Attentional Control and Brain Metabolite Levels in Methamphetamine Abusers

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Background: Methamphetamine abuse is associated with neurotoxicity to frontostriatal brain regions with concomitant deleterious effects on cognitive processes.

Methods: By using a computerized measure of selective attention and single-voxel proton magnetic resonance spectroscopy, we examined the relationship between attentional control and brain metabolite levels in the anterior cingulate cortex (ACC) and primary visual cortex (PVC) in 36 currently abstinent methamphetamine abusers and 16 non–substance-using controls.

Results: The methamphetamine abusers exhibited reduced attentional control (i.e., increased Stroop interference) compared with the controls ($p = .04$). Bonferroni-adjusted comparisons revealed that ACC levels of *N*-acetyl aspartate (NAA)– creatine and phosphocreatine (Cr) were lower and that levels of choline (Cho)–NAA were higher in the methamphetamine abusers compared with the controls, at the adjusted p value of .0125. Levels of NAA-Cr, but not of Cho-NAA, within the ACC correlated with measures of attentional control in the methamphetamine abusers ($r = -.41$; $p = .01$) but not in controls ($r = .22$; $p = .42$). No significant correlations were observed in the PVC (methamphetamine abusers, $r = .19$; $p = .28$, controls, $r = .38$; $p = .15$).

Conclusions: Changes in neurochemicals within frontostriatal brain regions including ACC may contribute to deficits in attentional control among chronic methamphetamine abusers.

Key Words: Anterior cingulate cortex, attention, imaging, methamphetamine, MRS, NAA, Stroop

In the past decade, the use of the stimulant methamphetamine has increased in the general population, with worldwide abuse of amphetamines surpassing that of cocaine and opiates combined (United Nations Office on Drugs and Crime 2004). Admissions to substance-abuse treatment programs in the United States with amphetamines as the primary substance of abuse increased fivefold in the decade for which latest figures are available, 1992–2002 (Office of Applied Studies, Substance Abuse and Mental Health Services Administration 2004). Although in the past methamphetamine abuse has been concentrated in the western United States, many indicators point to abuse and dependence spreading across the country, into urban and rural areas in the south and northeast (Community Epidemiology Work Group 2004; National Drug Intelligence Center 2005). Compounding the problem is evidence that psychostimulants, such as methamphetamine, are neurotoxic to dopaminergic frontostriatal brain regions, with corresponding deficits in selective attention and cognitive control (Ernst *et al.* 2000; Nordahl *et al.* 2003; Salo *et al.* 2005; Sim *et al.* 2002; Simon *et al.* 2000).

Damage after methamphetamine abuse to frontostriatal brain regions such as the striatum, prefrontal cortex, and anterior cingulate cortex (ACC) may contribute to the wide range of cognitive deficits observed in methamphetamine-abusing subjects (Ricaurte *et al.* 1984; Villemagne *et al.* 1998). Although early studies reported that acute doses of methamphetamine administered to drug-naive subjects actually produced improvements in cognitive processing (Kornetsky *et al.* 1959; Seashore and Ivy 1953; Soetens *et al.* 1995), it now is known that continued use of methamphetamine produces neural damage as well as cognitive sequelae of that damage (Nordahl *et al.* 2003; Rogers *et al.* 1995; Volkow *et al.* 2001). Cognitive impairments have been observed in methamphetamine abusers, with increased performance deficits appearing on tasks that require the suppression of task-irrelevant information (Salo *et al.* 2005), decision making (Kalechstein *et al.* 2003; Paulus *et al.* 2003), and working memory (McKetin and Mattick 1998). Any combination of the cognitive impairments described in this paragraph may contribute and promote maintenance of the maladaptive actions that are associated with drug-seeking behavior (Porrino and Lyons 2000; Robbins and Everitt 1999).

Study Rationale

The goal of the present study was to examine the relationship between attentional control, as measured by a computerized single-trial version of the Stroop Attention Task, and brain metabolite levels, via proton magnetic resonance spectroscopy (MRS). The Stroop task is a powerful test of selective attention that requires subjects to engage cognitive control to inhibit a prepotent but task-irrelevant response (i.e., word reading) and execute the task-relevant response (color naming; Henik and Salo 2004; MacLeod 1991; Stroop 1935). Although several studies have used MRS to examine the effects of methamphetamine on neurometabolites (Ernst *et al.* 2000; Nordahl *et al.* 2002, 2005; Taylor *et al.* 2000), none to our knowledge have linked neurochemical levels to behavioral measures of cognition, such as the Stroop task. Thus, the current study generates novel information about the relationship between neurometabolite levels within targeted regions of interest and measures of cognition. This approach is essential to examine the degree to which metham-

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Received February 2, 2006; revised July 11, 2006; accepted July 13, 2006.

0006-3223/07/$32.00
doi:10.1016/j.biopsych.2006.07.031

phetamine abuse alters cognitive control, and it will provide empirical data regarding the relationship to the underlying neural mechanisms supporting these processes. Because deficits in top-down attentional control may promote drug-seeking behavior, the understanding of how substance abuse affects both brain function and cognitive control may guide substance-abuse interventions.

Magnetic Resonance Spectroscopy

Recent studies have validated the use of MRS in detecting neuronal damage in both animals and human beings, with evidence linking proton MRS biochemical markers with actual lesion extent (Cecil *et al.* 1998; Sager *et al.* 2001). Magnetic resonance spectroscopy is based on the same physical principles as magnetic resonance imaging (MRI) and produces patterns of signals, or spectra, that allow for the visualization of diverse group of markers of cellular integrity and function. These markers include the following: (1) *N*-acetyl aspartate (NAA), which is believed to be present almost exclusively in neurons and their dendritic and axonal processes (Gonen *et al.* 2000; Simmons *et al.* 1991; Tsai and Coyle 1995); (2) creatine + phosphocreatine (Cr), which reflects high-energy phosphate metabolism (Chang *et al.* 1996) and often serves as a reference for other peaks on the assumption that its concentration is relatively constant; and (3) choline (Cho), which increases in signal intensity with membrane synthesis and turnover (Miller *et al.* 1999; Tedeschi *et al.* 1996). Furthermore, MRS-measured Cho does not vary in signal intensity between gray matter (GM) and white matter (WM), thus allowing for the measurement of change across the cortical surface (Lim *et al.* 1998).

Study Hypotheses

On the basis of our previous findings (Salo *et al.* 2002), we hypothesized that methamphetamine-abusing subjects would exhibit reduced levels of attentional control on the Stroop attention test (i.e., increased Stroop interference) compared with controls. We sought to replicate our previous findings of abnormally low NAA-Cr levels in the ACC, but not in primary visual cortex (PVC; Nordahl *et al.* 2002, 2005). Furthermore, we predicted that our measure of attentional control (i.e., the Stroop task) would correlate with reduced NAA-Cr and elevated Cho-NAA metabolite ratios in the ACC but not with metabolite ratios in the PVC, a region that receives relatively little dopamine innervation (Eberling *et al.* 2002; Hall *et al.* 1994).

Table 1. Characteristics of Research Participants

	Control Subjects (n = 16)	Methamphetamine Abusers (n = 36)
Age in y, Mean (SEM)	32.19 (1.79)	36.92 (1.56)
Females	8	23
Subject's Education in y, mean (SEM)	14.44 (.44)	13.22 (.24)[a]
Parental Education in y, mean (SEM)	14.38 (.58)	13.71 (.56)
NART	112.1 (1.21)	107.47 (.91)[b]
Race		
White (Non-Hispanic)	8	29
White (Hispanic)	5	5
African American	1	1
Other	2	1
Right-handed	13	33

NART, National Adult Reading Test.
[a]Significantly different from control group, $p < .05$.
[b]Significantly different from control group, $p < .01$.

Table 2. Self-reported Drug Use

	Methamphetamine Abusers (n = 36)
Methamphetamine Use	
Duration in y, mean (SEM)	12.37 (1.22)
Months Abstinent, Mean (SEM)	19.87 (5.37)
Age of First Use in y, Mean (SEM)	19.33 (1.01)
Tobacco Smokers	30

Methods and Materials

Subjects

Two groups were studied: 36 methamphetamine-abusing subjects and 16 age-matched non–substance-abusing control subjects.[1] The methamphetamine abusers were recruited from substance-abuse treatment centers and residential-housing programs in the Sacramento area and met DSM-IV criteria for lifetime methamphetamine dependence, as determined by using the Structured Clinical Interview (First *et al.* 1995). Random urine screens were performed at the referring sites.[2] For the methamphetamine subjects, inclusion criteria were as follows: (1) lifetime diagnosis of methamphetamine dependence according to DSM-IV criteria and (2) age between 18 and 55 years.

The controls were recruited from the local community. Controls met the same criteria as the patients, except for the history of methamphetamine dependence. Exclusion criteria for both groups were as follows: (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 (e.g., stroke or seizure disorder); (5) reported history of a seropositive test for human immunodeficiency virus; (6) severe hepatic, endocrine, renal disease, or history of loss of consciousness of more than 30 min; (7) compound skull fracture or clear neurological sequelae of head trauma; and (8) metal implantation that would preclude MRI procedure. All subjects signed informed consent that was approved by the University of California, Davis Institutional Review Board and were paid a modest stipend for their participation in the study.

On average, the controls and methamphetamine abusers did not differ in age [$F(1,50) = 3.21$, $p = .08$] or years of parental education [$F(1,50) = .61$, $p = .44$] but did differ in years of education [$F(1,50) = 6.92$, $p = .01$] and estimates of premorbid intelligence, as assessed by the National Adult Reading Test (NART; Nelson 1982) [$F(1,50) = 8.71$, $p = .005$] (Table 1). All methamphetamine abusers had been drug abstinent a minimum of 3 weeks. Substance use characteristics of the methamphetamine abusers are outlined in Table 2.

Stroop Attention Test

Apparatus. Stimuli were presented on a 14-in. video graphics array color monitor and by an IBM-compatible, computer-

[1]The cognitive data have not been published previously; however, a subset of the imaging data from 14 of the methamphetamine abusers and 7 of the controls were reported in our previous study (Nordahl *et al.* 2005).

[2]Drugs screened in the random urine toxicology included the following: alcohol, amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine, cocaine, benzodiazepines, barbiturates, tetrahydrocannabinol, morphine, codeine, hydro- and oxycodone.

controlled stimuli presentation and data collection system. Voice responses were recorded via a voice-operated relay interfaced to the microcomputer. Response timing was to 1-msec resolution and was controlled by the 8253 chip. Stimulus timing was tied to the vertical synchronous pulse.

Stimuli. Four colors were used in this experiment: red, green, blue, and yellow. The incongruent stimuli were created by printing each of the four color names in the three other ink colors. The congruent stimuli were created by printing each of the four color names in its own color. The neutral stimuli consisted of strings of "X"s printed in one of the four colors of ink. Each letter within the stimulus words was upper case and subtended 1° vertically. The width of each word display varied as a function of the word presented (range, 3–6 letters; approximately 2.4–5.4 visual degrees).

Procedure. Subjects were instructed to say aloud as rapidly as possible the color of ink that the words were printed in, while ignoring the word itself. They were given instructions to discourage a speed–accuracy tradeoff in that they were instructed to respond as quickly as possible without making too many errors. Each trial began with a fixation point of 500 msec, followed by the stimulus at the center of the screen. The onset of the subject's voice triggered the voice-operated relay switch (recorded by the computer to the nearest msec) and terminated the stimulus display on the screen. The experimenter then typed in the first letter to record the subject's response, which also initiated the subsequent trial. The fixed time interval between the subjects' response and the next trial was 494 msec. Any response–stimulus interval that exceeded 494 msec was excluded from the analysis. These excluded trials accounted for less than 1% of the trials and did not differ between groups. There were two blocks of trials, each one composed of 162 stimuli: a practice block was administered but was not included in the analysis.

Magnetic Resonance Spectroscopy

Image Acquisition. 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, Wisconsin) with a gradient specification of 40 mT/m 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 GM of the anterior cingulum and primary visual cortex (PVC). *N*-Acetyl aspartate values were expressed as ratios of Cr, whereas Cho values were expressed as ratios of both Cr and NAA (Frederick *et al.* 1997; Kattapong *et al.* 1996; Tedeschi *et al.* 1996).

Sagittal Scout Sequence: The midsagittal slice of a sagittal fast spin echo (FSE) sequence (repetition time (TR) = 2500 msec, echo time (TE) = 85 msec, slice thickness = 3 mm, skip = 1.5 mm, number of excitations (NEX) = 1, time < 2 min) was used to compute axial slice positions on the basis of the identification of the AC-PC line.

Axial FSE Sequence: 19 oblique slices (FOV = 24 cm, 256 × 256 matrix, TR = 3500, TE = 17/115, echo train length = 20, slice thickness = 5 mm, skip = 0 mm, NEX = 2) were acquired parallel to the anterior commissure-posterior commissure line (AC-PC) line. These data covered the entire brain and permitted the online selection of the voxels for MRS sampling.

Single-Voxel MRS Sequence: Localized brain spectra were collected by using a long TE point-resolved spectroscopy (PRESS) sequence (Bottomley 1987) that is 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 three slices formed by a succession of spatially selective 90°-180°-180° radiofrequency pulses (RF) pulses. The following parameters were used for data acquisition: pulsed sequence database (psd), PROBE-P; orientation, axial-oblique (AC-PC line); TE, 144 msec; TR, 1500 msec; number of spectral

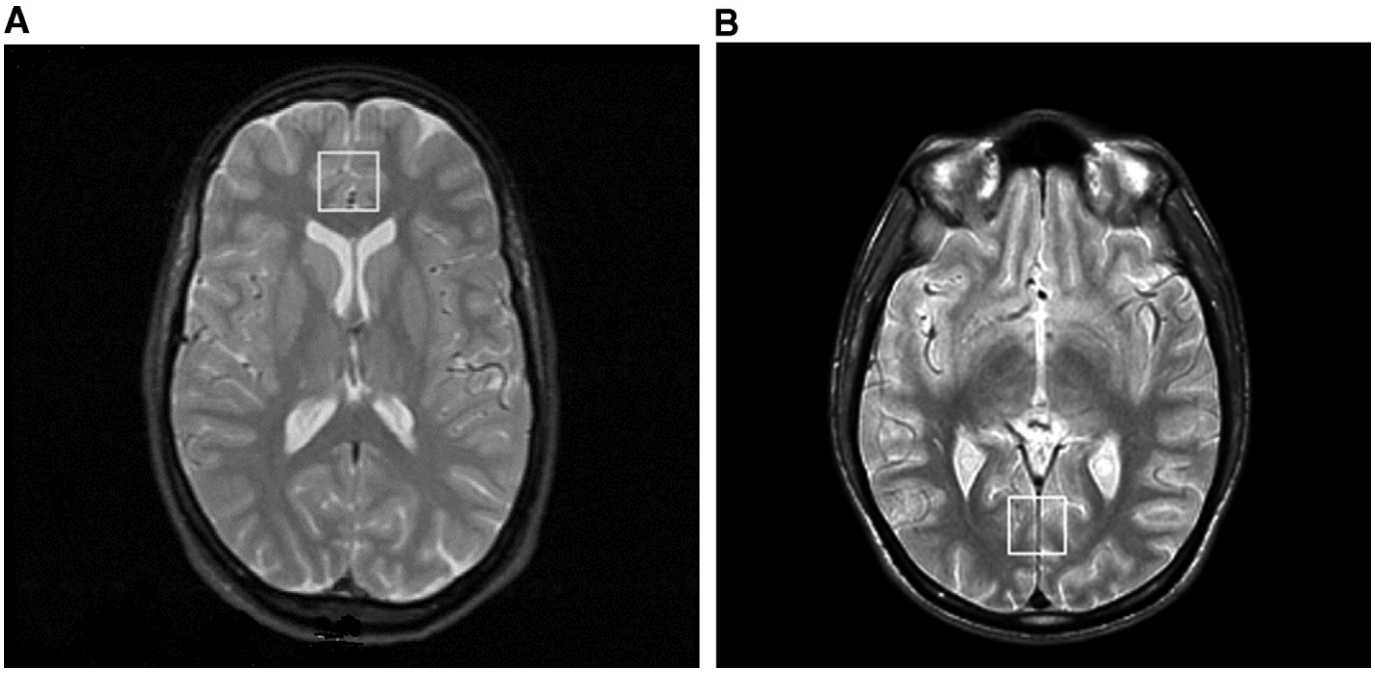

Figure 1. Oblique-axial proton density magnetic resonance image showing the typical location (white box) of the voxel sampling the anterior cingulate cortex (**A**) and the primary visual cortex (**B**). In each panel, the left side of the figure is the right side of the brain.

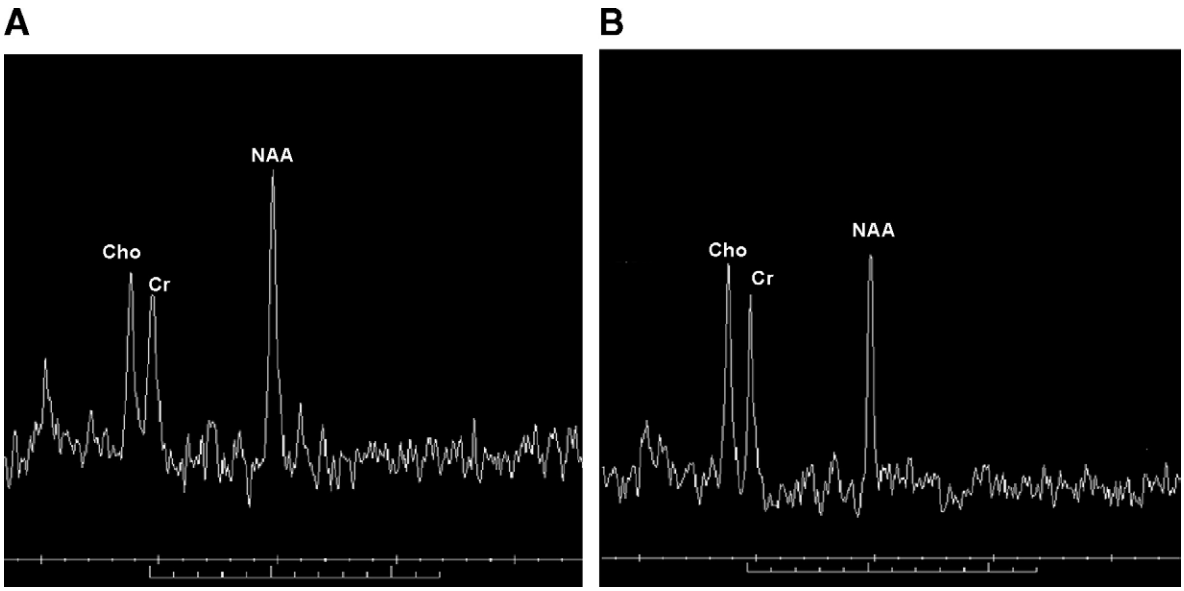

Figure 2. Representative anterior cingulate cortex spectra from one control subject (**A**) and one methamphetamine abuser (**B**). Cho, choline; Cr, creatine + phosphocreatine; NAA, *N*-acetyl aspartate.

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 min 54 seconds.

Voxels of dimension 2 × 2 cm in plane and 1 cm thick were placed sequentially by using a priori rules (described two paragraphs below) in the anterior cingulum and in the control region, the PVC. Before the 128 spectra, two baseline-reference lines of time-domain data were acquired with the PRESS sequence (PROBE/SV manual; GE Medical Systems) without application of RF excitation pulses. These data were used for estimation of the noise in the SNR calculation of individual spectral peaks. Sixteen lines of time-domain data (2 frames with 8-direction phase cycling per frame) then were acquired with the PRESS sequence without application of water suppression. The time-domain data was used for phase alignment of the water-suppressed lines acquired later in the scan to effectively correct for effects of eddy currents and to provide more accurate quantification of the spectral peak areas. The average water-suppressed time-domain data was subtracted from the average water-unsuppressed time-domain data to generate water-only time-domain data. To eliminate any residual water signal from the water-suppressed time-domain data, these water-only data were optimally scaled and subtracted from the line of water-suppressed data. The resulting water-suppressed time-domain data then were Fourier transformed to create final spectra.

Automatic prescan was performed before each MRS scan and consisted of an automated first-order shimming procedure within the defined voxel and of an automated RF pulse-flip angle-optimization procedure for optimal RF-pulse–based water suppression. Automatic prescan routinely produced a line width of 4 Hz or less. A line width of ≤6 Hz was deemed adequate for all scans. The automatic water-suppression optimization typically provided 99% suppression, using a flip angle of 135 ± 30° for the last of the three RF pulses used for suppression.

We acquired 2048 complex time-domain data points using a spectral width of 2500 Hz. Zero filling was used to expand the time-domain data to 4096 points. The 128 lines of water-suppressed time-domain data were averaged, baseline corrected, phase corrected, apodized, and Fourier transformed to form the spectra from which areas under each resonance peak was derived by independent analysis of each peak. Reported metabolite ratios were calculated as ratios of the estimates of the area under the peak. Metabolite peaks for NAA (2.02 ppm), Cr (3.03), and Cho (3.21 ppm) easily were identified in all of the spectra.

Placement of Voxels. Anterior Cingulum: The cingulum voxel (Figure 1A) was placed at the midline and included samples from both left and right hemispheres. The voxel abuts posteriorly upon 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 (Figure 2).

Primary Visual Cortex: This voxel (Figure 1B) was placed in the midline and included tissue from both the left and right occipital hemispheres. The bias of the sampling was anterior nearly to the point of sampling some ventricle, to ensure exclusion of signal 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 (Figure 3).

Data Analysis

Stroop Attention Test. Mean reaction times (RTs) for correct responses for every condition were computed for each subject.[3] Analysis of variance (ANOVA) procedures for repeated measures were used to analyze the data in a 2 × 3 mixed ANOVA, with group as a between-subjects factor (patients vs. controls) and with word type (incongruent vs. congruent vs. neutral) as within-subject variable. To calculate Stroop interference, the mean RT of all neutral trials was subtracted from the mean RT of all incongruent trials. To calculate Stroop facilitation, the mean RT of all congruent trials was subtracted from the mean RT of all neutral trials. Incorrect responses were not included within the analysis of variance for RT. Further analyses were planned to examine the effect of error responses on within- and between-

[3]Analysis of median values revealed similar group differences.

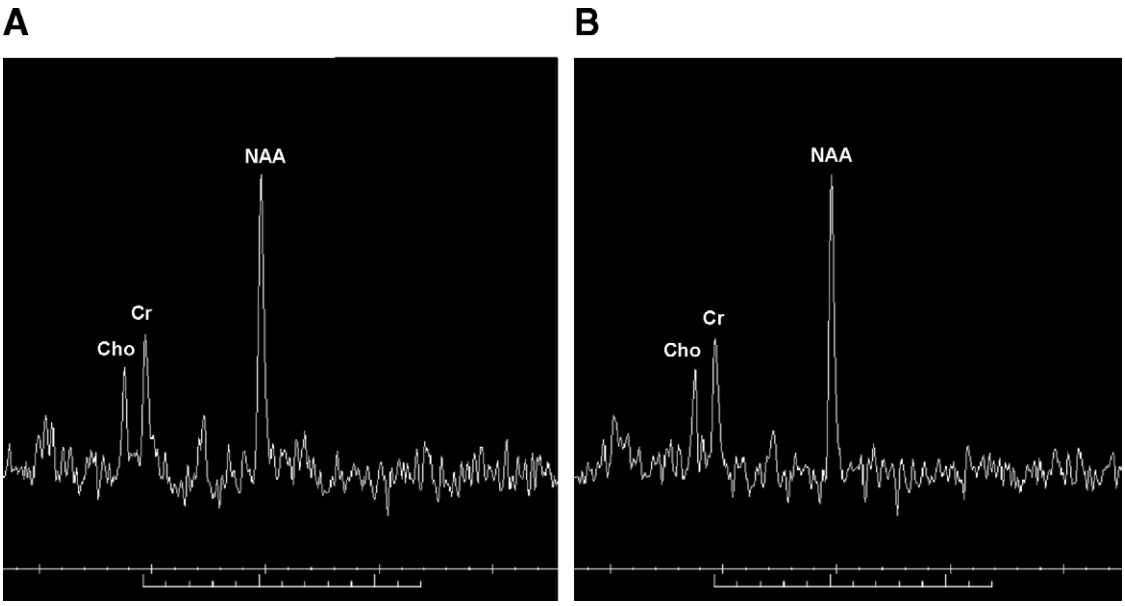

Figure 3. Representative primary visual cortex spectra from one control subject (**A**) and one methamphetamine abuser (**B**). Cho, choline; Cr, creatine + phosphocreatine; NAA, *N*-acetyl aspartate.

trial effects. Planned comparisons of interference (incongruent minus neutral) and facilitation (neutral minus congruent) were performed across all subjects. All values presented are two-tailed unless otherwise specified.

MRS Metabolites. On the real part of the phase-aligned spectra, quantitative analysis was performed on each resonance peak (Cho, Cr, and NAA) by using analysis software provided on the MRI system (PROBE/SVQ; GE Medical Systems). A narrow-frequency window was set around the residual water resonance and each of the metabolite resonances. Each resonance was apodized a second time to effect line-width normalization on the basis of the width of the Cr resonance and to effect a line-shape transformation from Lorentzian to Gaussian. By normalizing the line widths, direct measurement of the heights of the processed resonances then was 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 curve by the Marquardt-Levenberg method. The maximum value of the Gaussian fit was assumed to represent the relative metabolite concentration in arbitrary units determined by the analysis software, from which NAA-Cr, Cho-Cr, and Cho-NAA ratios were computed. Only the metabolite ratios, and specifically not the relative metabolite values, were relied on for the main statistical conclusions presented.

Only MRS data with adequate quality of shim (main field homogeneity) were included for statistical analysis. Because of the gray–white variability in Cr values, we examined the Cr values for the primarily gray regions, the ACC and PVC, because these Cr values were to be used in the normalization process. Next, we examined NAA-Cr, Cho-Cr, and Cho-NAA values in the ACC and PVC. All values presented are two tailed unless otherwise specified.

Segmentation of ACC and PVC Voxels

Fully automated brain segmentation procedures were performed to separate the axial slices into the three components: WM, GM, and cerebrospinal fluid (CSF; Cohen *et al.* 1992; Pfefferbaum *et al.* 1999, 2000). Similar to the segmentation algorithm described by other investigators (Cohen *et al.* 1992; Pfefferbaum *et al.* 1999, 2000), our algorithm was based on the short-echo (proton density, PD) and the long-echo (T2) MRI images (or dual-echo FSE images) of the same-slice location. The CSF–brain tissue separation was performed similarly to the procedure in Cohen *et al.* (1992) by using intensity-shifted T2 image subtracted by PD image. This image-math technique (e.g., image subtraction) was applied to further enhance the CSF separation in the T2 image.[4] Images were acquired 5 mm apart to obtain corrected Cr values for both cortical tissue and CSF. By this method, we were able to obtain estimates of GM and WM for the ACC, a voxel in which we observed differences in Cho ratios as well as the PVC.

Results

Behavioral Results

Reaction-time Analyses. Analyses revealed main effects of Stroop word type [$F(2,100) = 366.08$, $p = .0001$] as well as an interaction between group and word type [$F(2,100) = 3.84$, $p = .02$]. Planned comparisons revealed that RT interference (incongruent minus neutral) differed significantly between the methamphetamine abusers and controls [$F(1,50) = 4.28$, $p = .02$; one tailed], with the methamphetamine abusers exhibiting increased Stroop interference compared with controls (Figure 4). Facilitation effects did not differ between groups (control = 28.3 msec; methamphetamine abusers = 27.5 msec; $F < 1$, $p = .95$). No other main effects or interactions reached significance. We also performed analyses of covariance, controlling for differences in age, education, and NART scores. The group differences in interference endured with age ($p = .04$), education ($p = .04$), and NART scores ($p = .05$) as covariates.

Error Analyses. Although error trials were not included in the RT analyses, further analyses examined the effect of error responses on within- and between-trial effects. Analyses re-

[4]The WM–GM separation is based on PD image only, not a sum of T2 and PD image.

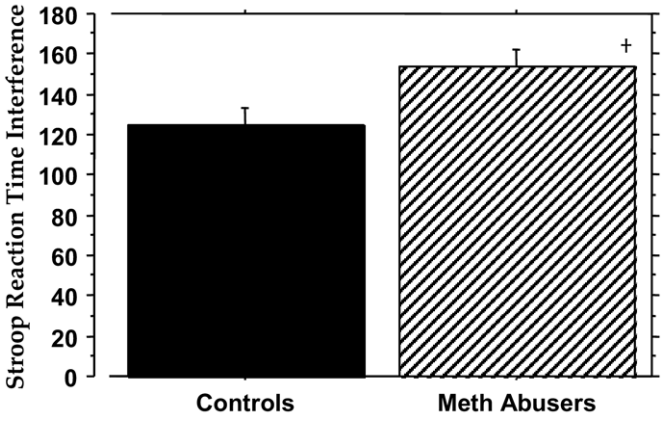

Figure 4. Group differences in mean Stroop reaction-time (RT) interference between 36 methamphetamine abusers (155 ± 8.51 msec) and 16 controls (125 ± 9.01 msec). †Significantly different from control group, $p < .05$.

vealed a main effect of word type [$F(2,100) = 40.51$, $p = .0001$]. Additional comparisons revealed that all groups made significantly more errors in the incongruent condition (13%) than in either the neutral condition (2%) or the congruent condition (1%). No other effects were significant. Analyses revealed no evidence of a speed–accuracy trade-off for both groups (methamphetamine abusers: $r = .14$, ns; controls: $r = .31$, ns). In fact, a positive correlation indicates fewer errors with faster RTs.

Imaging Results

The creatine values did not differ between the methamphetamine abusers and the control group in either the anterior cingulum or the PVC [ACC: $F(1,50) = .03$, $p = .87$; PVC: $F(1,49) = .37$, $p = .55$]. By using information from the segmented regions, the Cr values were adjusted to control for CSF signal. No group differences were observed for the adjusted Cr values [ACC: $F(1,50) = .001$, $p = .97$; PVC: $F(1,49) = 1.66$, $p = .20$; Table 3].

There were significantly lower values for the ACC NAA-Cr and higher values of the ACC Cho-NAA in the methamphetamine-abusing subjects compared within the controls [ACC NAA-Cr: $F(1,50) = 7.79$, $p = .007$; ACC Cho-NAA: $F(1,50) = 9.12$, $p = .004$]. Group differences for NAA-Cr endured when age ($p = .006$), education ($p = .008$), and NART scores ($p = .005$) were used as covariates. In a similar pattern group, differences in ACC Cho-NAA endured with the same covariates: age ($p = .001$), education ($p = .006$), and NART ($p = .005$). Values of ACC Cho-Cr did not differ significantly between the groups [$F(1,50) = 1.40$, $p = .24$]. No metabolite abnormalities were noted for the control region, the PVC [NAA-Cr: $F(1,49) = 1.61$, $p = .21$; Cho-Cr: $F(1,49) = .01$, $p = .92$; and Cho-NAA: $F(1,49) = .56$, $p = .46$].[5] We applied Bonferroni corrections for the four planned comparisons, which were tested one tailed because of the hypothesized directions (lower NAA-Cr and higher Cho-NAA in the ACC but not the PVC of the patients than controls); the required p value for statistical significance was $.05/4 = .0125$. The statistically significant comparisons in the ACC remained significant.

Correlations with Cognitive Measures and Metabolite and Usage Patterns

A significant correlation was observed between Stroop RT interference and NAA-Cr levels in the methamphetamine abusers. Longer RTs in the Stroop task (i.e., greater interference) correlated with lower NAA-Cr levels in the ACC ($r = -.41$, $F = 6.98$; $p = .01$) but not in the PVC ($r = .19$; $F = 1.21$; $p = .28$). No significant correlations were observed between Stroop RT interference and ACC Cho-Cr ($r = .04$; $F = .05$; $p = .83$) or ACC Cho-NAA ($r = .26$; $F = 1.64$; $p = .12$). Furthermore, we tested for statistical differences between the slopes of the two regions (ACC and PVC) with NAA-Cr ratios and found that they differed significantly ($p = .005$; one-tailed). None of the correlations reached statistical significance in the controls (Figure 5).

By using linear multiple-regression techniques, we also examined correlations between usage patterns, metabolite ratios, and the cognitive measures in the methamphetamine abusers. Correlations between Stroop RT interference and ACC NAA-Cr endured when age ($F = 4.42$; $p = .02$), years of use ($F = 3.76$; $p = .02$), and months of drug abstinence ($F = 6.98$; $p = .005$) were used as covariates. As expected, no correlation emerged for years of usage or months of abstinence with any of the PVC metabolite ratios.

Discussion

This current study is one of the first MRS studies in methamphetamine abusers to examine the relationship between changes in neurometabolites and cognitive performance. Consistent with our previous behavioral studies, we observed group differences in Stroop RT interference between methamphetamine abusers and non–substance-using controls (Salo *et al.* 2002). Group analyses also revealed abnormally low NAA-Cr and elevated Cho-NAA levels in the ACC of methamphetamine-abusing subjects compared with control subjects, but not in PVC. A significant correlation between abnormally low NAA-Cr ratios in the ACC and reduced levels of attentional control (i.e., increased Stroop interference) also was observed in the methamphetamine abusers but not in the control subjects. Because levels of NAA have been found to correlate with measures of selective attention in other studies, these findings suggest that neuronal integrity within the ACC may be essential to regulate cognitive mechanisms that monitor conflict and that the integrity of the ACC may be compromised after long-term methamphetamine abuse (Grachev *et al.* 2001).

Exposure to high doses of methamphetamine and related stimulants has been shown to cause long-term changes in the dopaminergic system (Ricaurte *et al.* 1980, 1982, 1983, 1984; Wagner *et al.* 1980). Medial frontal brain regions, such as the ACC, are relatively rich in dopamine innervation and thus may be particularly vulnerable to the neurotoxic effects of long-term methamphetamine abuse (Paus 2001). The role of the ACC within a neural control network has been described as one of

Table 3. Regional Metabolite Values

Metabolite Ratios, Mean (SEM)	Control Subjects ($n = 16$)	Methamphetamine Abusers ($n = 36$)
ACC: NAA-Cr	1.79 (.04)	1.62 (.04)[a]
ACC: Cho-Cr	1.25 (.05)	1.31 (.03)
ACC: Cho-NAA	.70 (.02)	.82 (.02)[a]
PVC: NAA-Cr	2.10 (.05)	2.01 (.05)
PVC: Cho-Cr	.65 (.02)	.65 (.02)
PVC: Cho-NAA	.31 (.01)	.33 (.01)

ACC, anterior cingulate cortex; Cho, choline; Cr, creatine + phosphercatinine; NAA, *N*-acetyl aspartate; PVC, primary visual cortex.
[a]Significantly different from controls, $p < .01$.

[5]Primary visual cortex metabolites were missing for one methamphetamine abuser.

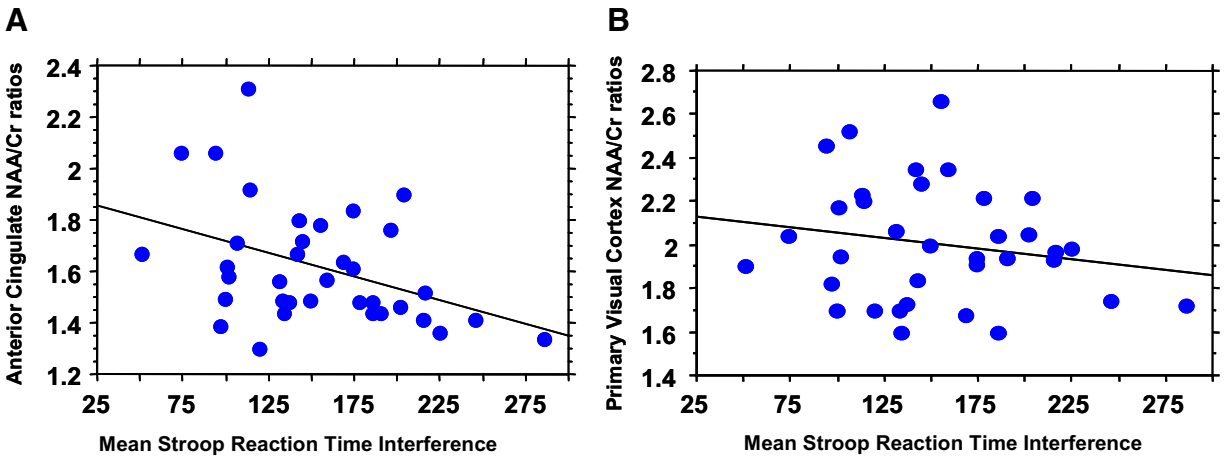

Figure 5. Correlation between NAA-Cr metabolite levels in the anterior cingulate cortex ($r = .41$; $p = .04$; **A**) and primary visual cortex (r = ns; **B**) and mean Stroop reaction-time interference in 36 methamphetamine abusers.

monitoring actions and contributing to the control of goal-related behavior (Carter *et al.* 1999; Kerns *et al.* 2004; MacDonald *et al.* 2000). Thus, abnormal ACC function may result in dysregulation of goal-directed behavior in that substance-abusing individuals often continue to use drugs when the use is associated with a negative outcome (Kalivas and Volkow 2005). These behaviors may be characterized as impulsive in nature because they reflect actions associated with immediate reward without consideration of the long-term negative consequences associated with drug use (Chambers *et al.* 2001).

Addiction is thought to be caused by long-lasting changes in the brain that result in a compromised ability to exercise top-down control (Kalivas and Volkow 2005). Thus, it is noteworthy that NAA-Cr, and not Cho-NAA values correlated with our measure of attentional control. Recent studies suggest that NAA is synthesized within the mitochondria and that decreases in NAA correlate with reductions in adenosine triphosphate (Manji *et al.* 2000). Thus NAA can be regarded as a marker of neuronal viability as well as a neuronal marker (Grachev *et al.* 2001; Ohrmann *et al.* 2004; Tsai and Coyle 1995). In contrast, alterations in Cho levels may represent a short-term pattern of response to neuronal injury and not sustained neural changes that support cognitive regulation (Nordahl *et al.* 2005; Pennypacker *et al.* 2000).

Limitations

One key limitation is a lack of baseline in the current study. To minimize the possibility that group differences were caused by pre-existing abnormalities in the methamphetamine subjects, 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 abuse or dependence of other substances was greater than 5 years before time of study. It also is possible that chronic tobacco use may potentiate the effects of methamphetamine by degrading the ability of the brain to metabolize dopamine (Fowler *et al.* 1996a, 1996b). To minimize the effects of nicotine on our results, we included a subsample of controls (32%) who were chronic smokers (Brody *et al.* 2004; Durazzo *et al.* 2006; Table 2). We also conducted post hoc analyses on those methamphetamine abusers who were smokers ($n = 30$) versus those who were nonsmokers ($n = 6$) and found no group differences in the metabolites of interest within the ACC (NAA-Cr, $p = .49$; Cho-Cr, $p = .63$; and Cho-NAA, $p = .85$) or PVC (NAA-Cr, $p = .54$; Cho-Cr, $p = .28$; and Cho-NAA, $p = .41$).

Conclusion

Models of drug addiction have proposed the existence of two synergistic mechanisms that promote drug-seeking behavior (Chambers *et al.* 2001; Jentsch and Taylor 1999). These models propose that drug-seeking behavior may result both from the increased saliency of the positive rewards associated with drug use as well as a diminished capacity to control drug-seeking behavior at the cognitive level. The data in the current study would support this model by linking neurochemical abnormalities within medial frontal regions, such as the ACC, and reduced attentional control.

In our imaging studies published elsewhere of methamphetamine abusers, we have reported evidence of neurochemical changes in the ACC with sparing in the PVC (Nordahl *et al.* 2002). We also have reported evidence of normalization of select neurometabolites across periods of abstinence (i.e., Cho), whereas other metabolite changes appear more stable (i.e., NAA; Nordahl *et al.* 2005). Future longitudinal studies linking neurochemical changes across periods of abstinence to cognition will contribute to the knowledge of how brain recovery is linked to behavior. This study provides an important direction for our imaging work in substance abusers by linking neurochemical changes in the methamphetamine abusers with behavioral regulation. Increased knowledge about the neural mechanisms underlying behaviors that promote and sustain substance use will help to guide treatment strategies and will constitute an important contribution to the neuroscience of drug addiction.

This work was supported by National Institute on Drug Abuse Grants DA16293-01 (RS), DA14359 (TEN), and DA10641 (GPG).

We thank John Ryan for his expert technical assistance with magnetic resonance data collection. We also are very appreciative of the support of Cameron Carter, M.D.

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