

Metabolic Abnormalities in Abstinent Methamphetamine Dependent Subjects

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Abstract

Introduction: Chronic methamphetamine use results in persistent neuropsychological deficits in abstinent methamphetamine dependent (AMD) subjects. We examined the hypothesis that elevated concentration of cerebral glutamate (Glu), an excitatory neurotransmitter and neurotoxin, occurs in human AMD.

Materials and Methods: We examined 40 subjects, 18 of whom were AMD, abstinent more than 3 weeks and 22 were age matched controls. A Structured Clinical Interview was applied to exclude AMD with comorbid depression. We used TE-Averaged technique of MRS to uniquely identify and quantify the glutamate resonance at 2.35 ppm on a 3T clinical MR scanner. Statistics, including Bonferroni correction for multiple MRS variables were applied.

Results: Glu was significantly higher in frontal white matter of AMD (+19%, $P = 0.01$) and N-acetylaspartate (NAA), an axonal marker, was lower (-14%, $P = 0.004$). No significant MRS abnormalities were detected in posterior gray matter. Significant correlations were observed between NAA and Glu ($P = 0.002$ for AMD and $P = 0.06$ for controls in the posterior gray matter and $P = 0.01$ for controls and not significant for AMD in the frontal white matter).

Conclusion: Our results demonstrate a significant excess of glutamate in frontal white matter of AMD subjects and offer support for the hypothesis that methamphetamine abuse may exert its long-term neuro-toxicity via glutamate.

Keywords: (3–5) methamphetamine, proton MRS, glutamate, white matter, TE-average

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Introduction

Methamphetamine, an increasingly prevalent drug of abuse in humans, is an excitatory neural stimulant. Direct evidence that glutamate (Glu), the dominant excitatory neurotransmitter in the human brain, plays any role in addiction or contributes to the long-lasting neuropsychological effects of methamphetamine abuse is lacking.¹⁻⁴ However, in preclinical studies, the vesicular glutamate transporter has been shown to facilitate accumulation of Glu after exposure to methamphetamine.⁵ Attempts to explore the impact of methamphetamine abuse on human brain glutamate have been hampered by the absence of a reliable technique for glutamate quantification. Most early studies used proton MRS which at low magnetic fields (typically 1.5 Tesla) relies upon a combined measurement of glutamate and glutamine (termed Glx). These two brain metabolites, although closely linked in the glutamine-glutamate cycle of glutamate neurotransmission, represent separate cell populations and their cerebral concentrations can change independently and in opposite directions.^{6,7} The detection of uncontaminated Glu has improved with high field ¹H MRS⁸⁻¹¹ and the MRS-Glx assay has now been superseded by a specific improvement in proton MRS at higher magnetic fields, such as 3 Tesla, whereby brain glutamate can be clearly separated.^{8,11}

While the acute neuropsychological effects of methamphetamine are global and include psychosis, mania and delirium,¹² the long-term deleterious effects of methamphetamine appear to be regionally selective. Neuroimaging studies with positron emission tomography (PET) and functional MRI reveal metabolic and physiological abnormalities in the frontal brain of substance abusers,¹³⁻¹⁶ and it has been suggested that the frontal brain structures are closely involved in craving and continued drug use.^{17,18}

In addition, it has been hypothesized that brain damage resulting from methamphetamine abuse outlives the duration of drug use and can be documented objectively by structural and chemical changes in abstinent methamphetamine dependent subjects.¹⁹⁻²⁴ Specifically, enlarged brain volume was observed in recently abstinent (<4 months) methamphetamine users and relatively normal volume with longer than 20 months of abstinence. MRS of N-acetylaspartate (NAA), an axonal marker in white matter and a neuronal marker in gray matter, indicates a significant

decrease in NAA levels in abstinent methamphetamine dependent subjects^{20,24-26} whose abstinence periods were between 8 to 20 weeks, consistent with neuronal and/or axonal damage which lasts beyond the immediate period of drug abuse. Furthermore, reduction of Glx (glutamine plus glutamate), in the frontal brain was observed in early abstinent methamphetamine users, followed by relatively normal Glx concentration after 2 months of abstinence.¹⁹

If glutamate is an important component of the cycle of methamphetamine abuse, craving, abstinence, and recovery, then significant changes in brain glutamate concentration should be detectable and may persist when drug abuse ends. This study aimed to determine whether brain glutamate is increased in frontal white matter of AMD subjects.

Materials and Methods

Participants

The study was approved by the Internal Review Boards of Huntington Memorial Hospital and Keck School of Medicine, University of Southern California. All subjects gave their written consent before participating in the study. A total of 25 AMD subjects were recruited, 7 subjects were excluded from the study due to depressive symptoms (N = 6) and HIV seropositive (N = 1). Twenty-two age and gender-matched controls with no history of drug abuse were also recruited.

Participants who completed the entire study received modest cash compensation to cover their transportation and other inconveniences. Based on prior studies on the effects of drug abstinence on brain metabolite concentrations, referenced in the Introduction section, AMD subjects were subdivided according to duration of abstinence, defined as 'short-term' (3-8 weeks: N = 7) and 'long-term' (more than 20 weeks: N = 11). All subjects were recruited by the principle investigator (NS) and were screened for eligibility by a research psychiatrist (OA).

Control subjects

Non-drug users were recruited from the local community by word-of-mouth and excluded if they reported any lifetime alcohol or other substance abuse or met DSM-IV criteria for any other axis I diagnosis, including depression.



Methamphetamine dependence and abstinence

Subjects were recruited from local drug rehabilitation centers and Drug Enforcement Administration Centers in Los Angeles County (“half-way houses”) created for individuals recently released from incarceration for drug-related charges. Other sources of subject recruitment were drug counseling centers, which have random and routine weekly urine screenings. Methamphetamine can be detected in the urine between 2–7 hours after intake and up to 4 days. Accurate determination of abstinence was nevertheless subjective relying on a self-reporting questionnaire and the Structured Clinical Interview for DSM-IV (SCID).²⁷ Drug-use history was obtained by an additional questionnaire that inquired about the number of years of methamphetamine use, frequency of use, amount used each time, the last time of usage (abstinence period), as well as any other illicit drug use. Consistent with the majority of clinical surveys, a small minority, 4 of 18 AMD subjects, reported occasional use of other recreational drugs cocaine (N = 1), marijuana (N = 2) and alcohol (N = 1). In each case, the second drug use was minor and abstinence period was longer than a one-year. Thus, all abstinent methamphetamine dependent participants in the present study, listed methamphetamine as their primary drug of choice, and none met the DSM-IV criteria for dependence on any other substance within the past year. Current methamphetamine users were intentionally excluded from our cohort to avoid drug-induced psychosis, a common feature of active methamphetamine users, which is a possible confound in MRS results. Also excluded were any AMD subjects who met DSM-IV criteria for any major depressive disorder. Co-morbid depression could have introduced a significant confound, since in several prior published studies a variety of MRS abnormalities, including Glx, have been reported.²⁸

Neuropsychological testing was conducted by a trained, supervised research coordinator (MH). Test battery was designed to test the following cognitive domains: pre-morbid intelligence using the National Adult Reading Test (NART); attention and psychomotor speed using Symbol Digit Modalities, the Grooved pegboard, and the Trail Making A tests; cognitive flexibility and response inhibition, using

the Trail Making Test B and the Stroop Interference; learning and memory, using the Rey Auditory Verbal Learning Test (RAVLT).²⁹

MR spectroscopy

Examinations were performed on a GE 3T whole body MR scanner, as previously described¹¹ using an 8-channel head coil for signal reception and body coil for transmission. High resolution, axial T1-weighted spoiled gradient echo images were acquired and used for image segmentation of voxel composition, and for prescription of single voxel MR spectroscopy. Two 8 cc volumes were placed in the right frontal white matter (FWM) and the posterior gray matter (PGM). For the specific purposes of the present study, brain glutamate and other metabolites were determined by using multiple echo times (TE) single voxel PRESS sequence (TE-Averaged PRESS, GE HealthCare, software version 12.0) with acquisition of 32 echo times in one scan session starting from TE = 35 ms and ending at TE = 190 ms. Spectral bandwidth was set to 5000 Hz (standard for GE 3T) with the number of spectral points at 2048 and the repetition time at 1.5 s. Sixteen scans of unsuppressed water free induction decays (FIDs) were acquired together with the suppressed FIDs (eight averages for each echo time) and used for eddy current correction and internal water scaling. Total acquisition time for MRI and two voxel MRS examinations was 40 minutes per subject. To avoid chemical shift dependent spatial mis-registration for Glu, the transmit frequency was centered close to that of the refocused glutamate C4 proton resonance, at 2.35 ppm. Magnetic field homogeneity within the prescribed voxel was performed using the manufacturer’s AUTOSHIM routine and accepted when line width at half maximum height of unsuppressed water resonance was 6–10 Hz. This was within accepted limits for both voxels in all of the 40 subjects, so that no MRS data was rejected on the grounds of poor shimming. Modern MRI employs parallel imaging technology, which results in added complexity for MRS studies. The present study employed the standard 8-channel head coil. As a result, there were eight separate single-voxel TE-Averaged point-resolved spectroscopy data sets for each brain location. To maximize the MRS signal, these raw data sets were then combined in the time domain^{30,31} on the basis of



coil sensitivity. Coil sensitivity was determined from the unsuppressed water signal intensities of the individual coils and weighted by the sum of the squares of the signal intensity from each coil.

MRS data analysis

After MRI and MRS, raw data was stored for quantitative analysis. The eighty spectra were processed blind to their diagnosis. Twenty-two controls (10 F, 12 M; 31.9 ± 9.7 years) provided a single group. The eighteen AMD subjects (7 F, 11 M; 35.0 ± 9.3 years) were analyzed initially as a single group.

Quantitative MRS assay of glutamate

Metabolite concentrations were quantified using a modification of the standard LCModel³² developed in this Laboratory and also previously described.¹¹ A set of *in vitro* reference solutions (basis sets) was prepared from which spectra were acquired using the same MRS data acquisition as *in vivo*. The standard solutions were 50 mM N-acetylaspartate (NAA), 100 mM creatine (Cr), 50 mM choline (Cho), 100 mM glutamate, 100 mM glutamine (Gln), and 150 mM myo-inositol (mI). All standard solutions were adjusted to pH 7.2 with 0.1 M NaOH. To allow for differences in MR-scanner characteristics, which include variations in hardware and software, between the acquisition of the basis sets and the *in vivo* spectra, the LCModel uses a calibration factor to scale the *in vitro* basis set to the *in vivo* spectra. The calibration factor was obtained by using the LCModel to estimate the known concentration of the NAA solution employing the same basis set that was used for *in vivo* quantification. The same calibration factor was then used for all data sets. Metabolite concentrations were corrected for T2 relaxation using the following correction factor: $f = \exp(TE_{\text{avg}} * (1/T2_{\text{met}} - 1/T2_{\text{in vitro}}))$, where TE_{avg} is the averaged TE (112.5 ms), $T2_{\text{met}}$ is T2 relaxation of the metabolite, and $T2_{\text{in vitro}}$ is the T2 of the *in vitro* reference solution. A total of six spectra were excluded from the final analysis due to glutamate signal-to-noise ratio ≤ 4 (insufficient information), percent standard deviation (%SD) $> 30\%$, and technical failures.

MRI segmentation

Because the metabolite concentrations of white and gray matter are known to differ, it was necessary

to define possible differences in voxel composition between AMD subjects and controls from which a correction could be applied, based upon the relative contributions of each tissue type to the nominal 'white' and 'gray' matter. An automated MRI threshold segmentation tool BioImage Suite from Oxford Center for Functional MRI of the Brain (FAST^{33,34}) was applied to the images of each subject. The MRI-segmentation method also allowed for the calculation of the contribution of cerebrospinal fluid (CSF), yielding similar results to that of the T2-7 point technique.³⁵ Brain metabolite concentrations in the voxel were corrected for CSF-partial volume, within which the important metabolite concentrations were deemed to be zero on the basis that cerebral metabolites are below the limits of MRS-detection, using $\text{Glu}_{\text{corr}} = (\text{Glu} * 100) / (100 - \text{percent CSF})$.

Statistical analysis

A statistical analysis was conducted using Statview software (version 5.0, SAS Institute). For comparison of the mean values of the metabolite concentrations between AMD subjects and controls, the Student's *t*-test was used. The Bonferroni correction was then applied to the MRS data to correct for possible Type 1 error where metabolite measurements are potentially interdependent. Nonparametric correlations techniques were used to examine the relationship between metabolite concentrations for each of the voxel locations and years of methamphetamine use, total methamphetamine use and duration of abstinence. In addition, correlations between neurochemical abnormalities and abnormalities in individual neuropsychological tests were performed. In all analyses, the level of significance was set at $P < 0.05$.

Results

Demographic characteristics of study participants are given in Table 1. There were no significant differences in age (mean = 35 years old, 95% Confidence interval = 30.7–39.4 for AMD subjects and mean = 31.9 years old, 95% Confidence interval = 27.9–35.9 for controls) and sex composition ($P = 0.47$) between AMD subjects and healthy controls.

Figure 1 illustrates the locations of the FWM and the PGM voxels examined in this study. Representative TE-Averaged MRS brain spectra from a control and an AMD subject are shown in

Table 1. Demographics of participants in MRS study.

	Abstinent methamphetamine dependent (N = 18)	Controls (N = 22)	P
Age (years)	35.0 ± 9.3	31.9 ± 9.7	NS
Gender (female/male)	7/11	10/12	NS
Education (years)	13.0 ± 3.0	16.0 ± 2.2	<0.05
Years of meth used	10.2 ± 6.7	N/A	N/A
Total meth used (gm)	4448 ± 5120	N/A	N/A
Duration of abstinence	All (N = 18) Short duration, 3–8 weeks (N = 7) Long-term duration, >20 weeks (N = 11)	N/A	N/A

Demographics apply to 40 subjects who completed the study with appropriate MRS protocol and for whom MRS data is provided in Table 2. Four methamphetamine dependent subjects reported mild poly-drug use, none of whom reached criteria for dependency on any substance other than methamphetamine. Values are mean ± standard deviation.

Abbreviations: NS, not significant, N/A, not applicable.

Figure 2. The resulting spectra differ in overall appearance from the conventional single echo time acquisitions at short (TE 35 ms) echo times. In the TE-Averaged data acquisition of the present study, myo-inositol was the only resonance of importance which was not captured, while the glutamate resonance at 2.35 ppm appears as a well-resolved single peak in all four spectra. Glutamate appears more prominent in the representative spectrum from the FWM of an AMD subject (Fig. 2D) than in the FWM of a representative control subject (Fig. 2C).

Differences between AMD and control subjects, which were not readily discerned in individual spectra, became apparent and statistically significant after quantitative MRS analysis (Table 2). Glutamate was significantly increased by 19% in the FWM of

the AMD group compared to controls (6.8 mM vs. 5.7 mM, $P = 0.01$). NAA was significantly reduced (8.0 mM vs. 9.3 mM, $P = 0.004$). No significant differences between AMD and control subjects were found in the PGM. There were no significant differences between controls and AMD subjects for Cho and Cr in the FWM or the PGM.

Clinical characteristics and metabolite concentrations

The effects on brain glutamate found for the AMD group as a whole was observed. However, when the group was subdivided on the basis of duration of abstinence, only short-term abstinence reach statistical significance after the Bonferroni correction was applied (Table 2). During short-term abstinence 3–8 weeks; (8.2 vs. 9.3; $P = 0.03$), the 12% reduction of NAA of the FWM was significant, but the 16% reduction was persistently observed after >20 weeks long-term abstinence ($P = 0.01$) suggesting, as others have observed, a long-term axonal (or neuronal) deficit in AMD subjects. The impression that both neurochemical changes persist and are directly related to the duration of abstinence is supported by a plot of metabolite concentration versus weeks of abstinence for AMD subjects (Fig. 3) which shows a significant correlation for Glu (Spearman correlation, $r = -0.49$, $P = 0.05$) and a trend for NAA ($r = -0.31$, $P = 0.21$). No correlation was observed for years of usage. In addition no significant correlations were observed between the posterior gray matter with either years of usage or abstinent period.

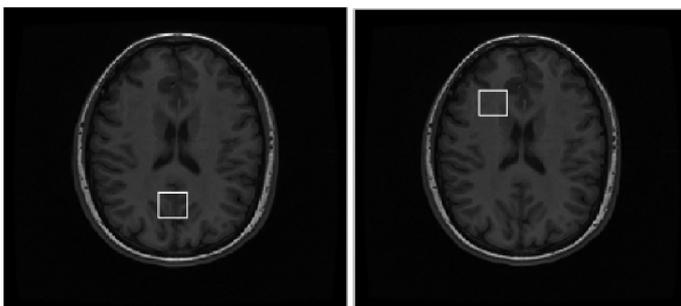


Figure 1. Axial images showing locations of the MRS voxels. $2 \times 2 \times 2 \text{ cm}^3$ voxels were reproducibly prescribed using MRI landmarks in axial slices. Posterior gray matter (left) and frontal white matter (right). Segmentation indicates the fractional contribution of gray matter, white matter and cerebrospinal fluid in the FWM = 77% white, 18% gray and 5% CSF and in PGM = 18% white, 68% gray and 14% CSF. No differences in the proportions of white and gray matter were detected between AMD subjects and controls. Corrections to final metabolite concentrations in Table 2 were applied for the relative contribution of CSF but not for the differences in voxel composition between white and gray matter.

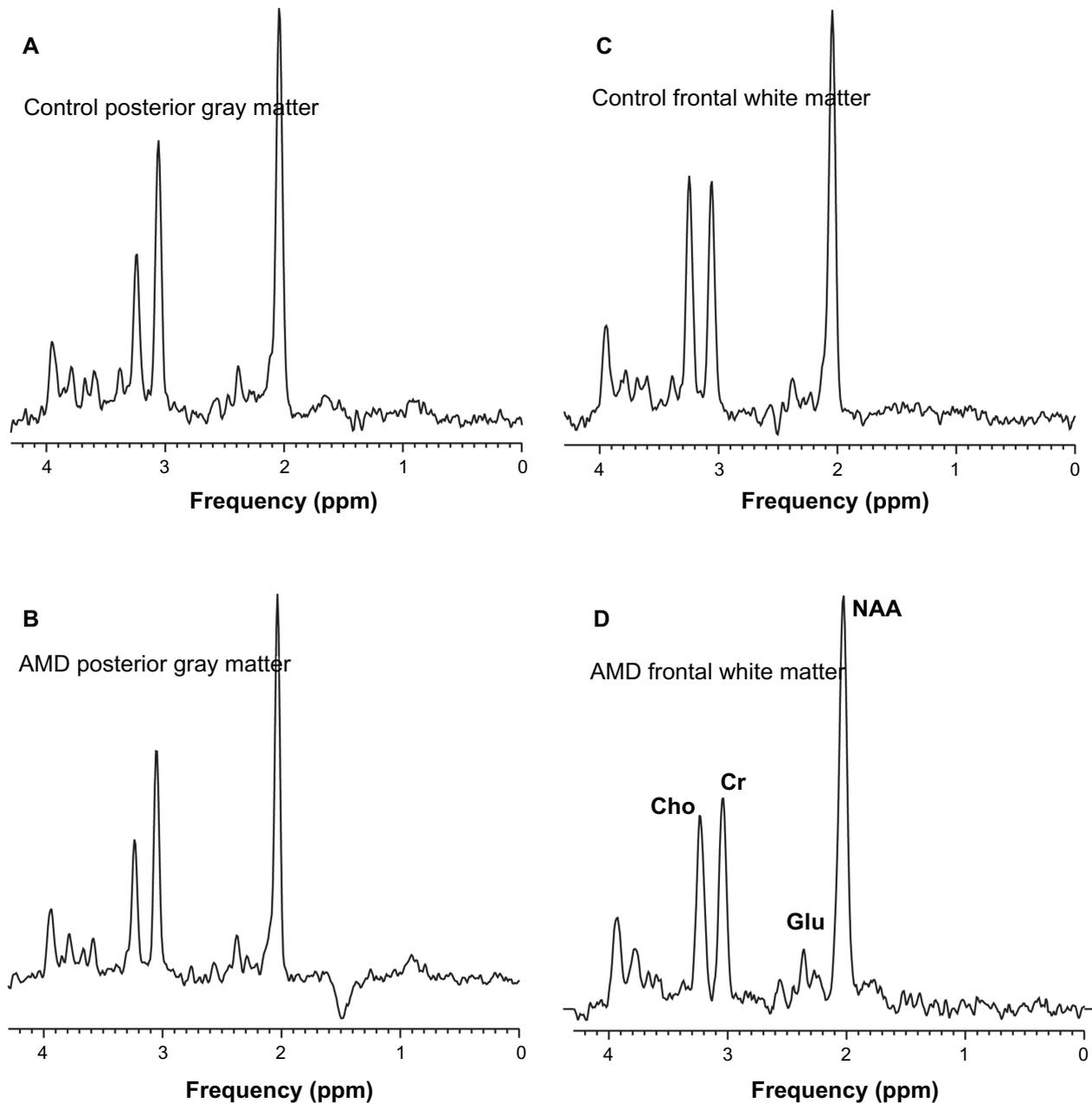


Figure 2. Representative TE-Averaged MRS spectra from an abstinent methamphetamine dependent (AMD) subject and a control. MRS was performed in localized brain regions indicated on axial MRI as frontal white matter and posterior gray matter. The expected differences between MRS of gray matter and white matter are noted. Based on our observation that there are no significant differences in choline concentration between the two groups, spectra were scaled to choline amplitude for comparative purposes.

Relationship between NAA and Glu

Since both of the metabolites impacted in the FWM are believed to be at their highest concentrations in neuronal and axonal tissue, rather than glia, a possible relationship exists between NAA and Glu. In the FWM (Fig. 4) of AMD subjects and of controls, plots of Glu vs. NAA were parallel but different. When plotted on the same axes, NAA was markedly reduced in AMD subjects compared to controls

(Pearson correlation, $r = 0.15$, $P = 0.57$ in AMD and $r = 0.59$, $P = 0.01$ in controls). There were no significant correlations in the PGM of AMD subjects and controls. Interestingly, when all subjects were combined a significant correlation between the two metabolite concentrations ($\rho = 0.78$, $P = 0.001$) was found as expected, since both glutamate and NAA are predominantly concentrated in neurons (and axons).

Table 2. Cerebral metabolite concentrations in the frontal white matter and the posterior gray matter of abstinent methamphetamine dependent subjects and controls.

Subject	Duration of abstinence (weeks)	Number of subjects (N)	Frontal white matter [#]				Posterior gray matter [#]			
			NAA	Cr	Cho	Glu	NAA	Cr	Cho	Glu
Controls		N = 22	9.3 ± 0.9 ^a (8.9–9.7)	6.8 ± 0.9 (6.4–7.2)	2.0 ± 0.3 (1.9–2.1)	5.7 ± 1.0 (5.3–6.1)	10.0 ± 2.0 ^a (9.2–10.8)	8.8 ± 1.4 (8.2–9.4)	1.2 ± 0.3 (1.1–1.3)	8.0 ± 1.7 (7.3–8.7)
AMD	All	N = 18	8.0 ± 1.2 (7.5–8.5)	6.1 ± 0.3 (5.9–6.2)	1.8 ± 0.4 (1.6–2.0)	6.8 ± 1.4 (6.2–7.2)	9.7 ± 2.0 (8.8–10.6)	8.4 ± 2.0 (7.5–9.3)	1.2 ± 0.3 (1.1–1.3)	7.4 ± 2.0 (6.5–8.3)
	P (all AMD vs. controls)		0.004	0.08	0.25	0.01	0.41	0.30	0.95	0.61
	3–8 (short-term)	N = 7	8.2 ± 1.0 (7.5–8.9)	6.2 ± 0.9 (5.5–6.8)	1.9 ± 0.4 (1.6–2.2)	7.1 ± 1.5 (6.0–8.2)	10.1 ± 2.0 (8.6–12.6)	8.7 ± 2.0 (7.2–10.2)	1.3 ± 0.3 (1.1–1.5)	7.8 ± 3.0 (7.6–8.0)
	P (short-term vs. controls)		0.03 ^c	0.12	0.49	0.03 ^c	0.93	0.94	0.38	0.82
	>20 (long-term)	N = 11	7.8 ± 1.4 ^b (7.0–8.6)	6.1 ± 1.1 (5.5–6.7)	1.9 ± 0.5 (1.6–2.2)	6.6 ± 0.9 (6.1–7.1)	8.9 ± 2.3 ^b (7.5–10.3)	7.9 ± 2.3 (6.5–9.2)	1.1 ± 0.3 (0.9–1.3)	7.6 ± 2.4 (6.2–9.0)
	P (long-term vs. controls)		0.01	0.12	0.27	0.06	0.24	0.16	0.53	0.57

[#]Metabolite concentrations are given in mM, mean ± standard deviations (SD) and 95% confidence interval of the mean (in parenthesis). P values refer to differences in metabolite concentrations from control (Bonferroni corrected). N = number of subjects. Of the 80 spectra acquired and analyzed, technical failures resulted in exclusion of 6 spectra as indicated. ^aMissing 2 spectra from WM and 2 spectra from GM in controls, ^bMissing 1 spectrum from WM and 1 spectrum from GM in long-term AMD group, ^cP values were before the Bonferroni correction and they were not significant after correction.

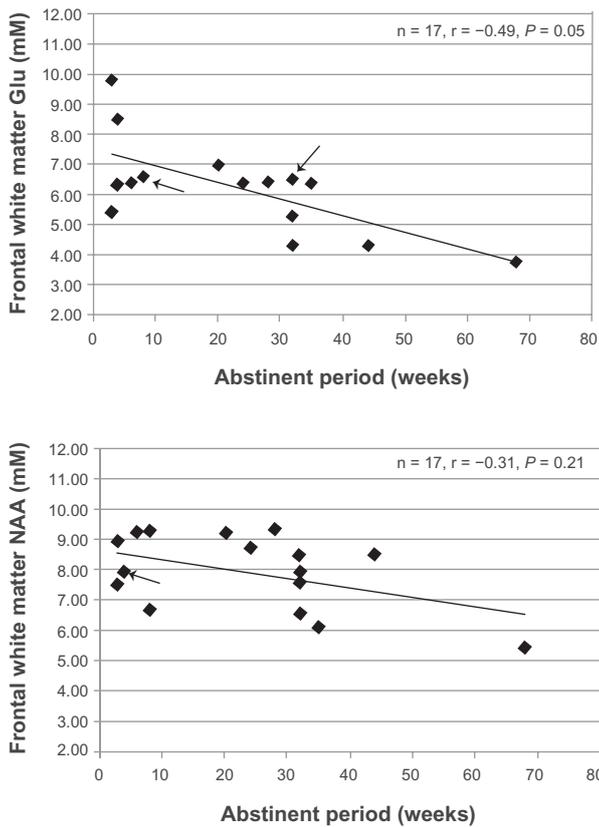


Figure 3. Correlations between MRS determined cerebral metabolites and duration of abstinence in weeks. Glutamate in the frontal white matter (Fig. 3, top) which is elevated in AMD subjects decreased significantly and was weakly correlated ($P = 0.05$) with duration of abstinence. NAA (Fig. 3, bottom) was decreased in AMD and the decrease showed a significant correlation ($P = 0.21$) with duration of abstinence. Six subjects had overlapping values, indicated by arrows.

Correlations between neuropsychological tests and cerebral glutamate concentration

As previously reported by others,³⁶ there is a significantly positive correlation between the FWM NAA and memory tasks of RAVLT [the delayed recall: $\rho = 0.6$, $P = 0.03$, $N = 12$, and long-term retention: $\rho = 0.7$, $P = 0.003$, $N = 12$]. However, there are no significant correlations between neuropsychological test results and glutamate concentration in the FWM or the PGM in AMD subjects.

Gray-white matter composition within voxels of interest

The size and locations of the two voxels studied are shown in Figure 1. FWM and PGM voxels differed significantly in their relative composition with

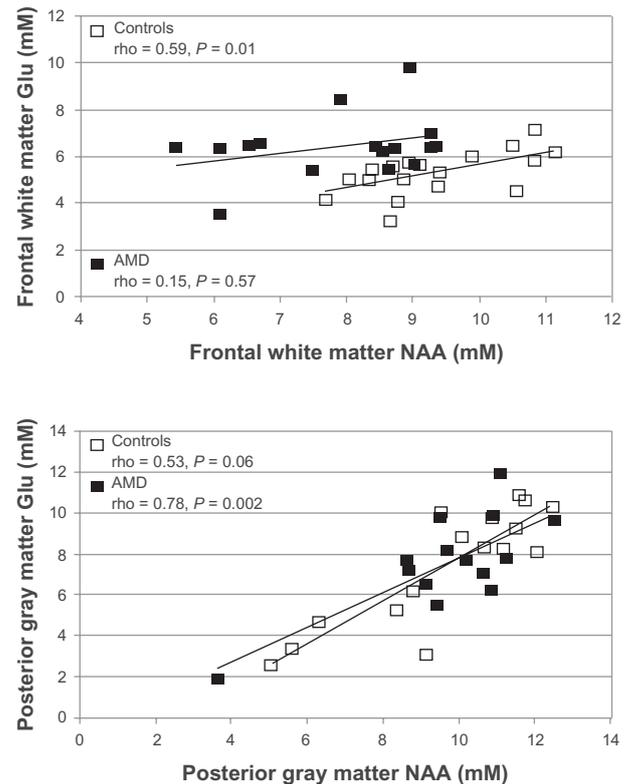


Figure 4. Correlation between concentrations of glutamate and NAA in the frontal white matter (top) and posterior gray matter (bottom) of AMD subjects and controls. In the frontal white matter, there are no correlations between NAA and Glu in AMD subjects ($P = 0.57$) but significant correlation in controls ($P = 0.01$) (Top). In posterior gray matter linear relationship between NAA and Glu is defined the separation between groups reflects the persistent abnormalities in both metabolites in AMD subjects (bottom). The lines overlap indicating no neurochemical differences between the two groups in the posterior gray matter.

greater contribution from CSF in GM ($14\% \pm 2\%$, comparable to a previous report by Zhang and colleagues³³ accounted for by the mid-line prescription of the posterior cingulate gyrus which includes the cerebral falx). The CSF contribution to the selected FWM voxel was much lower ($5\% \pm 1\%$). Corrections were applied to the MRS findings as described in the methods section assigning zero concentrations for both NAA and Glu to CSF.

MRI segmentation showed the altered proportions of white and gray matter in each of the prescribed brain location: the FWM comprised = 77% WM and 18% GM, <5% CSF and the PGM comprised = 68% GM and 20% WM, 12% CSF. When groups were compared, there was no increase in WM contribution to the FWM or PGM voxels of AMD subjects, compared to controls. Therefore no corrections were applied to the metabolite concentrations.



Correction for transverse relaxation time (T2)

T2 relaxation times determined from eighteen AMD and eight control subjects were comparable. Averaged transverse relaxation times in the FWM (T2 in ms, standard deviation in parentheses) were NAA = 214 ms,⁴¹ Cr = 143 ms,²⁸ Cho = 196 ms;⁶¹ and for the PGM: 181 ms,⁸ 133,¹⁰ 173 ms.²¹ *In vitro* T2 relaxation times for NAA = 472 ms, Cr = 302 ms and Cho = 212 ms. These values were used to correct for T2 relaxations for these three metabolites. However, due to strong J coupling of Glu resonances, it was not possible to determine T2 of Glu directly using a single exponential decay fit. A two-dimensional fitting procedure has been reported for calculating T2 from the multiple echo times data set.³⁷ Glu concentration was corrected for *in vivo* T2 using previously reported values³⁸ and assuming identical T2 in AMD subjects and controls.

Discussion

To examine the hypothesis that excess glutamate may contribute to dysfunction in methamphetamine dependent subjects and that the effect of methamphetamine dependence may persist during abstinence, glutamate concentration was determined in the FWM of AMD subjects. We measured N-acetyl aspartate, a well recognized MRS-marker of neurons and axons in white matter as a surrogate for neuronal/axonal injury. A novel feature of this study was the use of an MRS technique which has been optimized for single-line detection of glutamate resonance at 2.35 ppm to quantify cerebral glutamate concentration in the same brain locations. To avoid the psychosis, mania, and other psychiatric confounds associated with active methamphetamine use, we chose to examine methamphetamine dependent subjects during a carefully documented period of abstinence and to compare them to age-matched healthy, non-drug users as controls. The choice of brain locations was dictated by the use of the 'posterior' brain as a control for the expected principal target of neuropsychological and neurochemical deficits in AMD subjects, the frontal brain.

The main findings of this study were a statistically significant elevation (+19%) of Glu in the FWM of AMD subjects and a statistically significant reduction

(-14%) in NAA, confirming an observation by others of long-term neuronal/axonal injury in AMD subjects. The abnormalities in both metabolites appear to be confined to the FWM. No statistically significant differences exist in concentrations of either metabolite in the PGM. Although statistically weaker, abnormal metabolites in AMD subjects appear to persist in proportion to the duration of abstinence. A rich clinical literature reports various neurocognitive impairments associated with short and long-term exposure to methamphetamine, including deficits in decision-making,^{39,40} learning difficulties, problem-solving, and difficulty in maintaining attention.^{36,41,42} It is also suggested that these impairments are largely the result of frontal and subcortical damage. A significant correlation between low NAA concentration in the FWM and memory tasks was observed in the AMD subjects. Although neuropsychological deficits have been reported in methamphetamine users,^{36,43} limited studies correlate such deficits with metabolite concentrations. Salo and colleagues⁴⁴ found a significant correlation between low NAA/Cr ratio and attention control. Taylor and his colleagues⁴⁵ also observed a poorer neurocognitive performance in stimulant dependent subjects with lower NAA levels. These observations reflect the impact of methamphetamine on cognition. No correlation of neuropsychological test results with elevated brain glutamate was found in the present study.

Previous studies reported abnormal Cho level in abstinent methamphetamine dependent subjects. Sekine and colleagues²⁶ found evidence of abnormally high Cho/Cr ratio in the basal ganglia of 13 AMD subjects compared with 11 controls. Nordahl and colleagues⁴³ also found evidence of elevated Cho/Cr and Cho/NAA ratios in the anterior cingulate cortex in 16 AMD subjects. The lack of congruity between the studies was probably reflecting a different voxel of interest location.

This is the first report of increased glutamate concentration in the FWM of human brain associated with dependence on an excitatory drug, methamphetamine, although animal studies have predicted such a finding.^{46,47} NAA is often reported as a neuronal marker; therefore reduction of NAA indicates reduced neuronal integrity, the result of either neuronal cell death or functional impairment. Such a view is based on



previous immunohistochemical studies on the whole brain using NAA-specific antibodies.⁴⁸ NAA is also a major metabolite in axons, where it is concentrated, after being synthesized in and transported from the neuron. We observed significant positive correlations between NAA and Glu in AMD subjects and controls suggest that the majority of Glu, measured by proton MRS, is of neuronal and axonal origin. However, the significant correlation was lost in the FWM of AMD subjects, in which we observed reduced NAA and increased Glu compared to normal subjects. It is not known with certainty within which structures of white matter the impact of brain injury on the distribution of glutamate may be. Because of the cross-sectional design of the present study, we cannot address the issue of causality. Longitudinal studies, covering the period of methamphetamine abuse and abstinence, are urgently required to answer the tempting speculation as to whether in AMD subjects the persistent elevation of glutamate in white matter may be the cause of neuronal and axonal injury, noted here as a reduction in the MRS marker NAA. However, it is possible that our observation reflects abnormalities in non-neuronal cells, microglia and macroglia (astrocytes), within the FWM brain region. White matter abnormalities have been well documented in AMD subjects.^{15,21,49–52} In a study by Sung and colleagues, elevated myo-inositol, a marker of glial cells, was observed in AMD subjects, from which glial cell proliferation in response to the neuronal toxicity from methamphetamine was concluded. In our recent study,⁵² using a novel 13 C MRS method, we reported direct evidence of abnormalities in glial oxidative mechanism in frontal brain of AMD subjects.

Another explanation for the present findings concerns the current view of white matter function that axons communicate with the neurons of gray matter via chemical messengers, has recently been amplified by the discovery of transmitter function of oligodendrocytes, the glial cells which are responsible for myelin formation.⁵³ One of these messengers is Glu. Glu is released in an activity-dependent manner from white matter; glial cells within fiber tracts express Glu receptors and Glu transporters which are present to remove Glu, preventing Glu excitotoxicity.^{54,55} Therefore, a possible explanation for elevated Glu in the white matter could be a result of impairment of Glu receptors or Glu transporters in white

matter. Recently, a dysfunction of Glu transporters was reported in preclinical studies after exposure to methamphetamine.⁵⁴ Based upon our present findings, when the putative neurotoxin, glutamate, returns to normal further neurological damage will be limited. As part of the process of glutamatergic neurotransmission, Glu releases from the neuron and is taken up by astrocytes, where it is converted to glutamine (Gln) by a glutamine synthetase enzyme, glutamine is then transported back to the presynaptic neuron, and reconverted to Glu.⁵⁶ Elevation of the Glu level indicates less conversion of Glu to Gln, and therefore suggests less glutamatergic activity. Increased Glu has been demonstrated in preclinical studies in repeated injections of methamphetamine in rodent striatum.^{46,57} In a recent study, McFarland et al⁴⁷ demonstrated that increased Glu release in the pre-frontal cortex and accumbens is required for the reinstatement of cocaine seeking in rats. Our observations provide preliminary evidence in humans, which supports regional cerebral impairment in glutamate metabolism or in glutamatergic neurotransmission, as a contributor to the persistent neurochemical abnormalities and cognitive deficits, which result from long-term methamphetamine dependence.

Limitation in MRS study

Our study has a number of limitations. Glu concentrations reported in this study are approximate concentrations due to: first, we did not measure T1 of Glu using the TE-Averaged method, we had to compromise between measurement time and signal-to-noise for glutamate detection. Using TR = 6 s (for expected T1 of 1.2 s) and longer for T1 measurement would not have been feasible because scan time would have been too long. In addition, our simplifications approach to perform T2 correction. Second, possible confounding factors in our results are the characteristics of AMD subjects which include an average of 10 years of drug use, a wide range in the estimated amount of drug use of 4448 ± 5120 g and abstinence ranging from 3 weeks–122 weeks. In addition, AMD subjects had an average of 3 years of education less than the controls. This difference was statistically significant ($P < 0.05$). We noted this difference and discussed its possible impact on our MRS neurochemical results, since it has been shown that subjects' educational levels contributed to a small and significant increase in



NAA concentration of white matter.⁵⁸ No equivalent data relating brain glutamate with education level is available. We conclude that this difference in education levels between the AMD subjects and controls is unlikely to be the explanation for the substantial and statistically significant elevation in Glu of the FWM, which appears to recover towards normal over the duration of abstinence.

Conclusion

The present results define increased concentrations of glutamate, a known neurotoxin and may, therefore, offer a novel mechanism for neurological injury in methamphetamine abuse. This study lends support to the growing body of data available from studies in animals, implicating glutamate in a methamphetamine-dependent neurological and neuropsychiatric disorder.

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Disclosures

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

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