Published in final edited form as:

Bioanalysis. 2012 July ; 4(14): 1787–1804. doi:10.4155/bio.12.129.

Preclinical ^1H -MRS neurochemical profiling in neurological and psychiatric disorders

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Abstract

The ongoing development of animal models of neurological and psychiatric disorders in combination with the development of advanced nuclear magnetic resonance (NMR) techniques and instrumentation has led to increased use of *in vivo* proton NMR spectroscopy (^1H -MRS) for neurochemical analyses. ^1H -MRS is one of only a few analytical methods that can assay *in vivo* and longitudinal neurochemical changes associated with neurological and psychiatric diseases, with the added advantage of being a technique that can be utilized in both preclinical and clinical studies. In this review, recent progress in the use of ^1H -MRS to investigate animal models of neurological and psychiatric disorders is summarized with examples from the literature and our own work.

Proton magnetic resonance spectroscopy (^1H -MRS) has many advantages when it comes to the investigation of **neurological disorders**, especially since it is a completely non-invasive method capable of measuring neurometabolite concentrations *in vivo* [1–5]. There are other methods available, such as microdialysis [6,7] or fast-scan cyclic voltammetry (FSCV) [8], that are capable of measuring *in vivo* metabolite levels in the brain, but they require the insertion of a probe with a semipermeable membrane (for microdialysis) or an electrode (for FSCV) into the brain for neurochemical quantification. In addition, these methods might measure different pools of metabolites; while ^1H -MRS measures signals from everywhere inside a selected voxel (total volume $\sim 8\ \mu\text{l}$) and, therefore, both intra-cellular and extracellular metabolites, microdialysis may only measure global (within a single brain region [200–400 μm diameter probe]) extracellular neurochemicals and FSCV may measure local (a few synapses within a brain region [5–10 μm diameter probe]) metabolite concentrations. However, considering the non-invasiveness of ^1H -MRS, it has a notable advantage for clinical translatability and is an ideal tool for parallel experimental paradigms using animals and humans [9]. Since ^1H -MRS experimental methods are similar regardless of whether a human or preclinical animal model subject is being analyzed, it may be

possible for preclinical data to give additional insight into clinical analysis. Preclinical *in vivo* ^1H -MRS provides clues to the underlying pathophysiology of neurological and **psychiatric disorders** as well as suggest possible interventions, as it is possible to scan some brain regions in animal models, such as the nucleus accumbens [10,11], which are very difficult to analyze in humans because of their size and location. In addition, ^1H -MRS-based neurochemical analysis can be used for longitudinal studies [2,9]. Because experimental animals do not need to be sacrificed for the measurement of metabolites when using ^1H -MRS, the same subject can be analyzed multiple times. This allows for experimental paradigms, such as before and after treatment analyses, instead of a case/control paradigm. Scanning the same animal multiple times in an experiment also permits the use of more powerful statistical modalities, such as repeated measures analysis of variance (ANOVA) or a paired analysis.

To maximize spectral sensitivity and resolution it is necessary to minimize the objects movement during ^1H -MRS acquisition. Therefore, in animal studies anesthetics are typically used along with a triggering system to ensure that the signal is acquired only between breaths. This may be less preferable than other *in vivo* approaches in which animals move freely. Therefore, when interpreting the results of ^1H -MRS in animal models, investigators should consider the possibility of different neurochemical levels between awake (freely moving) and asleep (anesthetized) animals as well as the potential effect of the anesthetic itself on the observed biochemical process. The acquisition time for one animal ^1H -MRS spectrum could be longer than an hour especially if the voxel is small ($<10\ \mu\text{l}$), which is typical in preclinical studies using mice. Nonetheless, preclinical *in vivo* ^1H -MRS is a useful technique that provides reliable data and is a powerful method to examine neurochemical changes in the brain. Several excellent reviews have already been published on the techniques and applications of ^1H -MRS for neurochemical profiling [4,12-15] and, in this review we focus on the application of ^1H -MRS in neurological and psychiatric disorders.

^1H -MRS = proton nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy in medical practice and research is better known as MRS. This technique plays a prominent role in *in vivo* neurochemical studies in spite of its inherent low sensitivity compared with other commonly used analytical methods for the analysis of neurochemicals such as UHPLC or LC-MS/MS. Its main disadvantage – low sensitivity – is inseparable from its main advantage – non-invasive interrogation. In addition to the non-invasive probing of *in vivo* chemical compositions, MRS is useful in various studies of the brain, primarily for its ability to detect subtle physicochemical changes in neurological tissue, such as intracellular pH [16] or molecule dynamics [17–19]. Analyzed subjects can be scanned repetitively, since exposure is limited to nonionizing electromagnetic field and magnetic field gradients without known hazards. However, subjects cannot be scanned continuously as heating may occur due to the coupling between the applied radiofrequency (RF) field and the highly dielectric biological tissue.

Simplified principles

NMR detects signals from the motion of atomic nuclei, which is induced in a RF coil when nuclei are placed in an external magnetic field and excited by the RF field. This signal can be detected from many (usually stable) nuclei, but for biological studies the most useful include hydrogen (^1H), carbon (^{13}C), phosphorous (^{31}P) and nitrogen (^{15}N). The receptivity of the nucleus is proportional to $\gamma^{1/4} I(I+1)$ where γ is the magnetogyric ratio and I is the spin quantum number of the nucleus. At a given field, the receptivity of ^1H is an order of magnitude larger than of ^{31}P , the next most sensitive nuclei in biological systems ($\gamma_{\text{H}}/\gamma_{\text{P}} = 2.5$, $I_{\text{H}} = I_{\text{P}}$). Similarly, the S:N ratio (SNR) of a given nuclei is proportional to $\omega_0^{7/4}$, where

ω_0 is the resonance frequency (directly proportional to the external magnetic field, B_0 , $\omega_0 = \gamma B_0$). This suggests that going from $B_0 = 7$ T ($\omega_0, {}^1\text{H} = 300$ MHz) to 16.4 T (700 MHz) for a given nuclei will improve the sensitivity by approximately four-times (Figure 1A). This is indeed the case in high-resolution NMR and microimaging where the principal source of noise is the RF coil. With larger coils, such as in human imaging and spectroscopy, the subject itself is the principal source of noise and then SNR increases linearly with the field, $\text{SNR} \propto B_0$. Thus, in general $\text{SNR} \propto B_0^\alpha$, where α smoothly decreases from 1.75 to 1 with the increase of the coil diameter. Thus, to maximize sensitivity for *in vivo* studies, proton NMR (also known as ${}^1\text{H}$ -MRS) is most commonly performed with the smallest coil at the highest field available.

For *in vivo* neurochemical profiling the most important principles are:

- The linearity of ${}^1\text{H}$ -NMR spectroscopy (which is the basis for quantification of the spectra);
- The lack of chemical interference (the signal from a given species is independent from the presence of other chemical species);
- The robustness of the *in vivo* chemical shifts (the position of spectral line vary little or not at all from tissue to tissue).

Thus, irrespective of the presence of other molecules, each metabolite produces the same distinct spectral pattern with an integral intensity directly proportional to the concentration of the metabolite. Elements of the spectral patterns are somewhat sensitive to numerous physicochemical parameters (temperature, pH, salt and paramagnetics), but in live neurological systems these are well regulated so that one metabolite gives almost the same *in vivo* spectral profile regardless of the brain region or biological species. This enables quantification of spectra by linear combination of patterns of individual metabolites (as implemented in LCModel algorithm [20–24]) and also helps create extensive data banks with spectral properties of common metabolites [20–24]. These principles are particularly important for *in vivo* ${}^1\text{H}$ -MRS where heavy spectral overlap precludes observation of individual lines while prior knowledge of the peak positions and spectral linearity enables unique decomposition of overlapped lines into individual components (Figure 2F).

In addition, scalar coupling constants, which define line multiplicity, are very useful in identifying metabolites by advanced multidimensional methods [25]. However, when the spectral patterns of all sought components are already known, the coupling constants have little use and may even become a burden. Namely, a signal from a particular metabolic fragment, instead of being concentrated in a single line with a given line width, splits into several lines with separation commensurate to an *in vivo* line width. The total effect is that the resulting signal is several times broader than in the absence of coupling and the SNR is several times smaller (Figure 1A). Thus, novel methods for homonuclear ${}^1\text{H}$ decoupling such as CT-PRESS[26] are certainly welcome.

The higher the field, the better the spectrum?

Increasing the static magnetic field improves both sensitivity and spectral dispersion, thus, there is a natural tendency to strive for higher magnetic field strengths. However, in practice, the spectral improvement with B_0 is not as dramatic as theoretically expected [27] for several reasons. First, for all metabolites, T1 relaxation times tend to increase with B_0 field strength [27], which leads to either prolonged repetition time during the data acquisition or reduced signal for a given repetition time. Second, T2 relaxation times (inversely proportional to the line widths, $\Delta\omega$, $T2 \propto 1/\delta\omega$) decrease with the field strength thus leading to line broadening. For example, it was found empirically that in a human brain, at the fields

1.5–9.4 T, the line width of the NAA methyl group increases roughly by 1.35 Hz/T [27], which results in a minimal linewidth of 9.5 Hz at 7 T and 22 Hz at 16.4 T (extrapolated). This line width increment with the field strength translates into constant line widths of 0.03 ppm. Since the line separation, expressed in ppm, is also constant, increasing the field strength does not increase the spectral resolution since the ratio of line width and line separation is constant irrespective of the field. However, the scalar coupling constant is virtually independent of the applied field, thus, the constant line splitting of J Hz, when expressed in ppm units scales with the field by $J(\text{ppm}) = J(\text{Hz})/[42.576 \times B_0(T)]$, where J is the coupling constant (in ppms or Hz) and B_0 is the magnetic field strength in teslas. The effective line broadening due to the scalar coupling is inversely proportional to the applied field. This is very important for resolving overlapping multiplets, for example, of glutamine and GABA at 2.35 ppm (Figure 1A). At fields below 7 T, their chemical shift difference of 0.08 ppm is commensurate with the coupling constants 7.5 Hz ($2 \times J(\text{ppm}) = 0.05$ ppm) (Figure 1B), whereas at 16.4 T the chemical shift difference exceeds the coupling constant splitting separating clearly equivalent CH_2 groups in Glu and GABA (Figure 1C). These arguments are qualitative since the multiplet pattern changes with the ratio of coupling constant and chemical shift difference (in Hz) among coupling partners (transformation from strong to weak coupling).

In ^1H -MRS spectra of the human brain it was found that, with appropriate gradient coil design and shimming procedures, it is possible at 7 T to reach line widths that are independent of the voxel size [27]. Thus, the observed line width is really an intrinsic line width. Shimming in the brain of preclinical animal models is much more difficult and such narrow lines are obtained only by dedicated equipment (and spectroscopists) [12,28,29]. In addition to the equipment design and spectroscopist's skills, the line width critically depends on the brain region selected. For example, in the cervical spinal cord, it seems more challenging to reach the line width of nucleus accumbens.

The SNRs in imaging and spectroscopy are proportional to each other, proportionality constant being a function of the spectral line width [30]. At constant line widths they both have the same field dependence, B_0^α . However, the increase of spectral line width with increased field strength (or constant line width when expressed in ppms) affects spectroscopy only. Extrapolating observed linear dependence of the line width with the applied field [27] to fields above 7 T, the field effect on line broadening offsets the dependence of spectroscopic SNR to $\text{SNR}_{\text{sp, in vivo}} \propto B_0^{\alpha-1}$. Also, increased dielectric losses and more complex coil design at higher frequencies may further offset the field gain, scaling down overall spectroscopic SNR improvement. For example, with $\alpha = 1.75$ (typical for 20 mm [diameter] RF coils), in going from 7 to 16.4 T, one would expect a twofold improvement of $\text{SNR}_{\text{sp, in vivo}}$ (more precisely 1.89). However, with similar equipment (Bruker Avance, 7 and 16.4 T vertical bore systems, 20 mm coils), the SNR improvement in the mouse brain is on the order of 40–50% (Figure 1B & C).

In summary, higher fields are better, but the improvement in the $\text{SNR}_{\text{sp, in vivo}}$ is less impressive than the improvement in imaging SNR, although sufficient to warrant getting the field as high as possible. Besides SNR improvement, the major advantage to minimizing the effects of scalar coupling on the apparent line width is that this allows for better quantification of heavily overlapped lines and easier identification of new metabolites or other small molecules, such as drugs or anesthetics.

Data acquisition

Given the variability of the metabolite concentrations throughout the brain (or any other organ), determined metabolite concentrations represent an average value of the

concentrations over the volume of interest (VOI). Smaller VOI apparently leads to smaller concentration gradients. Ideally, one would divide the whole brain into the smallest possible volume elements (voxels) and then record spectra from all voxels in parallel. This spectroscopic imaging was proposed many years ago and is possible in principle, but rarely used *in vivo* simply because of the prohibitive length of the experiment. A lighter version with the voxel array restricted to a plane is frequently used in human MRS. Such an approach is also possible in mice [12,29], but still used rarely because of the difficulties in shimming large brain areas and the relatively low spatial resolution compared with a single voxel method. In serial scans and for better spatial localization of the voxel, it is more efficient to record the spectrum from a single voxel placed in the brain region of interest. Most localized spectroscopy methods generate rectangular VOI by slice-selective pulses in three orthogonal directions; particularly popular are STEAM, PRESS and LASER, which were reviewed and compared recently [13]. In the mouse brain, the voxel dimensions are between 2 and 3 mm with volumes of 8–27 μl . Most often, the voxel is cubic, but it may be a cuboid or rectangular prism to follow brain morphology.

The concentration of water in brain tissue is approximately 47 M since our brains are 75–95% water [31]. In terms of NMR signals (two equivalent protons), the concentration of water is approximately 94 M, whereas the concentration of metabolites in the brain is less than 10 mM. Observing a relatively weak metabolite signal in the presence of a strong water signal is challenging, and the water signal must be suppressed. The efficiency of water suppression critically depends on shimming to the narrowest line, which can then be saturated by RF irradiation. Water can be eliminated completely with excellent shims. However, when spectra are quantified in the time domain, it is sufficient to scale the water signal down to the range of the metabolite signals.

During scanning, mice are anesthetized with vaporized anesthetics (isoflurane or halothane), placed in an RF coil in the magnet and body temperature maintained at 37°C. First, a scout image is recorded for voxel positioning and then the magnetic field is shimmed on the positioned voxel only (Figure 2A & B). Typically 256–4000 scans are recorded, which could last from 15 min to 2 h.

Data processing & spectral quantification

MRS is an intrinsically linear method, which means that the integral of observed spectral lines grows proportionally with the concentration of respective metabolites. When spectral lines are well resolved (high-resolution NMR spectroscopy of tissue extracts or *in vivo* MRS at long echo times), it is possible to integrate lines separately. In fully relaxed spectra, ratios of integrals weighted by a number of equivalent spins (three for methyl, two for methylene and so on) are equal to the ratios of respective concentrations. However, the line overlapping in *in vivo* spectra at short echo times is so severe that it is hard to separate the contribution of individual lines in a complex spectral envelope without deconvolution (Figure 2C). Much more robust spectral deconvolution is possible with time domain data than with spectra, thus current methods integrate *in vivo* spectra in the time domain. The most popular methods are LCModel (Figure 2D, e & F) [20] and AMARES [32], implemented in the jMRUI package [33]. In addition, irrespective of the ways spectra were integrated, obtained integrals have to be further corrected and calibrated [2,14].

Mouse versus human brain

Despite the fact that mice and other laboratory rodents are very different from humans, we share approximately 99% of the same genes and molecular pathways. This makes rodents a very suitable model organism to study various human diseases in great detail. However,

even though mice are widely used in research, there are questions about how accurately they model human diseases. There are several examples of successful treatments in mouse models of human disorders that subsequently failed in human clinical trials or even exacerbated the human disease [34]. Other species, such as dogs, cats, pigs, sheep and non-human primates, are genetically closer to humans, but the two main factors that limit their widespread use are: higher costs and potential ethical problems and concerns. In summary, despite their limitations, mice are the most suitable models of disease; they are small, breed quickly and require relatively inexpensive maintenance. Finally, after several decades of research in numerous disease models, scientists learned a lot about mouse genetics and biology. This further allowed relatively fast production of genetically altered mice to study very specific aspects of a disease, or production of 'humanized' mouse that produces a human version of certain proteins of interest.

Figure 3 shows equivalent MRI cross-sections through human (Figure 3A) and mouse (Figure 3B) brain with the areas of the striatum highlighted. The whole mouse brain is smaller than the standard voxel in the human brain ($2 \times 2 \times 2$ cm). The mouse brain is less than 10 mm in diameter, with typical voxels only a millimeter from bone (skull) or air (ear, throat), making shimming more difficult than in human studies. These difficulties, caused by the small size of the mouse brain, are compensated by the much higher SNR offered by a higher magnetic field available for mice spectroscopy. With the appropriate equipment and a skilled spectroscopist high quality mouse spectra can be obtained in approximately three-times longer time than in humans (15 min in mice vs 5 min in humans), but in three orders of magnitude smaller voxel volumes (8 μ l in mice vs 8 ml in humans).

Common metabolites

In vivo spectra typically present about 20 readily observable metabolites. These are mostly determined by their concentrations and spectral patterns (multiplicity and number of equivalent spins). Detailed NMR properties of common metabolites are well known (e.g., [24,35]) and given the lack of interference in the MRS properties of neat compounds, these are directly portable to their metabolic mixtures. The most easily detected are metabolites with the *N*-methyl or acetyl groups because the three methyl protons are equivalent and remote from others (thus, give a single sharp line). Spectroscopic properties of commonly observed metabolites and their biological roles were extensively reviewed [12,13,35] and will not be repeated here.

Applications of ^1H -MRS

Psychiatric disorders

Alcoholism, also known as alcohol dependence, is one of the most devastating addictive disorders worldwide with craving, loss of control of drinking behavior, physical dependence and tolerance phenotypes [36–40]. The MRS technique has been used to explore underlying metabolite changes in the brain following acute or chronic ethanol exposure. Several essential brain metabolites are correlated with different stages of excessive ethanol use, either immediately following ethanol administration or during ethanol withdrawal. Therefore, specific changes in the metabolic profile of brain regions could be a biomarker to diagnose or define different stages of addiction. Metabolites consistently altered by ethanol include glutamate/glutamine and GABA [10,41–44], NAA [44] (see [3] and references therein), and choline-containing compound (Cho) (see [3,45–48] and references therein).

Owing to its non-invasive nature, using brain MRS to assess alcohol use disorders is an excellent application of the direct translation of animal studies into a clinical setting. With both human and rodent MRS readily available, genetically engineered animal models can

provide molecular signatures of human disorders including alcohol use disorders. A recent publication highlights the importance of this translatability and describes the MRS-based glutamate:glutamine ratio as a potential marker for alcohol withdrawal [9]. Increased extra cellular glutamate levels are highly associated with chronic ethanol treatment and withdrawal in several brain regions including the striatum and amygdala [49,50]. The type 1 equilibrative nucleoside transporter (ENT1)-null mouse model is one that mimics chronic ethanol treatment. ENT1 is an ethanol-sensitive nucleoside transporter that regulates adenosine levels in response to acute ethanol treatment [51]. ENT1-null mice show reduced ataxic and hypnotic effects of acute ethanol intoxication as well as increased ethanol drinking compared with wild-type littermates [6,7,52,53]. Constitutively increased glutamate levels in the nucleus accumbens (NAc) of ENT1-null mice are responsible for such behaviors [6,7,10,52–54]. Treatment to alleviate the hyperglutamatergic state using pharmacological agents, such as acamprosate (Campral®), an US FDA-approved, clinically used drug to treat alcoholism, alters several key metabolites including glutamate in various brain regions using *in vivo* MRS [10,44]. Both studies used a PRESS sequence with a repetition time (TR) of 1768 ms and an echo-time (TE) of 10 ms, with analyses of metabolites completed using LCModel [20,21]. Acute acamprosate treatment (200 mg/kg intraperitoneal [ip.]) decreases glutamate+glutamine (Glx) and glutamine levels, and increases taurine and Cho levels compared with the saline control group in the NAc of ENT1 null mice, but not in their wild-type littermates [10]. Furthermore, daily treatment with acamprosate for 4 days (200 mg/kg/12h, ip.) to mice withdrawn from alcohol increases GABA levels and decreases Cho levels in the medial prefrontal cortex (mPFC), as well as decreases glutamate levels in the NAc of ENT1-null mice [44].

Another rodent model used in MRS analysis is the alcohol-preferring rat model [55,56]. Alcohol-preferring rats have been widely used for alcohol use disorder studies (see [57,58] and references therein). One group showed that alcohol-preferring rats with thiamine deficiency show characteristics of Wernicke's encephalopathy, a disease commonly associated with alcoholism caused by lesions in thalamus, hypothalamus and inferior colliculi, with a significant decrease in NAA levels in the thalamus using single-voxel 2D *J*-resolved MRS with a Chemical Shift Selective (CHESS) sequence (TR = 2 s, TE1 = 35 ms, Δ TE = 10 ms, total 16 TE steps). Thiamine treatment recovers this NAA deficiency phenotype in these rats [56].

Other studies using rats exposed to chronic alcohol treatment have shown possible correlations between NAA and Cho levels with the alcoholic phenotype [9,47,48,59]. Short-term forced alcohol consumption (one-bottle choice with 16–40 weeks of exposure) increases Cho levels, whereas long-term alcohol treatment (>40 weeks of exposure) decreases these levels in the thalamus using a STEAM sequence with TR = 3 s and TE = 68 ms [48,59]. Chronic alcohol exposure can also be achieved by intermittent exposure to alcohol (16–17 h of vapor ethanol intoxication plus 7–8 h of withdrawal per day) for a period of time (days to weeks) [9,44,60–62]. Chronic alcohol exposure for 45 days decreases NAA levels and glutamate:glutamine ratio in the rat mPFC [9] as measured by MRS with a PRESS sequence (TR = 4 s, TE = 4 ms) and analyzed by LCModel.

There have been other efforts to generate alcohol withdrawal symptoms using a vapor ethanol chamber [9,44]. Alcohol withdrawal symptoms are generated by withdrawal from chronic alcohol exposure for a certain period of time (usually 8–12 h after alcohol exposure), and an alcohol withdrawal seizure-like phenotype can be measured by a handling-induced convulsion assay [63]. When mice are withdrawn from chronic alcohol, their GABA levels are reduced and Cho levels are elevated in the mPFC, while glutamate

levels are increased in the NAc [44]. Another group has also shown that ethanol withdrawal in rats causes increased glutamate levels in the rat mPFC [9].

Taken together, a number of investigations using *in vivo* MRS have clearly shown that several brain metabolites and/or neurotransmitters, including glutamate/glutamine, GABA, NAA and Cho, are involved in different brain regions at differential stages of alcohol use disorders and are strongly correlated with human alcohol use disorder patients. In general, glutamate, glutamine, Glx and NAA are increased in the NAc. Furthermore, Cho is increased in the mPFC while NAA and GABA are decreased in the brain region (Table 1).

Mood disorders, including bipolar disorders and major depressive disorder, are the leading contributors to disabling psychiatric disorders worldwide [40,64,65]. Several brain metabolites in humans and rodents have been shown to be correlated with mood disorders, including glutamate [66–69], NAA [69–71], Cho [72,73] and creatine [66]. A few rat models have been established to study mood disorders [69]. One of these models for studying major depression is the chronic mild stress rat model [69,71,74,75]. This rat model is generated by exposing rats to 2–8 weeks of mild stressors, including food and/or water deprivation, 45° cage tilt, wet or soiled cage and overnight illumination [69,71,76]. These rats experience reduced NAA levels, increased NAAG levels and increased glutamate levels in the hippocampus as measured with a PRESS sequence (TR = 2500–5000 ms, TE = 13.20 ms; 7.9.4 T) [69,71]. Several studies utilized MRS to understand metabolic changes after pharmacological treatment of mood stabilizers and antidepressants [77–79]. Mood stabilizers, both valproate and carbamazepine, increased NAA levels and decreased glutamate levels in the frontal cortex, but not in hippocampus or striatum tissue extracts of rats using NOESY or noesy-pr1d (1D NOESY with presaturation) pulse sequences [77,78]. Also the antidepressant phenelzine decreased glutamate levels and increased GABA levels in the neocortex using a fully adiabatic PRESS sequence (TE = 15 ms; 11.7 T) [79]. Therefore, a number of investigations have utilized *in vivo* MRS to understand the correlation between several brain metabolites and disease phenotypes of mood disorders as well as with drug treatments. The consensus seems to be that glutamate is increased in the hippocampus while NAA is reduced in this brain region (Table 1).

Schizophrenia is a major psychiatric disorder characterized by hallucinations, delusions, disorganized thought processes, emotional flattening and social dysfunction [80–84]. A number of studies have shown the association of reduced NAA levels [81,85,86], increased NAAG levels [85] and reduced Cho levels [87] with disease phenotypes in several brain regions by MRS. Glutamate is also associated with the schizophrenic phenotype; however, the trend is rather complex [88]. Some MRS studies have shown elevated glutamate levels in the prefrontal cortex and dorsomedial striatum (caudate) [89–93], whereas other studies have shown reduced glutamate levels in the anterior cingulate, prefrontal cortex, thalamus and hippocampus [94–98], or no differences in the same brain regions [99–102]. One study highlights the overall heterogeneity of schizophrenia, as no significant differences were found in Glx levels of the white or gray matter of patients with schizophrenia compared with healthy controls, but did find significant correlations between cognitive function and Glx levels in schizophrenia [103]. Contradictory results of glutamate may rise from the smaller sample sizes of human and animal subjects ($n < 20$) and the heterogeneity of schizophrenia with different degrees of dysfunction, therefore, the implication of glutamate in schizophrenic remains unclear. Moreover, there have been a few studies regarding the relationship between glutathione (GSH; an antioxidant) and schizophrenia utilizing MRS. In these studies, lower GSH levels are associated with schizophrenic symptoms [104–107]. There are two distinct rodent models used to study schizophrenia, one utilizing a genetically modified mouse model, such as a mutant mouse model [87] or a genetic deletion mouse model [107], and the other using pharmacological agents, such as *N*-methyl-D-aspartate

(NMDA) receptor antagonists, to induce schizophrenic phenotypes [93,98]. The chakragati (ckr) mouse is a transgenic insertional mutant that exhibits behavioral abnormalities similar to schizophrenia [108-110]. The ckr mutant mouse (heterozygous or homozygous) shows elevated Cho:NAA ratios compared with wild-type mice [87] using a PRESS sequence (TR = 1500 ms; TE = 100 ms; 4.7 T). Recently, Duarte *et al.* using SPECIAL (TR = 4s; TE = 2.8 ms; 14.1 T) spectroscopy demonstrated that glutamate-cysteine ligase modifier subunit (GCLM)-null mice – one model of schizophrenia – show reduced GSH levels but increased glutamate, glutamine, NAA, *myo*-inositol, lactate and alanine levels in the anterior cortex [107]. Furthermore, NMDA receptor antagonists are commonly used to induce schizophrenic phenotypes in rodents. One of these agents, ketamine, a noncompetitive NMDA receptor antagonist, has been shown to induce an increase in glutamate levels in rat cerebral cortex (PRESS: TR = 4 s; TE = 20 ms; 4.7 T) [93], whereas phencyclidine, another non-competitive NMDA receptor antagonist, reduces glutamate levels, but increases glutamine levels in the rat prefrontal cortex (STEAM: TR = 4.5 s; TE = 2 ms; 9.4 T) [98]. More interestingly, phencyclidine also increases glucose levels and decreases lactate levels in the same brain region. Taken together, it remains unclear how glutamate and/or glutamine contribute to schizophrenia, but other metabolites, such as NAA, NAAG and Cho, have shown strong correlations to the disease phenotype. Overall, it has been found that Cho is increased in the brain of models of schizophrenia while glutamate is increased in the neocortex and glutamine is increased in the mPFC. Furthermore, glutamate is reduced in the mPFC of models of schizophrenia (Table 1).

Neurological disorders

Multiple sclerosis (MS) is a chronic inflammatory disease of the CNS, with only partially effective available treatments and an unknown cause. Several MRS studies in human MS patients have demonstrated significant changes in concentrations of many brain metabolites, most importantly in NAA, Cho, lactate, *myo*-inositol and glutamate/glutamine [111,112]. Consistently, studies using preclinical animal models of MS and MRS indicate changes in NAA, Cho and GABA (Table 2). The two most commonly studied animal models of MS are experimental autoimmune encephalomyelitis (EAE) and Theiler's murine encephalomyelitis virus (TMEV) [113]. Almost two decades ago Gravenmade and Minderhoud appreciated the importance of utilizing MRS as a tool to evaluate and monitor MS progression in preclinical animal models of MS. The authors were enthusiastic about the noninvasiveness of MRS and emphasized that MRS findings in animal models correlated with histopathology [114]. In one of the earliest MRS studies, Richards used a zero quantum NMR technique and showed that mobile lipids were associated with demyelination in the brains of monkeys with EAE [115]. Another group used MRS (TR = 1.4 s; TE = 420 ms; 4.7 T) to study acute EAE in guinea pigs and showed significant increases in choline levels 2 weeks post-immunization [116]. In addition, *in vitro* MRS further revealed changes in several brain metabolites, such as increased choline, phosphorylcholine and betaine levels, as well as decreased NAA, NAAG, aspartate and inositol levels. Preece *et al.* performed high-resolution *in vitro* MRS (11.8 T) of spinal cord extracts from Biozzi antibody high (AB/H) mice with induced EAE [117]. The chronic phase of EAE in this mouse strain was characterized by pathological changes in the white matter of the spinal cord, extensive inflammation and demyelination that resolves during remission [118]. The authors observed increased levels of taurine and *N*-trimethyl compounds but decreased levels of *myo*-inositol. In addition, they studied the neurochemical profile of the same mouse strain after infection with Semliki Forest virus, which is known to cause demyelinating encephalitis [119]. Similarly, MRS of CNS tissue extracts showed elevated levels of *N*-trimethyl compounds and decreased levels of NAA. The main conclusion of this study was that changes in *N*-trimethyl and *N*-acetyl MRS signals were related to myelin degradation (demyelination) and subsequent neuronal dysfunction. Hart *et al.* used high-resolution MRS (9.4 T) and pattern

recognition techniques to study the correlation between urine composition and disease progression in an EAE marmoset model of MS as well as to compare the MR spectral composition of urine in MS patients, patients with other neurological disorders and healthy controls [120]. The authors showed that changes in the chemical composition of the urine were in association with the progression of inflammatory demyelination in both the marmoset EAE model and in MS patients. The novel findings from this study provide support for the use of MRS to monitor biomarkers of CNS white matter degradation. In a very recent study, Smolinska *et al.* used a combination of high-resolution MRS (18.7 T) and pattern recognition methods to investigate the metabolic profile of cerebrospinal fluid in early EAE-induced neuroinflammation in rats [121]. This group found that the MRS metabolic profile in EAE-affected rats was quite different than that in healthy rats and rats with peripheral inflammation. The most significant changes were in the levels of creatine, arginine and lysine. Since the authors were able to confirm these results with a high accuracy in a different group of animals, the findings from this study are important for the development of MRS for molecular diagnosis of early phases of human MS.

To our knowledge, the Rodriguez laboratory at Mayo Clinic (MN, USA) is the only group that utilizes MRS to study the virus-induced model of MS. Pirko *et al.* (PRESS: TR = 3 s; TE = 120 ms; 7 T) showed that NAA levels in the parahippocampal area of C57/BL6 mice were decreased at 3 and 7 days post-infection (dpi) compared with non-infected mice and then recovered by 45 dpi [122]. This recovery of NAA levels was correlated with tissue repair in this area. More recently, the Rodriguez laboratory performed longitudinal brainstem MRS in two mouse strains with different reparative phenotypes post-TMEV infection [2]. They found that NAA levels decreased in both mouse strains as demyelination progressed (PRESS: TR = 2 s; TE = 100 ms; 7 T). However, NAA levels diverged at the late stages of disease. SJL mice, in which disease continued to worsen, show a further drop in NAA levels at the last studied timepoint post-infection (270 dpi). Conversely, FVB mice that feature spontaneous remyelination, recovered NAA to levels close to normal. This finding is important and suggests that brainstem MRS could potentially be used as a surrogate marker for spinal cord health/integrity. In the most recent study, the same group investigated pathologic differences between two almost identical strains of mice with the only difference being a genetic deletion of β 2-microglobulin (β 2m) and, therefore, complete absence of CD8⁺ T cells among other cell types [123]. Having shown that β 2m-deficient mice featured significantly increased remyelination compared with β 2m-competent mice, the authors performed a longitudinal MRS study to compare brainstem NAA levels between these two strains. This study confirmed previous findings in SJL and FVB mice and demonstrated that post-infection NAA levels significantly increased in β 2m-deficient mice compared with β 2m-competent mice at chronic timepoints. These studies warrant and encourage further use of MRS in animals of MS.

Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disorder characterized by the selective loss of upper and lower motor neurons. NAA, GABA and glutamate are the metabolites that are most notably altered in preclinical animal models of ALS using MRS (Table 2). The most commonly studied mouse model of ALS is a transgenic mouse that carries the human G93A SOD1 mutation. Niessen *et al.* proposed MRS to detect neurochemical changes in the brain of ALS patients and demonstrated that observed changes may become important surrogate markers for early disease diagnosis and could potentially evaluate a response to treatment [124]. By performing high-resolution *in vitro* MRS (TR = 10 s, 8.5 μ s pulse length; 14.1 T) of spinal cord extracts, this group demonstrated reduced NAA levels after several months, as well as reduced glutamate and GABA 15–20 days, before the average onset of the disease [124]. Other studies tested whether a creatine-rich diet alters disease presentation or progression [1]. By using both *in vivo* and *in vitro* MRS (*in vivo* PRESS: TR = 2200 ms; TE = 144 ms; *in vitro* TR = 12 s; 12

μ s pulse width; 9.4 T), the authors demonstrated that increased creatine levels in the brain has a protective effect on NAA loss in the brainstem but not in the motor cortex [1]. This group suggested that monitoring of multiple metabolite markers by MRS may be a useful technique to prospectively monitor the effects of future therapy.

Huntington's disease (HD) is another progressive neurodegenerative disease that progresses to death in 15–20 years from the time of onset. NAA, Cho, glutamate and glutamine are among the metabolites that are most commonly altered in preclinical animal models of HD (Table 2). There are a limited number of MRS studies in R6/2 mice, a transgenic mouse model of HD. Tsang *et al.* utilized high-resolution magic angle spinning MRS (NOESY, 14.1 T) of various brain regions as well as other non-CNS tissues [125]. They showed robust differences in various brain metabolites, among which increased levels of glycerophosphocholine (GPC) were the most striking. Another study utilized *in vivo* MRS (STEAM: TR = 5 s; TE = 2 ms; 9.4 T) to assess the striatal neurochemical profile in R6/2 mice at different stages of the disease [126]. The authors found that levels of creatine, GPC and glutamine increased significantly, as early as within 8 weeks, whereas NAA levels were decreased. 4 weeks later, glutamate, creatine, *myo*-inositol and other metabolites decreased, which is consistent with impaired neurotransmission, neural integrity and energy demand. Individual HD transgenic animals were clearly distinguished from littermate controls. Similarly, a significant decrease of striatal NAA levels were independently reported in patients with HD using a PRESS sequence (TR = 2 s; TE = 68, 136 and 272 ms; 4.7T) [127]. Zacharoff *et al.* proposed MRS to detect markers of HD in animal models, as changes in brain metabolites preceded brain shrinkage [128]. Therefore, prospective monitoring of brain metabolites may serve as a surrogate measure of disease progression and treatment efficacy in both mouse models of HD and patients. To test the efficacy of CGS21680 in the treatment of HD, Chou *et al.* showed that this novel compound prevented huge increases in choline levels in the striatum using MRS with a PRESS sequence (TR = 1500 ms; TE = 62 ms; 4.7 T) [129]. Another group studied five different mouse models of HD that differed in the number of CAG repeats in the huntingtin protein using MRS (PRESS: TR = 2 s; TE = 136 and 272 ms; 4.7 T) [130]. These authors demonstrated that the degree of neurochemical changes in these mice was strongly influenced by the number of CAG repeats. For example, R6/2 mice with 150 CAG repeats in the huntingtin protein showed the most severe metabolite changes, most importantly, a profound decrease of NAA levels in striatum and cortex.

Alzheimer's disease (AD) is a neurodegenerative disease that represents the most common cause of dementia in the elderly population. MRS has already been utilized to quantify brain metabolites in AD patients [131,132]. A significant reduction in NAA levels in the hippocampus of AD patients were observed, while there were no changes of Cho levels. Here, we will further discuss the use of preclinical animal models to study altered metabolites by MRS (Table 2). Dedeoglu *et al.* used MRS (PRESS: TR = 2200ms; TE = 144 and 272 ms; 4.7 T) to study the APPTg2576 transgenic mouse model of AD very late in the disease and showed decreased cortical NAA, glutamate and glutathione, but increased taurine as compared with the wild-type controls [133]. A study performed by another independent group on a higher field strength (LASER: TR = 3 s; TE = 28 ms; 9.4 T) showed similar results in the APP-PS1 mouse, a double transgenic mouse model of AD [134]. NAA and glutamate were decreased, however, *myo*-inositol, instead of taurine, was profoundly increased in this mouse model. This was of particular importance as the MRS neurochemical profile of APP-PS1 mice is equivalent to human AD patients. Von Kienlin *et al.* studied a third transgenic mouse model of AD (PS2APP) (STEAM: TR = 2 s; TE = 8 ms; 4.7 T) and found that not only were NAA and glutamate levels significantly decreased in older animals, but also the levels of these metabolites were inversely correlated with the plaque load in the frontal cortex [135].

Parkinson's disease (PD) is yet another neurodegenerative disease characterized by the progressive, severe loss of dopaminergic neurons in the substantia nigra pars compacta. As this area sends efferent projections to the striatum, dopamine levels decrease significantly in this brain region [136]. An MRS study in PD patients (STEAM: TR = 4500 ms; TE = 5 ms; 4 T) demonstrated that GABA levels in the substantia nigra were threefold higher than in the cortex [137]. Consistently, studies using preclinical animal models of PD and MRS indicate changes in GABA as well as NAA, glutamate and glutamine (Table 2). Systemic administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toxin is commonly used as a mouse model of PD. Koga *et al.* performed MRS (PRESS: TR = 3 s; TE = 146 ms; 7 T) in the striatum of MPTP-intoxicated mice to identify metabolite changes very early following MPTP injection [138]. As early as 2 h post-MPTP treatment, MRS revealed a profound increase in lactate levels, which returned to baseline levels within 5 h. This study provided support for a mitochondrial hypothesis, in which initial mitochondrial energy crisis is accounted for subsequent dopaminergic neurotoxicity. A more recent study showed significant increases of glutamate, glutamine and GABA in the striatum of MPTP-treated mice by MRS (PRESS: TR = 4 s; TE = 8.8 ms; 9.4 T), confirming the concept of glutamatergic hyperactivity following loss of dopamine [139]. Another group performed MRS imaging (BEVR, Binomial Excitation with Volume-selective Refocusing: TR = 4 s; TE = 33 ms; 7 T) to assess the efficacy of treatment with Copaxone (Cop-1) immune cells in protecting the nigrostriatal dopaminergic pathway in MPTP-treated mice [140]. These authors first showed that in all MPTP-intoxicated animals, NAA levels were significantly decreased in the substantia nigra pars compacta compared with nontreated animals. However, NAA recovered only in animals treated with Cop-1 splenocytes and stayed low in a control group treated with ovalbumin splenocytes. This study highlights the benefits of MRS as a noninvasive method to evaluate the therapeutic efficacy of pharmacological agents in an animal model of neurological disease.

MRS has also been used to study rodent models of brain tumors. An early MRS imaging (nonlocalized 2D *J* and correlation spectroscopy) study of the rat glioma using Spin-Echo Correlation Spectroscopy (SECSY) pulse sequence (4.7 T) revealed significant differences in metabolite levels as compared with contralateral normal brain tissue [141]. The most important metabolite changes in the tumor were inversion of creatine+phosphocreatine/choline-containing compounds ratio, very low levels of NAA and the presence of a huge lactate peak. In a more recent study, Doblas *et al.* utilized MRS (PRESS: TR = 2500 ms; TE = 24 ms; 7 T) to assess metabolite levels in seven rat and mouse models of glioma [142]. These authors showed that the metabolic profiles differed in most of the studied glioma models and that this was sufficient to distinguish them from each other. This is a truly important finding as rodent glioma models were conventionally considered to be similar. Furthermore, the study demonstrated that the metabolite profile of a glioma model induced by ethyl-nitrosourea was similar to controls and, therefore, not recommended for use, whereas the GL261 model showed major differences in metabolite levels as compared with all other models. Hekmatyar *et al.* used MRS (LASER: TR = 3 s; TE = 60 ms; 7 T) to study a transgenic mouse model of medullo-blastoma and showed the metabolite profile to be similar to that observed in human medullo-blastoma [143]. Of interest, tumors showed high levels of taurine, glycine and choline-containing compounds.

Conclusion & future perspective

Neurochemical profiling by NMR spectroscopy is very useful for studies of various animal models used in neuroscience. Its major limitation is low sensitivity compared with other methods used to quantify extracted neurochemicals, such as UPLC and LC-MS/MS. This low sensitivity of MRS could be improved in several different ways. The use of cryo-coils and cooled preamplifiers as well as parallel data acquisition by multiple coils and receivers

would dramatically improve sensitivity. Another possibility is to increase spin polarization. Recent developments in hyperpolarization have opened an avenue for wider *in vivo* use of other nuclei [16,17].

Increasing the sensitivity of MRS would expand the applications of MRS in several ways. Besides enabling observation of less abundant metabolites (and nuclei) increased sensitivity would accelerate data acquisition and thus enable recording changes of metabolite concentrations in real time. This would open an avenue for ^1H , ^{13}C and ^{31}P *in vivo* metabolomics and fluxomics using MRS [144,145].

Further advances in method development, especially in spectroscopic imaging, would enable spatiotemporal monitoring of neurochemical profiles on large brain areas or better spatial and spectral resolution with arbitrarily shaped voxels.

Acknowledgments

We would like to thank L Dacy for assistance in preparing the manuscript.

Financial & competing interests disclosure

This research was funded by the Samuel Johnson Foundation for Genomics of Addiction Program at Mayo Clinic and by grants from the National Institutes of Health (NIH) to D-S Choi (P20 AA017830-Project 1) and to SI Macura (P20 AA017830-Project 3). Further support was provided to I Pirko (R01 NS058698) and M Rodriguez (R21 NS073684 and R01 NS032129). This work was also supported by (R01 GM092993, R01 NS024180 and R01 NS048357) and the National Multiple Sclerosis Society (CA 1060A11). The authors acknowledge with thanks support from the Applebaum, Hilton and Peterson Foundations, the Minnesota Partnership Award for Biotechnology and Medical Genomics and the European Regional Development Fund - Project FNUSAICRC (No. CZ.1.05/ 1.1.00 / 02.0123). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. No writing assistance was utilized in the production of this manuscript.

Key Terms

Neurological disorder	Disorder of the nervous system (brain, spinal cord and peripheral nervous system), which includes multiple sclerosis, amyotrophic lateral sclerosis, Huntington's disease, Alzheimer's disease and Parkinson's disease.
Psychiatric disorder	Disorder with psychological and behavioral abnormalities associated with disability or distress, which includes addictive disorder (such as alcoholism), mood disorders (bipolar, depression) and schizophrenia.
Nuclear magnetic resonance	Phenomenon that appears when atomic nuclei, such as hydrogen (proton; ^1H), carbon (^{13}C), nitrogen (^{15}N) and phosphorous (^{31}P), are placed in an external magnetic field and are exposed to radio frequencies.
Nuclear magnetic resonance spectroscopy	Technique to determine the physical, chemical and biological properties of molecules using nuclear magnetic resonance.
<i>In vivo</i> magnetic resonance spectroscopy	Non-invasive method to measure metabolite concentrations <i>in vivo</i> , which is often associated with magnetic resonance imaging.

Key Term Alcoholism	Disorder with physical dependence to alcohol with phenotypes such as craving, loss of control over drinking, physical dependence and tolerance.
Key Term Multiple sclerosis	Autoimmune, chronic inflammatory disease of the CNS, which is caused by damage to the myelin sheath that covers and protects axons.

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Executive summary

Background

- The ongoing development of preclinical animal models of neurological and psychiatric disorders has led to the use of advanced ^1H -MRS techniques for brain metabolite analyses.

^1H -MRS = proton nuclear magnetic resonance spectroscopy

- ^1H -MRS is an ideal method for studying neurological and psychiatric disorders as it is capable of *in vivo* and longitudinal neurochemical analyses with the added advantage of providing data that can be translated into clinical studies.

Simplified principles

- ^1H -MRS detects signal from the motion of protons (hydrogen nuclei), which is induced in a radiofrequency coil when nuclei are placed in an external magnetic field and excited by radiofrequency field.

The higher the field, the better the spectrum?

- Increasing the static magnetic field improves both sensitivity and spectral dispersion, thus, there is a natural tendency to strive for higher magnetic field strengths. However, in practice, the spectral improvement with B_0 does not seem as dramatic as theoretically expected.

Common metabolites

- *In vivo* spectra typically present approximately 20 readily observable metabolites at the same time.

Applications of ^1H -MRS

- Because of its noninvasive nature, using ^1H -MRS to assess neurological and psychiatric disorders is an excellent tool for the direct translation of animal studies into a clinical setting.

Figure 1. Comparison of ^1H -NMR spectra recorded at 7 T (300 MHz) and 16.4 T (700 MHz)
 All spectra are recorded from the $2\text{ mm} \times 2\text{ mm} \times 2\text{ mm}$ voxel using PRESS at 37°C with TE 10 ms, TR 2 s and NA 512; **(A)** spectra from a 0.5% agar gel phantom sample with Cr, GABA, NAA and Glu (50 mM each); Ins and Gln (25 mM each); and, Cho and Tau (10 mM each). Both spectra are shimmed to approximately 3 Hz. When presented on the same ppm scale, lines in the 700 MHz spectrum are narrower (7/3-times). At constant line width the sensitivity improves with the field strength, in our example about 2.7-times. For the same reason, multiplets in the 700 MHz spectrum look narrower. Multiplet narrowing generally increases apparent line separation across the spectrum. The Glu/GABA overlapping triplets at 2.3 ppm (green bar) are completely resolved at 700 MHz. **(B)** The 300 MHz *in vivo* spectra of mouse brain and the phantom from **(A)**. To guide the eye, the phantom spectrum is line broadened by LB 20 Hz. It is obvious that the *in vivo* spectrum resembles the line-broadened phantom spectrum. **(C)** The 700 MHz *in vivo* spectrum, at the same apparent line width is much better resolved. Notable is separation of Glu/GABA lines at 2.3 ppm, which were otherwise unresolved at 7 T.
 Cho: Choline; Cr: Creatine; GABA: γ -aminobutyric acid; Gln: Glutamine; Glu: Glutamate; GPC+PCh: Choline-containing compounds; Gua: Guanosine; Ins: *myo*-inositol; Lac: Lactate; NAA: *N*-acetylaspartate; PCr: Phosphocreatine; Tau: Taurine.

Figure 2. Metabolite quantification of the mouse nucleus accumbens using *in vivo* 16.4 T MRS and LCModel

(A) Representative RARE images of 1.0 mm thick coronal and sagittal brain sections where the 8 μ l VOI is centered on the nucleus accumbens. Scale bar = 2 mm. (B) Location of the voxel on a cartoon coronal and sagittal brain slices from the mouse brain atlas illustrating the anatomical location of the nucleus accumbens. Scale bar = 2 mm. (C) Representative brain metabolite spectrum acquired using *in vivo* 16.4 T recorded with a PRESS sequence, as short as possible echo time (10 ms) and number of scans and repetition time that give reasonable SNR (NA 3072, TR 2000). (D) LCModel best fit of the brain metabolite spectrum in (C) with (E) Glu (red), Gln (green), GABA (blue) and NAA (purple) separated out and (F) the residual difference. Ala: Alanine; Cr: Creatine; GABA: γ -aminobutyric acid; Gln: Glutamine; Glu: Glutamate; GPC+PCh: Choline-containing compounds; Gua: Guanosine; Ins: *myo*-inositol; Lac: Lactate; NAA: *N*-acetylaspartate; PCr: Phosphocreatine; Tau: Taurine; VOI: Volume of interest. Figure adapted with permission from [10]. Anatomic representation of brain sections were adapted with permission from [146] © Elsevier.

Figure 3. Comparison of human and mouse brain with the striatum in field of view

The human brain is roughly an order of magnitude larger in linear size or three orders of magnitude larger in volume. (A) A typical voxel in human studies (white square, $2 \times 2 \times 2 \text{ cm}$) is placed in the area of the striatum. Scale bar = 2 cm. False color highlights the caudate nucleus (red), putamen (green) and nucleus accumbens (blue). The typical 8 ml volume voxel is larger than the whole mouse brain, and three orders of magnitude larger than (B) a typical voxel in the mouse brain (white square, $2 \times 2 \times 2 \text{ mm}$). Scale bar = 2 mm. False color highlights the dorsal medial striatum (analogue to the caudate nucleus; red), dorsal lateral striatum (analogue to the putamen; green) and nucleus accumbens (blue). VOI: Volume of interest.

Table 1Common brain metabolite changes in various animal models of psychiatric disorders using *in vivo* MRS

Metabolites	Alcoholism [3,9,10,44,48,56,59]	Mood disorders [69-71]	Schizophrenia [87,97,98]
<i>N</i> -acetyl aspartate (NAA)	▲ (NAc) ▼ (mPFC)	▼ (Hipp)	
Choline-containing compounds (Cho)	▲ (mPFC)		▲ (Ctr)
γ-aminobutyric acid (GABA)	▼ (mPFC)		
Glutamate	▲ (NAc, mPFC)	▲ (Hipp)	▲ (Neo)
			▼ (PFC)
Glutamine	▲ (NAc)		▲ (PFC)
Glx (glutamate+glutamine)	▲ (NAc)		

Ctr: Center of the brain (between the rostral corpus callosum and posterior thalamus); Hipp: Hippocampus; mPFC: Medial prefrontal cortex; NAc: Nucleus accumbens; Neo: Neocortex (or cerebral cortex); PFC: Prefrontal cortex.

Table 2

Common brain metabolite changes in various animal models of neurological disorders using MRS

Metabolites	Multiple sclerosis [2,116,117,121,122]	ALS [1,124]	Huntington's disease [125-130]	Alzheimer's disease [133-135]	Parkinson's disease [139,140]
<i>N</i> -acetyl aspartate (NAA)	▼ (PH, bs) ▲ (CSF)	▼ (bs, sp, mCtx)	▼ (Str, Ctx, Cbl, bs)	▼ (bs)	▼ (SNc)
Choline-containing compounds (Cho)	▲ (WB)		▲ (Str)		
γ-aminobutyric acid (GABA)		▼ (sp)			▲ (Str)
Glutamate		▼ (mCtx)	▼ (Ctx, Cbl)	▼ (Ctx)	▲ (Str)
Glutamine			▲ (Str, Ctx, Cbl, bs)		▲ (Str)
Glx (glutamate+glutamine)			▲ (Str)		▲ (Str)

ALS: Amyotrophic lateral sclerosis; bs: Brainstem; Cbl: Cerebellum; CSF: Cerebrospinal fluid; Ctx: Cortex; mCtx: Motor cortex; PH: Parahippocampus; SNc: Substantia nigra pars compacta; sp: Spinal cord; Str: Striatum; WB: Whole brain.