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Brain metabolite concentrations across cortical regions in healthy adults

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Abstract

Magnetic resonance spectroscopy (MRS) can provide *in vivo* information about metabolite levels across multiple brain regions. This study used MRS to examine concentrations of N-acetylaspartate (NAA), a marker of neuronal integrity and function, and choline (Cho) which is related to the amount of cell membrane per unit volume, in anterior cingulate cortex (ACC) and parieto-occipital cortex (POC) in healthy individuals. Data were drawn from two experiments which examined glutamatergic and GABAergic signaling in schizophrenia and bipolar disorder. After controlling for gray matter percentages, NAA/Creatine (Cr) was 18% higher in POC than in ACC ($p < 0.001$); Cho/Cr was 46% lower in POC than in ACC ($p < 0.001$). There was an effect of study ($p < 0.001$ for both metabolites), but no region by study interaction (NAA $p = 0.101$, Cho $p = 0.850$). Since NAA is localized to the intracellular space, these data suggest that ACC neuronal compartment is reduced as compared with POC, or that there is a lower concentration of NAA per cell in the ACC than POC, or both. Since elevated Cho suggests more cell membrane per unit volume, reduced NAA in ACC appears to be coupled with increases in overall cell membrane compartment. These findings are consistent with a number of previous studies using proton MRS which found increasing NAA and decreasing Cho moving caudally, and with post mortem anatomical studies which found neurons in more widely spaced bundles in ACC when compared to parietal and occipital cortices. MRS may be a useful tool for studying physical properties of the living human brain.

1. Introduction

Magnetic resonance spectroscopy (MRS) can provide *in vivo* information about brain metabolite levels across multiple brain regions. One metabolite commonly quantified in MRS studies is N-acetylaspartate (NAA). NAA is highly abundant in the brain and its MRS signal can be reliably quantified. NAA is synthesized in neuronal mitochondria and is

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largely confined to neurons. The NAA signal has been used as a marker of neuronal integrity in healthy participants and a number of patient populations (for reviews see Steen et al., 2005; Capizzano et al., 2007). An increase in NAA may indicate an increase in spine/synapse number, neuron size, neuron density, or some combination of these. A second commonly quantified metabolite is Choline (Cho), which among other things, is a precursor for CDP choline, a mononucleotide involved in the synthesis of phospholipid structural components of cell membranes (Kennedy and Weiss, 1956; Chida and Shimizu, 1973). The metabolites which contribute to the Cho signal in MRS are not certain, however one typical interpretation in changes in Cho is a change in neuronal membrane composition (Govindaraju et al., 2000). Increased Cho is thought to signal either an increase in the amount of cell membrane per unit volume as in the case of highly membrane-dense cancerous tumors, or an increase in the release of Cho during the breakdown of myelin as in the case in neurodegenerative disorders (for review see Rudkin and Arnold, 1999).

Variations in NAA and Cho levels may provide structural and chemical information on brain regions of interest. We have focused on two such regions in our studies (Ongur et al., 2010b; Ongur et al., 2010a): the anterior cingulate cortex (ACC) and the parieto-occipital cortex (POC). ACC plays a role in cognitive functions such as reward anticipation, decision making, empathy, and emotion (Phillips et al., 2003; Walton et al., 2007; Walton and Mars, 2007). Parieto-occipital cortex (POC) is a central node in the dorsomedial visual stream, and is thought to be responsible for integration of visual information necessary for behaviors such as reaching and grasping (Chapman et al., 2002; Busan et al., 2009). The two regions are anatomically and functionally connected as part of the default mode network (Gusnard and Raichle 2001). The anatomical characteristics of these two regions of cortex have been described postmortem in humans (von Bonin and Mehler, 1971; Kenan-Vaknin et al., 1992; Schlaug et al., 1995; Vogt et al., 1995; Di Rosa et al., 2008) and non-human primates (Hof and Morrison, 1995; Melchitzky et al., 1998; Lewis and Van Essen, 2000), as well as a number of other animal models including rodents (Akers and Killackey, 1978; Gabbott and Bacon, 1996), bats (Morgane et al., 1988), and even dolphins (Morgane et al., 1988).

In this study MRS was used to examine NAA and Cho concentrations in ACC and POC in healthy individuals. The data are drawn from two previously published studies that examined glutamatergic and GABAergic signaling in schizophrenia and bipolar disorder (Ongur et al., 2008; Ongur et al., 2010b; Ongur et al., 2010a). The current analyses have been presented previously in abstract form.

2. Results

Gender, age, education, and employment status were not related to NAA/Creatine (Cr) levels (gender Pearson's $R = -0.126$, $p = 0.158$; age Pearson's $R = -0.164$, $p = 0.096$, education Pearson's $R = 0.075$, $p = 0.290$, employment status Pearson's $R = 0.056$, $p = 0.347$). Grey matter percent (GM%) was positively correlated with NAA/Cr (Pearson's $R = 0.290$, $p < 0.05$), and cerebro-spinal fluid percent (CSF%) was negatively correlated with NAA/Cr (Pearson's $R = -0.599$, $p < 0.001$). White matter percent (WM%) was not correlated with NAA/Cr (Pearson's $R = 0.095$, $p = 0.255$). Gender, age, and employment status were not related to Cho/Cr levels (gender Pearson's $R = -0.034$, $p = 0.394$; age Pearson's $R = -0.199$, $p = 0.056$, employment status Pearson's $R = 0.152$, $p = 0.143$). Education was positively correlated with Cho/Cr (Pearson's $R = 0.241$, $p < 0.05$). GM% was positively correlated with Cho/Cr (Pearson's $R = 0.702$, $p < 0.001$), and CSF% (Pearson's $R = -0.481$, $p < 0.001$) and WM% (Pearson's $R = -0.579$, $p < 0.001$) were negatively correlated with Cho/Cr.

NAA/Cr was 18% higher in POC than in ACC (Fig. 1A). After controlling for gray matter percentages, there was a significant main effect of brain region ($F(1,45) = 29.355$, $p < 0.001$), and an effect of study ($F = 15.878$, $p < 0.001$), but no region X study interaction ($F = 2.797$, $p = 0.101$). Pairwise comparisons revealed that NAA/Cr was significantly higher in POC than in ACC (difference = 0.270, 95% CI = 0.170 – 0.371, $p < 0.001$). Cho/Cr was 46% lower in POC than in ACC (Fig 1B). After controlling for gray matter percentages, there was a significant main effect of brain region ($F(1,45) = 29.392$, $p < 0.001$), and an effect of study ($F = 30.252$, $p < 0.001$), but no region X study interaction ($F = 0.036$, $p = 0.850$). Pairwise comparisons revealed that Cho/Cr was significantly lower in POC than in ACC (difference = 0.388, 95% CI = 0.244 – 0.533, $p < 0.001$). Together these data are consistent with the hypothesis that the density of cell membrane per unit volume is reduced in POC over ACC while neuronal intracellular volume is expanded, possibly pointing to a reduction in complexity of neuronal profiles.

NAA/Cr within ACC was positively correlated with Cho/Cr in ACC (Pearson's $R = 0.301$, $p < 0.05$), and with Cho/Cr in POC (Pearson's $R = 0.691$, $p < 0.001$); NAA/Cr within POC was positively correlated with Cho/Cr in POC (Pearson's $R = 0.722$, $p < 0.001$) and with Cho/Cr in ACC (Pearson's $R = 0.616$, $p < 0.001$).

3. Discussion

NAA/Cr was significantly higher in POC than in ACC in healthy adults (Fig. 1A). Since NAA is localized to the intracellular space, these data suggest either that ACC neuronal compartment is reduced (e.g. reduced neuronal size or number) as compared with POC, or that there is a lower concentration of NAA per cell in the ACC than POC, or both. An increase in neuron number in POC over ACC is consistent with postmortem anatomical studies in monkeys, which found more densely packed neurons in POC when compared to surrounding areas (Hof and Morrison, 1995; Lewis and Van Essen, 2000). Postmortem human studies found more widely spaced bundles of larger neurons (possibly resulting in fewer neurons per unit volume) in ACC when compared to other brain areas including parietal and occipital cortices (von Bonin and Mehler, 1971; Kenan-Vaknin et al., 1992; Schlaug et al., 1995; Di Rosa et al., 2008).

Cho/Cr was significantly lower in POC than in ACC in healthy adults (Fig 1B). This is in line with previous postmortem data which found lower Cho levels in occipital cortex than both frontal and parietal cortices (Nitsch et al., 1993). Decreased Cho signals a reduction in the amount of cell membrane per unit volume. Coupled with a finding of increased NAA in POC as compared with ACC, this pattern is consistent with the possibility that the density of cell membrane per unit volume is reduced while neuronal intracellular volume is expanded, possibly pointing to a reduction in complexity of neuronal profiles.

There are several caveats to this study. One is that the data are from two separate, previously conducted studies which used different acquisition parameters and voxel sizes. However, because there was no region by study interaction, the regional differences presented here are not likely due to these methodological distinctions. A second is the large values of Cramer-Rao Lower Bounds (CRLB's: a measure of variability) for Cho in the second study. This is caused by the different technique used in the second study (MEGAPRESS), which acquires only one 68 msec spectrum, while the technique used in the first study (J-resolved) acquires many spectra and averages them. The spectral averaging used in the J-resolved technique results in much greater detail and more certainty in fitting. Consequently, CRLB's are lower. Finally, the negative correlation between Cho/Cr and WM% is surprising. A close examination of the data indicates that this finding is not convincing as this correlation arose from several cases of high WM having low Cho/Cr (data not shown, 8 out of 50 cases).

Finally, because ACC and POC are anatomically and functionally connected as part of the default mode network, we ran additional posthoc correlational analysis of NAA/Cr versus Cho/Cr within each region and NAA/Cr versus Cho/Cr between both regions. However, these results should be interpreted with caution as we performed a total of 18 correlations. We did not correct for multiple comparisons because any correlation in this preliminary analysis may be significant. Thus, the levels of each metabolite were correlated with one another within individual subjects although we reported at the group level that NAA levels are elevated and Cho reduced in POC as compared with ACC. This pattern implies there is both an individual subject and a brain region effect on NAA and Cho levels.

The current findings and the interpretation of these findings presented here are consistent with previously published proton MRS studies (Pouwels and Frahm, 1997, 1998). Both studies used a short echo-time stimulated echo acquisition mode (STEAM) sequence at 2 Tesla in healthy young adults to measure NAA and Cr in frontal, parietal, and occipital voxels, and (Pouwels and Frahm, 1998) also measured Cho in these same regions. They found that NAA increased and Cho decreased as they moved caudally with the highest and lowest metabolite concentrations, respectively in primary visual cortex. They found no differences in Cr levels throughout the brain (Pouwels and Frahm, 1997, 1998). Similarly, a study by Baker et al., (2008), used a PRESS sequence for acquisition at 3 Tesla in healthy young adults to measure NAA, Cho and Cr in frontal, parietal, and occipital WM and GM separately. This report found that WM NAA did not change across brain regions, but GM NAA increased from frontal to occipital brain regions. Cho decreased from frontal to occipital regions in both GM and WM (Baker et al., 2008). In contrast to our study, the study by Baker et al., (2008) found that WM Cr decreased as they moved caudally, but GM Cr increased as they moved caudally. However, our study did not separate GM from WM, precluding a direct comparison.

The current findings, together with other literature suggest that MRS may be a useful tool, complementary to other approaches, for studying the cellular/physical properties of the living human brain. MRS may provide *in vivo* measures reflecting the brain's cellular properties, which may allow investigators to examine regional and/or inter-individual differences in the brain's cellular compartments.

4. Methods

Data from healthy adult participants included in two larger experiments were pooled in this study (Ongur et al., 2008; Ongur et al., 2010b; Ongur et al., 2010a). See Table 1 for participant characteristics. All participants were recruited following approval by the McLean Hospital institutional review board (IRB); all participants read and signed an IRB approved Informed Consent Form, and completed an Informed Consent Survey to ensure that they understood experimental procedures. Participants were excluded if they had significant neurological or medical problems, current substance abuse, a history of substance dependence (except for nicotine dependence), or any DSM-IV Axis I disorders as assessed using the Structured Clinical Interview for DSM-IV Disorders (First et al., 1997). All participants underwent a clinical structural brain scan on a 3 Tesla Trio MR scanner (Erlangen, Germany) which was read by a radiologist. Those with structural abnormalities of the brain were excluded from the study. All proton MRS scans were conducted on a Varian 4 Tesla MR scanner (Varian/UnityInova, Varian Inc., Palo Alto, CA).

4.1 Study 1

All methods were described in more detail previously (Ongur et al., 2008; Ongur et al., 2010b). Briefly, a standard point-resolved spectroscopy sequence (PRESS) was modified for the current multi-echo proton MRS protocol and employed a four-pulse water suppression

enhanced through T_1 -effects (Ogg et al., 1994); 48 TE-stepped spectra were acquired (TE increased from 30–500 ms in 10-ms steps). Voxels were $2 \times 2 \times 2$ cm. ACC voxels were placed along the midline, just superior and anterior to the genu of the corpus callosum (see Ongur et al., 2008 for visual depiction of voxel placement). POC voxels were placed along the midline, posterior to the splenium of the corpus callosum and superior to the calcarine sulcus. Total scan time ranged from 75 to 90 minutes. See Table 1 for spectral signal to noise ratios (SNRs).

All MRS processing was fully automated, and real (not magnitude) spectra were used. Blind to brain region, an MR physicist (JEJ) evaluated all spectra, and excluded those with low signal to noise and/or low spectral resolution (2 from ACC and 1 from POC). During post processing, each voxel was zero-filled, Gaussian filtered, and Fourier transformed. For 2D fitting, commercial spectral fitting package LCModel (version 6.0-1) was used (Provencher, 1993). See Table 1 for Cramer-Rao Lower Bounds (CRLBs), an estimate of the variance associated with fitting.

FMRIB's Automated Segmentation Tool (Oxford Centre for Functional Magnetic Resonance Imaging of the Brain, Oxford, United Kingdom) was used for tissue segmentation of T_1 -weighted images into gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF). See Table 1 for GM, WM, and CSF percentages.

As T_2 relaxation times were available for these data, they were used to rule out the effect of differing T_2 times between compartments on calculated NAA and Cho levels. Each metabolite was first normalized to its corresponding T_2 value (in ACC: NAA = 196, Cho = 190, Cr = 146; and in POC: NAA = 240, Cho = 238, Cr = 124). Resulting values of NAA and Cho were then normalized to Cr. Univariate ANOVAs in SPSS with normalized NAA and Cho as dependent variables, region as a fixed factor, and GM% as a covariate yielded similar finding (NAA region $F(1,26) = 30.956$, $p < 0.001$; Cho region $F(1,26) = 10.530$, $p < 0.01$) as pooled data that were not normalized to T_2 values.

4.2 Study 2

All methods were described in more detail previously (Ongur et al., 2010a). Briefly, the MEGAPRESS (Mescher et al., 1998) sequence acquired two sets of spectra: one PRESS acquisition at TE = 68 ms with selective Gaussian refocusing RF pulses centered at 1.9 ppm GABA resonance ("on" scan) and one with the editing RF pulses centered at 20000Hz off-resonance ("off" scan). Voxels were $23 \times 22 \times 33$ mm. ACC voxels were placed anterior to the genu of the corpus callosum, with the ventral edge oblique and aligned with the dorsal corner of the genu, and positioned on the midline on axial images (see Ongur et al., 2010a for voxel placement). POC voxels were placed on midsagittal images. The ventral edge of the voxel was aligned with the dorsal boundary of the splenium of the corpus callosum, covering posterior cingulate and retrosplenial cortices, and positioned on midline in axial images. See Table 1 for MEGAPRESS SNRs.

All MRS processing was fully automated. For each voxel, the "off" scans were averaged to yield a standard PRESS (TE = 68 ms) proton MRS spectrum. Blind to brain region, an MR physicist (JEJ) evaluated all spectra, and excluded those with low signal to noise and/or low spectral resolution (2 from ACC and 5 from POC). Commercial spectral fitting package LCModel (version 6.0-1) was used (Provencher, 1993). See Table 1 for CRLBs.

FMRIB's Automated Segmentation Tool (Oxford Centre for Functional Magnetic Resonance Imaging of the Brain, Oxford, United Kingdom) was used for tissue segmentation of T_1 -weighted images into GM, WM, and CSF. See Table 1 for GM, WM, and CSF percentages.

4.3 Analysis of pooled data

All data analysis was carried out using SPSS software. Data are expressed as means $\pm$ standard deviations. To ensure that regional differences were not due to variance in MR signal (eg. proximity to the head coil, signal quality), regional differences in NAA levels were normalized to Cr. The effect of age, gender, education, employment status, GM%, WM %, and CSF% on NAA/Cr and Cho/Cr was investigated using separate Pearson's R correlations (uncorrected for multiple comparisons so we could detect any meaningful relationships). The effect of region on NAA/Cr and Cho/Cr levels was investigated using a univariate ANOVA with Bonferroni correction for multiple comparisons. NAA/Cr and Cho/Cr were dependent variables, region and study were fixed factors, and GM% was a covariate. In addition, separate Pearson's R correlations (uncorrected for multiple comparisons so we could detect any meaningful relationships) were run between NAA/Cr and Cho/Cr within each region, between NAA/Cr in ACC/Cr and Cho/Cr in POC, and between Cho/Cr in ACC and NAA in POC.

Research Highlights

- NAA/Cr was 18% higher; Cho/Cr was 46% lower in POC than in ACC (P<0.001 for each).
- This suggests lower membrane density, and higher neuronal volume in POC.
- This could point to a reduction in the complexity of neuronal profiles.
- These findings are consistent with previous anatomical and proton MRS studies.
- MRS may provide *in vivo* measures reflecting the brain's cellular properties.

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Figure 1. Expression of N-acetylaspartate / Creatine (A), and Choline / Creatine (B) in anterior cingulate cortex and parieto-occipital cortex. *** $p < 0.001$

Table 1

Participant characteristics, signal to noise ratios (SNR), grey matter percentage (GM%), white matter percentage (WM%), cerebro-spinal fluid percentage (CSF%), and Cramer-Rao lower bounds (CRLBs) for N-acetylaspartate (NAA), creatine (Cr), and choline (Cho).

Study 1		Study 2	
Participant N	11 male 9 female	10 male 6 female	
Participant Age	31.15 ± 8.0 years		37.2 ± 10.1 years
	ACC	POC	ACC POC
SNR	26.80 ± 6.49	37.20 ± 5.93	4.00 ± 0.65 5.83 ± 1.19
GM%	79.16% ± 4.99%	71.22% ± 7.64%	61.60% ± 5.51% 55.54% ± 5.53%
WM%	16.28% ± 5.62%	26.88% ± 8.18%	23.96% ± 2.22% 32.64% ± 4.31%
CSF%	4.56% ± 2.81%	1.90% ± 1.30%	14.44% ± 4.74% 11.82% ± 5.61%
CRLBs			
NAA	3.45 ± 0.69	2.40 ± 0.91	4.19 ± 1.05 2.22 ± 0.97
Cr	3.35 ± 0.49	2.67 ± 0.62	3.06 ± 0.57 2.07 ± 0.27
CHO	3.30 ± 0.57	3.47 ± 1.06	29.38 ± 9.18 17.00 ± 8.26