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T_2 relaxation effects on apparent *N*-acetylaspartate concentration in proton magnetic resonance studies of schizophrenia

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ABSTRACT

Over the past two decades, many magnetic resonance spectroscopy (MRS) studies reported lower *N*-acetylaspartate (NAA) in key brain regions of patients with schizophrenia (SZ) compared to healthy subjects. A smaller number of studies report no difference in NAA. Many sources of variance may contribute to these discordant results including heterogeneity of the SZ subject populations and methodological differences such as MRS acquisition parameters, and post-acquisition analytic methods. The current study reviewed proton MRS literature reporting measurements of NAA in SZ with a focus on methodology. Studies which reported lower NAA were significantly more likely to have used longer echo times (TEs), while studies with shorter TEs reported no concentration difference. This suggests that NAA quantitation using MRS was affected by the choice of TE, and that published MRS literature reporting NAA in SZ using a long TE is confounded by apparent differential T_2 relaxation effects between SZ and healthy control groups. Future MRS studies should measure T_2 relaxation times. This would allow for spectral concentration measurements to be appropriately corrected for these relaxation effects. In addition, as metabolite concentration and T_2 relaxation times are completely independent variables, this could offer distinct information about the metabolite of interest.

1. Introduction

Spatially resolved magnetic resonance spectroscopy (MRS) has proved to be a powerful and non-invasive tool for the investigation of the neurochemistry of the working healthy and pathological brain. MRS has been used as an investigational tool in schizophrenia (SZ) research following the development of spatially selective pulse sequences and water suppression techniques in the late 1980s which enabled the in vivo detection of brain metabolite resonances.

One brain metabolite commonly examined in MRS studies is *N*-acetylaspartate (NAA). NAA is a free amino acid that is biosynthesized in neuronal mitochondria. It is found almost exclusively in neurons, including axons and dendrites, and is considered a marker for neuronal viability and integrity. Changes in NAA concentrations could be caused by changes in neuronal density or neuronal dysfunction (such as changes in glucose metabolism or mitochondrial function). A number of proton MRS (^{1}H MRS) studies have reported reduced NAA in the frontal and temporal lobes and other structures of patients with SZ. However, a lesser number of studies report no difference in NAA between patients with SZ and healthy controls (HCs). What are the possible origins of the disparate findings? There are many potential sources of variance which may contribute to these conflicting results including differences in clinical and demographic characteristics (such as medication status or duration of illness), and also the choice of specific MRS acquisition parameters, techniques, and analytic methods (Sanches et al., 2004).

The fundamental principle underlying proton MRS is that for each MRS-visible metabolite, the fundamental frequency at which the nucleus of each hydrogen atom (proton) resonates is shifted by a small amount (measured in parts-per-million, ppm) from the basic resonant frequency of a single, isolated, proton. Chemically identical hydrogen nuclei within an MRS-visible metabolite experience similar local magnetic fields and nuclear spin–spin interactions and therefore have a characteristic chemical shift along the resonance frequency axis, which results in a spectral peak that is a chemical signature of that group of protons within that metabolite. The peak intensity or area under the spectral peak

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is proportional to the number of nuclei contributing to that peak, which is determined by the concentration of that metabolite within a selected volume of interest (voxel) (Jansen et al., 2006).

As discussed below, several MRS acquisition parameters and subject tissue characteristics ultimately affect the measured spectral peak area. When these myriad factors are properly accounted for, or held constant, a "raw" peak integral (area under the peak) is obtained which is proportional to the concentration of the metabolite of interest. These raw spectral measurements reflect absolute metabolite concentrations, which may then be further normalized into conventional units or expressed as dimensionless concentration ratios to some within-subject reference metabolite such as creatine (Cr). The use of metabolite (or water) ratios does correct for differences in excitation within a voxel of interest. However, when using this method, if a change in normalized data is observed, it is impossible to tell whether the numerator (the metabolite of interest) or the denominator (the reference metabolite, often Cr) is changing (Jansen et al., 2006). In early MRS studies, the creatine spectral peak (Cr–PCr) was commonly chosen as the reference metabolite as it was hypothesized to be constant and comparable between brain regions or participant populations; however, it has been demonstrated that this assumption does not always hold, even in healthy individuals. In fact, coefficients of variation are higher in ratio studies than in absolute quantification studies (Schirmer and Auer, 2000; Li et al., 2003). The assumption of uniform concentration of a reference metabolite is even more unreliable in abnormal populations such as patients with SZ (Ongur et al., 2010b). Therefore, although the use of a reference metabolite such as Cr was common in the early MRS literature, in recent years this practice has diminished in favor of absolute concentration measures with the caveat that normalization to the absolute water reference peak is still common practice as discussed below.

However, there are a number of other methodological considerations that affect spectral quantification as well, including radiofrequency coil properties, calibration procedures, spectral fitting methods, voxel corrections for fractional cerebral spinal fluid (CSF)/gray matter/white matter content, macromolecule suppression, and spectral editing techniques (Jansen et al., 2006). The acquisition of a spatially resolved spectroscopic signal for a metabolite of interest requires the selection of a significant number of spectrometer acquisition parameters. Each of these parameters has an impact on the characteristics of the spectrometer signals used to excite the specific brain region being analyzed, and in the resultant spectrum obtained from the excitation echoes. The conversion of an integrated area under a spectral peak for a specific resonance line to a metabolite concentration requires a number of approximations. A general expression for this relationship between signal intensity I and metabolite concentration $[M]$ is:

$$I = c_1 N[M] V B_1(r) L \sin(\theta) \exp\left(\frac{-\mathrm{TE}}{T_2^{(*)}}\right)\left(1-\exp\left(\frac{-TR}{T_1}\right)\right) \quad (1)$$

where I=signal intensity, c_1=constant, N=number of equivalent atoms per molecule, $[M]$=metabolite concentration, V=volume, $B_1(r)$=reception field distribution, L=function of radiofrequency coil loading, θ=RF excitation tip angle, TE=acquisition delay or echo time (depending upon method), T_2^*=spin–spin transverse relaxation time including static field effects, TR=pulse repetition time, and T_1=spin–lattice relaxation time.

The goal in MRS experiments is to hold values of c_1, N, V, B_1(r), and θ constant, to the extent possible, or, when necessary, to correct for variations. For example, L (the amount of power necessary to transmit the signal) and the signal-to-noise ratio (SNR) are dependent on the volume of the object near the coil (i.e. the size and tissue composition of the head being examined) and by the electrical impedance of the coil when "loaded" with a participant's head. Larger, denser objects require more transmitted power to achieve a constant flip angle θ. As the size of the participant's head cannot be controlled, this is a source of variability, although some experimenters attempt to control for this by measuring the power received by the coil and the SNR and calculating the volume of the head (Jansen et al., 2006). T_1 is assumed to be constant, and in most proton MRS experiments, T_1 variability is considered to have a negligible effect, especially at a longer TR. Saturation of longitudinal magnetization due to repeated pulses in standard MRS pulse sequences also tends to reduce T_1 effects. Most TRs for these experiments are in the range of 1500–3000 ms, and the T_1 for NAA at 1.5 and 3 T is ~1300–1400 ms (Rutgers and van der Grond, 2002; Traber et al., 2004). This review assumes that T_1 is not variable between groups, but that could be an interesting topic of a future study, especially one focused on phosphorus MRS findings as the variability would manifest as a TR-dependence (long vs. short) in the observed MRS signal.

Thus, after eliminating all other terms of the above equation as sources of variance, this review will focus on the possibility that differential NAA concentration measurements between experiments could be due to the selection of long versus short TEs during signal acquisition because long TE experiments are more sensitive to any differences in T_2 relaxation times between healthy control (HC) and SZ groups. The T_2 relaxation time reflects the mean decay time of the MR signal or free-induction decay (FID) for a given metabolite, and different metabolites have different T_2 relaxation times. Mobile molecules will have longer T_2 times (longer FIDs) than less mobile molecules. Therefore, if the local micro-environment in which the metabolite of interest resides is altered, then relaxation times (and therefore measures of metabolite concentrations) may also be affected. This is especially important when normalizing metabolites that are intracellular only (i.e. NAA) to molecules which are found in both the intracellular and extracellular space (i.e. Cr) as changes in the relaxation times of metabolites in these two compartments could be differentially affected by an abnormal environment. Studies that normalize to water rather than Cr do not avoid this problem either, as previous studies have found schizophrenia-related changes in water proton relaxation times (Andreasen et al., 1991; Williamson et al., 1992; Supprian et al., 1997; Pfefferbaum et al., 1999; Aydin et al., 2007; Ongur et al., 2010b). For instance, some studies have found that within groups of patients with SZ, T_2 relaxation times of intracellular metabolites (Cr+phosphocreatine, choline containing compounds) are reduced compared to that of HC subjects (Ongur et al., 2010b). The authors suggest that this could be due to a decrease in neuronal cell volumes and/or increased macromolecule concentrations resulting in increased metabolite–macromolecule interactions and more rapid loss of transverse magnetization (decreased T_2 relaxation time) (Ongur et al., 2010b).

The existence of significant discrepancies in the MRS research literature examining SZ has motivated this review of these assumptions and analysis of the published results. These questions were tangentially addressed in an insightful review and meta-analysis by Steen et al. (2005) which concluded that some of the inconsistency in findings on NAA within the literature are due to many of these studies being underpowered. The present review focuses instead on the published proton MRS literature reporting proton MRS measurements of NAA in SZ research with a focus on the choice of TE. These analyses reveal evidence that certain analytic assumptions may not hold in comparisons of quantitative spectroscopic data between patients with SZ and HCs.