RESEARCH ARTICLE

Glutamatergic Neurometabolites during Early Abstinence from Chronic Methamphetamine Abuse

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Abstract

Background: The acute phase of abstinence from methamphetamine abuse is critical for rehabilitation success. Proton magnetic resonance spectroscopy has detected below-normal levels of glutamate+glutamine in anterior middle cingulate of chronic methamphetamine abusers during early abstinence, attributed to abstinence-induced downregulation of the glutamatergic systems in the brain. This study further explored this phenomenon.
Methods: We measured glutamate+glutamine in additional cortical regions (midline posterior cingulate, midline precuneus, and bilateral inferior frontal cortex) putatively affected by methamphetamine. We examined the relationship between glutamate+glutamine in each region with duration of methamphetamine abuse as well as the depressive symptoms of early abstinence. Magnetic resonance spectroscopic imaging was acquired at 1.5 T from a methamphetamine group of 44 adults who had chronically abused methamphetamine and a control group of 23 age-, sex-, and tobacco smoking-matched healthy volunteers. Participants in the methamphetamine group were studied as inpatients during the first week of abstinence from the drug and were not receiving treatment.
Results: In the methamphetamine group, small but significant (5–15%, $P<.05$) decrements (vs control) in glutamate+glutamine were observed in posterior cingulate, precuneus, and right inferior frontal cortex; glutamate+glutamine in posterior cingulate was negatively correlated ($P<.05$) with years of methamphetamine abuse. The Beck Depression Inventory score was negatively correlated ($P<.005$) with glutamate+glutamine in right inferior frontal cortex.
Conclusions: Our findings support the idea that glutamatergic metabolism is downregulated in early abstinence in multiple cortical regions. The extent of downregulation may vary with length of abuse and may be associated with severity of depressive symptoms emergent in early recovery.

Keywords: methamphetamine, abstinence, glutamate, magnetic resonance spectroscopy, depression

Introduction

Methamphetamine abuse, a major illicit drug problem (Gonzales et al., 2010), is resistant to treatment. Psychosocial interventions, the core of contemporary treatment for stimulant-use disorders, relieve only a portion of sufferers (Rawson et al., 2004), but effective medications are unknown (Shoptaw et al., 2009). Most stimulant abusers enter treatment in the first 7 to 10 days of abstinence

(Elkashef et al., 2007), the acute phase of withdrawal (Zorick et al., 2010). In this acute phase, risk of noncompliance is high, crucially affecting engagement, retention, and treatment outcome (Brecht et al., 2000). For this reason, neuroimaging at our center (London et al., 2004; Thompson et al., 2004; Berman et al., 2008; Tobias et al., 2010; Morales et al., 2012) and a few others (Kim et al., 2005 2009; Ernst and Chang 2008) has focused on characterizing the brain in acute abstinence from methamphetamine to inform development of novel and improved avenues to remediation.

Proton magnetic resonance spectroscopy (MRS) is a noninvasive neuroimaging technique used in several studies, mainly of longer term abstinence from methamphetamine (Ernst et al., 2000; Nordahl et al., 2002 2005; Sekine et al., 2002; Chang et al., 2005; Salo et al., 2007 2011a 2011b; Sung et al., 2007; Taylor et al., 2007; Sailasuta et al., 2010; Cloak et al., 2011; Howells et al., 2014). MRS assays the concentrations of several key metabolites potentially affected by methamphetamine in the living human brain. Ernst and Chang (2008) partly characterized the metabolic state of the brain in early abstinence with MRS. They identified one putative feature of this state: below-normal levels of glutamate+glutamine (Glx). Glx is a signal forming part of the MR spectrum; the magnitude of this signal is proportional to the sum of the concentrations of the amino acid and excitatory neurotransmitter glutamate (Glu) and the closely related neutral amino acid glutamine (Gln). The structural affinity of Glu and Gln renders them challenging to distinguish with MRS; hence, they are often assayed as a combined entity, Glx. Ernst and Chang (2008) showed evidence for a depletion of these glutamatergic compounds in methamphetamine abuse. In the first 2 months of abstinence, they measured below-normal Glx in anterior middle cingulate. (The anterior middle cingulate is located just caudal and dorsal to the portion of the cingulate that caps the genu of the corpus callosum; O'Neill et al., 2009). The authors deduced that low Glx corresponded to a downregulation of brain Glu-Gln systems induced in early abstinence, perhaps a rebound from elevated levels of these compounds prevailing in times of active abuse. Their interpretation was supported by a positive correlation between Glx and length of abstinence (ie, recovery of Glx) across their sample as well as by within-subject increases in Glx observed longitudinally during the course of abstinence. Multiple neurochemical interpretations of these findings were offered, including methamphetamine-associated disturbances in the Krebs cycle and/or the neuronal-astrocytic Glu-Gln cycle as well as cell damage and microglial activation.

Here, we further explore low Glx in early abstinence from methamphetamine. We sought to determine if the putative Glx deficit manifests in cortical regions beyond anterior middle cingulate. Thereby, we targeted regions implicated in methamphetamine abuse in prior work using other neuroimaging modalities. One such region was the medial parietal cortex. Using [18]FDG-PET, Volkow et al. (2001) established that methamphetamine-abusing subjects abstinent for 2 weeks to 2 years had above-normal absolute cerebral metabolic rate of glucose (CMRglc) in medial parietal cortex. We later showed that research participants who had chronically used methamphetamine and then maintained abstinence for 4 to 7 days showed elevated relative CMRglc in medial parietal cortex and adjacent posterior middle cingulate cortex (London et al., 2004) and that CMRglc increased during the first month of abstinence from methamphetamine, especially in medial parietal cortex (Berman et al., 2008). Given the metabolic coupling between Glu and CMRglc in brain (Sibson et al., 1998), such effects might also influence the MRS Glx peak. We divided the medial parietal target into posterior cingulate cortex and the immediately dorsal precuneus, a division motivated by our earlier structural MRI work that had revealed below-normal gray-matter volume in methamphetamine-abusing subjects in posterior cingulate, but not precuneus (Thompson et al., 2004). A second target region was inferior frontal cortex, selected because the right inferior frontal cortex has been assigned a special role in behavioral response inhibition (Aron et al., 2014), which is impaired in subjects during early abstinence from chronic methamphetamine abuse (Monterosso et al., 2005). In addition, we have observed a deficit in gray matter of right inferior frontal gyrus in these subjects (Thompson et al., 2004; Tabibnia et al., 2011). This deficit partly recovers in the course of longer abstinence (Morales et al., 2012). We hypothesized that Glx would be below normal in dorsal posterior cingulate, precuneus, and inferior frontal cortex and that Glx in these regions would be negatively correlated with duration of methamphetamine abuse. If low cortical Glx is a feature of early abstinence from methamphetamine, then it might contribute to behavioral symptoms during early abstinence, such as depressive symptoms (Newton et al., 2004; Zorick et al., 2010). We hypothesized that Glx in our target regions would be negatively correlated with the severity of depressive symptoms, measured by the Beck Depression Inventory (BDI; Beck et al., 1996). We tested these hypotheses in an inpatient sample of early (4–7 days) abstinent methamphetamine-abusing subjects and age-and sex-matched healthy control subjects.

Methods

Subjects

Forty-four methamphetamine-abusing and 24 healthy control subjects (Table 1), recruited via local print and radio ads,

Table 1. Clinical Characteristics of Study Subjects.

	Methamphetamine (n = 44)	Control (n = 24)	P
Sex			0.833
Female	19	11	--
Male	25	13	--
Age, yr	33.0±9.4	33.0±7.4	0.989
Depression, BDI Score	13.3±11.5	1.8±2.5	<0.0005
Years Meth Abuse	11.1±7.8	0	--
g/week Meth	1.9±1.4	0	--
Pack-Years Tobacco	10.9±11.6	9.3±8.0	0.508
Fagerström Score	3.0±2.2	3.5±2.3	0.474
day/month Marijuana	0.7±1.2	0.4±1.3	0.439
drink/week Ethanol	1.6±2.3	2.6±2.5	0.113

Shown are subject numbers or group mean±standard deviation. P values are for χ^2 (sex) or two-way independent t-tests (all other variables).

were enrolled into 2 groups: methamphetamine and control. Inclusion criteria for both groups were: (1) moderate or above intelligence (score >85 on the Shipley Institute of Living Scale; Zachary 1985), (2) right-handedness (score >20 on a modified Edinburgh Handedness Test), (3) taking no prescription medications or herbal products (eg, *Ginkgo biloba*) expected to affect brain function, and (4) no current Axis I psychiatric disorders, including substance dependence disorders other than nicotine dependence (or methamphetamine dependence for the methamphetamine group). Absence of psychiatric disorders was established by in-person screening with the Structured Clinical Interview for DSM-IV. All participants with a history of notable head trauma, neurological disease, cardiovascular or pulmonary disease, or other significant major medical conditions, such as seropositivity for HIV, were excluded. Because of the high prevalence of tobacco smoking among methamphetamine-abusing subjects, an effort was made to match the 2 groups for cigarette smoking. Recent smoking was verified by carbon monoxide levels in expired air ≥10 ppm (Micro smokerlyzer; Bedfont Scientific Ltd, Kent, UK) and the presence of urinary cotinine (≥200 ng/mL by Accutest NicAlert strips; JANT Pharmacal Corporation, Encino, CA). Mean nicotine dependence (Fagerström score), cumulative lifetime exposure (pack-years) to tobacco, marijuana consumption (days in past 30 days), and ethanol consumption did not differ significantly between groups (Table 1). After receiving a detailed explanation of the study, all subjects gave written informed consent to participate.

Participants in the methamphetamine group resided in the UCLA General Clinical Research Center inpatient unit during their participation. They all denied a desire to seek treatment yet were willing to remain abstinent from methamphetamine for the course of the study and were paid for their participation, including remaining abstinent from methamphetamine. These participants had to satisfy DSM-IV criteria (established by Structured Clinical Interview) for methamphetamine dependence and demonstrate use of methamphetamine within 3 days of enrollment, as verified by a positive urine toxicology screen. Thereafter, subjects were under regular supervision of medical staff and underwent twice-weekly random urine screens to confirm abstinence from illicit drugs of abuse. Methamphetamine

abuse variables, including duration of abuse in years and current intensity of methamphetamine abuse in grams per week (Table 1), were solicited by structured interview at the time of enrollment. Participants in the control group were matched to the methamphetamine group for age and sex (Table 1). MR scans were performed within 4 to 7 days of admission, and participants in both groups evidenced negative urine screens on the day of the scan. In the same timeframe, the BDI was administered to assess depressive symptoms. In early abstinence, methamphetamine-dependent subjects exhibit depressive symptoms and symptoms of psychosis, both of which largely resolve within a week of abstinence, although drug craving does not significantly decrease from the time of initiating abstinence until the second week (Zorick et al., 2010). The study was approved by the UCLA Office for Protection of Human Subjects.

Magnetic Resonance Acquisition

Proton MRS imaging (MRSI), the multivoxel version of MRS, and prescriptive structural MRI were acquired together in a single session. Acquisitions were performed on a 1.5-T magnetic resonance scanner (Siemens Sonata) using a standard quadrature head coil. In addition to MRSI, scanning sequences included a localizer scout and a high-resolution T1-weighted whole-brain MRI. Water-suppressed PRESS MRSI (repetition time = 1500 ms, echo time = 30 ms, number of excitations = 8, in-plane resolution: 11×11 mm^2, slab thickness: 12 mm for medial parietal or 9 mm for inferior frontal with nominal voxel size 1.4 or 1.1 cc, respectively) was acquired from three 2-dimensional slabs (Figure 1). The first slab was oriented sagitally and straddled the longitudinal midline sampling posterior cingulate cortex ventrally and precuneus dorsally. The posterior cingulate was a mixture of dorsal and ventral posterior cingulate cortex, as defined by Vogt (O'Neill et al., 2009). The second and third slabs were sagittal-oblique (roughly parallel to the ipsilateral temple) in orientation and sampled the left, respectively right, peri-Sylvian region, including inferior frontal gyrus and other nearby cortex. Each of the 3 MRSI slabs was followed by an identical acquisition (only 1 number of excitation) with water-suppression turned off.
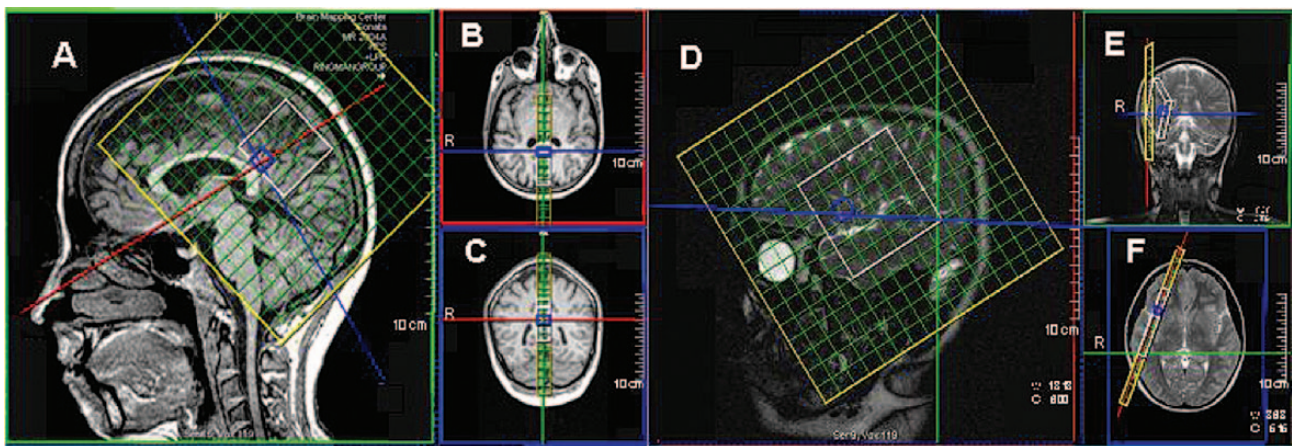

Figure 1. Sagittal (A), axial-oblique (B), and coronal-oblique (C) magnetic resonance imaging (MRI) of the brain showing "medial parietal" magnetic resonance spectroscopy imaging (MRSI) slab. The white square in a is A 12-mm–thick PRESS-select volume, consisting of 4 x 4 MRSI voxels, each 11×11 mm^2 in-plane; the blue square within the white square is one such voxel. The rear edge of the slab was aligned parallel to the parieto-occipital sulcus; the lower edge was parallel or tangent to the dorsal margin of the isthmus or splenium of the corpus callosum. The most ventral voxel row samples posterior cingulate (blue square) and dorsal rows sample mainly precuneus. Sagittal-oblique (D), coronal (E), and axial-oblique (F) MRI depicting positioning of 9-mm–thick MRSI slabs in peri-Sylvian region. The PRESS volume was aligned parallel to the temple, set approximately 2 cm deep into the brain, sized to anatomy, and rotated counter-clockwise parallel to the Sylvian fissure to sample inferior frontal cortex (blue square in D-F).

Table 2. Subject Numbers, MRSI Voxel Tissue Composition and Glx Levels in Target Cortices.

	Posterior Cingulate		Precuneus	
	Methamphetamine	Control	Methamphetamine	Control
subjects	39	17	30	15
gray matter	77.1±2.9	77.5±3.6	73.9±3.3	73.2±2.9
white matter	8.2±4.0	8.2±5.7	7.3±3.5	9.1±3.8
CSF	14.7±4.1	14.2±4.1	18.7±4.0	17.5±4.6
Glx	12.6±1.5*	13.7±1.4	12.2±1.7*	13.2±1.3
	Left Inferior Frontal Cortex		Right Inferior Frontal Cortex	
	Methamphetamine	Control	Methamphetamine	Control
subjects	41	23	38	20
gray matter	73.5±4.9	72.8±5.4	73.4±4.3	73.8±5.3
white matter	10.7±5.9	12.1±5.4	10.7±5.2	10.7±6.0
CSF	15.8±5.4	15.0±4.9	15.8±5.0	15.3±7.5
Glx	12.4±2.2	12.7±2.3	12.7±2.5*	14.6±2.6

Listed are numbers of subjects with usable Glx data in each target cortex.
Gray matter-, white matter-, CSF-content expressed as percent of total MRSI voxel volume.
Glx levels corrected for voxel CSF-content.
Tissue composition and Glx values are group means ± standard deviation.
Significant between-group comparisons (*$P<0.05$, *post-hoc* t-test following R-ANOVA)

Magnetic Resonance Postprocessing

The whole-brain T1-weighted volume of each subject was transformed into ICBM152 stereotaxic space, skull-stripped, and segregated into gray-matter, white-matter, and cerebrospinal fluid (CSF) subvolumes using the Statistical Parametric Mapping 5 software suite. Each whole-brain tissue subvolume was converted into a binary mask and transformed back into native space.

MRSI spectra were automatically fit with LCModel, yielding fitted peaks with absolute metabolite levels referenced to the unsuppressed water resonance and expressed in IU. Fitted peaks included Glx as well as other metabolites, which were not evaluated in this study. Numerous weaker resonances, in particular lipids and macromolecules, were included in the fit. The MRSI Voxel Picker (MVP) software suite (Seese et al., 2011) was used for MRI/MRSI co-processing. The T1-weighted volume and the gray-matter, white-matter, and CSF binary masks were imported into MVP as well as the MRSI raw data file and LCModel output. For each subject's MRSI slab, MVP reconstructed the T1-weighted volume and displayed it in a guided user interface in register with the corresponding MRSI PRESS volume in its plane of acquisition. MVP similarly reconstructed each mask and computed the volume percent gray matter, white matter, and CSF in each MRSI voxel. Then MVP corrected the LCModel-derived levels of each metabolite for voxel CSF content. Quality control of MRSI spectra was also automatically implemented by MVP supplemented by operator inspection. Only those spectra that exhibited linewidth ≤0.1 ppm and signal-to-noise ratio ≥5 were retained. Furthermore, within each voxel, only those Glx values were retained that were considered reliable by LCModel (standard deviation of metabolite signal <20%). Voxels were selected by a diagnosis-blind operator on the MVP guided user interface in left and right hemispheres for inferior frontal cortex, defined using protocols developed at our center (Blanton et al., 2004). Posterior cingulate and precuneal voxels comprised a roughly equal mixture of left and right hemispheres. Within each structure, MVP averaged the metabolite levels for all voxels that contained ≥60 volume percent gray matter and that satisfied the above quality-control criteria.

Statistics

Not all the subjects of Table 1 had usable Glx data for all 4 brain regions. In some cases, data were missing, because the scanning session was aborted early due to technical difficulties or subject compliance. This most affected the medial parietal slab, which was run late in the session. Other data were rejected, because they failed to meet the above-listed quality-control criteria. Consequently, the 2 subject groups were compared for sex, age, and smoking not just for the overall sample (Table 1) but also for each of the 4 regions individually using only the subjects who had usable Glx data for that region. The numbers of subjects with usable data for each region are given in Table 2. The χ^2 test was used to compare sex and independent t test was used to compare all other between-group variables. To assess possible confounds due to partial-volume effects, volume percent gray matter, white matter, and CSF were also compared between groups using independent t test in each individual region. Any of the aforementioned variables significantly differing between the groups was applied as a covariant in subsequent analyses in the relevant brain regions. Finally, mean BDI score was compared between groups for the overall sample using independent t test (Table 1). Mean BDI score was expected to be much higher for the methamphetamine group than for the control group, as acute depression is an established clinical feature of methamphetamine withdrawal (Newton et al., 2004; Zorick et al., 2010). Therefore, BDI score was not used as a covariate in subsequent analyses.

An omnibus approach to multiple comparisons was taken for between-group tests of mean Glx. A repeated-measures analysis of variance was performed with Glx as measure, "region" as within-subjects factor (4 levels for the 4 cortical regions), and "diagnosis" as between-subjects factor (2 levels: methamphetamine and control). This was followed by posthoc t tests comparing mean Glx between the 2 groups in each region. For any region showing a significant between-group difference in Glx, we then ran, within the methamphetamine group, a Spearman correlation of Glx vs duration of methamphetamine abuse in years partialling age. Finally, we ran a Pearson correlation in the

region within the methamphetamine group of Glx vs BDI score. The criterion for statistical significance for all tests was $P < .05$.

Results

Clinical Characteristics

In the overall sample (Table 1), there were no significant between-group differences in sex or mean age, pack-years of cigarettes, or Fagerström score. When retested for subjects having usable Glx data in each of the 4 cortical regions, there were again no significant between-group differences. Therefore, it was not necessary to use any of these variables as a covariate in subsequent analyses. As expected, mean depressive symptoms, as assessed by BDI score, were higher for the methamphetamine (13.3 ± 11.5) than for the control (1.8 ± 2.5) group ($t_{50.0} = 6.3$, $P < .0005$).

MRSI Voxel Tissue Content

There were no significant between-group differences in volume percent gray matter, white matter, or CSF in any region (Table 2). When the 2 subject groups were divided into male and female subgroups and then compared by diagnosis, there were also no significant differences in volume percent gray matter, white matter, or CSF in any region. Note that these volume percentages are amounts of gray matter, white matter, and CSF in the MRSI sampling volume, which typically occupy only a fraction of the target brain region. Thus, these measures are unsuitable to quantify atrophy or other volumetric effects on the region as a whole. Their purpose, rather, is to ensure that apparent between-group differences in metabolite levels are not confounded by between-group differences in MRSI voxel tissue composition.

Regional Glx Levels and Correlations with Duration of Methamphetamine Abuse and BDI

Repeated-measures analysis of variance of Glx levels across the 4 cortical regions yielded a significant main effect of diagnosis ($F_{1,36} = 4.8$, $P = .035$) (Table 2, Figure 2). Posthoc examination of the 4 target regions revealed significantly lower mean Glx for the methamphetamine than the control group in posterior cingulate (39 methamphetamine subjects/17 controls with usable data, 8.1% deficit, $t_{32.5} = 2.7$, $P = .011$), precuneus (30/15, 7.9%, $t_{35.0} = 2.2$, $P = .031$), and right inferior frontal cortex (38/20, 12.8%, $t_{37.7} = 2.6$, $P = .013$), but not in left inferior frontal cortex (Figure 2). When the 2 subject groups were divided into male and female subgroups, similar regional deficits in Glx were observed for the methamphetamine relative to the control group in both sexes in posterior cingulate (male 8.0% deficit, female 7.5%), precuneus (male 6.6%, female 9.7%), and right inferior frontal cortex (male 15.1%, female 9.2%), but not in left inferior frontal cortex (male 3.1% surplus, female 7.2% deficit). For males and females together, there was a notable (15.4%) right > left asymmetry in inferior frontal cortex in Glx within controls (paired $t_{20} = 3.5$, $P = .014$) that was not seen in the methamphetamine group ($t_{37} = 0.8$, $P = .444$).

Within the methamphetamine group, Glx in posterior cingulate decreased with increasing years of abuse ($df = 36$, $r = -0.42$, $P = .008$, partial age) (Figure 2). This relationship was not significant in the other 3 regions. In right inferior frontal cortex, Glx in
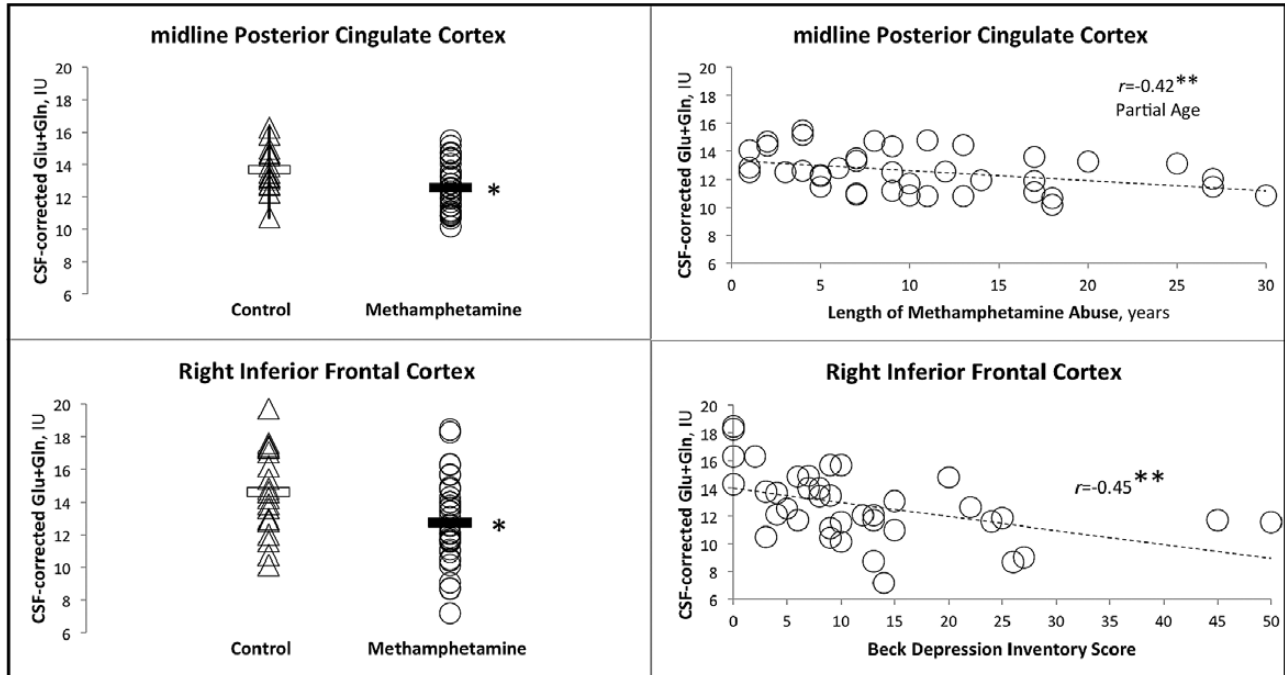

Figure 2. (Left) Levels of Glx (glutamate [Glu] + glutamine [Gln]) measured by magnetic resonance spectroscopy imaging (MRSI) in midline (left + right) posterior cingulate cortex (upper panel) and right inferior frontal cortex (lower panel) in early (4–7 days) abstinent methamphetamine abusers (circles) and age-and sex-matched healthy controls (triangles). Horizontal bars denote group means. Note lower mean Glx in the methamphetamine group in both cortices, 8.1% in midline posterior cingulate ($t_{32.5} = 2.7$, $P = .011$), and 12.8% in right inferior frontal cortex ($t_{37.7} = 2.6$, $P = .013$), suggesting local downregulation of brain glutamatergic systems in early abstinence. A similar Glx deficit (not shown) was observed in precuneus (7.9%, $t_{35.0} = 2.2$, $P = .031$). (Right, upper) Within the methamphetamine sample, Glx in posterior cingulate decreases with increasing years of abuse ($df = 36$, $r = -0.42$, $P = .008$, partial age). (Lower) In right inferior frontal cortex, Glx in the methamphetamine sample decreases with increasing depressive symptoms on the Beck Depression Inventory (BDI; $df = 38$, $r = -0.45$, $P = .005$). Metabolite levels in IU corrected for voxel CSF content. *$P < .05$, **$P < .01$.

the methamphetamine group decreased with increasing depressive symptoms on the BDI ($df=38$, $r=-0.45$, $P=.005$) (Figure 2). This relationship was not significant in the other 3 regions.

Discussion

Cortical Glx Deficits

This is 1 of only 2 proton MRSI studies of the brain in subjects who had chronically abused methamphetamine; the other is by Howells et al. (2014). As noted, several studies used single-voxel proton MRS to investigate effects of methamphetamine abuse on neurometabolites (Ernst et al., 2000; Nordahl et al., 2002 2005; Sekine et al., 2002; Chang et al., 2005; Salo et al., 2007 2011a 2011b; Sung et al., 2007; Taylor et al., 2007; Sailasuta et al., 2010; Cloak et al., 2011; Howells et al., 2014). Most studies predominantly examined patients who had been long-term abstinent from methamphetamine. Similar to Ernst and Chang (2008) and Yang et al. (2013), the former finding diminished Glx in anterior cingulate of methamphetamine-abusing humans and the latter finding it in methamphetamine-exposed monkeys, we focused on the acute phase of abstinence. We used short echo-time MRSI to assay Glx from multiple cortices at high spatial resolution. Poorly accessible inferior frontal cortex was sampled with the help of an innovative sagittal-oblique slab prescription. Voxel tissue-composition was determined and used to mitigate partial-volume effects, yielding high voxel tissue purity (Table 2).

This study yielded 3 major findings. The first was modestly (7–13%) lower levels of Glx in acute abstinence from methamphetamine abuse (vs in the control group) in 3 cerebral cortical regions: posterior cingulate, precuneus, and right inferior frontal cortex. Second, in one of these regions, posterior cingulate, Glx decreased with increasing years of methamphetamine abuse. Third, in another region, right inferior frontal cortex, Glx decreased with increasing depressive symptoms. Taken together, these findings support the notion advanced by Ernst and Chang (2008) of a downregulation of brain glutamatergic systems in the early days of withdrawal from methamphetamine and suggest that this phenomenon is not limited to the anterior middle cingulate.

The finding of a deficit in Glx in posterior cingulate, precuneus, and right inferior frontal cortex of methamphetamine abusers (Table 2, Figure 2) extends prior work with imaging modalities other than MRSI. In posterior cingulate and precuneus, [18]FDG-PET previously demonstrated elevated glucose metabolism in methamphetamine-abusing subjects following up to 14 days (London et al., 2004) or 14 days to 2 years (Volkow et al., 2001; Berman et al., 2008) abstinence from drug. In combination with these findings, the present results reinforce the view that methamphetamine abuse is associated with dysfunction in brain areas, such as medial parietal cortex, with sparse dopaminergic innervation (Descarries et al., 1987). Glx in the methamphetamine group also was below control levels in the right inferior frontal cortex. Notably, this deficit reflected the absence of a right>left excess of Glx seen in the controls (Table 2). The right inferior frontal gyrus has been linked to response inhibition (Aron et al., 2014; but see Swick et al., 2008), a cognitive function thought to be impaired in methamphetamine abuse (Monterosso et al., 2005; for review, see Baicy and London 2007), and to methamphetamine craving as well as emotion regulation (Tabibnia et al., 2011). Although we are unaware of previous MRS studies sampling inferior frontal cortex in subjects who had abused methamphetamine, in our previous published work, we have observed deficits in gray matter in this region in such subjects

(Thompson et al., 2004; Tabibnia et al., 2011). We did not observe Glx effects in left inferior frontal cortex. The present findings add to evidence that inferior frontal cortex is particularly affected by methamphetamine abuse and/or withdrawal and support our previous observations of a lateralization of methamphetamine-associated pathology (Thompson et al., 2004; Morales et al., 2012), perhaps related to the imputed lateralization of function (motor language, response inhibition) in this region.

Our second major finding was that, within the methamphetamine group, Glx in posterior cingulate decreased with increasing years of abuse. In anterior middle cingulate, Ernst and Chang (2008) found no significant relationship between Glx and methamphetamine abuse, operationalized as the logarithm of cumulative lifetime exposure. This negative result fortified their argument that low Glx levels are a more direct effect of withdrawal than of abuse per se. Our result does not disagree with this interpretation, because if a drop in cortical Glx is induced by withdrawal, a greater drop might be seen following more severe prewithdrawal abuse. It also may be noteworthy that although Ernst and Chang (2008) assessed Glx in the anterior middle cingulate cortex, the finding here was related to an observation in posterior cingulate.

The observation that Glx in the inferior frontal cortex of the right hemisphere was negatively associated with BDI score is of interest in the context of recent research that has been uncovering links between depression and central glutamatergic systems, including rapid responses to treatments for otherwise refractory depression (Sanacora et al., 2011; Zarate et al., 2013). These treatments include most notably NMDA receptor antagonists such as ketamine. Within the narrower context of methamphetamine use disorders, severity of depressive symptoms positively covaried with relative CMRglc in subgenual and pregenual anterior cingulate cortices during early abstinence from the drug (London et al., 2004), whereas BDI score covaried with relative CMRglc in right inferior frontal cortex within a healthy control group. Cortical volume in right inferior frontal gyrus and in multiple right-hemisphere, cingulate subregions also was below control values in a partially overlapping sample of methamphetamine users (Thompson et al., 2004).

Since the discovery of the "catastrophic reaction" following left-hemisphere middle cerebral artery infarcts (Goldstein 1939), the inferior frontal cortex has been linked with the lateralization of emotion in as yet unclear ways, although the pars orbicularis of this region has been implicated in inhibitory control in multiple modalities, including emotion regulation (Tabibnia et al., 2011). The right hemisphere is thought to dominate over the left in the expression of all or of only negative emotions (Hellige, 1993), with right-hemisphere overactivation possibly generating negative emotions (Davidson 1992), or depression impairing right-hemisphere function (Tucker, 1987). The present Glx result adds to evidence associating depression with inferior frontal lateralization. Moreover, it points to a linkage between depression in acute withdrawal from methamphetamine and Glu metabolism, although depressive symptoms may predate and possibly predispose an individual to methamphetamine use. As conventional serotonergic agents perform poorly in treating the depression of methamphetamine withdrawal, the poor response to serotonergic treatment correlating with high craving for methamphetamine and relapse (Zorick et al., 2011), glutamatergic alternatives might be useful for treating this condition. We are unaware of published or ongoing clinical trials or case studies involving glutamatergic drugs for methamphetamine use disorders, although there is some relevant preclinical work (Carroll, 2008; Iijima et al., 2013).

Ernst and Chang (2008) reported reduced Glx in anterior middle cingulate of methamphetamine users, including measurements during the early abstinence phase examined in the present study. Since the anterior middle cingulate was not sampled in the present study, it is not known whether the methamphetamine sample exhibited below-normal Glx in anterior middle cingulate along with the other brain regions, although it is possible. In any case, the mechanisms proposed by Ernst and Chang (2008) to explain reduced Glx in anterior middle cingulate of patients with methamphetamine use disorder also likely apply in the cortical regions investigated here. Briefly, the mechanisms include loss of glutamatergic neurons, decreased Glu synthesis by the Krebs cycle (inhibited mitochondrial function), reduced astrocyte uptake and recycling of Glu as Gln, and increased demand for Glu and Gln as amino acids to repair cell structures damaged by methamphetamine. Thus, there may be multiple, possibly overlapping, pathophysiological pathways to low cortical Glx following methamphetamine abuse. Regarding the medial parietal regions, posterior cingulate, and precuneus particularly, as discussed by Volkow et al. (2001), animal studies indicate that the parietal cortex is especially sensitive to methamphetamine toxicity (Pu et al., 1996), including damage to pyramidal glutamatergic cells (Commins and Seiden, 1986; Ryan et al., 1990). Methamphetamine also upregulates Glu receptors in parietal cortex (Eisch et al., 1996), increasing their sensitivity to glutamatergic excitotoxicity. These factors may contribute to regional drop in Glx. A further factor is methamphetamine toxicity mediated by σ-receptors (Kaushal and Matsumoto, 2011), which have high density in posterior cingulate (Stone et al., 2006).

This study has limitations. MRSI was acquired at 1.5 T, making segregation of Glx into Glu and Gln more difficult. Although we believe that presentation of MRS Glu results is defensible even at 1.5 T, we opted for Glx as a metric of regional glutamatergic metabolism in this report, since the motivating results of Ernst and Chang (2008) and Yang et al. (2013) were expressed as Glx. Further work at high field strength is nonetheless encouraged. Due to incomplete acquisition and rejection of data not meeting spectral quality or tissue-content standards, Glx values were missing for a number of subjects in some brain regions. Actual subject numbers analyzed for each region are indicated in Table 2. Potential confounds arising from this matter were addressed by confirming that the 2 subject groups were still balanced for sex, age, smoking, and MRSI voxel tissue-content in each region individually after accounting for these missing values. Whereas single-voxel MRS typically offers more rapid acquisition with higher signal-to-noise ratio, MRSI has the advantage of smaller voxel size and hence lower chance of confounding of results due to partial-volume effects. Our study also has certain uncommon strengths, one of these being matching of subject groups for tobacco smoking. Such matching may be critical for any study of glutamatergic compounds in methamphetamine abuse. Our work (O'Neill et al., 2014) suggests that cigarette smoking, extremely prevalent among methamphetamine abusers, is associated with low Glx in at least one brain region, the thalamus. Overall, the present investigation supports the notion of downregulation of glutamatergic metabolism in multiple cerebral cortices in very early abstinence from methamphetamine; low Glx may be associated with the depressive component of the methamphetamine withdrawal syndrome, which is presumably an important driver of relapse.

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Statement of Interest

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References

Aron AR, Robbins TW, Poldrack RA (2014) Inhibition and the right inferior frontal cortex: one decade on. *Trends Cogn Sci* 18:177–185

Baicy K, London ED (2007) Corticolimbic dysregulation and chronic methamphetamine abuse. *Addiction* 102(Suppl 1):5–15.

Beck AT, Steer RA, Ball R, Ranieri W (1996) Comparison of Beck Depression Inventories -IA and -II in psychiatric outpatients. *J Person Assess* 67:588–97.

Berman SM, Voytek B, Mandelkern MA, Hassid D, Isaacson A, Monterosso J, Miotto K, Ling W, London ED (2008). Changes in regional cerebral metabolism during early abstinence from chronic methamphetamine abuse. *Molec Psychiatry* 13:897–908.

Blanton RE, Levitt JG, Peterson JR, Fadale D, Sporty ML, Lee M, To D, Mormino EC, Thompson PM, McCracken JT, Toga AW (2004) Gender differences in the left inferior frontal gyrus in normal children. *NeuroImage* 22:626–636.

Brecht ML, von Mayrhauser C, Anglin MD (2000) Predictors of relapse after treatment for methamphetamine use. *J Psychoactive Drugs* 32:211–220.

Carroll FI (2008) Antagonists at metabotropic glutamate receptor subtype 5: structure activity relationships and therapeutic potential for addiction. *Ann NYAS* 1141:221–232.

Chang L, Ernst T, Speck O, Grob CS (2005) Additive effects of HIV and chronic methamphetamine use on brain metabolite abnormalities. *Am J Psychiatry* 114:65–79.

Cloak CC, Alicata D, Chang L, Andrews-Shigaki B, Ernst T (2011) Age and sex effects of choline compounds in the anterior cingulate cortex of adolescent methamphetamine users. *Drug Alcohol Depend* 119:207–215.

Commins DL, Seiden LS (1986) Alpha-methyltyrosine blocks methylamphetamine-induced degeneration in the rat somatosensory cortex. *Brain Res* 365:15–20

Davidson RJ (1992) Emotion and affective style: hemispheric substrates. *Psych Sci* 3:39–43.

Descarries L, Lemay B, Doucet G, Berger B (1987) Regional and laminar density of the dopamine innervation in adult rat cerebral cortex. *Neurosci* 21:807–824.

Eisch AJ, O'Dell SJ, Marshall JF (1996) Striatal and cortical NMDA receptors are altered by a neurotoxic regimen of methamphetamine. *Synapse* 22:217–225

Elkashef AM, Rawson RA, Anderson AL, Li S-H, Holmes T, Smith EV, Chiang N, Kahn R, Vocci F, Ling W, Pearce VJ, McCann M, Campbell J, Gorodetzky C, Haning W, Carlton B, Mawhinney J, Weis D (2007) Bupropion for the treatment of methamphetamine dependence. *Neuropsychopharmacology* 33:1162–1170.

Ernst T, Chang L (2008) Adaptation of brain glutamate plus glutamine during abstinence from chronic methamphetamine use. *J Neuroimmun Pharmacol* 3:165–172.

Ernst T, Chang L, Leonido-Yee M, Speck O (2000) Evidence for long-term neurotoxicity associated with methamphetamine abuse: a 1H MRS study. *Neurology* 54:1344–1349.

Goldstein K (1939) *The organism*. New York: American Books.

Gonzales R, Mooney L, Rawson RA (2010) The methamphetamine problem in the United States. *Annu Rev Public Health* 31:385–398.

Hellige JB (1993) *Hemispheric asymmetry*. Cambridge, MA: Harvard University Press.

Howells FM, Uhlmann A, Temmingh H, Sinclair H, Meintjes E, Wilson D, Stein DJ (2014) ^{1}H-magnetic resonance spectroscopy (^{1}H-MRS) in methamphetamine dependence and methamphetamine induced psychosis. *Schiz Res* 153:122–128.

Iijima M, Koike H, Chaki S (2013) Effect of an mGlu2/3 receptor antagonist on depressive behavior induced by withdrawal from chronic treatment with methamphetamine. *Behav Brain Res* 246:24–28.

Kaushal N, Matsumoto RR (2011) Role of sigma receptors in methamphetamine-induced neurotoxicity. *Curr Neuropharmacol* 9:54–57.

Kim I-S, Kim Y-T, Song H-J, Lee J-J, Kwon D-H, Lee HJ, Kim M-N, Yoo D-S, Chang Y (2009) Reduced corpus callosum white matter microstructural integrity revealed by diffusion tensor eigenvalues in abstinent methamphetamine addicts. *Neurotoxicol* 30:209–213.

Kim SJ, Lyoo IK, Hwang J, Chung A, Sung YH, Kim J, Kwon D-H, Chang KH, Renshaw PF (2005) Prefrontal grey-matter changes in short-term and long-term abstinent methamphetamine abusers. *Int J Neuropsychopharmacol* 8:1–8.

London ED, Simon S, Berman SM, Mandelkern MA, Lichtman AM, Bramen J, Shinn AK, Miotto K, Learn J, Dong Y, Matochik JA, Kurian V, Newton T, Woods R, Rawson R, Ling W (2004) Mood disturbances and regional cerebral metabolic abnormalities in recently abstinent methamphetamine abusers. *Arch Gen Psychiatry* 61:73–84.

Monterosso JR, Aron AR, Cordova X, Xu J, London ED (2005) Deficits in response inhibition associated with chronic methamphetamine abuse. *Drug Alcohol Depend* 79:273–277.

Morales A, Lee B, Hellemann G, O'Neill J, London ED (2012) Gray-matter volume in methamphetamine dependence: cigarette smoking and changes with abstinence from methamphetamine. *Drug Alcohol Depend* 125:230–238.

Newton TF, Kalechstein AD, Duran S, Vansluis N, Ling W (2004) Methamphetamine abstinence syndrome: preliminary findings. *Am J Addiction* 13:248–255.

Nordahl TE, Salo R, Possin K, Gibson DR, Flynn N, Leamon M, Galloway GP, Pfefferbaum A, Spielman DM, Adalsteinsson E, Sullivan E (2002) Low N-acetyl-aspartate and high choline in the anterior cingulum of recently abstinent methamphetamine-dependent subjects: a preliminary proton MRS study. *Psychiatry Res Neuroimaging* 116:43–52.

Nordahl TE, Salo R, Natsuaki Y, Galloway GP, Waters C, Moore CD, Kile S, Buonocore MH (2005) Methamphetamine users in sustained abstinence: a proton magnetic resonance spectroscopy study. *Arch Gen Psychiatry* 62:444–452.

O'Neill J, Sobel TL, Vogt BA (2009) Localizing cingulate subregions-of-interest in magnetic resonance images guided by cytological parcellations. In: *Cingulate neurobiology and disease* (Vogt BA, ed), pp 587–617. Oxford: Oxford University Press.

O'Neill J, Tobias M, Hudkins M, Oh E, Hellemann G, Nurmi E, London ED (2014) Thalamic glutamate decreases with cigarette smoking. *Psychopharmacology (Berl)* 231:2717–2724.

Pu C, Broening HW, Vorhees CV (1996) Effect of methamphetamine on glutamate-positive neurons in the adult and developing rat somatosensory cortex. *Synapse* 23:328–334.

Rawson RA, Marinelli-Casey P, Anglin MD, Dickow A, Frazier Y, Gallagher C, Galloway GP, Herrell J, Huber A, McCann MJ, Obert J, Pennell S, Reiber C, Vandersloot D, Zweben J, Methamphetamine Treatment Project Cooperative Authors (2004) A multi-site comparison of psychosocial approaches for the treatment of methamphetamine dependence. *Addiction* 99:708–717.

Ryan LJ, Linder JC, Martone ME, Groves PM (1990) Histological and ultrastructural evidence that D-amphetamine causes degeneration in neostriatum and frontal cortex of rats. *Brain Res* 518:67–77.

Sailasuta N, Abulseoud O, Hernandez M, Haghani P, Ross BD (2010) Metabolic abnormalities in abstinent methamphetamine dependent subjects. *Subst Abuse* 2010:9–20.

Salo R, Nordahl TE, Natsuaki Y, Leamon MH, Galloway GP, Waters C, Moore CD, Buonocore MH (2007) Attentional control and brain metabolite levels in methamphetamine abusers. *Biol Psychiatry* 61:1272–1280.

Salo R, Buononcore MH, Leamon M, Natsuaki Y, Waters C, Moore CD, Galloway GP, Nordahl TE (2011a) Extended findings of brain metabolite normalization in MA-dependent subjects across sustained abstinence: a proton MRS study. *Drug Alcohol Depend* 113:133–138.

Salo R, Nordahl TE, Buononcore MH, Natsuaki YT, Moore CD, Waters C, Leamon MH (2011b) Spatial inhibition and the visual cortex: a magnetic resonance spectroscopy imaging study. *Neuropsychologia* 49:830–838.

Sanacora G, Treccani G, Popoli M (2011) Towards a glutamate hypothesis of depression: an emerging frontier of neuropsychopharmacology for mood disorders. *Neuropharmacol* 62:63–77.

Seese RR, O'Neill J, Hudkins M, Siddarth P, Levitt J, Tseng B, Wu KN, Caplan R (2011) Proton magnetic resonance spectroscopy and thought disorder in childhood schizophrenia. *Schiz Res* 133:82–90.

Sekine Y, Minabe Y, Kawai M, Suzuki K, Iyo M, Isoda H, Sakahara K, Ashby CR Jr, Takei N, Mori N (2002) Metabolite alterations in basal ganglia associated with methamphetamine-related psychiatric symptoms: a proton MRS study. *Neuropsychopharmacol* 27:453–461.

Shoptaw SJ, Kao U, Heinzerling K, Ling W (2009) Treatment for amphetamine withdrawal. *Cochrane Database Syst Rev* 2:1–27.

Sibson NR, Shen J, Mason GF, Rothman DL, Behar KL, Shulman RG (1998) Functional energy metabolism: in vivo 13C-NMR spectroscopy evidence for coupling of cerebral glucose consumption and glutamatergic neuronal activity. *Devel Neurosci* 20:321–330.

Stone JM, Årstad E, Erlandsson K, Waterhouse RN, Ell PJ, Pilowsky LS (2006) [^{123}I]TPCNE—A novel SPET tracer for the sigma-1 receptor: first human studies and in vivo haloperidol challenge. *Synapse* 60:109–117.

Swick D, Ashley V, Turken AU (2008) Left inferior frontal gyrus is critical for response inhibition. *BMC Neurosci* 9:102.

Sung YH, Cho SC, Hwang J, Kim SJ, Kim H, Bae S, Kim N, Chang KH, Daniels M, Renshaw PF, Lyoo IK (2007) Relationship between N-acetyl-aspartate in gray and white matter of abstinent methamphetamine abusers and their history of drug abuse: a proton magnetic resonance spectroscopy study. *Drug Alcohol Depend* 88:28–35.

Tabibnia G, Monterosso JR, Baicy K, Aron AR, Poldrack RA, Chakrapani S, Lee B, London ED (2011) Different forms of self-control share a neurocognitive substrate. *J Neurosci* 31:4805–4810.

Taylor MJ, Schweinsburg BC, Alhassoon OM, Gongvatana A, Brown GG, Young-Casey C, Letendre SL, Grant I, HNRC Group (2007) Effects of human immunodeficiency virus

and methamphetamine on cerebral metabolites measured with magnetic resonance spectroscopy. *J Neurovirol* 13:150–159.

Thompson PM, Hayashi K, Simon SL, Geaga JA, Hong MS, Sui Y, Lee JY, Toga AW, Ling W, London ED (2004) Structural abnormalities in the brains of human subjects who use methamphetamine. *J Neurosci* 24:6028–6036.

Tobias MC, O'Neill J, Hudkins M, Bartzokis G, Dean AC, London ED (2010) White-matter abnormalities in brain during early abstinence from methamphetamine abuse. *Psychopharmacol* 209:13–24.

Tucker DM (1987) Hemisphere specialization: a mechanism for unifying anterior and posterior brain regions. In: *Duality and unity of the brain* (Ottoson D, ed). London: MacMillan Press.

Volkow ND, Chang L, Wang GJ, Fowler JS, Franceschi D, Sedler MJ, Gatley SJ, Hitzemann R, Ding YS, Wong C, Logan J (2001) Higher cortical and lower subcortical metabolism in detoxified methamphetamine abusers. *Am J Psychiatry* 158:383–389.

Yang S, Belcher AM, Chefer S, Vaupel DB, Schindler CW, Stein EA, Yang Y (2013) Withdrawal from long-term methamphetamine self-administration 'normalizes' neurometabolites in rhesus monkeys: a ^{1}H MR spectroscopy study. *Addict Biol* doi:10.1111/adb.12078.

Zachary RA, Paulson MJ, Gorsuch RL (1985) Estimating WAIS IQ from the Shipley Institute of Living Scale using continuously adjusted age norms. *J Clin Psychol* 41:820–831.

Zarate C, Duman RS, Liu G, Sartori S, Quiroz J, Murck H (2013) New paradigms for treatment-resistant depression. *Ann NYAS* 1292:21–31.

Zorick T, Nestor L, Miotto K, Sugar C, Hellemann G, Scanlon G, Rawson R, London ED (2010) Withdrawal symptoms in abstinent methamphetamine-dependent subjects. *Addiction* 105:1809–1818.

Zorick T, Sugar C, Hellemann G, Shoptaw S, London ED (2011) Poor response to sertraline in methamphetamine dependence is associated with sustained craving for methamphetamine. *Drug Alcohol Depend* 118:500–503.