Methamphetamine Users in Sustained Abstinence

A Proton Magnetic Resonance Spectroscopy Study

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Background: Abnormal patterns of metabolite levels have been detected by magnetic resonance spectroscopy in frontostriatal regions of individuals meeting *DSM-IV* criteria for methamphetamine dependence, but less is known about the effects of drug abstinence on metabolite levels.

Objective: To assess the effects of long-term methamphetamine use and drug abstinence on brain metabolite levels.

Design: To assess regional specific metabolite levels using magnetic resonance spectroscopy imaging techniques in 2 groups of currently abstinent methamphetamine users: methamphetamine users who recently initiated abstinence and methamphetamine users who had initiated abstinence more than 1 year prior to study.

Setting: Participants were recruited from outpatient substance abuse treatment centers.

Participants: Eight methamphetamine users with sustained abstinence (1 year to 5 years) and 16 recently abstinent methamphetamine users (1 month to 6 months) were compared with 13 healthy, non–substance-using controls.

Main Outcome Measures: Magnetic resonance spectroscopy measures of *N*-acetylaspartate–creatine and phosphocreatine (NAA/Cr), choline–creatine and phosphocreatine (Cho/Cr), and choline–*N*-acetylaspartate (Cho/NAA) ratios were obtained in the anterior cingulate cortex as well as in the primary visual cortex, which served as a control region.

Results: The absolute values of Cr did not differ between controls and methamphetamine users. Methamphetamine users had abnormally low NAA/Cr levels within the anterior cingulate cortex, regardless of the time spent abstinent ($F_{2,34} = 12.61$; $P<.001$). No NAA/Cr group differences were observed in the primary visual cortex ($F_{2,33} = 0.29$; $P = .75$). The Cho/NAA values for the anterior cingulate cortex were abnormally high in the methamphetamine users who recently initiated abstinence but followed a normal pattern in the methamphetamine users who had initiated abstinence more than 1 year prior to study ($F_{2,34} = 7.31$; $P = .002$).

Conclusions: The relative choline normalization across periods of abstinence suggests that following cessation of methamphetamine use, adaptive changes occur, which might contribute to some degree of normalization of neuronal structure and function in the anterior cingulum. More research is needed to elucidate the mechanisms underlying these adaptive changes.

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E XPOSURE TO HIGH DOSES OF methamphetamine has been shown to cause long-term changes in the dopaminergic system[1-9] as well as in the serotonergic system.[10-15] (Of the 15 studies cited, 13 involved rats and 2 involved monkeys.) Abnormalities have been observed in brain regions subserving selective attention, such as the striatum, frontal cortex and anterior cingulum cortex (ACC), and amygdala,[5,8] as well as regions subserving memory, such as the hippocampus, cortex, and the striatum.[15-17] Multiple techniques, including postmortem immunohistochemical staining, measurement of neurotransmitter levels, and in vivo measurements of transporter protein levels, have been used to infer the presence of neuronal damage or degeneration following methamphetamine exposure in animals.[10,18,19]

Recent animal and human studies suggest that neuronal changes associated with long-term methamphetamine use may not always be permanent but may partially re-

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cover with prolonged abstinence.[16,20-25] A series of positron emission tomography studies have tracked neuronal changes as a function of methamphetamine abstinence in human methamphetamine users. In 1 of the first positron emission tomography studies, McCann et al[21] reported striatal dopamine transporter abnormalities in methamphetamine-dependent subjects who had remained abstinent for approximately 3 years. In contrast, Volkow et al[23,26] reported evidence of striatal dopamine transporter normalization in detoxified methamphetamine-dependent subjects followed longitudinally. Wang et al[24] examined glucose metabolism using positron emission tomography and reported normalized thalamic metabolism following protracted abstinence (>12 months) relative to reduced metabolism assessed after a shorter abstinence interval (<6 months). In contrast, persistent decreases were observed in striatal metabolism (most accentuated in the caudate and nucleus accumbens). Whereas the recovery of thalamic glucose metabolic abnormalities could reflect adaptive compensatory responses to the damage or alterations to the dopamine system, lowered metabolism in striatal regions might reflect long-lasting changes in dopamine cell activity. Consistent with the in vivo findings of others,[23,24,26] Wilson et al[25] observed abnormally low postmortem striatal dopamine transporter protein levels and abnormally low dopamine and tyrosine hydroxylase levels in the presence of normal levels of both dopamine decarboxylase and the vesicular monoamine transporter (type II). Because the vesicular monoamine transporter is known to be abnormally low in patients with Parkinson disease, Wilson et al[25] interpreted these findings as reflecting that the damage found in methamphetamine users might be reversible.

Thus, some neuronal changes following methamphetamine exposure in human methamphetamine users could be caused by (1) normal physiological processes such as up- or down-regulation of receptors in response to differential levels of neurotransmitters, (2) transient damage or neuronal alteration, or (3) prolonged damage.[21,23,24,26] Until recently, research on neuronal recovery following long-term drug abstinence has been sparse and limited to a few investigative techniques. More research using a broad range of neuroscience methods is critical if we are to discover how the human brain responds following the insults of drug abuse. One technique that shows potential in detecting neuronal changes is magnetic resonance spectroscopy (MRS).

MAGNETIC RESONANCE SPECTROSCOPY

Recent studies have validated the use of MRS in detecting neuronal damage in animals, with evidence linking proton MRS biochemical markers with actual lesion extent.[27,28] Magnetic resonance spectroscopy allows for the visualization of a diverse group of markers of cellular integrity and function, including those of living neurons (N-acetyl compounds) comprising mainly N-acetylaspartate (NAA), high-energy metabolic products (creatine and phosphocreatine [Cr]), cell membrane synthesis or degradation (choline [Cho]), and glia (myo-inositol, choline).

The NAA signal, the most prominent and well-studied peak on the proton spectrum, represents the N-acetyl compounds, comprising several N-acetylated moieties, predominantly N-acetylaspartate, and a significant but much smaller signal from N-acetylaspartylglutamate. A putative measure of the amount of neuronal loss is assessable from the level of the amino acid derivative, NAA, which is believed to be present almost exclusively in neurons and their dendritic and axonal processes.[29-31] Several clinical conditions associated with neuronal damage or tissue loss have demonstrated region-specific low NAA via proton spectroscopy. Examples include Alzheimer disease,[32-34] seizure disorder,[35] multiple sclerosis,[29] human immunodeficiency virus-associated brain disease,[36,37] alcoholism,[38] brain tumors,[39,40] brain infarction,[41] and head trauma.[42]

The Cho signal is primarily generated by free choline, phosphocholine, and glycerophosphocholine with only minimal contributions from acetylcholine.[43] The Cho signal increases in signal intensity with membrane synthesis and turnover.[44,45] Because Cho is an index of cellular density in brain tumors and a possible marker of increased glial density associated with age and disease, it may be an ideal marker to track changes consistent with neuronal recovery associated with drug abstinence. For elderly individuals, MRS-measured Cho values do not vary in signal intensity between gray and white matter, thus allowing for the measurement of change across the cortical surface.[46] However, there is evidence of gray-white matter variability in Cho signal in young adults, with the young adults showing higher Cho values in white than in gray matter.[47] The voxels sampled in this study are predominantly gray matter.

The Cr signal reflects high-energy phosphate metabolism.[48] Spectroscopy studies often use Cr as a reference for other peaks on the assumption that its concentration is relatively constant. From in vivo MRS, higher Cr values have been found in gray matter than white matter,[36,46,49-51] with significant regional variation, maximal in the cerebellum.[47,48,52,53] Owing to the gray-white variability in Cr values, we compared the Cr values for the ACC and primary visual cortex (PVC) voxels between the controls and the subjects who used methamphetamine.

In the past few years, several studies have used MRS to examine the effects of substance abuse on the brain.[54-57] Ernst et al[54] reported an inverse correlation between frontal white NAA values and the total amount of methamphetamine usage in a group of currently abstinent methamphetamine users. In 2 subsequent studies, abnormally low anterior cingulate NAA/Cr ratios were found in the ACC.[55,57] Sekine et al[56] found evidence of an abnormally high Cho/Cr ratio in the basal ganglia of 13 abstinent methamphetamine users compared with 11 healthy controls. Furthermore, the elevation in the Cho/Cr ratio was significantly correlated with the duration of methamphetamine use and with the severity of residual psychiatric symptoms. Nordahl et al[55] found evidence of elevated Cho/Cr and Cho/NAA ratios in the ACC in methamphetamine users who had been abstinent for not longer than 3 months (reanalysis of published data set).

Table 1. Characteristics of Research Participants			
Characteristic	**Control Subjects (n = 13)**	**Recently Abstinent (n = 16)**	**Distantly Abstinent (n = 8)**
Mean ± SEM age, y	34.69 ± 2.29	37.19 ± 2.26	36.50 ± 2.80
Women	8	11	4
Mean ± SEM subject education, y	14.62 ± 0.62	13.44 ± 0.34	12.88 ± 0.35*
Mean ± SEM parent education, y	14.77 ± 0.80	14.75 ± 0.76	12.75 ± 0.94
Mean ± SEM National Adult Reading Test score	111.42 ± 2.31	109.33 ± 1.45	107.88 ± 1.49
Race, No.			
White (non-Hispanic)	7	14	7
White (Hispanic)	3	0	1
African American	2	1	0
Other	1	1	0
Right-handed, No.	12	15	6

*Significantly different from control group, $P<.03$.

Table 2. Self-reported Drug Use		
Methamphetamine Use	**Recently Abstinent (n = 16)**	**Distantly Abstinent (n = 8)**
Mean ± SEM duration, y	8.81 ± 1.08	13.63 ± 2.91
Mean ± SEM time abstinent, m	2.95 ± 0.37	37.50 ± 5.87*
Mean ± SEM age, y	37.19 ± 2.26	36.50 ± 2.80
Mean ± SEM age at first use, y	22.38 ± 1.66	18.88 ± 3.24
Tobacco smokers, No.	9	7
Mean ± SEM National Adult Reading Test score	109.33 ± 1.50	107.88 ± 1.49

*Significantly different from recently abstinent group, $P<.001$.

STUDY RATIONALE

One goal of the current study was to replicate and extend our initial findings[55] obtained in men to a larger sample that contained a robust subsample of female methamphetamine users. A second goal was to examine the effects of sustained drug abstinence on metabolite levels in the hypothesized region of interest, the ACC. We tested the hypothesis that methamphetamine users who recently initiated abstinence would exhibit via proton MRS abnormally low ratios of ACC NAA/Cr and abnormally high Cho/Cr or Cho/NAA ratios compared with controls. Low NAA/Cr ratios and high Cho/Cr or Cho/NAA ratios would be consistent with neuronal changes or overt damage with increased membrane turnover in the ACC. We further hypothesized that no group differences in metabolite levels would be observed in the PVC, a region that receives relatively little dopamine innervation.[58,59]

METHODS

SUBJECTS

Two groups were studied: 24 methamphetamine users and 13 matched control subjects. (None of the subjects was scanned in the previous study.[55]) The methamphetamine user group was recruited from 3 substance abuse treatment centers (1 inpatient clinic and 2 outpatient clinics) and met *DSM-IV* criteria for lifetime methamphetamine dependence determined from the Structured Clinical Interview.[60] Random urine screens were performed at the referring sites. (Drugs screened in the random urine toxicology included alcohol, amphetamine, methamphetamine, methylenedioxy-methamphetamine, cocaine, benzodiazepines, barbiturates, tetrahydrocannabinol, morphine, codeine, hydrocodone, and oxycodone.) For the purposes of analyses, the methamphetamine users were divided into 2 groups: 8 distantly abstinent methamphetamine users (1 year to 5 years) and 16 recently abstinent methamphetamine users (1 month to 6 months). On average, the controls and the recently and distantly remitted methamphetamine groups did not differ in age ($F_{2,34}=0.314$; $P=.73$), years of parents' education ($F_{2,34}=1.50$; $P=.24$), or estimates of premorbid intelligence[61] ($F_{2,32}=0.808$;

$P=.45$) but did differ marginally in years of education ($F_{2,34}=3.15$; $P=.055$) (**Table 1**). For the subjects who used methamphetamine, inclusion criteria were lifetime diagnosis of methamphetamine dependence according to *DSM-IV* criteria and ages between 20 and 50 years.

The controls were recruited from the local community. Controls met the same criteria as the methamphetamine users, except for the history of methamphetamine dependence. Exclusion criteria for both groups were (1) substance dependence other than methamphetamine (except nicotine) within the past year; (2) alcohol abuse within the past 5 years; (3) treatment or hospitalization for non–drug-related *DSM-IV* Axis I psychiatric disorders; (4) medical or neurological illness or trauma that would affect the central nervous system (eg, stroke or seizure disorder); (5) severe hepatic, endocrine, or renal disease or a history of loss of consciousness of longer than 30 minutes; (6) compound skull fracture or clear neurological sequelae of head trauma; and (7) metal implant or any other indication that would preclude a magnetic resonance imaging (MRI) procedure.

Of the 24 methamphetamine users tested, 16 had a history of tobacco abuse (range, 1-37 pack-years). Although none of the subjects met dependence or abuse criteria for illicit substances other than methamphetamine in the past 5 years, there was a history of past substance abuse (>5 years since last use) for the following substances: cocaine, n = 2 (8%); sedatives, n = 5 (21%); hallucinogens, n = 7 (29%); and alcohol, n = 8 (33%) (**Table 2**).

PROCEDURE

Single voxel 1H-MRS and structural MRI scans were acquired with a neuro-optimized 1.5-T GE Signa NV/i MRI system (GE Medical Systems, Waukesha, Wis) having a gradient specification of 40 mT/m (millitesla per meter) peak and 150 mT/m per millisecond slew rate and running LX 84M4 operating system software. Proton MRS measures of interest were NAA, Cho, and Cr. Voxels of interest were the ACC and PVC, both regions containing predominately gray matter. The NAA values were expressed as ratios of Cr, whereas the Cho values were expressed as ratios of both Cr and NAA.[45,62-64]

We used the midsagittal slice of a sagittal fast spin echo sequence to compute axial slice positions based on the identification of the anterior commissure–posterior commissure line. The following parameters were used: repetition time, 2500 milliseconds; echo time, 85 milliseconds; slice thickness, 3 mm; skip, 1.5 mm; number of excitations, 1; time, less than 2 minutes.

We acquired 19 slices parallel to the anterior commissure–posterior commissure line. These data covered the entire brain and permitted the online selection of the voxels for MRS sampling. The following parameters were used: field of view, 24

cm; 256 × 256 matrix; repetition time, 3500 milliseconds; echo time, 17/115 milliseconds; echo train length, 20; slice thickness, 5 mm; skip, 0 mm; number of excitations, 2.

Localized brain spectra were collected using a long echo time, point-resolved spectroscopy sequence[65] available on our MRI system (Probe/SV, GE Medical Systems). The sequence generates a spin echo signal within a cubical volume defined by the intersection of 3 slices formed by a succession of spatially selective 90°-180°-180° radiofrequency pulses. The following parameters were used for data acquisition: pulsed sequence database, PROBE-P; orientation, axial-oblique (anterior commissure–posterior commissure line); echo time, 144 milliseconds; repetition time, 1500 milliseconds; number of spectral points, 2048; spectral bandwidth, 2500 Hz; total number of repetitions, 128; phase cycling, 8; center frequency, water; extended dynamic range, on; water suppression optimization, on; spatial saturation pulses, on; scan time, 4 minutes 54 seconds. Prior to the 128 spectra, 8 baseline reference spectra were acquired with the point-resolved spectroscopy sequence without excitation pulses, followed by 16 spectra acquired with the point-resolved spectroscopy sequence but without prior water suppression. Voxels of dimension 2 cm × 2 cm in plane and 1 cm thick were sequentially placed using a priori rules in the ACC and the control region, the PVC.

Prescanning was performed prior to each MRS scan and included an automated first-order shimming procedure within the defined voxel and an automated radiofrequency pulse flip angle optimization procedure for optimal water suppression (Probe/SV Manual, GE Medical Systems). Prescanning routinely produces a line width of 4 Hz or less, and automatic water suppression optimization typically provides 99% suppression using a flip angle of the last of the 3 radiofrequency pulses used for suppression, of 135° ± 30°. A line width of 6 Hz or less was deemed adequate for all scans.

We used a spectral width of 2500 Hz and 2048 complex signal acquisition points, with zero filling to 4096 points. A spectral width of 2500 Hz, which is very wide relative to the spectral range of the metabolites of interest (about 300 Hz), ensured that signals from background macromolecules were separated as well as possible and did not alias into the signals from the metabolites. The water signals of the 16 non–water-suppressed spectra served as reference for the phase correction of the water-suppressed spectra that followed in the scan. The 128 spectra were averaged, baseline corrected, phase corrected, and apodized to form the spectra from which spectral peaks and areas were derived by separate analysis of the individual resonances. Phase correction aligned the metabolite signals along the known inphase (real part of complex spectra) direction. Metabolite peaks for NAA (2.02 ppm), Cr (3.03 ppm), and Cho (3.21 ppm) were easily identified in all of the spectra.

The ACC voxel (**Figure 1**; for spectra, see **Figure 2**) was placed at the midline and included samples from both left and right hemispheres. This voxel abuts posteriorly on the anterior portion of the corpus callosum. The caudate was well-formed visually at this level, but the sampling was near the superior portion of the putamen, at a level with dense striatal connections. The sampling of the ACC contained the terminal portion of axons synapsing in the ACC, including the neuropil, which is primarily gray matter.[15]

The PVC voxel (**Figure 3**) was sampled in the midline and included tissue from both the left and right occipital hemispheres. The placement of the sample was anterior nearly to the point of sampling some ventricle to ensure exclusion of signals from posterior scalp lipids. This voxel was acquired at a level sufficiently inferior so that the superior portion of the voxel did not sample parietal cortex.

Fully automated brain segmentation procedures were performed to separate the axial slices into the 3 components: white
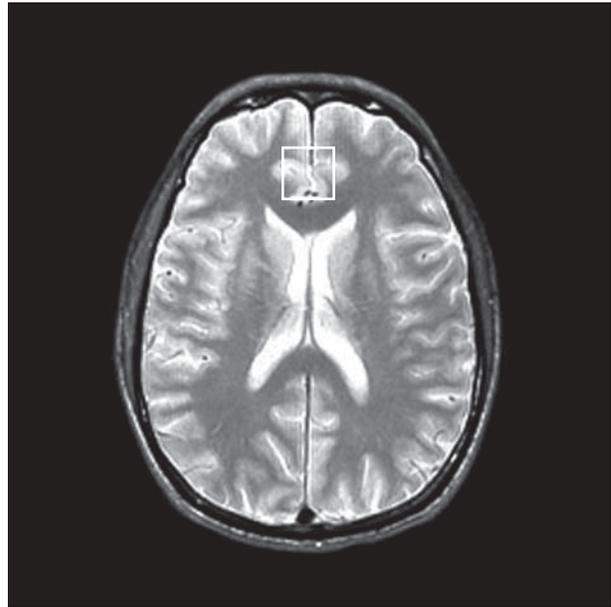

Figure 1. Oblique-axial slice proton density magnetic resonance image showing the typical location (white box) of the voxel sampling the anterior cingulate cortex. The left side of the figure is the right side of the brain.
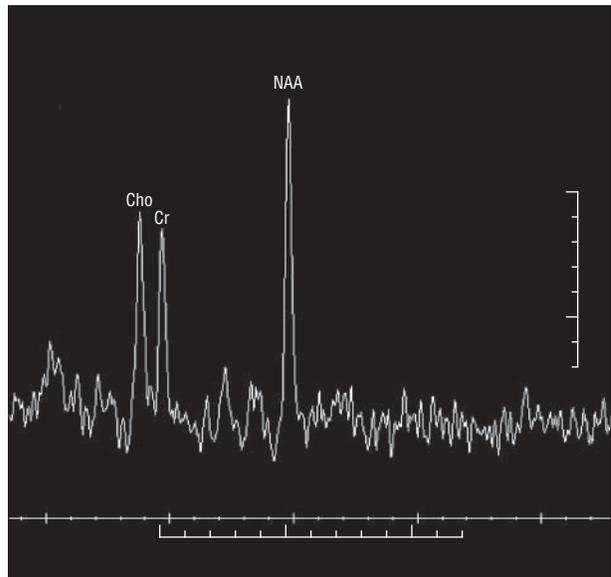

Figure 2. Representative proton magnetic resonance–extracted single spectrum acquired from the anterior cingulate cortex voxel of 1 subject. Cho indicates choline; Cr, creatine and phosphocreatine; and NAA, *N*-acetylaspartate.

matter, gray matter, and cerebral spinal fluid.[47,66,67] Similar to the segmentation algorithm described by others,[47,66,67] our algorithm was based on the short echo (proton density) and the long echo (T2-weighted) MRI images (or dual-echo, fast spin echo images) of the same slice location. We performed the cerebral spinal fluid–brain tissue separation similarly to that in Cohen et al[66] by using the intensity-shifted T2-weighted image minus the proton density image. This "image math" technique (eg, image subtraction) was applied to further enhance the cerebral spinal fluid separation in the T2-weighted image.[4] (We remark that gray-white matter separation is based on the proton density image only and not a sum of the T2-weighted image and the proton density image.)
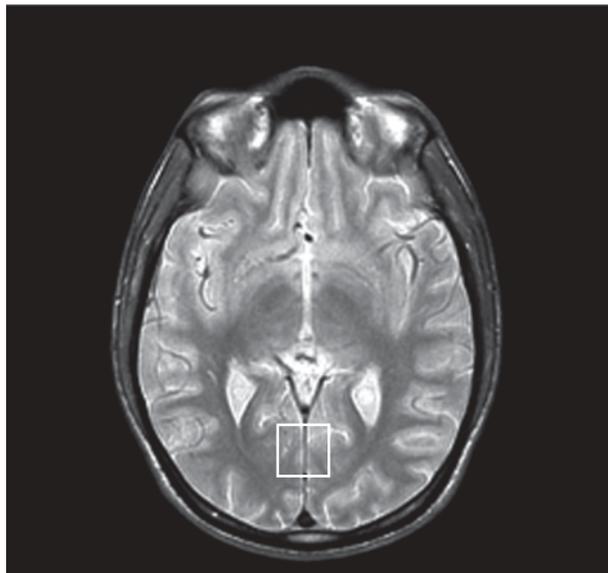
Figure 3. Oblique-axial slice proton density magnetic resonance image showing the typical location (white box) of the voxel sampling the primary visual cortex.

Table 3. Regional Metabolite Values

Metabolite Value	Recently Abstinent (n = 16)	Distantly Abstinent (n = 8)	Controls (n = 13)
Anterior cingulate cortex NAA/Cr	1.53 ± 0.03	1.56 ± 0.07	1.83 ± 0.05*
Anterior cingulate cortex Cho/NAA	0.89 ± 0.03	0.74 ± 0.05	0.73 ± 0.04†
Primary visual cortex NAA/Cr	1.95 ± 0.07	2.01 ± 0.08	2.02 ± 0.08
Primary visual cortex Cho/NAA	0.35 ± 0.01	0.33 ± 0.02	0.34 ± 0.02
Primary visual cortex Cho/Cr	0.68 ± 0.03	0.66 ± 0.03	0.68 ± 0.04

Abbreviations: Cho/Cr, choline–creatine and phosphocreatine; Cho/NAA, choline–*N*-acetylaspartate; NAA/Cr, *N*-acetylaspartate–creatine and phosphocreatine.

*Significantly different from recently abstinent and distantly abstinent groups, $P<.001$.

†Significantly different from recently abstinent group, $P<.01$.

MRS ANALYSIS

We performed quantitative analysis on each resonance (Cho, Cr, and NAA) separately using analysis software (GE Medical Systems) on the MRI system. The analysis was done using only the real part of the spectra. We set a narrow frequency window around the residual water resonance and each of the metabolite resonances. Each resonance was apodized a second time to effect line width normalization based on the width of the Cr resonance and to effect a line shape transformation from a Lorentzian to a Gaussian distribution. Because we normalized the line widths, directly measuring the heights of the processed resonances was then equivalent to measuring areas under the unprocessed resonances. All line widths were transformed to 1.0 Hz. Each processed resonance was then curve fit to a Gaussian using the Levenberg-Marquardt method. The maximum value of the Gaussian fit was assumed to represent the relative metabolite concentration, from which the NAA/Cr, Cho/Cr, and Cho/NAA ratios were computed.

STATISTICAL ANALYSIS

Only MRS data with adequate quality of shim (main field homogeneity) were included for statistical analysis. All values presented are 2-tailed unless otherwise specified. For the group comparisons, absolute Cr values were examined for the primarily gray regions, the ACC and PVC. We divided these absolute Cr values into the respective metabolites as a part of the normalization process.[62] Using information from segmentation of the brain, we adjusted the Cr values for the ACC voxel and the PVC voxel to control for cerebral spinal fluid signal. These corrected Cr values were compared between the subject groups.

Using linear multiple regression, we examined correlations between relative metabolites, years of usage, and months of abstinence in the methamphetamine users. In addition, we used nonparametric correlational techniques to examine the relationship between years of usage and relative metabolites separately for each of the 2 groups of methamphetamine users.

RESULTS

GROUP DIFFERENCES IN REGIONAL METABOLITE VALUES

The absolute Cr values did not differ between the methamphetamine users who recently initiated abstinence, the methamphetamine users who had initiated abstinence more than 1 year prior to study, or the control group, either in the ACC or the PVC (ACC, $F_{2,34} = 0.27$, $P = .77$; PVC, $F_{2,32} = 1.13$, $P = .33$). No group differences were observed for the adjusted Cr values (ACC, $F_{2,34} = 0.11$, $P = .89$; PVC, $F_{2,32} = 0.93$, $P = .40$). There were significantly lower values for the ACC NAA/Cr in both the short- and long-term abstinent groups of methamphetamine users compared with the controls ($F_{2,34} = 12.61$; $P<.001$). In addition, there was a significant interaction between group and normalized ACC Cho values (Cho/Cr, $F_{2,34} = 4.14$, $P = .03$; Cho/NAA, $F_{2,34} = 7.31$, $P = .002$). Planned comparisons revealed that the ACC Cho/NAA values were statistically greater for the methamphetamine users who recently initiated abstinence compared with both the controls ($F_{1,27} = 14.1$, $P<0.001$) and the methamphetamine users who had initiated abstinence more than 1 year prior to this study ($F_{1,22} = 7.7$; $P = .01$). The ACC Cho/NAA values did not differ between the control subjects and the methamphetamine users who had initiated abstinence more than 1 year prior to this study ($F_{1,19} = 0.055$; $P = .82$). No metabolite abnormalities were noted for the control region, the PVC (NAA/Cr, $F_{2,33} = 0.29$, $P = .75$; Cho/Cr, $F_{2,33} = 0.15$, $P = .86$; Cho/NAA, $F_{2,33} = 0.50$, $P = .61$). (Primary visual cortex metabolite values were not available from 1 control subject.)

METABOLITE CORRELATIONS WITH YEARS OF USE, LENGTH OF REMISSION, AND OTHER METABOLITES

There were no significant differences in years of methamphetamine use ($F_{1,22} = 3.6$; $P = .07$) or ages of first use ($F_{1,22} = 1.15$; $P = .30$) between recently and distantly abstinent methamphetamine users. All comparisons are presented as 2-tailed values (**Table 3**).

Using multiple regression techniques, we examined the relationship between months of abstinence, years of usage, and relative metabolites. Months of abstinence correlated inversely with ACC Cho ratios (Cho/Cr, t = −2.60, P = .02; Cho/NAA, t = −3.09, P = .006) but not with ACC NAA/Cr ratios (t = 1.05, P = .31). Years of usage correlated with ACC Cho/NAA ratios (t = 2.08; P = .05) but not with Cho/Cr ratios (t = 0.47; P = .65). Years of usage correlated inversely with NAA/Cr ratios (t = −2.78; P = .03). As expected, no correlation emerged for years of usage, months of abstinence, and any of the PVC metabolite ratios (P > .28) (**Figure 4**).

Spearman correlations were carried out to examine the relationship between metabolite ratios and years of use separately in the 2 groups of methamphetamine users. Among the methamphetamine users who recently initiated abstinence, no significant correlations were observed between years of usage and any of the metabolite values (ACC NAA/Cr, P = .71; ACC Cho/Cr, P = .99; ACC Cho/NAA, P = .39; PVC NAA/Cr, P = .64; PVC Cho/Cr, P = .43; PVC Cho/NAA, P = .60). We observed a similar pattern among the methamphetamine users who had initiated abstinence more than 1 year prior to this study (ACC NAA/Cr, P = .33; ACC Cho/Cr, P = .15; ACC Cho/NAA, P = .11; PVC NAA/Cr, P = .45; PVC Cho/Cr, P = .18; PVC Cho/NAA, P = .32).

SEX ANALYSES

Because this study extended our previous findings to a larger group that included both men and women, it was relevant to examine sex in terms of the dependent metabolite values. No sex differences in any of the metabolite ratios were observed: ACC NAA/Cr (F = 0.029; P = .59), ACC Cho/Cr (F = 0.246; P = .62), and ACC Cho/NAA (F = 0.039; P = .85).

COMMENT

In recent years, there has emerged compelling evidence from both animal and human studies that methamphetamine creates changes within frontal-striatal regions of the brain.[5,8,10,17,55,57] The data in this study from both male and female methamphetamine users replicate our initial findings of abnormally low NAA/Cr levels in the ACC of male methamphetamine users.[55] Abnormal ACC NAA/Cr levels were observed across all methamphetamine users, regardless of length of methamphetamine abstinence. Sustained low ACC NAA/Cr levels in the presence of normal ACC Cho/NAA levels suggest that some neurochemical changes that occur as a result of long-term methamphetamine exposure might be more permanent than others. In addition, the findings that ACC NAA/Cr levels correlate with years of usage, whereas ACC Cho/NAA levels correlate with months of abstinence, further indicate that the changes in the relative metabolite values (NAA/Cr and Cho/NAA) might involve different processes associated with methamphetamine use. Further, the finding that abnormally high Cho/NAA levels within the ACC of methamphetamine users observed early in remission (1-6 months) return to normal levels fol-
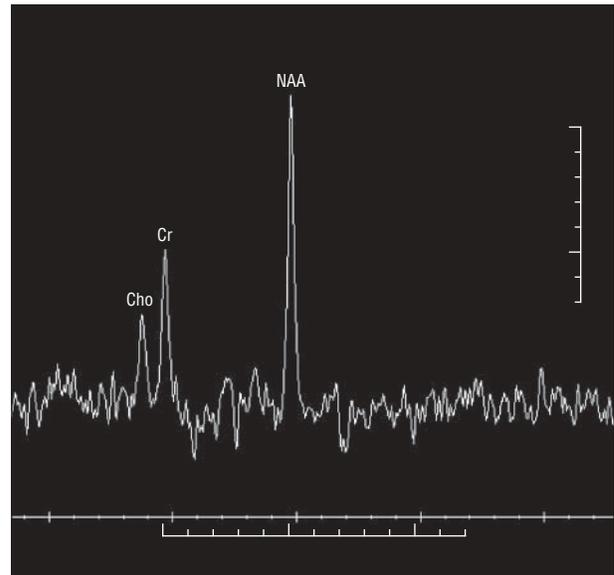

Figure 4. Representative proton magnetic resonance–extracted single spectrum acquired from the primary visual cortex of 1 subject. Cho indicates choline; Cr, creatine and phosphocreatine; and NAA, *N*-acetylaspartate.

lowing extended remission periods may represent an adaptive response to overt damage. Findings from animal studies may provide viable models for interpreting our data because a number of animal studies have reported cellular changes occurring in the first year following exposure to methamphetamine.[10,15,68,69]

Pennypacker et al[69] reported that following exposure to methamphetamine, neurons in rats expressed genes to adapt to changes in the postinjury state. The postinjury response is mediated by certain transcription factors that are induced or modified to alter transcription of these genes over a period of days to months. One initial consequence of this response on the molecular level is a reactive gliosis occurring between 14 and 21 days postinjury, which can lead to early elevations in Cho levels. Because Cho is found in glial tissue, this might account for the relatively elevated Cho levels observed in subjects who were studied in the first month of remission. Subsequently, at 2 to 8 months following exposure, axonal sprouting begins, occurring primarily in anterior brain regions with less evidence of sprouting and membrane turnover in posterior brain regions such as occipital cortex.[10,15]

According to this model, in the first 8 months following methamphetamine exposure, 3 processes may contribute to elevated Cho levels: (1) the release of Cho-containing compounds associated with acute damage to membrane, (2) gliosis, and (3) membrane biosynthesis. The time course of this regeneration phase, which includes reactive gliosis, increased membrane turnover, and axonal sprouting, may provide 1 explanation for our findings of elevated Cho levels in methamphetamine users in early remission (1-6 months). In contrast, longer remission periods may be characterized by less membrane synthesis and turnover, potentially explaining the normalized relative Cho values measured in the affected regions. In addition, those subjects in later remission may

no longer be experiencing axonal sprouting and may instead be experiencing pruning, as mistargeted axons undergo a natural pruning process.[70]

LIMITATIONS

This study has limitations, including the lack of a pre–methamphetamine-use baseline. To minimize the possibility that group differences were due to preexisting abnormalities in the methamphetamine users, we excluded those who had non–drug-related Axis I disorders. Nonetheless, history of drug abuse other than methamphetamine, a common comorbidity of such individuals, could have contributed to the metabolite abnormalities. To minimize such effects, we studied subjects whose primary drug of choice was methamphetamine and whose alcohol abuse happened more than 5 years prior to the time of study. It is also possible that chronic tobacco use might potentiate the effects of methamphetamine by degrading the ability of the brain to metabolize dopamine.[71,72] To minimize the effects of nicotine on our results, we included a subsample of controls (n = 6; 46%) who were chronic smokers.[73]

Some studies have found persistent neuronal terminal abnormalities as a result of long-term methamphetamine use, whereas others have reported evidence of normalization following extended periods of abstinence.[21,23,24,26] The findings within the present study suggest that adaptive changes, as evidenced by reduced Cho/Cr and Cho/NAA ratios, may occur across periods of extended drug abstinence. In the early periods following methamphetamine exposure, the brain may might undergo several processes leading to increased membrane turnover. The relative Cho normalization across periods of abstinence suggests that when drug exposure is terminated, adaptive changes occur, which may contribute to some degree of normalization of neuronal structure and function. More research using a longitudinal design is needed to elucidate the mechanisms underlying these adaptive changes.

CLINICAL IMPLICATIONS AND FUTURE DIRECTIONS

The understanding of how the human brain can recover or partially recover as a function of extended drug abstinence has important implications both for the neurobiology of addiction and substance abuse treatment. If damaged or altered neuronal tissue partially normalizes across extended periods of abstinence, this finding would be clinically salient. Researchers studying motor neuron disease[74] and multiple sclerosis[75-77] are using serial Cho and other MRS metabolite values to follow the clinical course of an illness as well as to study treatment effects, an approach that might be of value for methamphetamine users. Additional longitudinal studies of the long-term effects of methamphetamine use are needed to further understand the underlying physiological changes of stimulant drugs on the human brain.

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