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J-difference edited MR spectroscopy measures of γ -aminobutyric acid before and after acute caffeine administration

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Abstract

Purpose: To investigate potential effects of acute caffeine intake on *J*-difference edited MR spectroscopy (MRS) measures of the primary inhibitory neurotransmitter γ -aminobutyric acid (GABA).

Methods: *J*-difference-edited MEGA-PRESS and conventional PRESS data were acquired at 3T from voxels in the anterior cingulate (ACC) and occipital area (OCC) of the brain in 15 healthy subjects, prior to and after oral intake of a 200-mg caffeine dose. MEGA-PRESS data were analysed with the MATLAB-based Gannet tool to estimate GABA + macromolecule (GABA+) levels, while PRESS data were analysed with LCModel to estimate levels of glutamate (Glu), glutamate+glutamine (Glx), N-acetylaspartate (NAA), and myo-inositol (mI). All metabolites were quantified with respect to the internal reference compounds creatine (Cr) and tissue water, and compared between the pre- and post-caffeine intake condition.

Results: For both MRS voxels, mean GABA+ estimates did not differ before and after caffeine intake. Slightly lower estimates of mI were observed after caffeine intake in both voxels. NAA, Glu and Glx did not show significant differences between conditions.

Conclusion: Mean GABA+ estimates from *J*-difference edited MRS in two different brain regions are not altered by acute oral administration of caffeine. These findings may increase subject recruitment efficiency for MRS studies.

Keywords

Caffeine; Magnetic resonance spectroscopy; GABA; MEGA-PRESS; spectral editing

Introduction

γ -aminobutyric acid (GABA) is the most important inhibitory neurotransmitter in the human central nervous system. Its non-invasive detection and quantification with magnetic resonance spectroscopy (MRS) has received increasing attention in behavioural and clinical research throughout recent years ¹. At 3 Tesla, *J*-difference edited MRS methods such as MEGA-PRESS ^{2,3} are widely used to detect GABA. A GABA-editing MEGA-PRESS acquisition consists of two sub-experiments ('ON' and 'OFF') which differ in the way the GABA spin system is treated: The 'ON' experiment applies frequency-selective RF editing pulses to the 1.9-ppm resonance of the GABA molecule, whereas the 'OFF' experiment does not. Subtraction of the two sub-experiments cancels out any signal unaffected by the editing pulse in the 'ON' experiment, and reveals the 3-ppm GABA resonance which is coupled to the 1.9-ppm GABA resonance.

Edited MRS measurements of GABA levels have been linked to behavioural indicators of brain function such as tactile discrimination thresholds ⁴, working memory ⁵, time perception ⁶, and cognitive performance in the elderly ⁷, as well as to characteristics of neural oscillations and BOLD signals ^{8–10}. Brain GABA levels may further be altered in a number of neuropsychiatric disorders, for example autism spectrum disorder (ASD), Tourette's, amyotrophic lateral sclerosis (ALS), hepatic encephalopathy (HE), and attention-deficit/hyperactivity disorder (ADHD).

Due to the low abundance of GABA (1–2 mmol/L), a number of studies investigated the reproducibility and repeatability of edited MRS measures at 3T. Inter- and intra-subject coefficients of variation were determined to be on the order of 10%, indicating good short-term and long-term stability and reliability of GABA estimates under normal conditions ^{11–16}. Several factors have been identified as potential sources of additional variance of GABA estimates, although several of these studies feature small sample sizes (resulting in low statistical power), or use creatine as the internal reference standard, which may itself be affected by confounders, e.g. age. The results may therefore be rather indicative of potential effects, warranting further investigation. Potential sources of variance include measurement-intrinsic effects such as scanner drift ^{17,18}, biological confounders such as gender ¹⁹, age ^{7,20,21} and menstrual cycle ^{22–24}, or intake of common psychoactive substances such as alcohol, nicotine and caffeine. Systematic effects of chronic alcohol abuse ^{25–27} and acute alcohol intake ²⁸ on MRS metabolite estimates have been previously shown.

It currently remains unclear whether the acute intake of everyday doses of caffeine directly prior to a measurement affects MRS estimates of GABA. Caffeine is widely used as a recreational drug to increase cognitive performance and attention, most notably in the form of coffee, tea, energy drinks, or pills. Administration of caffeine has been shown to alter brain activity and connectivity patterns in attention-related networks ^{29–32}, influence measures of cerebral perfusion ^{33,34}, affect neurotransmitter metabolism in rodents and cell cultures ^{35,36}, and cause significant changes to MRS measures of brain lactate ³⁷. Subjects participating in neurological and neuroscientific studies are routinely instructed to abstain from drinking energy drinks or coffee for a given period of time prior to the examination.

The everyday nature and widespread consumption make it, however, difficult to reliably control whether these instructions have been followed. Therefore, it is worthwhile to investigate whether acute caffeine consumption needs to be considered as a potential source of variance for MRS estimates of GABA, or whether caffeine consumption should be used as an exclusion criterion for MRS studies of GABA.

In the present study, repeated MEGA-PRESS acquisitions were performed in healthy adult subjects in two separate brain regions (anterior cingulate cortex, occipital lobe), prior to and after oral administration of a common caffeine dose.

Methods

15 healthy adult male subjects (mean age: 23.7 ± 1.8 y, 12 non-smokers) were included. Written informed consent was obtained from each subject. Subjects were instructed to abstain from consuming caffeine on the day of the measurement. A quantitative assessment of regular caffeine consumption habits, or acute alcohol or nicotine intake prior to the measurement, was not performed. The study was approved by the local institutional review board in accordance to the Declaration of Helsinki. All measurements were performed on a clinical whole-body 3T MRI (Siemens MAGNETOM Trio A TIM System, Siemens Healthcare AG, Erlangen, Germany), using a 12-channel head matrix coil for receive and the body coil for transmit.

Data acquisition protocol

For optimal repeated placement of the spectroscopic volumes, a vendor-proprietary 'Auto Align Head Scout' was acquired prior to each measurement. This function performs a basic anatomical normalization, and allows reloading of the voxel coordinates from previous scans. The scout was followed by a high-resolution 3D anatomical transversal T_1 -weighted magnetization prepared gradient echo (MP-RAGE) scan (TR/TE = 1950/4.6 ms; isotropic resolution of 1 mm; 176 slices).

MRS volumes were placed in specific anatomical locations. The first spectroscopic volume (anterior cingulate cortex, 'ACC voxel') was placed in the central frontal lobe. The caudal face was aligned parallel to the anterior dorsal edge of the corpus callosum (Fig. 1a). The second spectroscopic volume (occipital cortex, 'OCC voxel') was placed in the central occipital lobe to include as much of the visual cortex as possible. The caudal face of this volume was aligned along the cerebellar tentorium (Fig. 1b). In all cases, as much cortical volume as possible was included within the voxel and, on the other hand, unwanted lipid contamination from the skull was avoided. After localizing the target volumes, MEGA-PRESS spectra were acquired (ACC: dimensions = (40 (AP) x 30 (RL) x 20 (FH)) $\text{mm}^3 = 24$ ml, OCC: dimensions = (30 x 30 x 30) $\text{mm}^3 = 27$ ml; 256 averages, i.e. 128 ON and 128 OFF transients; TR = 2140 ms; TE = 68 ms; spectral width = 1200 Hz; 1024 data points, Fig. 1c). The bandwidth (full-width half-maximum) of the Gaussian-shaped editing pulses was specified on the exam card to be 44 Hz, corresponding to an actual FWHM bandwidth of approximately 64 Hz after Hanning filtering of the pulse (personal communication, Dr. Sinyeob Ahn, Siemens Healthcare). The editing pulses were applied at 1.9 ppm (in the 'ON' experiment) and 7.5 ppm (in the 'OFF' experiment). Due to the limited spectral selectivity

of the 1.9 ppm editing pulse in the ON acquisition, the 1.7 ppm macromolecular (MM) resonance is partially affected by this pulse, resulting in a notable contribution of MM signal to the 3-ppm peak. Therefore, all estimates of GABA levels in this manuscript are referred to as “GABA + macromolecules” (GABA+).

A water-unsuppressed MEGA-PRESS reference scan (8 averages, TE = 68 ms) was acquired for each volume by setting the ‘water suppression’ exam card option to ‘RF OFF’ to maintain sequence timing.

A regular PRESS scan (128 averages; TR = 2140 ms; TE = 30 ms; spectral width = 1200 Hz; 1024 data points) and an unsuppressed water reference (8 averages, TE = 30 ms) were also acquired from the same spectroscopic volume.

The order of MRS acquisitions was randomized in both sessions to avoid any systematic bias, which may result from increased subject motion towards the end of the scan, systematic thermal scanner drift^{17,18}, or caffeine effect duration.

After completion of the pre-caffeine-challenge acquisition, the subjects left the scanner for oral administration of an over-the-counter caffeine pill with a dose of 200 mg caffeine (one cup of coffee roughly equals 120 mg of caffeine). They were repositioned in the scanner after 25 minutes in order to begin with MRS acquisition after 30 minutes, a common time interval chosen in previous neuroimaging investigations of caffeine effects on blood flow and brain activity^{29–34}. For the post-caffeine-challenge acquisition, the measurement protocol was repeated.

Post-processing and metabolite quantification

MEGA-PRESS data were analysed with the MATLAB-based tool Gannet 2.0³⁸, including spectral registration³⁹ for frequency-and-phase correction of the individual transients, automated rejection of corrupted transients, zero-filling to 32,768 data points, 3-Hz exponential line broadening, and single-Gaussian fitting of the 3-ppm GABA+ resonance in the MEGA-PRESS difference spectrum.

PRESS data were pre-processed with FID-A⁴⁰, including spectral registration for frequency-and-phase correction of the individual transients, and automated rejection of corrupted transients. Neither zero-filling nor line broadening were applied to allow correct estimation of Cramér-Rao lower bounds (CRLB). The pre-processed PRESS spectra were analysed with LCModel v6.3⁴¹, including eddy-current correction using the unsuppressed water reference. The default LCModel 30-ms PRESS basis set was used for the analysis, containing simulated resonances of alanine (Ala), aspartate (Asp), creatine (Cr), GABA, glucose (Glc), glutamate (Glu), glutamine (Gln), glutathione (GSH), glycylphosphocholine and phosphocholine (GPC, PCh), lactate (Lac), myo-inositol (mI), N-acetylaspartate (NAA), N-acetylaspartylglutamate (NAAG), scyllo-inositol (Scy), taurine (Tau), lipids (Lip), and macromolecules (MM).

The tissue composition of the spectroscopic volumes was determined by co-registering each volume to the corresponding T₁-weighted anatomical image (i.e., within the pre- and post-caffeine acquisitions) and subsequent segmentation into gray matter (GM), white matter

(WM), and cerebrospinal fluid (CSF). These steps were carried out within the Gannet toolbox, using the 'Coregister' and 'New Segment' functions of the Statistical Parametric Mapping toolbox (SPM8) ⁴².

All metabolites of interest (GABA+ from MEGA-PRESS difference spectra; NAA, Cr, Glx (= Glu+Gln), Glu, mI from LCModel analysis of PRESS) were quantified with respect to two different reference standards: (a) the internal creatine signal (metabolite/Cr ratio), and (b) the internal water signal from the unsuppressed water scan. Relative water densities of 0.78 (GM), 0.65 (WM) and 0.97 (CSF) and an MR-visible water concentration of 55.5 mol were assumed ⁴³. T_1/T_2 relaxation times of tissue water and metabolites were adapted from literature; tissue water: gray matter 1331/110 ms, white matter 832/79.6 ms, CSF 4160/500 ms; GABA+ 1310/88 ms; NAA 1410/271 ms; Cr 1135/141 ms; Glu 1220/181 ms; Glx 1080/181 ms; mI 1090/197 ms ^{44–49}. As no exact T_2 values for the combined Glx measure were found in literature, it was assumed to be identical to the T_2 of Glu, which is present at more than twice the concentration of Gln in the brain⁵⁰. Water-scaled metabolite estimates are provided in institutional units (i.u.).

Spectral quality and data acquisition quality was assessed for each MRS scan with three metrics: the GABA+ peak fit error output by Gannet for the MEGA-PRESS and the CRLB (as indicated by the LCModel output) for the PRESS scans; the number of averages rejected by the automated spectral alignment routines in Gannet and FID-A; and the frequency standard deviation (before alignment) of the residual water peak and the full-width half-maximum (FWHM, after alignment) of the 3.0 ppm creatine peak.

Statistical analysis

Normal distribution of metabolite levels, tissue composition, and spectral quality metrics was tested with a one-sample Kolmogorov-Smirnov test within each spectroscopic volume (ACC and OCC) and each condition (pre- and post-caffeine). Group differences of metabolite levels, tissue composition, and spectral quality metrics between conditions were tested for significance with a paired two-tailed t-test for normal distributed data, and with the Wilcoxon-Mann-Whitney test for non-normal distributed data. In all cases, the significance level was $\alpha = 0.05$. All statistical analyses were performed with IBM SPSS Statistics for Windows, version 22 (IBM Corp., Armonk, NY, USA).

Results

No MEGA-PRESS spectra from the ACC had to be discarded. MEGA-PRESS spectra from the OCC voxel had to be discarded for the pre-caffeine condition in one subject, and for the post-caffeine condition in another subject (both due to extensive subject motion), i.e. 13 complete datasets were included in the paired MEGA-PRESS analysis. In all remaining cases, the GABA+ fit errors for the MEGA-PRESS scans were smaller than 13%. No PRESS spectra had to be discarded, i.e. 15 complete datasets were included in the paired PRESS analysis. In all cases, the CRLB for the PRESS scans were smaller than 3% (NAA), 3% (Cr), 8% (Glx), 7% (Glu), and 6% (mI).

No significant differences in tissue composition between the pre- and post-caffeine challenge acquisitions were observed in the ACC voxel (GM: $52.4\% \pm 2.7\%$ vs. $52.6\% \pm 2.6\%$; WM: $36.5\% \pm 4.4\%$ vs. $36.3\% \pm 4.3\%$; CSF: $11.1\% \pm 2.2\%$ vs. $11.3\% \pm 2.2\%$) and the OCC voxel (GM: $57.9\% \pm 3.1\%$ vs. $58.2\% \pm 2.8\%$; WM: $34.0\% \pm 3.4\%$ vs. $33.7\% \pm 3.3\%$; CSF: $8.1\% \pm 1.7\%$ vs. $8.1\% \pm 1.8\%$). No differences in spectral quality metrics between the pre- and post-caffeine challenge acquisition were observed.

The mean GABA-edited MEGA-PRESS difference spectra across all included participants (black line) and the standard deviations (shaded gray area) are shown in Fig. 2a (ACC voxel, pre-caffeine), Fig. 2b (ACC voxel, post-caffeine), Fig. 2c (OCC voxel, pre-caffeine), and Fig. 2d (OCC voxel, post-caffeine). The mean PRESS spectra across all included participants (black line) and the standard deviations (shaded gray area) are shown in Fig. 3a (ACC voxel, pre-caffeine), Fig. 3b (ACC voxel, post-caffeine), Fig. 3c (OCC voxel, pre-caffeine), and Fig. 3d (OCC voxel, post-caffeine). The higher mean lipid contribution and therefore larger standard deviation in the PRESS spectra for the OCC voxel result from few datasets with residual lipid contamination. These datasets still showed consistently low CRLBs in all cases, and were therefore not excluded.

Quantitative results of the MEGA-PRESS difference spectra analysis are shown in Figure 4a (GABA+/Cr) and Figure 4b (water-scaled GABA estimates). Mean GABA+/Cr estimates were 0.088 ± 0.014 (pre-caffeine) and 0.0942 ± 0.017 (post-caffeine, $P = 0.23$) for the ACC voxel, and 0.100 ± 0.010 (pre-caffeine) and 0.101 ± 0.008 (post-caffeine, $P = 0.83$) for the OCC voxel. Mean water-scaled GABA+ estimates were 1.285 ± 0.228 i.u. (pre-caffeine) and 1.360 ± 0.259 i.u. (post-caffeine, $P = 0.33$) for the ACC voxel, and 1.425 ± 0.166 i.u. (pre-caffeine) and 1.428 ± 0.093 i.u. (post-caffeine, $P = 0.95$) for the OCC voxel. No significant differences in GABA+ estimates were observed between the pre- and the post-caffeine conditions.

Results of the PRESS spectra analysis are summarized in Table 1. Two significantly decreased measures of mI/Cr (OCC, $p = 0.03$) and mI/water (ACC, $p = 0.04$) in the post-caffeine spectra were observed. However, significance for the comparisons disappears upon Bonferroni correction for multiple comparisons (4 comparisons for Cr ratios and 5 comparisons for water-scaling per voxel). Several measures of Glu and Glx appeared decreased in the post-caffeine condition, but did not reach statistical significance. No significant changes in metabolite estimates were observed for NAA, and Cr.

Discussion

In this study, the in-vivo brain GABA+ levels of 15 subjects have been assessed with J -difference edited MR spectroscopy before and after oral administration of a common dose of caffeine. Further, the levels of other major contributors to the un-edited brain MR spectrum (NAA, Glx, Cr, mI) have been analysed. The spectroscopic volumes that were chosen for this study – the anterior cingulate cortex (ACC) and the occipital lobe (OCC) – are regions of interest commonly investigated with MRS, and are known to be involved in attention-related processes, which have been shown to be affected by caffeine consumption.

The main observation of this study is that GABA+ estimates from *J*-difference edited MRS with MEGA-PRESS do not significantly change after acute caffeine intake. While subjects participating in neuroscientific studies are often routinely advised to refrain from acute consumption of caffeine prior to the study, it is difficult to reliably determine whether these instructions have been followed. Further, studies including edited MR spectroscopy measures regularly suffer from the particular susceptibility of the MEGA-PRESS method to subject motion, which makes it necessary in many cases to discard spectra due to insufficient quality. The results shown in this study demonstrate that acute caffeine intake may not need to be a hard rejection criterion for edited MRS measurements of GABA+, a finding that may help improve the efficiency of subject recruitment, and allow performing subject exclusion in a less strict fashion.

It should be noted that the narrow age range of participants in this study limits the applicability of these results. Elderly subjects or participants with disease may see different neurochemistry reactions to caffeine exposure. However, the primary purpose of this study was to explore potential effects on younger cohorts, as are routinely recruited from student populations as test subjects for method development, or neuroscientific investigations of the healthy brain. It should be further noted that the observations made in this study may only apply for the brain regions that were investigated. Since the GABAergic tone is a region-specific parameter, our findings may not be generalizable to MRS measurements in other brain regions, especially since caffeine is assumed to have region-specific effects on brain activity itself ⁵¹.

While the pharmacological effect of caffeine is assumed to be mainly mediated through blocking of adenosine receptors, it has also been demonstrated in rodent models to influence other ways of neurotransmission, including dopaminergic, glutamatergic, and GABAergic. It has been shown to modulate GABA release via NMDA receptor activation in retinal cells ³⁵ and via serotonergic mediation in thalamic cells ⁵², increase the expression of benzodiazepine-binding sites associated with cortical GABA_A receptors ⁵³, and suppress GABAergic inhibition in hippocampal pyramidal neurons ⁵⁴. However, direct increase of GABA levels after caffeine challenge has not been observed in the hypothalamus ³⁶. Our study provides evidence that, while neurotransmission and inhibitory activity might be modulated by caffeine via several pathways, the macroscopic bulk levels of GABA+ in defined regions of the human brain are not immediately influenced by it. It should be noted that caffeine may alter metabolic processes on time scales that are inherently inaccessible to MRS, as levels measured with MRS reflect a homeostatic average over the measurement time. It is entirely possible that production and consumption rates are affected by caffeine, while homeostatic balance is maintained.

The stability of the GABA+ measures with regard to caffeine consumption has been consistently observed for the tissue-water-scaled estimates and the GABA+-to-creatine ratio. In an additional analysis step, the water-scaled creatine estimates proved to be unchanged between the pre- and post-caffeine scans, indicating that potential alterations in the metabolite-to-creatine ratios would have been attributable to actual changes in the levels of the respective metabolite. However, no statistically significant differences were observed for NAA, Glx, and Glu between the pre- and post-caffeine acquisitions.

The discrepancy between the significant differences that were observed for the water-scaled (in ACC, but not in OCC) and the creatine-scaled estimates of mI (in OCC, but not in ACC), as well as a number of near-significant decreased measures of Glu and Glx may indicate caffeine-related effects in the respective systems. Increased sample sizes in future MRS studies of caffeine intake could provide sufficient power to solidify these findings, as to this date, no direct interactions between MRS-measured levels of mI, Glu, or Glx in the human brain and caffeine have been reported. However, caffeine administration in diabetic rats has been shown to restore abnormal mI concentrations in the hippocampus, suggesting that caffeine can actively influence cellular osmoregulation, a mechanism with pivotal involvement of mI⁵⁵. Glutamate release after caffeine ingestion has been reported in rat hypothalamus³⁶, but it is unknown whether this is a region-specific effect.

The peak plasma concentrations of caffeine are assumed to be reached after 30–120 min after oral intake, and brain caffeine levels remain stable for more than an hour afterwards^{56,57}. Several imaging studies that investigated interactions of caffeine with neuroimaging modalities were designed with 30- to 45-minute waiting time between ingestion of a 200-mg over-the-counter caffeine pill and data acquisition^{29–32,57–59}. The caffeine dose, ingestion method, and time difference were matched in the design of this study. The timing was adjusted by allowing for a 25-minute break before the subject re-enters the scanner, accounting for the repeated acquisition of the anatomical scan prior to the MRS measurement. While re-positioning of the subject between scans may be a further source of variance due to potential inconsistencies in MRS voxel placement, this study design was deliberately chosen to increase the degree of subject cooperation and limit susceptibility to motion, since the total scan time (including the 25-min waiting time between acquisitions) approached approximately two hours. Randomization of the order of MRS data acquisition as well as the use of the ‘Auto Align’ scout were included in the study design to minimize systemic bias in metabolite estimation due to increased subject motion towards the end of the scan.

A potential source of between-subject variance of metabolic reaction to oral caffeine ingestion may be subject-specific differences in caffeine absorption behaviour. As the study did not intend to fully elucidate caffeine pharmacodynamics, but rather whether acute intake may be a systemic source of bias for MRS levels of major metabolites, caffeine consumption habits of subjects were not assessed in detail. The lack of accounting for individual caffeine intake routine (as well as for bias due to potential acute consumption of nicotine and alcohol) is a major limitation of this study, as metabolic reactions and subsequent changes in metabolite levels may be different between subjects depending on their degree of habituation to caffeine. Future investigations would benefit from a stricter assessment of individual caffeine consumption habits. A more complex study design could feature control groups of subjects who never consume caffeine at all, or groups receiving a placebo between acquisitions. Finally, the relatively low sample size (15 subjects) of this study limits the detectable effect size. Based on 15% within-group variance in GABA estimates, changes of 15% can be detected with 94% power, while changes of 10% will only be detected with 67% power. Increasing the number of subjects will allow even subtler effects than this to be reliably detected.

Conclusion

In conclusion, this paper demonstrates that GABA+ estimates from *J*-difference edited MRS in two different brain regions are not altered by acute oral administration of caffeine. These findings may help design less strict exclusion and rejection criteria for neuroimaging studies that include edited MRS of GABA+, and therefore increase subject recruitment efficiency.

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Abbreviations:

GABA	γ -aminobutyric acid
MEGA-PRESS	Mescher-Garwood point resolved spectroscopy
ACC	anterior cingulate
OCC	occipital lobe
Glu	glutamate
Glx	glutamate+glutamine
mI	myo-inositol
ASD	autism spectrum disorder
ALS	amyotrophic lateral sclerosis
HE	hepatic encephalopathy
ADHD	attention-deficit/hyperactivity disorder
AP	anterior-posterior
RL	right-left
FH	foot-head
CRLB	Cramer-Rao lower bounds
GM	gray matter
WM	white matter
MM	macromolecules

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Figure 1:

(a) Exemplary placement of the anterior cingulate MRS volume ('ACC voxel'); (b) Exemplary placement of the occipital MRS volume ('OCC voxel'); (c) Sequence diagram of the Siemens WIP MEGA-PRESS implementation that was used to acquire the GABA+-edited data.

Figure 2:
Mean GABA-edited MEGA-PRESS difference spectra (black line) and standard deviation (gray-shaded area) across the included subjects for each condition: (a) ACC voxel pre-caffeine challenge; (b) ACC voxel post-caffeine challenge; (c) OCC voxel pre-caffeine challenge; (d) OCC voxel post-caffeine challenge.

Figure 3: Mean PRESS spectra (black line) and standard deviation (gray-shaded area) across the included subjects for each condition: (a) ACC voxel pre-caffeine challenge; (b) ACC voxel post-caffeine challenge; (c) OCC voxel pre-caffeine challenge; (d) OCC voxel post-caffeine challenge.

Figure 4:

(a) GABA+/Cr ratios (mean $\pm$ SD) for the ACC and OCC voxel pre- and post-caffeine challenge; (b) Water-scaled GABA+ concentration estimates (mean $\pm$ SD) for the ACC and OCC voxel pre- and post-caffeine challenge.

Table 1:

Quantitative results of PRESS spectra analysis with LCModel for five major metabolites (mean value, standard deviation in parentheses).

	ACC			OCC		
	Pre	Post	<i>P</i>	Pre	Post	<i>P</i>
NAA/Cr	1.388 (0.184)	1.397 (0.142)	0.87	1.532 (0.104)	1.525 (0.181)	0.81
Glu/Cr	1.183 (0.125)	1.146 (0.183)	0.39	1.053 (0.110)	1.000 (0.184)	0.22
Glx/Cr	1.573 (0.246)	1.517 (0.279)	0.44	1.475 (0.169)	1.383 (0.214)	0.07
mI/Cr	0.764 (0.057)	0.757 (0.103)	0.74	0.703 (0.050)	0.682 (0.049)	0.03
Cr/water	8.284 (0.051)	8.173 (0.936)	0.29	8.151 (0.670)	8.245 (1.130)	0.87
NAA/water	11.310 (1.780)	10.807 (1.540)	0.09	12.249 (1.280)	11.862 (2.000)	0.14
Glu/water	9.602 (1.267)	9.089 (1.646)	0.10	8.404 (1.120)	7.995 (1.660)	0.22
Glx/water	10.363 (1.745)	9.735 (1.760)	0.10	9.559 (1.280)	9.095 (2.240)	0.05
mI/water	5.869 (0.458)	5.654 (0.557)	0.04	5.329 (0.630)	5.199 (0.570)	0.25