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Adaptation of brain glutamate plus glutamine during abstinence from chronic methamphetamine use

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Abstract

Methamphetamine (METH) is a stimulant drug that is toxic primarily to dopaminergic and serotonergic neurons, and may lead to inflammatory changes in the brain. Additionally, the glutamatergic system is altered following METH exposure. Therefore, concentrations of brain glutamate+glutamine (GLX) were assessed during abstinence from chronic METH abuse. 25 subjects with a history of METH-dependence (age 31.8 ± 7.4 years, 14 females and 11 males) and 28 control subjects without a history of drug abuse (age 32.6 ± 8.8 years, 14 females) were enrolled. Twelve of the METH subjects were followed and rescanned 5-months later. METH users had used the drug 5.9 ± 1.7 times / week, for 109 ± 69 months, and had been abstinent for 2.1 ± 3.0 months. GLX was measured in the basal ganglia and frontal gray and white matter, using proton magnetic resonance spectroscopy. While overall GLX concentrations at baseline were similar between METH and control subjects, METH users with ≤ 1 month of abstinence showed reduced frontal gray matter GLX ($p=0.01$). Time of abstinence correlated positively with GLX in frontal gray ($p<0.0001$) and white matter ($p=0.03$). After 5-months, changes in frontal gray matter GLX showed a trend to correlate inversely with duration of abstinence ($p=0.07$). Subjects with craving symptoms had lower frontal gray matter GLX than those without craving (-8% , $p=0.05$). These findings suggest dynamic abnormalities in brain GLX in recently abstinent methamphetamine users, with a depletion of the glutamatergic system in METH users within the first two months of abstinence, and some normalization during prolonged abstinence. Since craving may contribute to relapse, medications that normalize GLX may minimize craving in METH users.

Keywords

Methamphetamine; addiction; glutamate; glutamine; spectroscopy; brain

Introduction

Methamphetamine (METH) is an illicit stimulant drug commonly abused in the U.S. and worldwide. In addition to the acute effects of METH, such as the transient massive release of dopamine and glutamate, the drug has also been associated with long-lasting brain changes in humans. For instance, chronic but abstinent METH users were shown to have reduced dopamine transporters and altered glucose metabolism on positron emission tomography

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(Volkow et al, 2001b); however, these abnormalities appeared to normalize with prolonged abstinence in some brain regions (Volkow et al, 2001a; Wang et al, 2004). Other neurochemical changes in human METH users were found with proton magnetic resonance spectroscopy (^1H MRS), which can quantify various neuro-metabolites in the millimolar concentration range. Specifically, the neuronal marker N-Acetyl-Aspartate (NA) or NA-to-total creatine ratio (NA/Cr) may be decreased in abstinent METH abusers (Ernst et al, 2000; Nordahl et al, 2002) and in relation to the total lifetime exposure to METH (Ernst et al, 2000; Sung et al, 2007). The neurotoxic effect of chronic METH exposure is further demonstrated by the associated neuroinflammatory responses, such as the activation of microglia, astroglia, and elevated mediators of inflammation including cytokines, chemokines, prostaglandins, interleukins, as well as factors associated with oxidative stress (Asanuma et al, 2003; Thomas et al, 2004; Yamamoto and Bankson, 2005).

Recent research found that the effects of METH are related not only to dopamine and serotonin, but also to other neurotransmitters such as glutamate (GLU). For instance, the striatum of rats showed prolonged increases in extracellular glutamate after METH administration (Nash and Yamamoto, 1992), and glutamate might be an important contributor to oxidative stress-mediated neurotoxicity of METH (Yamamoto and Bankson, 2005). Cocaine is another psychostimulant that may affect the GLU system similarly to METH. In rats, low basal GLU concentrations in the nucleus accumbens was associated with reinstatement of self-administration of cocaine, a model for relapse of cocaine use (Baker et al, 2003). Furthermore, reinstatement of cocaine-self administration in cocaine-dependent rats was prevented by administration of N-acetylcysteine, which stimulates the cystine/glutamate exchanger and increases basal GLU concentrations (Cornish and Kalivas, 2000). A recent open-labeled study further showed that human cocaine users who were treated with N-acetylcysteine had longer retention during a 4-week medication trial and reduced cocaine usage (Mardikian et al, 2007). However, little is known about changes in the glutamate system in human METH users.

Since the sum of glutamate plus glutamine (GLX) can be evaluated by ^1H MRS, we assessed the effect of abstinence from chronic METH abuse on the brain concentrations of GLX in a cross-sectional sample of METH users, and in a subset of these participants longitudinally after 5 months of abstinence. The GLX measured with ^1H MRS comprises both glutamate and glutamine from the intracellular and extracellular compartments, but these chemicals are largely sequestered intracellularly. Based on the preclinical studies, we hypothesized that GLX would be decreased in abstinent METH users, especially during early abstinence. Since craving for the drug is typically strong during early abstinence, we also expected that lower GLX levels may be associated with greater craving for METH.

Materials and Methods

A group of 25 subjects with a history of METH dependence (mean age 31.8 ± 7.4 years, 11 males, 14 females) and 28 control subjects with no history of drug dependence (mean age 32.6 ± 8.8 years, 14 males, 14 females) were screened and enrolled in the study. Each participant provided a written informed consent approved by our institutional review board prior to an extensive screening evaluation. The screening evaluation involved a detailed neuropsychiatric history and physical examination, screening blood tests, and urine toxicology screens, and ensured that all methamphetamine users fulfilled the following study criteria: 1) age 18 to 50 years; 2) history of methamphetamine dependence according to the Diagnostic Statistical Manual (DSM) IV criteria; 3) regular methamphetamine use for at least 12 months, at least 5 times/week, and at least 0.5 gram per day; 4) methamphetamine was the primary drug of choice; 5) no present or past alcohol abuse or dependence; 6) enrolled in a drug rehabilitation program; 7) negative urine toxicology screen for illicit drugs (amphetamines, cocaine, marijuana, benzodiazepine, barbiturates, and opiates) at baseline assessment. Subjects in both METH user

and control groups were excluded if they 1) were seropositive for HIV-1; 2) had a history of head trauma with loss of consciousness for more than 30 minutes; 3) had a history of substance dependence including alcohol (except for methamphetamine in the METH user group and nicotine in either group); 4) had any chronic medical, neurological or psychiatric illnesses (e.g. seizure disorders, depression, schizophrenia, hypertension, or diabetes); 5) were pregnant; or 6) had metallic implants contraindicated for the MR studies. All healthy control subjects were on no medications. A detailed drug use history was also obtained from each subject for the most common common illicit drugs, nicotine, and alcohol, using a standardized screening form. The total amount of lifetime exposure to METH was calculated by multiplying the average daily METH amount taken (in grams / day) with the number of days per week METH was used, times the total time period (in weeks) of METH use.

All subjects underwent a baseline ^1H MRS study. Additionally, ^1H MRS was repeated 5 months after the baseline scan in 12 of the 25 abstinent methamphetamine abusers, using identical techniques and identical voxel locations. These 12 subjects were followed carefully as outpatients by study personnel and the staff at the rehabilitation center; the remaining 13 subjects were lost to follow-up. Craving was assessed by self-report on the day of the MRS examination, using a visual analog scale (VAS, 0-100; 100=strongest craving).

Localized ^1H MRS was performed on a 1.5 Tesla GE scanner, using a quadrature head coil. Three voxels were evaluated in the medial frontal gray matter (anterior cingulate), frontal white matter, and in the basal ganglia (Figure 1; volume approximately 5-6 cm³). Spatial localization of spectra was achieved using a point-resolved spectroscopy (PRESS) sequence (Bottomley, 1987) with the gradient order optimized for minimizing artifacts in the frontal lobe (Ernst and Chang, 1996). Data were acquired at short echo-time (TE=30ms), relatively long TR (3 seconds), and 128 averages. These parameters allow measurement of coupled resonances, in particular GLX, and minimize the effects of T1 and T2 relaxation on metabolite values. Metabolite concentrations, including N-acetylaspartate, myoinositol and glutamate+glutamine (GLX), were determined relative to the MR-visible brain water, using a well-validated technique that corrects for the T2-decay of the water signal and for the presence of cerebrospinal fluid (CSF) in each MRS voxel (Ernst et al, 1993). This was achieved by measuring the unsuppressed fully relaxed water signal in each voxel at 10 different echo times, and fitting the resulting water amplitudes to a double-exponential decay model. The spectra were processed with a well-validated semi-automatic program (Kreis et al, 1993) that involved a water-attenuation filter, mild apodization (0.5 Hz), zero filling and DC baseline correction (determined from data points around 2.75 ppm). Peak areas were estimated by fitting a Lorentzian to the four major metabolite peak, and by automatic integration of the β - γ -GLX signals between 2.2 and 2.4 ppm. Peak areas were then referenced to the brain-water signal from the T2-fit to determine metabolite concentrations corrected for the percentage of CSF in each voxel.

Statistical analyses were performed in StatView (version 5.0.1) and SAS (both SAS Institute Inc., Cary, NC). Comparisons of GLX concentrations between control subjects and METH users were performed using Student's t-tests. Linear regression analyses were performed between GLX concentrations and the total lifetime exposure to METH and the time since last METH exposure (both log-transformed). A non-linear exponential model was also fitted to the GLX data with time as independent variable, using SAS. Additional linear regression analyses were performed between the change in the GLX concentration over 5 months, and the time of abstinence at baseline examination (log-transformed). Furthermore, the relationship between GLX concentrations in voxels with significant regressions with duration of abstinence, and two other metabolites in each of these voxels (NA as a neuronal marker and mI as a glial marker) were assessed using linear regressions. Statistical significance was defined as $p < 0.05$.

Results

On average, the METH users had used the drug 5.9 ± 1.7 times per week and for 109 ± 69 months, and had stopped using METH 2.1 ± 3.0 months (range: 1 week to 12 months) prior to the study. All METH users smoked the drug in its crystalline form. Mean daily estimated METH use was 1.3 ± 1.2 grams (median: 1.0 gram), and the mean estimated cumulative lifetime exposure was $3,068 \pm 2,900$ grams (median: 2,079 grams). Although the METH subjects had lower educational attainment than the control subjects (12.4 ± 1.2 vs. 14.8 ± 1.5 years, $t_{51} = 6.3$), the average estimated verbal intelligence quotient did not differ between the two groups (METH: 98.4 ± 12.1 ; controls: 101.8 ± 11.8 , $t_{51} = 1.0$).

At baseline, perhaps due to the variation in duration of abstinence, the concentrations of GLX or NA were not significantly different between the METH users and the control subjects in any of the three brain regions studied. However, time of abstinence (after log transformation) showed a positive correlation with the GLX, but not the NA, concentration in the frontal gray matter ($r=+0.78$, $p<0.0001$, $F_{1,22}=35.1$, Figure 2A) and the frontal white matter ($r=+0.44$, $p=0.03$, $F_{1,20}=5.4$, Figure 2B), and a trend for correlation in the basal ganglia ($r=0.37$, $p=0.09$, $F_{1,22}=3.1$). Specifically, the GLX concentration was approximately 15% below normal levels in subjects who were within the first 2 weeks of abstinence, reached normal concentrations in subjects who were abstinent for 1-2 months, and exceeded normal levels in subjects with longer-term abstinence (see Figures 2A & B). The correlation between GLX concentration and duration of abstinence remained significant after correction for multiple comparisons (using Bonferroni's method) in the frontal cortex (anterior cingulate). Furthermore, the 16 METH users with ≤ 1 month of abstinence showed reduced GLX concentrations in the frontal gray matter (17.1 ± 1.3) compared to control subjects (18.5 ± 1.7 mM; $p=0.01$, $t_{41}=2.6$), but not in the basal ganglia or frontal white matter. No correlations were observed between the GLX concentrations in any of the brain regions and the logarithm of cumulative lifetime METH exposure.

The linear regression curves shown in Figure 2 represent a logarithmic relationship between the time of abstinence and the GLX concentration, which is not truly a realistic model, since the GLX concentration cannot increase indefinitely with time. Therefore, we refitted the data with a mono-exponential model (with a negative time-constant and a positive offset) that represents a gradual initial rise of the GLX concentration with time towards a "steady-state" level over longer time periods. The results of these fits are shown as red curves in the Figures. In the frontal gray matter, the best fit reveals a long-term "steady-state" concentration of 21.2 mM, with a 95% confidence interval (C.I., 19.4 to 22.9 mM) above the concentration in the control subjects (18.5 mM). The time constant of the initial rise is 2.3 months (95% C.I. 0.22 to 4.4 months), during which 63% of the initial rise would be completed. The overall increase in frontal gray matter GLX over time was 5.6 mM (95% C.I. 3.7 to 7.5 mM), which represents a 36% increase over the GLX value during early abstinence. In the basal ganglia, the "steady-state" concentration of GLX was 20.3 mM (C.I. 16.8 to 23.8 mM), the time constant 1.9 months (C.I. -3.2 to 6.9 months), and the overall rise 4.0 mM (C.I. -0.4 to 8.4 mM). In the white matter GLX, the non-linear fit yielded a steady-state concentration of 12.5 mM (95% C.I. 11.4 to 13.5 mM) and an overall rise of 10.1 mM; however, the latter value was not significantly different from zero (95% C.I. -9.0 to 29.3 mM).

Longitudinal changes in GLX concentrations over 5 months

If the scenario described in the previous paragraph (based on cross-sectional data from different METH subjects) is correct, then the GLX concentration should (1) increase over time in abstinent METH users with brief periods of abstinence, (2) be relatively unchanged over time in users with intermediate periods of abstinence, and (3) possibly decrease in users with longer periods of abstinence (if in fact there is gradual normalization after the initial phase of

overcompensation). Accordingly, longitudinal changes were evaluated in a sub-group (n=12) of these abstinent METH users, in whom follow-up data were available. The subjects were evaluated with ^1H MRS at baseline and again 5 months later, while being monitored weekly at the rehabilitation center to ensure that they remained abstinent. The baseline scan was performed within the first 2 months of abstinence in the majority of subjects (10/12). The 12 subjects in this group showed the same effect of time of abstinence (after log-transform) on the frontal gray matter GLX ($p=0.003$, $F_{1,11}=14.6$) and basal ganglia GLX ($p=0.005$, $F_{1,9}=14.9$) as the entire METH group. Additionally, there were no significant differences in any of the epidemiological or drug usage variables between subjects with and without a repeat scan, suggesting that the subsample of repeat subjects was representative for the entire METH group.

The 5-month change in GLX in the frontal cortex is illustrated in Figure 3A as 12 individual data pairs. Overall, GLX concentrations increased in subjects who were studied within 1 month of withdrawal, and were approximately zero in subjects with 1-2 months of abstinence. One subject showed markedly decreased GLX level after 10 months of “abstinence” (dark blue line); however, this participant had been abstinent for 5 months already at enrollment but had relapsed and began using METH just days before her repeat scan 5 months later and had a positive urine toxicology screen on the day of the follow-up scan. For the remaining 11 subjects who supposedly did not relapse, 5-month changes in the GLX concentration in the frontal gray matter, but not the basal ganglia, showed a trend to correlate inversely with the Log of duration of abstinence ($r=-0.56$, $p=0.07$, $F_{1,10}=4.0$; Figure 3B).

Effects of craving on GLX concentrations and relationship of GLX to other MRS markers

Of the 25 recently-abstinent METH dependent subjects recruited, 15 had symptoms of craving (defined as a craving VAS > 0 ; mean VAS= 34 ± 25), and 10 had no symptoms of craving for METH (VAS=0) at the time of the baseline study. The prefrontal gray matter GLX concentration was significantly lower (-8% ; $p=0.05$, $t_{1,22}=2.1$) in subjects who had any craving symptoms compared to subjects without craving for METH. This relationship remained significant when all METH scans (baseline and repeat) were included in the analysis ($p=0.025$, $t_{1,38}=2.3$). We also evaluated the relationship between the GLX concentrations and depression scores, but no significant correlations were found.

Furthermore, in the basal ganglia, the GLX concentration was correlated positively with the concentration of NA (a neuronal marker, $r=0.47$, $p=0.03$, $F_{1,20}=5.3$), but not with the glial marker mI. However, the basal ganglia NA concentration showed only a non-significant trend for correlation with the duration of abstinence ($r=0.29$, $p=0.17$, $F_{1,20}=2.1$).

Discussion

To our knowledge, this is the first ^1H MRS study demonstrating abnormal glutamate plus glutamine (GLX) levels in abstinent human METH abusers, and their relationship with time of abstinence. Specifically, the METH users showed reduced GLX during early abstinence, followed by relatively normal GLX concentrations after 1-2 months of abstinence, and possibly some overcompensation in the longer term, especially in the frontal gray matter. The recovery of the glutamate system appears to have a typical time constant of 2 months, and result in a 30% increase in GLX over the baseline levels. The cross-sectional findings are further supported by longitudinal GLX changes, with the largest 5-month increases in subjects who were initially scanned soon after cessation of METH. Together, these results suggest a scenario in which the glutamate / glutamine system is down-regulated in METH abusers during early abstinence.

Glutamate is synthesized *de novo* from ketoglutarate via the tricarboxylic acid (TCA) cycle. Glutamate in neurons has the dual roles of an amino-acid and that of the most abundant

excitatory neurotransmitter. Glutamate released during synaptic transmission into the extracellular space (ECS) is taken up by astrocytes and converted into glutamine. Astrocytes then release glutamine into the ECS, which is taken up by neurons and converted back into glutamate. The flux through this “glutamate-glutamine shuttle” represents 40-50% of the total flux from the TCA cycle (Morris and Bachelard, 2003; Shen et al, 1999).

One potential explanation for reduced GLX is a loss of glutamatergic neurons. In the basal ganglia, this interpretation has some support due to the positive correlation between GLX and the NA concentration, a putative neuronal marker. Prior MRS studies have consistently documented decreased NA in the frontal gray matter or basal ganglia regions (Ernst et al, 2000; Nordahl et al, 2002). However, our data and methods do not allow a clear conclusion that there is loss of glutamatergic neurons in either region in the current cohort of subjects.

Several other mechanisms may account for reduced GLX in abstinent METH users. First, reduced GLX might be the result of decreased *de novo* synthesis via the TCA cycle. In the rodent striatum, inhibition of mitochondrial function has been found within 2 hours after METH administration. This down-regulation of the mitochondrial electron transport chain is thought to be related to oxidative stress after METH-induced glutamate and dopamine release (Brown and Yamamoto, 2003). If a state of inhibited mitochondrial function persists after multiple exposures to METH, then a reduction in total glutamate plus glutamine is plausible, since the *de novo* ketoglutarate and eventually glutamate synthesis would likely be down-regulated as well.

Second, net glutamate output (incomplete recycling) by the glutamate-glutamine shuttle into the vasculature or ECS may reduce the total amount of glutamate plus glutamine. In animal studies, METH administration indeed has been associated with reduced glial glutamate uptake and / or recycling (Yamamoto and Bankson, 2005). Additionally, METH causes activation of glial cells (Hess et al, 1990; Pu and Vorhees, 1993), specifically microglia (Asanuma et al, 2003; Escubedo et al, 1998), which in turn may lead to inhibition of glutamate uptake (Zou and Crews, 2005) and glutamine synthesis (Muscoli et al, 2005). Inhibition of glial glutamate recycling also might increase extracellular glutamate and therefore lead to excitotoxicity.

Third, a reduction in GLX might be due to increased demand for glutamate as an amino-acid. Continuous cell damage due to METH-induced oxidative stress (Tata and Yamamoto, 2007) is likely to require increased repair to cell membranes and proteins synthesis. The latter would increase usage of glutamate as an amino-acid, and therefore reduce GLX, since amino-acids generally become invisible to MR measurements once they are integrated into larger molecules, such as proteins.

We speculate that all three of the above mechanisms contribute to the current finding of reduced GLX. This view is in agreement with a scenario developed by Yamamoto et al, in which METH exposure causes excessive release of dopamine and glutamate, leading to a feed-forward cycle of inflammation and oxidative stress, and associated glial activation and mitochondrial dysfunction. At autopsy, chronic METH users had higher concentrations of two aldehydes indicative of oxidative damage (Fitzmaurice et al, 2006).

Our human subjects showed GLX abnormalities in subcortical as well as cortical gray matter, whereas the METH neurotoxicity is essentially limited to the striatum in animals (Stephans and Yamamoto, 1996). However, rodents are typically exposed to METH for relatively short time periods (days to perhaps weeks), while all of the participants in our study had been abusing METH for years or even decades. It seems plausible that chronic METH exposure might eventually cause damage to the frontal cortex, where the density of dopaminergic synapses is lower but that of glutamatergic synapses is relatively high. Prior MRS studies indeed found lower levels of the neuronal marker NA, or NA/CR, in both the frontal and striatal brain regions

of chronic METH users (Ernst et al, 2000; Chang et al, 2005; Nordahl et al, 2005; Sung et al, 2007).

Alternatively, chronic over-stimulation of frontal areas due to METH abuse may induce a down-regulation of the glutamate system in order to reduce excitotoxicity (Ramonet et al, 2004). Since the neuronal TCA cycle flux is tightly coupled to neuronal stimulation (Patel et al, 2004), a persistent down-regulation of basal neuronal activity is likely to be associated with a reduction in the TCA cycle flux, which in turn may cause a decrease in the GLX concentration. In fact, withdrawal from self-administered cocaine, another stimulant, in rodents was associated with reduced intracellular glutamate concentrations in the nucleus accumbens (Keys et al, 1998). A down-regulated glutamate system may also be associated with the common symptoms of craving and particularly depression (Hasler et al, 2007). However, regressions between GLX concentrations and depression scores yielded no significant results.

In our study, a down-regulated state (neuroadaptation) of the glutamate system to attenuate excitotoxicity might have been revealed by the abrupt elimination of chronic METH exposure after the participants became abstinent by enrolling in a treatment program. Such a neuroadaptive state might reverse over the course of months. For instance, an [¹⁸F]fluoro-deoxy-glucose (FDG) positron-emission tomography (PET) study of 17 short-term abstinent METH users (4-7 days of abstinence) found reduced glucose metabolism relative to non-drug users in the anterior cingulate (same as our medial frontal voxel), but increased metabolism in the ventral striatum (close to our basal ganglia voxel) (London et al, 2004). Since the TCA cycle flux and glutamate homeostasis are coupled, this PET study provides some support for our finding of reduced GLX, in particular in the frontal gray matter (anterior cingulate). Conversely, another FDG PET study (Volkow et al, 2001c) found that longer-term abstinence (2 weeks and 5 months) in METH users was associated with increases in glucose consumption globally and in several cortical areas, but decreases in subcortical regions (Volkow et al, 2001c).

Another interesting observation is that subjects with symptoms of craving for METH had lower GLX in the frontal cortex compared to those without craving. In rats, low basal glutamate concentrations in the nucleus accumbens were associated with reinstatement of self-administration of cocaine (Baker et al, 2003). However, reinstatement could be prevented by administration of N-acetylcysteine, which stimulates the cystine/glutamate exchanger and increases basal GLU concentrations (Cornish and Kalivas, 2000). Since lower GLX is observed in those with craving, which may contribute to relapse of psychostimulant abuse, treatment with medications such as N-acetylcysteine might decrease craving and prevent relapse. Decreased cocaine usage and longer retention were observed in a recent open-labeled study of human cocaine users who received N-acetylcysteine (Mardikian et al, 2007). However, a clinical trial of N-acetylcysteine for treatment of METH abuse has not been performed and is needed.

Two technical limitations may affect our findings. First, the techniques employed to acquire and analyze the data cannot distinguish glutamate from glutamine at 1.5 Tesla field strength; therefore, our findings may reflect changes in either glutamate or glutamine, or both. However, as discussed above, several mechanisms in abstinent METH users are likely to decrease glutamate plus glutamine, which can be detected by our method. Second, apparent changes in GLX, as measured in this study, might be partly due to changes in lipid or macromolecule signals that co-resonate with GLX (Behar et al, 1994), perhaps due to cell membrane injury associated with METH-induced neurotoxicity. Future studies using techniques to eliminate these macromolecule and lipid signals are needed to validate our observations.

We conclude that ^1H MRS provides *in vivo* evidence for abnormalities in the glutamate and glutamine system of abstinent chronic METH abusers. These findings are consistent with a model of a down-regulated glutamatergic state immediately after METH withdrawal, and a gradual reversal over longer time periods. However, it is unclear whether changes in GLX after longterm METH use would reverse completely during continued abstinence, or whether some of the alterations in GLX would persist. The size of the effect and the non-invasive nature of MRS studies suggest that GLX, measured with ^1H MRS, may be a useful biomarker to study short-term changes in brain metabolism after cessation of METH abuse. Future studies may employ new MRS methods that specifically evaluate glutamate (Hurd et al, 2004) or glutamine separately, measure changes longitudinally both at the onset of abstinence and after longer periods (6 to 9 months) of abstinence, and further determine if the severity of the hypoglutamatergic state correlates with craving or withdrawal symptoms. Should these preliminary observations be validated, measurements of GLX or glutamate may provide an important surrogate marker for the monitoring of treatment effects in clinical trials or to determine which individuals might need more aggressive treatment approaches, such as adjunctive pharmacotherapy.

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References

- Asanuma M, Tsuji T, Miyazaki I, Miyoshi K, Ogawa N. Methamphetamine-induced neurotoxicity in mouse brain is attenuated by ketoprofen, a non-steroidal anti-inflammatory drug. *Neuroscience Letters* 2003;352:13–16. [PubMed: 14615038]
- Baker DA, McFarland K, Lake RW, Shen H, Tang X-C, Toda S, Kalivas PW. Neuroadaptations in cystine-glutamate exchange underlie cocaine relapse. *Nature Neuroscience* 2003;6:743–749.
- Behar K, Rothman D, Spencer D, Petroff O. Analysis of macromolecule resonances in ^1H NMR spectra of human brain. *Magnetic Resonance in Medicine* 1994;32:294–302. [PubMed: 7984061]
- Bottomley PA. Spatial localization in NMR spectroscopy in vivo. *Annals of the New York Academy of Sciences* 1987;508:333–348. [PubMed: 3326459]
- Brown JM, Yamamoto BK. Effects of amphetamines on mitochondrial function: role of free radicals and oxidative stress. *Pharmacol Ther* 2003;99:45–53. [PubMed: 12804698]
- Chang L, Ernst T, Speck O, Grob C. Additive effects of HIV and chronic methamphetamine use on brain metabolite abnormalities. *American Journal of Psychiatry* 2005;162:361–369. [PubMed: 15677602]
- Cornish J, Kalivas P. Glutamate transmission in the nucleus accumbens mediates relapse in cocaine addiction. *Journal of Neuroscience* 2000;20:RC89. [PubMed: 10899176]
- Ernst T, Kreis R, Ross BD. Absolute quantitation of water and metabolites in the human brain. I: compartments and water. *Journal of Magnetic Resonance* 1993;B102:1–8.
- Ernst T, Chang L. Elimination of Artifacts in Short Echo Time ^1H MR Spectroscopy of the Frontal Lobe. *Magnetic Resonance in Medicine* 1996;36:462–468. [PubMed: 8875419]
- Ernst T, Chang L, Leonido-Yee M, Speck O. Evidence for long-term neurotoxicity associated with methamphetamine abuse: A ^1H MRS study. *Neurology* 2000;54:1344–1349. [PubMed: 10746608]

- Escubedo E, Guitart L, Sureda FX, Jimenez A, Pubill D, Pallas M, Camins A, C J. Microgliosis and down-regulation of adenosine transporter induced by methamphetamine in rats. *Brain research* 1998;814:120–126. [PubMed: 9838075]
- Fitzmaurice PS, Tong J, Yazdanpanah M, Liu PP, Kalasinsky KS, Kish SJ. Levels of 4-hydroxynonenal and malondialdehyde are increased in brain of human chronic users of methamphetamine. *J Pharmacol Exp Ther* 2006;319:703–9. [PubMed: 16857724]
- Hasler G, van der Veen J, Tumonis T, Meyers N, Shen J, Drevets W. Reduced Prefrontal Glutamate/ Glutamine and {gamma}-Aminobutyric Acid Levels in Major Depression Determined Using Proton Magnetic Resonance Spectroscopy. *Archives of General Psychiatry* 2007;64:193–200. [PubMed: 17283286]
- Hess A, Desiderio C, McAuliffe WG. Acute neuropathological changes in the caudatenucleus caused by MPTP and methamphetamine: immunohistochemical studies. *Journal of Neurocytology* 1990;19:338–342. [PubMed: 1975269]
- Hurd R, Sailasuta N, Srinivasan R, Vigneron DB, Pelletier D, Nelson SJ. Measurement of brain glutamate using TE-averaged PRESS at 3T. *Magnetic Resonance in Medicine* 2004;51:435–440. [PubMed: 15004781]
- Keys A, Mark G, Emre N, Meshul C. Reduced glutamate immunolabeling in the nucleus accumbens following extended withdrawal from self-administered cocaine. *Synapse* 1998;30:393–401. [PubMed: 9826231]
- Kreis R, Ernst T, Ross BD. Absolute quantitation of water and metabolites in the human brain. II: metabolite concentrations. *Journal of Magnetic Resonance* 1993;B102:9–19.
- London E, Simon S, Berman S, Mandelkern M, Lichtman A, Bramen J, Shinn A, Miotto K, Learn J, Dong Y, Matochik J, Kurian V, Newton T, Woods R, Rawson R, Ling W. Mood disturbances and regional cerebral metabolic abnormalities in recently abstinent methamphetamine abusers. *Archives of General Psychiatry* 2004;61:73–84. [PubMed: 14706946]
- Mardikian P, LaRowe S, Hedden S, Kalivas P, Malcolm R. An open-label trial of N-acetylcysteine for the treatment of cocaine dependence: a pilot study. *Progress in Neuropsychopharmacology and Biological Psychiatry* 2007;31:389–394.
- Morris P, Bachelard H. Reflections on the application of ¹³C-MRS to research on brain metabolism. *NMR in Biomedicine* 2003;16:303–312. [PubMed: 14679497]
- Muscoli C, Visalli V, Colica C, Nistico R, Palma E, Costa N, Rotiroti D, Nistico G, Mollace V. The effect of inflammatory stimuli on NMDA-related activation of glutamine synthase in human cultured astroglial cells. *Neurosci Lett* 2005;373:184–188. [PubMed: 15619540]
- Nash JF, Yamamoto BK. Methamphetamine neurotoxicity and striatal glutamate release: comparison to 3,4-methylenedioxymethamphetamine. *Brain Research* 1992;581:237–243. [PubMed: 1356579]
- Nordahl T, Salo R, Possin K, Gibson D, Flynn N, Leamon M, Galloway G, Pfefferbaum A, Spielman D, Adalsteinsson E, Sullivan E. Low N-acetyl-aspartate and high choline in the anterior cingulum of recently abstinent methamphetamine-dependent subjects: a preliminary proton MRS study. *Psychiatry Research* 2002;116:43–52. [PubMed: 12426033]
- Nordahl TE, Salo R, Natsuaki Y, Galloway GP, Waters C, Moore CD, Kile S, Buonocore MH. Methamphetamine users in sustained abstinence: a proton magnetic resonance spectroscopy study. *Arch Gen Psychiatry* 2005;62:444–52. [PubMed: 15809412]
- Patel A, de Graaf R, Mason G, Kanamatsu T, Rothman D, Shulman R, Behar K. Glutamatergic neurotransmission and neuronal glucose oxidation are coupled during intense neuronal activation. *Journal of Cerebral Blood Flow and Metabolism* 2004;24:972–985. [PubMed: 15356418]
- Pu C, Vorhees CV. Developmental dissociation of methamphetamine-induced depletion of dopaminergic terminals and astrocyte reaction in rat striatum. *Brain Research. Developmental Brain Research* 1993;72:325–328. [PubMed: 8097974]
- Ramonet D, Rodriguez M, Fredriksson K, Bernal F, Mahy N. In vivo neuroprotective adaptation of the glutamate/glutamine cycle to neuronal death. *Hippocampus* 2004;14:586–594. [PubMed: 15301436]
- Shen J, Petersen K, Behar K, Brown P, Nixon T, Mason G, Petroff O, Shulman G, Shulman R, Rothman D. Determination of the rate of the glutamate/glutamine cycle in the human brain by in vivo ¹³C NMR. *Proceedings of the National Academy of Sciences USA* 1999;96:8235–8240.

- Stephans S, Yamamoto B. Methamphetamines pretreatment and the vulnerability of the striatum to methamphetamine neurotoxicity. *Neuroscience* 1996;72:593–600. [PubMed: 8861054]
- Sung Y, Cho S, Hwang J, Kim S, Kim H, Bae S, Kim N, Chang K, Daniels M, Renshaw P, Lyoo I. 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 and Alcohol Dependence* 2007;88:28–35. [PubMed: 17084995]
- Tata DA, Yamamoto BK. Interactions between methamphetamine and environmental stress: role of oxidative stress, glutamate and mitochondrial dysfunction. *Addiction* 2007;102(Suppl 1):49–60. [PubMed: 17493053]
- Thomas DM, Walker PD, Benjamins JA, Geddes TJ, Kuhn DM. Methamphetamine neurotoxicity in dopamine nerve endings of the striatum is associated with microglial activation. *Journal Pharmacology Experimental Therapeutics* 2004;311:1–7.
- Volkow N, Chang L, Wang G-J, Fowler J, Franceschi D, Sedler M, Gatley S, Miller E, Hitzemann R, Ding Y-S, Logan J. Loss of dopamine transporters in methamphetamine abusers recovers with protracted abstinence. *Journal of Neuroscience* 2001a;21:9414–9418. [PubMed: 11717374]
- Volkow N, Chang L, Wang G, Fowler J, Ding Y, Sedler M, Logan J, Franceschi D, Gatley J, Hitzemann R, Gifford A, Wong C, Pappas N. Low level of brain dopamine d(2) receptors in methamphetamine abusers: association with metabolism in the orbitofrontal cortex. *American Journal of Psychiatry* 2001b;158:2015–2021. [PubMed: 11729018]
- Volkow N, Chang L, Wang G, Fowler J, Franceschi D, Sedler M, Gatley S, Hitzemann R, Ding Y, Wong C, Logan J. Higher cortical and lower subcortical metabolism in detoxified methamphetamine abusers. *American Journal of Psychiatry* 158:383–389.
- Wang G, Volkow N, Chang L, Miller E, Sedler M, Hitzemann R, Zhu W, Logan J, Ma Y, Fowler J. Partial recovery of brain metabolism in methamphetamine abusers after protracted abstinence. *American Journal of Psychiatry* 2004;161:242–248. [PubMed: 14754772]
- Yamamoto BK, Bankson MG. Amphetamine neurotoxicity: cause and consequence of oxidative stress. *Critical Reviews in Neurobiology* 2005;17:87–118. [PubMed: 16808729]
- Zou JY, Crews FT. TNF alpha potentiates glutamate neurotoxicity by inhibiting glutamate uptake in organotypic brain slice cultures: neuroprotection by NF kappa B inhibition. *Brain Res* 2005;1034:11–24. [PubMed: 15713255]

Figure 1.
Location of MRS voxels in frontal cortex, frontal white matter (WM), and basal ganglia.

Figure 2.

Graphs showing relationship between time of abstinence and the GLX concentration in (A) frontal gray matter and (B) frontal white matter. The dotted black lines and the R and p-values represent the result of a linear regression. The red solid lines show the results of a mono-exponential model with a negative time-constant and a positive offset. This model is more realistic in that it represents a gradual initial rise in the GLX concentration with time towards a “steady-state” level over longer time periods. The equations show the fitted dependence of the GLX concentration (in mM) on time (in months). While the mono-exponential fit yielded a good result for the frontal gray matter GLX (A), the result was less robust for the frontal white matter (see Text).

(A)

(B)

Figure 3.

(A): Graph of frontal gray matter GLX concentrations in individuals with repeat MRS studies ($n=12$). The two measurements for each subject are connected by solid lines. The data point at the far right (at approximately 10 months; dark blue line) was obtained in a participant who was initially enrolled at 5 months of abstinence, but relapsed shortly prior to the repeat examination at 10 months. B) The bottom graph demonstrates the relationship between time of abstinence and the change in the GLX concentration in METH users over the course of 5 months, excluding the subject who relapsed.