor test-retest reliability [5]. Therefore, while an essential component of any treatment trial, these measures need to be supplemented with noninvasive neuroimaging to directly assess treatment effects on the brain.

Commensurate with the complexity of these diseases, there are a number of imaging modalities that can be utilized to monitor various aspects of the pathophysiology of neurodegeneration. Mechanistic investigations into the process of neurodegeneration uncovered that while the genetic and environmental triggers may be different for different neurodegenerative conditions, multiple pathogenic pathways are common to many of them. These include accumulation of aberrant or misfolded proteins, oxidative stress, mitochondrial injury, excitotoxicity, synaptic failure, altered metal homeostasis, axonal and dendritic transport failure, neurovascular deficits, and neuroinflammation [6–9]. These pathogenic processes are active for years and result in neuronal dysfunction, even prior to onset of overt clinical symptoms [10], and lead to the ultimate demise of neurons when the cells can no longer cope with the loss of homeostatic balance.

Table 1.1 lists examples of commonly used modalities to monitor these pathogenic events at multiple levels from the global network level down to the subcellular (axonal/dendritic/synaptic) and molecular levels. Only proton (^1H) MR modalities that can be performed with standard equipment are listed in the table. However, it should be noted that there are non-proton MR methods such as ^{31}P , ^{13}C , and ^{17}O MRS that can provide even more detailed mechanistic information on metabolic aspects of neurodegeneration [11–13]. Also note that some of these modalities have been substantially more validated and are used in modern diagnostic criteria [14], while others require further validation. Thus, rates of global and regional brain atrophy measured with *structural MRI* have been validated in longitudinal multi-site studies and now are being accepted as therapeutic outcome measures in some neurodegenerative diseases [2, 5, 15]. Structural MRI is advantageous because MR scanners are widely available in the clinical setting and volumetric measurements are based on relatively standardized and simple data acquisition with protocols that are part of routine clinical MRI.

While gross morphometric changes detectable by structural MRI have found wide utility and undoubtedly contributed to understanding of the temporal progression of neurodegeneration, microstructural damage in the tissue occurs earlier in the neurodegenerative sequence of events and is accessible by *diffusion MRI* [16]. Recent years marked a surge in development of methods to increase the reliability of tractography and to enhance understanding of the contributions to the diffusion MR signal in order to extract tangible quantitative properties of the tissue, such as axon diameter and density. In addition, functional network connectivity in the brain can now be relatively easily assessed using *resting state functional MRI* (rsfMRI), which measures spontaneous fluctuations in the blood-oxygen-level-dependent (BOLD) signal that are correlated across anatomically distinct brain regions [17]. Importantly, increasing evidence supports the involvement of structural and functional network connectivity deficits in progressive neurodegeneration [18, 19], and hence these methods are expected to find wide utility in neurodegenerative disease trials.

Among non-MR modalities, *positron emission tomography* (PET) has been used by many to monitor multiple pathophysiological events during neurodegeneration because it can detect early metabolic and cellular abnormalities, such as toxic protein accumulation [20], defects in glucose uptake [21], synaptic dysfunction [22], and, more recently, neuroinflammation [23] (Table 1.1). Importantly, new NIH and Alzheimer Association guidelines include amyloid ligand PET in the diagnostic criteria for AD [24]. The drawbacks to PET scans are that they are significantly more expensive and less readily available than MR and involve radiation exposure, which makes repeat scanning problematic.

With yet other methods available to assess other pathophysiological aspects of neurodegeneration, such as demyelination using *magnetization transfer MRI* [25], axonal transport deficiencies using *manganese-enhanced MRI* (MEMRI) [26], and iron content using various T_2 and susceptibility-based MRI methods [27], it is clear that multimodal investigations have the best chance to comprehensively evaluate progressive neurodegeneration and if interventions slow down or reverse the

Table 1.1 Neuroimaging modalities that can be utilized to assess pathophysiological events during neurodegeneration

Pathophysiological event	Imaging modality
Whole brain/network level	
Regional atrophy	Structural MRI
Reduced structural connectivity	Diffusion MRI
Reduced functional connectivity	Resting state fMRI
Cellular level	
Neuronal loss	<i>MRS (NAA, Glu)</i>
Reactive astrocytosis	<i>MRS (Ins, Gln)</i>
Microglial activation/inflammation	PET (TSPO), <i>MRS (Ins, Gln)</i> , DCE-MRI (BBB breakdown)
Neurovascular dysfunction	Perfusion MRI (ASL), fMRI
Subcellular level	
Axonal/dendritic transport failure	MEMRI
Demyelination	MT-MRI, diffusion MRI, <i>MRS (Cho)</i>
Axonal loss	Diffusion MRI, <i>MRS (NAA, Glu)</i>
Synaptic dysfunction	PET (dopamine), <i>MRS (Glu, Gln, GABA)</i>
Molecular level	
Toxic protein accumulation	PET (A β)
Impaired energetics/mitochondrial injury	PET (FDG), <i>MRS (Lac, NAA, Cr)</i>
Oxidative stress	<i>MRS (GSH, Asc)</i>
Altered metal homeostasis	Relaxometry, SWI (Fe)

The list is not meant to be comprehensive and only includes examples of commonly used modalities and measured compounds, e.g., amyloid β accumulation with PET

NAA N-acetylaspartate, *Ins* myo-inositol, *Gln* glutamine, *TSPO* translocator protein, *DCE-MRI* dynamic contrast-enhanced MRI, *BBB* blood-brain barrier, *ASL* arterial spin labeling, *MEMRI* manganese-enhanced MRI, *MT-MRI* magnetization transfer MRI, *Cho* choline, *Glu* glutamate, *GABA* γ -aminobutyric acid, *FDG* fluoro-deoxyglucose, *Lac* lactate, *Cr* creatine, *GSH* glutathione, *Asc* ascorbate, *SWI* susceptibility-weighted imaging, *Fe* iron

progression of pathology. PD presents a good example for a need for multimodal imaging. Namely, radionuclide imaging detects and monitors dopamine dysfunction with high specificity and sensitivity in PD, but alternative methods are needed to monitor non-dopaminergic aspects of the disease [22], as well as to monitor the nigral pathology [28], which is the hallmark of PD. This need has spurred interest in MR-based biomarkers for PD despite great success with PET/SPECT imaging of dopamine function.

Added Value of MRS in Clinical Research and Care in Neurodegenerative Diseases

When regional morphometric changes in conventional T_1 and T_2 MRI are typically observed in degenerative brain conditions and are easily accessible via routine clinical exams, the need for more specialized techniques such as *magnetic resonance spectroscopy* (MRS) can be questioned. The macrostructural changes however are the end point of a cascade of events that lead to neuronal death and atrophy, and it is widely accepted that the underlying cellular and biochemical changes start years before symptoms and irreversible structural damage detectable by conventional imaging [10]. Therefore, biomarkers and surrogate markers that are sensitive to these early pathological changes and that can gauge drug effectiveness objectively and quickly are still needed and can have a high impact on development of neuroprotective therapies. In addition, there are cases where standard CT or MRI evaluation does *not* demonstrate gross structural nervous system changes even in symptomatic patients, such as in PD [29] and ALS [30]. Methods that uncover chemical changes in the tissue, such as MRS, are ideally suited in these cases to assess pathological changes in the absence of gross morphological alterations.

MRS has been repeatedly shown to be sensitive to events preceding neuronal loss [31–33] and can be included in a standard brain MRI exam along with structural MRI. It has been a powerful complementary tool to conventional MRI for diagnosis and monitoring of disease progression and response to therapy because it can detect changes in cell density, cell type, or biochemical composition [34]. Namely, ^1H MRS enables quantification of endogenous neurochemicals, including potential markers of neuronal health (*N*-acetylaspartate (NAA), glutamate), glial proliferation (*myo*-inositol, glutamine), demyelination/increased membrane turnover (choline), deficits in energy metabolism (lactate, NAA, creatine), neurotransmitter abnormalities (glutamate, glutamine, GABA), and oxidative stress (glutathione and ascorbate) [35], and thereby can contribute to monitoring multiple pathogenic events in neurodegeneration alongside other neuroimaging modalities (Table 1.1). Furthermore, a number of clinical and preclinical applications have demonstrated that MRS can assess neuronal dysfunction and loss, as well as accompanying cellular processes, and thereby provide robust and noninvasive biomarkers of neurodegeneration [36–46], as detailed in this volume. Importantly, neurochemical alterations measured noninvasively by MRS are independent of [47] and precede atrophy [31–33] and therefore provide additional information over structural MRI, especially early in the neurodegenerative disease course.

Consistently, a recent international MRS consensus effort concluded that MRS is expected to contribute to patient management in neurodegenerative diseases [34]. In addition, consensus was reached that MRS is “clinic-ready” for diagnostic, prognostic, and treatment assessment of brain tumors, various neonatal and pediatric disorders, demyelinating disorders, and infectious brain lesions. The breakdown for the utility of MRS into the clinic-ready vs. promising disease categories primarily stemmed from the differences in effect sizes observed in these diseases.

Namely, clinic-ready applications involve large effect sizes such that biochemical changes in brain lesions are detectable reliably in individual patients. On the other hand, neurochemical changes are more subtle in the “promising” category of diseases that include neurodegenerative diseases, making robust and highly reproducible MRS acquisition and analysis protocols critical for clinical utility.

Perspectives: What Still Needs to Be Done

While standard MR hardware and software are highly optimized and automated for imaging protocols, MRS protocols that are currently provided by clinical scanner manufacturers are not state of the art. Namely, the data quality that can be obtained with the vendor-provided protocols is sufficient to quantify three to five metabolites (NAA, creatine, choline, and, depending on acquisition parameters, *myo*-inositol and lactate); however, the data quality typically does not allow quantification of metabolites such as glutamate, glutamine, GABA, and glutathione, metabolites of high interest for neurodegenerative conditions. Due to inadequacies of automated shimming protocols, many expert researchers utilize manual shimming on clinical hardware for best shimming results, which is not feasible or efficient in the clinical setting. In addition, vendor-provided localization sequences have several deficiencies such as large chemical shift displacement at 3 T and higher field strengths, poor water suppression performance, and generation of unwanted coherences, which prevent acquisition of consistently high-quality data that can reliably demonstrate subtle neurochemical alterations in disease. For example, in a systematic comparison of the standard vendor protocol (PRESS + advanced 3D shimming) vs. an in-house developed MRS protocol (sLASER + FASTMAP) with randomized acquisitions in the same scanning session at 3 T, we found that almost half of the spectra obtained with the conventional protocol (13/28) did not fit our quality criteria (water linewidth ≤ 10 Hz, [48]), whereas only 1/28 spectra obtained with the sLASER + FASTMAP protocol did not fit these criteria in the elderly population [49]. Note that this linewidth criterion was set to avoid bias in concentrations of some weakly represented metabolites [48]. These considerations underline the advantages of utilizing advanced MRS methodology at 3 T when investigating neurodegenerative diseases. Namely, high-quality MRS data with excellent within- and between-site reproducibility of 10–15 neurochemicals can be obtained with standard clinical 3 T hardware as long as optimized acquisition and analysis techniques are used, including robust protocols for B_0 and B_1 adjustment [48]. Figure 1.1 shows examples of 3 T spectra acquired with an advanced MRS protocol from brain regions that are affected by the progressive neurodegenerative diseases that are covered in this volume.

Another critical need for wider utility of advanced MRS methodology is *automation* of MRS acquisition and analysis protocols to make them “MR technologist friendly.” For example, one of the biggest challenges of single voxel MRS (SVS) in the clinical environment is the operator dependence of voxel selection. Methods

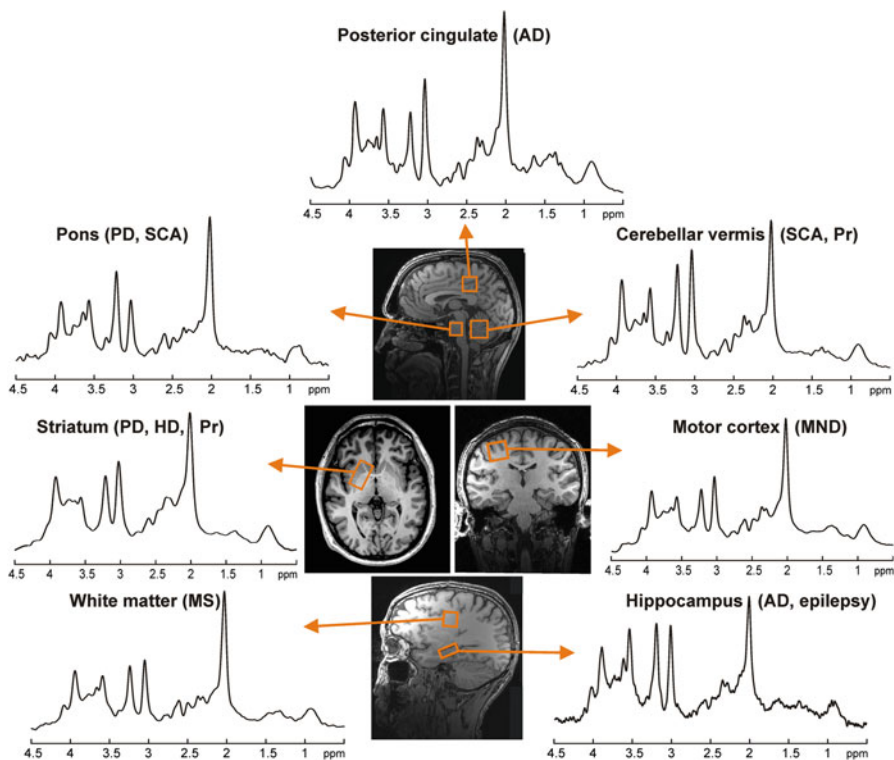


Fig. 1.1 Proton MR spectra (semi-LASER [57], TR/TE = 5000/28 ms) obtained at 3 T from brain regions that are of interest for various degenerative brain diseases as listed in *parentheses*. The spectra were processed and weighted identically (Gaussian apodization $\sigma = 0.13$ s) prior to Fourier transformation. Voxel positions are shown on T₁-weighted images. *AD* Alzheimer's disease, *PD* Parkinson's disease, *HD* Huntington's disease, *SCA* spinocerebellar ataxia, *MND* motor neuron disease, *MS* multiple sclerosis, *Pr* prion disease. Spectra and images courtesy of Drs. Dinesh Deelchand, James Joers, Pierre-Gilles Henry, Fanny Mochel, and Petr Bednařik

have been developed to automatically place the volume of interest (VOI) in longitudinal scans to match the VOI at the baseline scan of the same subject [50, 51]; however, atlas-based automatic voxel positioning to improve VOI consistency both between- and within-subjects has not yet been implemented for SVS. On the analysis end, spectral fitting using a metabolite basis set needs to be incorporated into the workflow on the scanner as data analysis is currently a major hurdle in the clinical utility of MRS [34].

Next, it will be important to *standardize* such automated, advanced MRS acquisition and analysis protocols across vendors and validate their multi-site reproducibility [48, 52]. In fact, lack of standardization has been an impediment for all biomarker discovery efforts [53] and its importance recognized for all neuroimaging modalities [54]. Clearly such standardization efforts would be most effectively pursued in collaboration with MR scanner manufacturers, and it is

encouraging that representatives from major manufacturers are involved in the MRS consensus effort [34], demonstrating their interest in improving the vendor-provided MRS packages in future products.

Finally, following standardization of acquisition and analysis methodology in multi-site settings [48, 52], the added value of MRS needs to be evaluated systematically for different neurodegenerative diseases and clinical questions [55] to provide sufficient data for future evidence-based medicine (EBM) assessments [56].

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