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^{1}H MRSI of Middle Frontal Gyrus in Pediatric ADHD

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1. Introduction

Attention Deficit Hyperactivity Disorder (ADHD) is a neurobehavioral condition affecting 3-9% of children and 4-6% of adults worldwide (McCracken, 1998; Kessler et al., 2006). Consistent inattention, impulsivity, and hyperactivity are the core symptoms of ADHD (Diagnostic and Statistical Manual of Mental Disorders, 2000). These symptoms (Jensen et al., 2001), along with impaired neurocognitive performance on multiple instruments (Aron et al., 2003; Bedard et al., 2003; Bedard et al., 2002), respond to stimulant treatment, popularly methylphenidate. Methylphenidate decreases synaptic dopamine reuptake in the brain (Seeman & Madras, 1998) in part by inhibiting the dopamine transporter (DAT) (Volkow et al., 1998). Therefore, neuroimaging studies of ADHD have targeted brain structures in dopamine-rich frontostriatal circuits (Cheon et al., 2003), especially since these circuits are implicated in attention and other neurocognitive functions impaired in ADHD. One such structure is the “dorsolateral prefrontal cortex” (DLPFC), target of the present investigation.

The human DLPFC is described by various authors as the dorsal half of the middle frontal cortex, the entire middle frontal cortex, or the middle frontal plus superior frontal cortex. This report concerns specifically the “middle frontal gyrus” and avoids the term “DLPFC”. Evidence from multiple modalities (neurocognitive, neuropharmacological, neuroimaging) in pediatric (Barkley & Grodzinsky, 1994; Loo et al., 2004; Weber et al., 2005; Weber et al., 2007; Barnett et al., 2009; Gau et al., 2009; Gau & Shang, 2010) and adult (Mehta et al.,

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2000; Moll et al., 2002; Moser et al., 2002; Owen et al., 1996; Zakzanis et al., 2005) ADHD implicates this region in ADHD, its dopaminergic treatment, or neurocognitive functions impaired in ADHD. But this region has been little studied using proton magnetic resonance spectroscopy (^{1}H MRS), a method that characterizes regional neurometabolism.

Previous MRS investigations of ADHD (reviewed in O'Neill et al., in press; Perlov et al., 2009) have emphasized basal ganglia (Liew & Yan, 2006; Carrey et al., 2003; Carrey et al., 2007), white matter (Fayed & Modergo, 2005; Yeo et al., 2003), and cingulate cortex (Colla et al., 2008; Kronenberg et al., 2008; Perlov et al., 2007; Courvoisie et al., 2004; MacMaster et al., 2003; Sparkes et al., 2001) rather than middle frontal gyrus. These studies have identified effects of ADHD or of stimulant treatment involving the metabolite signals for *N*-acetyl-aspartate+*N*-acetyl-aspartyl-glutamate (tNAA), glutamate+glutamine (Glx), creatine +phosphocreatine (Cr), and choline compounds (Cho). In several cases effects restricted to left or right cerebrum were found. It should be noted, however, that many of these studies deployed large MRS voxel sizes suffering from the 'partial-voluming problem' (inclusion of multiple different brain structures in a single acquisition volume) and/or expressed their results as metabolite ratios rather than absolute levels. Partial voluming makes assignment of effects to a single structure ambiguous while ratios make assignment of effects to a single metabolite signal ambiguous. One exception was Colla et al. (2008) who studied the cingulate using absolute metabolite levels and identified above-normal Cho in the subjects with ADHD in the left and in combined left + right anterior middle cingulate cortex (aMCC). This group used the magnetic resonance spectroscopic imaging (MRSI) variant of MRS which, in contrast to single-voxel MRS, acquires data from an array of multiple voxels simultaneously, typically with higher spatial resolution, i.e., smaller voxel-size. We are aware of only two MRS studies of DLPFC in any of its definitions (Soliva et al., 2010; Hesslinger et al., 2001). One (Soliva et al., 2010) measured below-normal Cr in right middle frontal gyrus using single-voxel MRS in a pediatric ADHD sample; the other, (Hesslinger et al., 2001) again using single-voxel MRS, found lower tNAA in left middle frontal gyrus in ADHD than in attention deficit disorder (ADD) or healthy controls in adult subjects. Thus, there is evidence for below-normal MRS metabolite levels in middle frontal gyrus in ADHD, but the region remains understudied.

The present study examined the bilateral middle frontal gyri in pediatric ADHD using MRSI and absolute metabolites. An unconventional coronal-oblique prescription was used to enable access to this anatomic site. As a further novelty, and in contrast to Colla et al., (2008), we used short echo-time (short-TE) MRSI, which measures more metabolites and higher levels of most metabolites relative to long-TE. Based on the above literature, we anticipated below-normal levels of tNAA and Cr in patients with ADHD, possibly more severely in the right hemisphere. These levels might also correlate with attentional functions.

2. Methods

Thirteen children meeting the DSM-IV diagnosis for ADHD (8 boys, 5 girls; mean age ± SD, 12.3 ± 1.5 years) and 13 healthy control subjects (8 boys, 5 girls; 12.2 ± 2.7 years) were recruited to participate. Four ADHD patients were of the Inattentive subtype, 7 of Combined subtype, and 2 were ADHD NOS. Subjects were screened for neurological, psychiatric, language, or hearing disorders by clinical interview and developmental history. Mean full-scale IQ (Wechsler, 1974) was 85.6 ± 18.1 for the ADHD group and 93.5 ± 19.0 for the control group. Of the ADHD subjects, one was undergoing treatment with oral methylphenidate and two with amphetamines. In the ADHD group 12 children were right-handed and 1 was left-handed; in the control group 11 children were right-handed and 2 were left-handed. The study was conducted in accordance with the policies of the Human

Subjects Protection Committees of the University of California, Los Angeles. Informed assents and consents were obtained from all subjects and their parents, respectively.

For 10 of the ADHD and 9 of the control subjects, Parts A and B of Trail Making Task (Reitan, 1979) were administered, the scores on which signify focused attention, concentration, and set-shifting.

Structural MRI and ^{1}H MRSI were acquired on a 1.5 T Siemens Sonata scanner and quadrature head coil. ADHD subjects were medication-free on the day of scanning. Two sagittal spoiled gradient recalled (SPGR) acquisitions were performed separately to diminish the likelihood of subject movement during scanning and were subsequently co-registered to each other and averaged yielding high-resolution T1-weighted whole-brain volumes. MRSI was acquired with PRESS (TR/TE = 1500/30 ms, NEX = 4, slab thickness = 9 mm, in-plane resolution = 11 × 11 mm^2). The MRSI slab (Figure 1) was coronal-oblique and sampled bilateral middle frontal ("dorsolateral prefrontal") cortex, mesial prefrontal cortex (pregenual anterior cingulate cortex, paracingulate portion of aMCC, or superior frontal cortex, depending on subject anatomy) and prefrontal white matter (mainly corona radiata) lying in between. Subjects with clinically relevant structural findings (e,g., congenital, traumatic, vascular, neoplastic, or infectious lesions of the brain or head visible on MRI) were liable for exclusion from the study. No subjects were excluded for this reason.

Using software and protocols developed at the UCLA Laboratory of Neuroimaging (LONI) (Blanton et al., 2004; Taylor et al., 2005), gyral volumes-of-interest (VOIs) consisting of cortex, superjacent sulcal CSF, and subjacent white matter were sketched manually for left and right middle frontal gyri using the sagittal T1-weighted MRI volume of each subject in multiplanar view (Figure 2). Independently, the entire brain was segregated into gray matter, white matter, and CSF subvolumes (Shattuck et al., 2001). By overlapping the VOIs with these whole-brain subvolumes, we divided each VOI into three separate "tissue-segmented VOIs". All personnel involved in region measurement received extensive specialized training in neuroanatomy. Intra- and interrater reliability were maintained at an intraclass correlation coefficient of at least 0.90 for each VOI.

MRSI data were post-processed automatically with the LCModel package (Provencher, 2001) yielding metabolite values for major resonances of tNAA (2.01 ppm), Glx (2.1-2.5 ppm), Cr (3.01 ppm), Cho (3.24ppm), and *myo*-inositol (mI; 3.54 ppm). Our self-designed MRSI Voxel Picker (MVP) program (O'Neill et al., 2006; Seese et al., 2011) was used for co-processing of MRI and MRSI data. MVP reconstructed the subject's whole-brain T1-weighted, gray matter, white matter, and CSF volumes, as well as each of the tissue-segmented VOIs, into the space of the MRSI slab. MVP then returned the tissue content (both on whole-brain and VOI bases) and CSF-corrected metabolite levels for each voxel in the slab. For each VOI, values were averaged together for all voxels containing at least 75% by volume gray matter plus white matter of the VOI in question, a signal-to-noise ratio of three or greater, and a full width at half maximum (FWHM) less than or equal to 0.1 ppm. Also, only metabolite peaks satisfying the LCModel criterion of less than or equal to 20% standard deviation were included in the average. MVP implemented these quality-control criteria automatically. Additionally, with the help of a guided user interface, all voxels that passed these criteria were manually inspected by raters blind to age, gender, and diagnosis. Voxels that showed significant artifact (i.e., contaminating signals from proximal extracranial tissue) were eliminated from analysis.

Given the modest number of subjects, non-parametric analyses were performed. This was achieved by rank-transforming the data prior to conducting the presently described T-tests and repeated-measures analyses-of-variance (R-ANOVA). Mean age, full-scale IQ, and

voxel tissue composition in each VOI were compared between the ADHD and healthy control groups using independent T-tests. To account for multiple comparisons in evaluating between-group differences in regional metabolite levels, an R-ANOVA was performed for each of the five metabolites (tNAA, Glx, Cr, Cho, mI) with the level of the metabolite as the dependent measure, Hemisphere as within-subjects factor (two levels: Left, Right), and Diagnosis as between-subjects factor (two levels: ADHD, Control). For metabolites showing a significant main effect or interaction involving diagnosis, R-ANOVA was followed-up with a *post-hoc* t-test in each VOI comparing metabolite levels between the two groups directly. For regional metabolite levels that differed significantly between ADHD subjects and controls, Spearman correlation was run on the (non-rank-transformed) data between the metabolite level and the standard scores on Trails A and Trails B within the subsample of ADHD subjects who underwent neurocognitive assessment. An α level of 0.05 (two-tailed) was considered statistically significant for all tests.

3. Results

The ADHD and control groups did not differ significantly in age ($t(20.1) = 0.28$, $p = 0.785$) or full-scale IQ ($t(20.0) = -0.98$, $p = 0.338$). Group mean ± standard deviation scores for the ADHD subsample undergoing neurocognitive assessment were 94.9 ± 12.4 (standard score, range: 74-118) on Trails A and 99.1 ± 23.7 (range: 52-126) on Trails B. For the control subsample the scores were 101.6 ± 9.8 (range: 81-113) on Trails A and 98.8 ± 10.3 (range: 78-113) on Trails B. The scores did not differ significantly between groups.

3.1. MRSI Voxel Tissue Composition and Metabolite Levels in Left and Right Middle Frontal Gyri

No significant between-group differences were found in the mean volume percent gray matter, white matter, or CSF of the MRSI voxels sampled in left or right middle frontal gyrus (all $p > 0.10$; Table 1).

Mean CSF-corrected metabolite levels in MRSI voxels sampled from left and right middle frontal gyri for the ADHD and control groups are listed in Table 1. Omnibus R-ANOVA found a significant main effect of Diagnosis ($F(1,15) = 9.6$, $p < 0.01$) and a significant Diagnosis-by-Hemisphere interaction ($F(1,15) = 12.9$, $p < 0.01$) for tNAA, a significant Diagnosis-by-Hemisphere interaction ($F(1,14) = 30.2$, $p < 0.0005$) for Cr, a significant main effect of Diagnosis ($F(1,12) = 4.6$, $p < 0.05$) and a significant Diagnosis-by-Hemisphere interaction ($F(1,15) = 40.3$, $p < 0.0005$) for Cho, and a significant main effect of Diagnosis ($F(1,9) = 7.6$, $p < 0.05$) and a significant Diagnosis-by-Hemisphere interaction ($F(1,9) = 8.8$, $p < 0.05$) for mI. There were no significant main effects or interactions for Glx.

In *post-hoc* independent T-tests, there were no significant between-group differences in metabolite levels in left middle frontal gyrus. In right middle frontal gyrus, tNAA was on average 27.4 % lower in the ADHD than in the control group ($t(22.7) = -4.4$, $p < 0.0005$). The tNAA level was below the control mean for 12 of 13 ADHD subjects (Figure 3). Cr was on average 34.9 % lower in the ADHD than in the control group ($t(22.1) = -4.2$, $p < 0.0005$). The Cr level was below the control mean for 11 of 13 ADHD subjects. Cho was on average 27.6 % lower in the ADHD than in the control group ($t(19.8) = -4.7$, $p < 0.0005$). The Cho level was below the control mean for 10 of 13 ADHD subjects. mI was on average 21.6 % lower in the ADHD than in the control group ($t(20.7) = -3.1$, $p < 0.005$). The mI level was below the control mean for 10 of 13 ADHD subjects. For the three ADHD subjects being treated with stimulants, values were in the midrange of metabolite levels within the ADHD group. Thus, the middle frontal gyrus (DLPFC) showed significant right-sided unilateral depletion of four major MRSI metabolites relative to the healthy control group (Table 1).

3.2. Correlations between MRSI Metabolite Levels and Neurocognitive Scores

Given that significant between-group differences were found in right middle frontal gyrus for the levels of tNAA, Cr, Cho, and mI, Spearman correlations were run for each of these regional metabolite values *versus* scores on Trails A and Trails B. For right middle frontal gyrus Cr in the ADHD group, significant positive correlations were found with Trails A ($r(10) = 0.64$, $p < 0.05$) and Trails B ($r(10) = 0.66$, $p < 0.05$; Figure 4) scores, implying better performance for higher Cr level. No significant correlations were found for tNAA, Cho, or mI (all $p > 0.05$). For right middle frontal gyrus Cr in the control group, a significant negative correlation was found with Trails B only ($r(9) = -0.81$, $p < 0.05$; Figure 4).

4. Discussion

This study found below-normal levels of four major MRSI metabolites (tNAA, Cr, Cho, and mI) in the middle frontal gyrus of children and adolescents with ADHD compared to age- and sex-matched healthy controls. Significant deficits in these neurometabolites were confined to the right cerebral hemisphere. Lower right cerebral levels of one neurometabolite (Cr) in ADHD subjects were related to worse performance on two neurocognitive tests of attention. These deficits may be related to ADHD or to pharmacologic treatment of ADHD subjects with methylphenidate.

Our finding of neurometabolite depletion in the middle frontal gyrus is consistent with evidence from a wide range of neuroscience modalities implicating this gyrus, or larger regions labeled “DLPFC” that contain this gyrus, in ADHD. To begin, several investigations of cortical thickness (CT) have demonstrated cortical thinning in the DLPFC in subjects with ADHD (Makris et al., 2007; Shaw et al., 2006; Narr et al., 2009); moreover, thinner medial prefrontal cortex has been associated with worse clinical outcome in ADHD (Shaw et al., 2006). In the present study, 9 of the subjects with ADHD and 7 control subjects were included in our 2009 report of decreased CT (Narr et al., 2009) in middle frontal gyrus, among other regions, in pediatric ADHD (Narr et al., 2009) Spatial working memory is a neurocognitive function that is impaired in pediatric ADHD (Gau et al., 2009), correlates with pediatric ADHD core symptoms (Barnett et al., 2009) and is affected by stimulants in pediatric ADHD patients (Gau et al., 2009) and adult healthy controls (Mehta et al., 2000). In normal adults scanned with $H_2{}^{15}O$-positron emission tomography (PET), regional cerebral blood flow (rCBF) is increased in right middle and right superior frontal cortices by spatial working memory tasks (Owen et al., 1996) and is decreased by methylphenidate in left middle frontal cortex (Mehta et al., 2000).Thus, patients with ADHD are impaired in a neurocognitive function that appears to be at least in part localized to middle frontal cortex; moreover a stimulant that treats ADHD improves this function and elicits neuroimaging responses in middle frontal cortex. Performance on the Trails, a test of visual attention and attentional set-shifting, is below-normal in pediatric ADHD (Barkley et al., 1994). This task has been linked to left DLPFC by functional magnetic resonance imaging (fMRI) in normal adults (Moll et al., 2002; Zakzanis et al., 2005), and by transcranial magnetic stimulation of the cortex in depressed adults (Moser et al., 2002). Using the novel technique of functional near-infrared spectroscopy (fNIRS) in healthy and ADHD children, Weber et al. (2007) showed that Trails increased cerebral blood volume and oxyhemoglobin concentration bilaterally in a region comprising middle frontal+superior frontal cortex. In this region on the left only, elevation of deoxyhemoglobin concentration during Trails was less for ADHD than for healthy controls. In this region on the right only, methylphenidate reduced cerebral blood volume and oxyhemoglobin concentration in ADHD (Weber et al., 2007). In power EEG in pediatric ADHD (Loo et al., 2004), methylphenidate-associated improvement in continuous performance task score correlated with increased β-power over bilateral middle frontal cortex. Methylphenidate increased β-amplitude over bilateral middle frontal cortex in responders to methylphenidate and decreased it in non-responders. Thus, our MRSI results

join evidence from diverse sources implicating the middle frontal and neighboring cortex in ADHD and/or its response to dopaminergic stimulants.

Our study found significant depression of MRSI metabolites only in the *right* middle frontal gyrus. Notably, some of the above-cited DLPFC findings were specific to the right cerebral hemisphere. In some cases this may have been due to lack of statistical power. But, it may as well reflect right-sided asymmetry in the pathology of ADHD. In particular, neglect of one visual hemispace is a syndrome that in animals is induced by lesions of dopaminergic cortical innervations (Iversen, 1984). In human neurological patients, neglect of the *left* hemispace is much more commonly observed and has been attributed to dominance of the right hemisphere in spatial attention (Mesulam, 1981). Clinical and experimental measures have frequently demonstrated such attentional asymmetries in pediatric ADHD (Iversen, 1984; Mesalum, 1981; Voeller & Heilman, 1988) and in mothers of ADHD children (Nigg et al., 1997). Dopamine agonists (Fleet et al., 1987), including methylphenidate for pediatric ADHD (Nigg et al., 1997; Sheppard et al., 1999), are effective in normalizing unilateral neglect. Right-hemisphere dominance of cortical attention functions may be related to right-sided preponderance in the striatum of dopamine D2 receptors (Larisch et al., 1998) and DAT (Larisch et al., 1998) in healthy controls and of DAT in ADHD (Laakso et al., 2000). Using resting-state BOLD fMRI, Yang et al. (2011) calculated lower amplitude of low-frequency fluctuations in pediatric ADHD patients than in controls in bilateral anterior and posterior middle cingulate cortex and specifically in right middle frontal cortex. In a longitudinal activation BOLD fMRI study using the continuous performance task (Epstein et al., 2009), the right middle frontal cortex was unique among brain regions in that local BOLD effect increased for the adolescent ADHD sample and decreased for the control sample at one-year follow-up relative to baseline.Hence, our MRSI findings of exclusively right-hemisphere neurometabolite depletion meet an expectation of specific right-sided effects emerging from prior neurocognitive and neuroimaging work in ADHD.

In a recent rigorous single-voxel MRS study of pediatric ADHD, similar to the present study, Soliva et al., (2010) measured below-normal tNAA (mean 35.9% vs. 27.4% in the present study), Cr (33.6% vs. 34.9%), and Cho (25.4% vs. 27.4%) in right middle frontal gyrus in their ADHD sample (Soliva et al., 2010). However only Soliva et al.'s (2010) Cr effect was statistically significant, possibly due to high standard deviations. Alternative methodology in our study, such as the smaller voxel sizes of MRSI, offline verification of voxel volume% middle frontal gyrus, etc. may have afforded lower standard deviations resulting in additional significant effects. The finding of quantitatively comparable deficits in multiple metabolite levels in the same brain region in two independent samples of pediatric ADHD obtained with two different scanning platforms and protocols (GE single-voxel MRS vs. Siemens MRSI) suggests these deficits are a feature of ADHD or of its treatment with stimulants.

Within a subsample of the ADHD group who had undergone neurocognitive assessment, lower levels of right middle frontal gyrus Cr correlated with worse performance on the Trail Making Task. This test assays, in compounded and overlapping fashion, multiple attentional functions, such as focused attention, concentration, and set-shifting. However, when a subsample of the control group underwent the same assessment, lower levels of right middle frontal gyrus Cr correlated with better performance on the task. These correlations suggest that the observed Cr deficits could potentially be in some manner related to neurophysiological factors producing or associated with the neurocognitive symptoms of ADHD, e.g., common signs of inadequate cerebral energy metabolism. If we assume Cr to represent energy consumption, healthy controls consume less energy (i.e., get more efficient) as they improve at the task, while ADHD patients must expend more energy in order to perform the task better. That significant correlations were not found for the other

neurometabolites may be due to the small number of ADHD subjects (10) for whom neurocognitive data were available. Interpretation of the Trails correlation is also limited by the fact that even non-parametric correlations in small samples are less informative. It is important to reiterate that further causative exploration is necessary before providing implications based on the correlational data.

As detailed in reviews (e.g. Storck & Renshaw, 2005; Ross & Blüml, 2001), there is evidence implicating each of the ^{1}H MRS metabolites in various cellular physiologic functions. In particular, NAA and NAAG likely serve to export metabolic water from the neuron and otherwise interact with cell energy metabolism. The well-known creatine-phosphocreatine buffer provides ATP for short-term cell energy demands. Choline compounds are building blocks for cell membranes and products of membrane degradation. mI is a key second-messenger linking neurotransmitter receptor activation to cell membrane synthesis and energetic function. Additionally, nearly all major MRS metabolites act as osmolytes helping to protect neurons and glia from becoming waterlogged or dehydrated. Given that simultaneous regional deficits in four different metabolite signals were recorded in this investigation, some type of osmotic disturbance or dysfunction is perhaps the most parsimonious explanation. Many SPECT (Amen & Carmichael, 1997; Kim et al., 2002, 2010; Kaya et al., 2002; Lorberboym et al., 2004), PET (Ernst et al., 2003), and perfusion MRI (O'Gorman et al., 2009) studies have measured, often lateralized, effects of ADHD on rCBF. The patterns of lateralization can be complex. Such phenomena have been observed as above- or below-normal rCBF in ADHD in one or more cortical regions in left or right hemisphere only (Amen & Carmichael, 1997; Kim et al., 2002, 2010; Kaya et al., 2002; Ernst et al., 2003; O'Gorman et al., 2009); positive correlation of rCBF with ADHD symptoms in a brain structure in one hemisphere and negative correlation in its contralateral homologue (Spalletta et al., 2001; Ernst et al., 2003); and different lateralized distribution of rCBF in medicated vs. unmedicated ADHD patients (O'Gorman et al., 2009). One scenario derivable from the ADHD rCBF (and above-cited fMRI and fNIRS) literature is that blood perfusion in right middle frontal cortex is above-normal in ADHD, leading to compensatory drops in intracellular metabolite molalities to correct the osmotic balance, and thus to the observed below-normal levels of MRS tNAA, Cr, Cho, and mI in right middle frontal cortex. Alternatively, a previously hypothesized defect in cortical astrocyte output of energetic substrate, e.g., lactate, in ADHD (Todd & Botteron, 2001; Russell et al., 2006) may result in decelerated cell energy metabolism, membrane synthesis, and neuronal acetyl-CoA synthesis leading to diminished levels of Cr, Cho, and NAA, respectively. Thus, simultaneous low levels of multiple metabolites could be due to the common role of these metabolites in cellular osmotic regulation and/or to their common dependence on energetic metabolism. However, further causative investigation is necessary to support such an implication.

4.1. Conclusions/Limitations

This was a small study and replication is needed. The sample was heterogeneous and too small to properly statistically control for gender, medication, and ADHD subtype effects. Nonetheless, it was notable that each effect was observed for 10-13 of 13 ADHD subjects. On average, the MRSI voxels sampled in our study contained roughly equal volumes of gray (~50%) and white (~40%) matter. Thus, we cannot say if below-normal metabolite levels in ADHD reflect concentrations in gray matter, white matter, or both. Future MRSI studies at high-field (the present study was performed at only 1.5 T) using smaller voxel-sizes may acquire regional voxels of higher tissue purity and thereby answer this question. As a further limitation, 3 ADHD subjects were undergoing stimulant treatment at the time of study. Although these agents act rapidly and subjects abstained from their medication on the day of scan and these subjects did not exhibit extreme metabolite levels, this does not rule-out

possible chronic effects of these drugs on the brain. Moreover, all the ADHD patients in Soliva et al., (2010) were currently being medicated with methylphenidate and were scanned 24 hr off-meds. As Soliva et al., (2010) found no statistically significant correlations between dose (mg/kg body weight) or length of time (months) on methylphenidate and any MRS metabolite level in right middle frontal gyrus, this suggests that the observed metabolite-level deficits in this brain region are associated with ADHD and not with stimulant treatment.

Future clinically oriented neuroimaging ADHD research could assess possible contributions of altered blood flow to putative neurometabolic disturbances in ADHD by means of contemporaneous assessment of rCBF and proton MRS metabolite levels in middle frontal cortex. In pediatric samples rCBF assay might be attempted via arterial spin labeling or specialized BOLD fMRI (Leontiev et al., 2007); in adults, radiotracer methods (e.g., PET and SPECT) represent additional ethically defensible options. Local astrocyte energetic dysfunction postulated for ADHD by Russell et al. (2006) might be probed by looking for a below-normal lactate signal in middle frontal cortex with long-TE proton MRS. The difficulty is that brain lactate at rest is typically at noise levels and in ADHD it is proposed to be even lower. Similar to work done in inferior frontal gyrus (Urrila et al., 2003), however, one could compare the augmented and now measurable lactate levels following demanding attention tasks in ADHD patients and controls. As suggested by Russell et al. (2006), investigations of potential therapeutic agents that target brain energetics rather than neurotransmitter function may yield therapeutic responses beyond those currently obtained with monoaminergic stimulants. Novel forms of therapeutic agents targeting astrocytes and the energy cycle could be tested. Impairment of normal rCBF in ADHD might suggest use of vasoactive agents.

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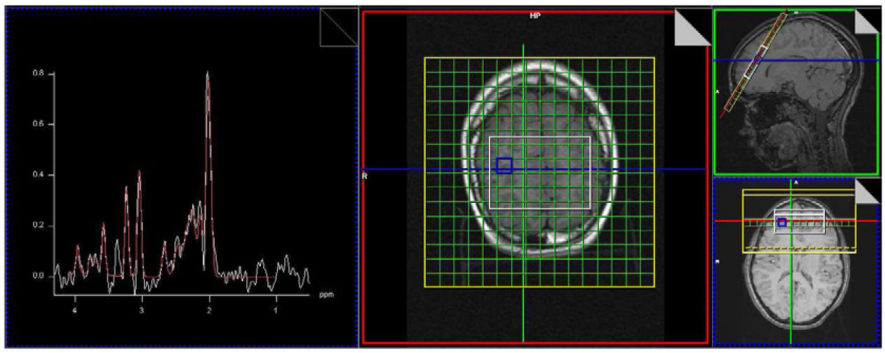

Figure 1.

(*Left*)^{1}H MRSI spectrum (PRESS TR/TE = 1500/30 ms) from voxel outlined in *blue* in the right middle frontal gyrus of a study subject. Note sharp peaks for tNAA (2.01 ppm), Cr +PCr (3.02 ppm), Cho (3.24 ppm), and mI (3.54 ppm) and shoulder for Glx (2.1-2.3 ppm). Coronal-oblique (*center*), sagittal (*right upper*), and axial (*right lower*) MRI depicting positioning of 9-mm thick "dorsolateral prefrontal cortex" MRSI slab (*green grid*). The coronal-oblique plane was acquired 43° counterclockwise to the genu-splenium line as seen in the sagittal plane. The PRESS volume (*white rectangle*) was oriented parallel to the aforementioned coronal-oblique plane then, in the sagittal plane, aligned parallel to the first cortical gyrus (paracingulate or superior frontal gyrus depending on subject) exterior to the rostral cingulate gyrus. Within the coronal-oblique plane, the PRESS volume was then sized and positioned to encompass as much lateral cortex as possible without contacting extracranial lipids. In addition to bilateral middle frontal ("dorsolateral prefrontal") cortex, the PRESS volume samples paracingulate or medial prefrontal cortex and prefrontal white matter.

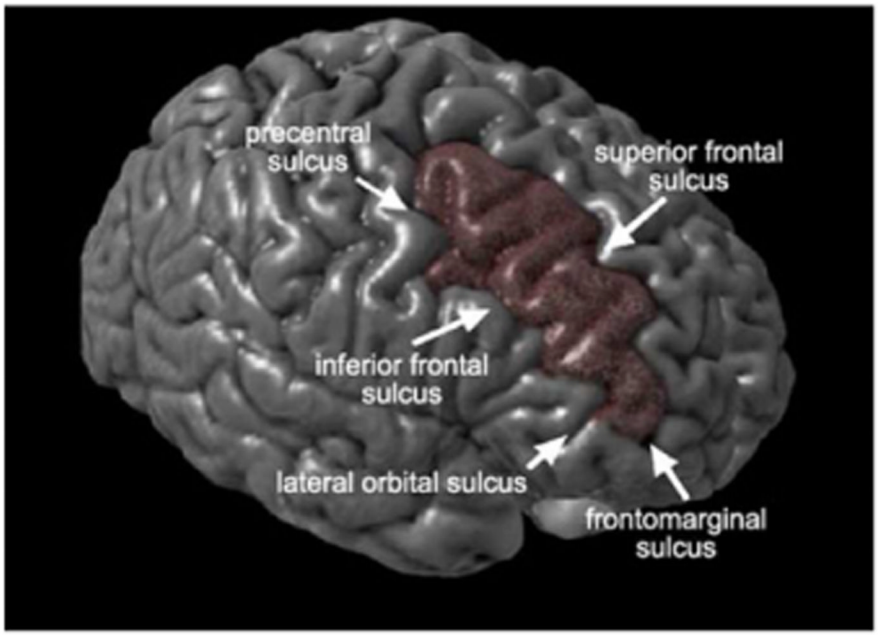

Figure 2.
Delineation of right middle frontal gyrus (black) with the methods of Blanton et al. (2004) and Taylor et al. (2005).

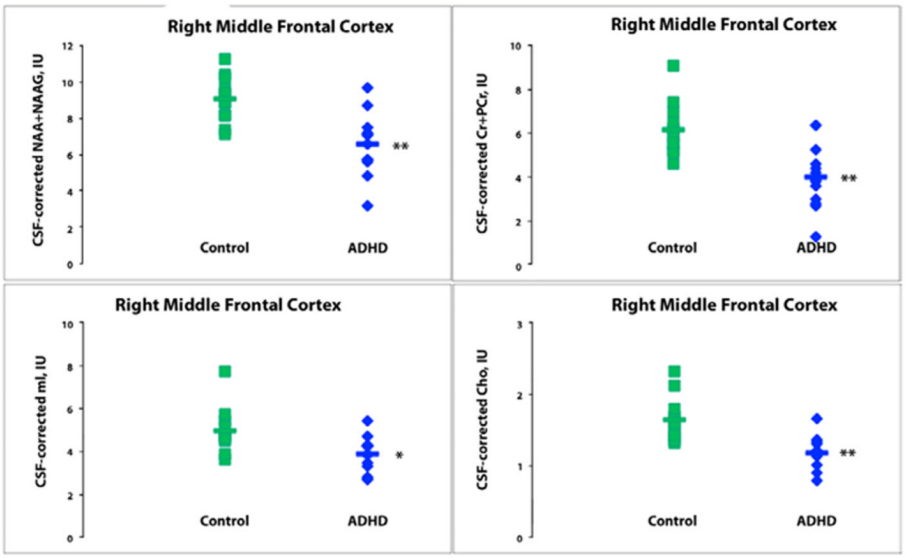

Figure 3.

Levels of CSF-corrected *N*-acetyl-aspartate+*N*-acetyl-aspartate-glutamate (NAA+NAAG), creatine+phosphocreatine (Cr+PCr), choline compounds (Cho), and myo-inositol (mI) for individual ADHD (*blue diamonds*) and control (*green squares*) subjects. *Horizontal bars* denote group means. *$p < 0.005$, **$p < 0.0005$ independent T-test following omnibus repeated-measures analysis-of-variance (data rank-transformed, therefore non-parametric).

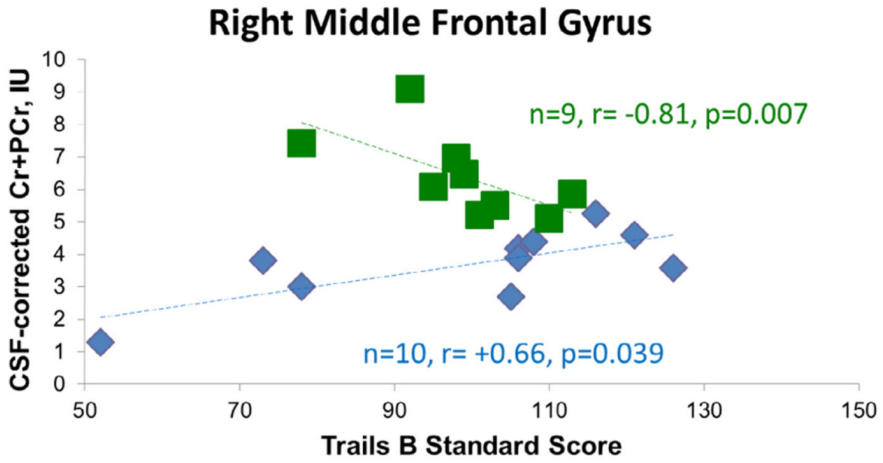

Figure 4.

Within a subsample (n = 10) of the pediatric ADHD (blue diamonds) and control (n = 9) subjects (green squares), correlations (Spearman) of right middle frontal gyrus Cr+PCr level with Trails B score signifying attention, concentration, and set shifting.

Table 1

Tissue composition (mean ± SD volume % gray matter, white matter, CSF) of MRSI voxels and CSF-corrected metabolite levels (Institutional Units, IU) for attention-deficit hyperactivity disorder (ADHD; n = 13) and healthy control (n = 13) groups. Voxels contained ≥ 75 volume % gray matter+white matter of left, respectively, right middle frontal gyrus.

Tissue Type/Metabolite	Left Middle Frontal Gyrus		Right Middle Frontal Gyrus	
	ADHD	Control	ADHD	Control
gray matter (vol%)	57.2 ± 17.9	51.2 ± 10.6	54.4 ± 11.4	53.0 ± 7.1
white matter (vol%)	39.1 ± 20.9	47.2 ± 13.0	44.3 ± 13.0	45.5 ± 8.2
CSF (vol%)	3.2 ± 4.3	1.6 ± 3.2	1.0 ± 1.2	1.2 ± 1.1
NAA+NAAG (IU)	6.5 ± 2.4	6.0 ± 1.1	**6.6 ± 1.7****	**8.9 ± 1.2**
Glu+Gln (IU)	11.4 ± 2.0	11.1 ± 2.4	12.1 ± 2.5	13.0 ± 1.6
Cr+PCr (IU)	4.0 ± 1.5	3.0 ± 0.6	**4.0 ± 1.4****	**6.1 ± 1.2**
Cho (IU)	1.1 ± 0.3	0.8 ± 0.2	**1.2 ± 0.2****	**1.6 ± 0.3**
mI (IU)	4.3 ± 2.5	2.8 ± 0.5	**3.9 ± 0.8***	**5.0 ± 1.1**

No significant between-group differences in tissue composition. CSF = cerebrospinal fluid

* $p < 0.005$,

** $p < 0.0005$

independent T-test following omnibus repeated-measures analysis-of-variance (data rank-transformed, therefore non-parametric). NAA = *N*-acetyl-aspartate, NAAG = *N*-acetyl-aspartyl-glutamate, Glu = glutamate, Gln = glutamine, Cr = creatine, PCr = phosphocreatine, Cho = choline-containing compounds, mI = *myo*-inositol