Test-retest reliability of key metabolite measurements in the anterior cingulate cortex using single voxel 1H Press at 3T

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Background

In vivo magnetic resonance spectroscopy (MRS) can be used to study aspects of brain chemistry and metabolism, and has been increasingly applied to the study of pathophysiological mechanisms in neurological and psychiatric disorders[1-3]. Despite such widespread use, very few studies have quantitatively examined the reliability of these MRS-derived indices. Moreover, it is possible that certain metabolites are influenced by the events preceding data acquisition (e.g., functional MRI task, or other affective or stressful conditions). The aim of this study was to examine the test-retest reliability of proton-MRS derived measures of commonly investigated metabolites (*N*-acetyl compounds [NAA], glutamate+glutamine [Glx], creatine+phosphocreatine [Cr], trimethylamines [TMA; which are predominantly choline compounds], and myoinositol [mI]) in a commonly investigated brain region – the anterior cingulate cortex (ACC). Taking the 1H-MRS before and after a cognitive challenge task allowed us to examine the influence of behavioural demands on the derived indices. Comparing test-retest variance in these metabolites with inter-subject variance allows us to determine how much of the group differences reported in the literature are due to individual (i.e., biological) versus methodological (i.e., instrumental) factors.

Methods

Participant and MRI Imaging Protocol: We acquired three single voxel spectra from the ACC within the same session (~22:00 minute session) from 10 subjects (3 male; mean age = 22.9 years). Subjects were asked to engage in a task of high cognitive load (an adapted Multi-Source Interference Task)[4,5] between the first and second MRS session in order to perturb the metabolites with a more dynamic nature (Figure 1A). MRI sequences were acquired in a single scanning session using a 3T Siemens Trio whole body scanner (software 15B, 32 channel matrix coil). Scanning sequences consisted of a scout localiser, T1-weighted anatomical, and three sets of ^{1}H-MRS, in that order. The ^{1}H-MRS was recorded using a short-echo point resolved spectroscopy sequence (PRESS; TR = 2000ms, TE = 30ms, NEX = 16; voxel size ~ 3.0cm^{3}) from the left dorsal ACC (Figure 1B).

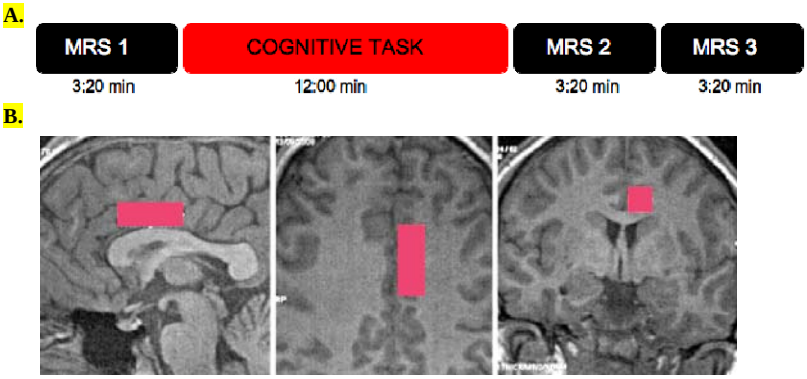
Statistical Analyses: Metabolite concentrations derived from ^{1}H-MRS were determined with LCModel software. This software used a library of reference spectra in a basis set recorded at 3T and calibrated using the tissue water signal as an internal standard. The LCModel fitting algorithm uses the multiple peaks contributing to an individual metabolite spectrum to estimate the tissue content of each metabolite. The residual signal corresponds to, and is fitted by, additional broad peaks representing unknown metabolites and other factors such as macromolecular components with short T1 relaxation times. Given the voxel used was identical for all three MRS acquisitions, we did not correct for tissue content. MRS parameters used for this study provided robust signals with a signal-to-noise ratio of 15.80 and a full-width-half-maximum of 7.6Hz. All metabolites showed good reliability of fit as judged from the average Cramer-Rao lower bounds (CRLB). The mean CRLB values (estimates of the standard deviation [SD] of the fit) for Cr, NAA, TMA, mI and Glx were 3.5, 3.9, 5.4, 6.1 and 8.6 respectively (all CRLB values were <20). The variability of key metabolites in the ACC was assessed using the coefficient of variation (CV). The appeal of the CV as a measure is that the standard deviations (SDs) of such metabolites generally increase or decrease proportionally with changes in the mean, so that division by the mean removes it as a factor in the variability. The CV is therefore a standardization of the SD that allows comparison of variability estimates regardless of the overall magnitude of metabolite concentration.

Results

Figure 1D illustrates that while all the metabolites were variable between subjects (range from 7-15%; blue bars), the test-retest variability was relatively smaller (range 2-8%; yellow bars). CV measures indicate that Cr (2.2%) NAA (4.0%), TMA (4.7%) and mI (5.5%) were especially stable across the three within subject acquisitions (examples of Cr and NAA are displayed on Figure 1C). However, the CV was higher for Glx (8.4%). These findings are also indicated in the ratio measures (displayed on the blue bars) of the two sources of variance (inter-subject : test-retest) for all metabolites.

Discussion

Our findings suggest that anterior cingulate measures of Cr, Cho, NAA, and mI can be reliably indexed in a flexible manner at 3T using short TEs. mI may be moderately reactive to (and interact with) the mental demands of the scanning protocol prior to the acquisition, while Glx showed a relatively large test-retest variability, suggesting patient-control differences in this metabolite must be interpreted cautiously when measured in uncontrolled situations.



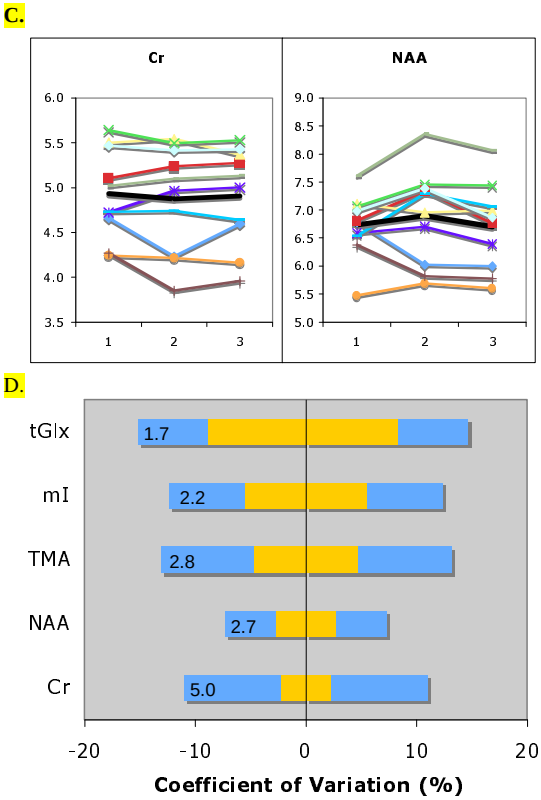


Figure 1: *A. Illustration of the scanning protocol with baseline (MRS 1) and 2 repeat test-retest scans (MRS 2 & MRS 3) following a cognitive challenge task; B. Placement of the voxel-of-interest in the ACC; C. Examples of metabolite concentrations across three within subject scanning sessions for Cr and NAA and; D. Variation around the mean of each metabolite as a function of test-retest (yellow bars) and inter-subject (blue bars) variability. The numbers on the blue bars are the inter-subject-to-test-retest ratio variability (higher values representing proportionally greater variance due to inter-individual variability.*

References

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