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Low *N*-acetyl-aspartate and high choline in the anterior cingulum of recently abstinent methamphetamine-dependent subjects: a preliminary proton MRS study

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Abstract

Studies based on animal models report that methamphetamine (MA) abuse diminishes dopamine (DA) and serotonin innervation in frontal brain regions. In this in vivo human study, we used proton magnetic resonance spectroscopy (MRS), which yields measures of *N*-acetyl-aspartate (NAA), a marker of living neurons, to examine frontal brain regions possibly affected by methamphetamine dependence (MD). We tested the hypothesis that MD subjects would exhibit abnormally low levels of NAA, referenced to creatine (Cr), in anterior cingulate gray matter. We further hypothesized that the primary visual cortex, which receives relatively less DA innervation than the frontal brain regions, would show normal NAA/Cr ratios in MD subjects. Subjects included nine MD men (mean ± standard deviation (S.D.) = 32.5 ± 6.4 years) and nine age-matched control men (mean ± S.D. = 32.7 ± 6.8 years). The MD subjects were MA-free for 4–13 weeks. Proton MRS metabolites were expressed as ratios of creatine; the absolute values of which did not distinguish controls and MD subjects. With regard to metabolite ratios, the MD men had significantly lower NAA/Cr in the cingulum (mean ± standard error (S.E.): control = 1.46 ± 0.03; MD = 1.30 ± 0.03; Mann–Whitney $P = 0.01$) but not in the visual cortex (mean ± S.E.: control = 1.64 ± 0.06; MD = 1.69 ± 11; Mann–

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Whitney $P=0.52$) relative to controls. These results provide evidence for NAA/Cr deficit that is selective to the anterior cingulum, at least with respect to visual cortex, in MD subjects. The neuronal compromise that these changes reflect may contribute to the attentional deficits and dampened reward system in MD.

Keywords: Methamphetamine; Imaging; MRS; *N*-acetyl-aspartate; Choline; Anterior cingulum

1. Introduction

Animal experiments of methamphetamine (MA) exposure have shown frontal lobe and other brain regional alterations to dopamine (DA) and serotonin (5-HT) systems (Axt et al., 1991; O'Hearn et al., 1988; Preston et al., 1985; Ricaurte et al., 1980, 1982, 1983, 1984; Seiden et al., 1975; Wagner et al., 1980; Woolverton et al., 1989; Zhou and Bledsoe, 1996). Some studies have noted that axonal damage occurs in conjunction with neuronal terminal abnormalities (Fischer et al., 1995; Cass and Manning, 1999; Axt and Molliver, 1991). Zhou and Bledsoe (1996) reported that frontal cortical areas of monkeys given MA were damaged at lower doses than parietal cortical areas.

Despite the utility of animal studies in understanding the neural changes associated with MA abuse, multiple factors mitigate against generalization of results from animals to humans. For example, doses and frequency of MA exposure clearly differ between human usage and controlled animal studies. Further, the preferred DA metabolic pathways are different in rats and in humans (Cooper et al., 1996). This latter observation is important as there is evidence that DA is excitotoxic and thus different metabolic pathways may lead to different locations and degrees of damage (Chiueh et al., 1994; Michel and Hefti, 1990). These differences indicate the importance of studying the effects of MA abuse and dependence upon neural structures within the human brain.

Consistent with the animal literature, recent human functional imaging studies have noted DA transporter abnormalities in methamphetamine-dependent (MD) individuals (McCann et al., 1998b, 2000; Volkow et al., 2001a) as well as 5-HT transporter abnormalities in methylenedioxymethamphetamine (MDMA) dependence (McCann et al., 1998a). McCann et al. (1998a) found lower serotonin transporter binding sites in the cortex of MDMA-abusing subjects with primary deficits occurring in a number of brain regions including the anterior cingulum. Consistent with this, Zhou and Bledsoe (1996) found damage to serotonin axons in the anterior cingulum of monkeys given MA.

In a proton magnetic resonance spectroscopy (MRS) study, Taylor et al. (2000) reported that MD subjects had abnormally low *N*-acetyl-aspartate (NAA) values referenced to creatine (Cr) in the anterior cingulum. As NAA is a neuronal marker, this reduction was interpreted as consistent with the presence of damage in anterior cingulum of MD individuals. Taylor et al. (2000) also found that anterior cingulate NAA/Cr values in the MD subjects correlated significantly with the following neuropsychological domains: abstraction—cognitive flexibility, attention—concentration, complex perceptual—motor, sensor, and global neuropsychological functioning.

Evidence linking lateral frontal white matter areas as sites of potential damage resulting from MD has also been reported. In a study of MD subjects who were currently abstinent, Ernst et al. (2000) reported an inverse correlation between frontal white NAA values and the total amount of MA usage. However, only a trend toward abnormally low (absolute) NAA values was noted in lateral frontal white matter of these same MD subjects. Iyo et al. (1997) also found perfusion deficits in frontal cortex and anterior brain regions, but not in the occipital cortex of MD subjects compared with controls.

Here, we tested the hypothesis that MD subjects would exhibit, via proton MRS, abnormally low levels of NAA, referenced to creatine, in the anterior cingulum, but not in the primary visual cortex. Low NAA/Cr ratios and high Cho/Cr ratios would be consistent with neuroaxonal dam-

age in these regions. MRS 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 NAA), high-energy metabolic products (creatine + phosphocreatine (Cr)), cell membrane synthesis or degradation (choline (Cho)), and glia (myo-inositol (mI)). NAA is believed to be present almost exclusively in neurons and their dendritic and axonal processes (Petroff et al., 1995; Simmons et al., 1991; Tsai and Coyle, 1995). Multiple preclinical studies report correlations between NAA levels and the extent of brain lesions (Cecil et al., 1998; Roffman et al., 2000; Sager et al., 2001).

Neuropsychiatric and neurodegenerative disorders associated with neuronal damage or loss have also exhibited abnormally low NAA levels in relevant brain regions via proton spectroscopy. High Cho/Cr ratios would be consistent with glial proliferation, which would give complementary information supporting the presence of neuronal damage. We further hypothesized that the primary visual cortex, which receives relatively less DA innervation than frontostriatal brain regions (e.g. Hall et al., 1994; Eberling et al., 2002), would show normal NAA/Cr ratios in MD subjects relative to controls. Correlation analyses examined relationships between ventral and dorsal lateral frontal white matter NAA/Cr values, age, and years of MA usage.

2. Methods

2.1. Subjects

Two groups were studied: nine MD subjects mean ± S.D. (32.5 ± 6.4 years) and nine age-matched control subjects (CN) (32.7 ± 6.8 years). All subjects were men. The controls were recruited from the local community. Seven of the MD subjects were recruited from treatment facilities and two MD subjects were referred by an outreach worker from a drug-restricted living site. On average the MD group had lower estimates of premorbid intelligence (National Adult Reading Test (NART—Nelson, 1982) I.Q. = 105.1 ± 3.9) than did the control group (116.9 ± 7.4; $z = -2.805$, $P = 0.005$). Interviews with the MD subjects, along with collateral information from the treatment facility staff and an experienced outreach worker familiar with the subjects, confirmed that the subjects were MA-free for 4–13 weeks (9.3 ± 3.3 weeks) (Table 1).

All controls and MD patients were interviewed by research psychiatrists or psychologists using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID—First et al., 1995). DSM-IV diagnoses were confirmed by the use of a clinical SCID, as well as measures of MA usage and paranoia associated with MA usage. For the MD subjects, inclusion criteria were (1) lifetime diagnosis of MD according to DSM-IV criteria; (2) age range between 20 and 50 years. Exclusion criteria for both groups were: (1) substance dependence other than MA (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 which would affect the CNS (e.g. stroke or seizure disorder); (5) severe hepatic, endocrine, renal disease, or history of loss of consciousness of over 30 min; (6) compound skull fracture or clear neurological sequelae of head trauma; and (7) metal implantation that would preclude MRI scanning. It is difficult to quantitate absolute amount of MA consumed as there is considerable variability among MA laboratories (Nordahl et al., in press). Controls met the same criteria as the patients, except for the history of MD.

2.2. Procedure

2.2.1. Image acquisition

In vivo proton MRS and structural magnetic resonance imaging scans were acquired using a quadrature head coil on a 1.5-T magnetic resonance imaging scanner (5.6 System software; GE Signa, Milwaukee, WI) with echo-speed gradient hardware (GE Medical Systems; Milwaukee, WI) (2.2-G/cm maximum gradient amplitude, and 185-μs minimum rise time). Proton MR spectroscopy measures of interest were NAA, Cho and Cr. Voxels of interest were the gray matter of the anterior cingulum and visual cortex and white matter of the dorsolateral and ventrolateral frontal

Table 1
Characteristics of the MA group

Subject no.	Age	Usage (years)	Weeks abstinent	Alcohol dependence	Other abuse	NART
1	27	8	4	No	–	114
2	39	12	9	No	–	105
3	28	10	11	No	Marijuana	N/A
4	43	28	12	8 years ago	–	107
5	23	7	9	No	–	105
6	28	11	5	No	Amyl-nitrate	N/A
7	29	19	13	No	–	102
8	35	21	9	No	Crack: 1990–1992	103
9	33	20	12.86	No	–	102
Mean ± S.D.	32.5 ± 6.4	15.1 ± 7.2	9.3 ± 3.3			105.4 ± 4.2

regions. Metabolites NAA and Cho were expressed as ratios of Cr (Frederick et al., 1997; Kattapong et al., 1996; Tedeschi et al., 1996).

2.2.1.1. Sagittal scout sequence. The midsagittal slice of a sagittal gradient echo recalled series (TR = 50 ms, TE = 6 ms, flip angle = 30°, slice thickness = 5 mm, skip 2.5 mm, NEX = 1, time < 3 min) was used to compute axial slice positions based on the identification of the AC–PC line.

2.2.1.2. Axial fast spin echo sequence. Fifty-three oblique slices (FOV = 24 cm, 256 × 256 matrix, TR = 8000, TE = 17/98, echo train length = 8, slice thickness = 3 mm, skip = 0 mm, NEX = 1) were acquired parallel to the AC–PC line. These data covered the entire brain and permitted the on-line selection of the voxels for MRS sampling.

2.2.1.3. Single-voxel MRS. Localized brain spectra were collected using the GE Probe/SV acquisition protocol available on our scanner. The sequence is based on the point resolved spectroscopy (Bottomley, 1987). The following parameters were used for data acquisition: TR/TE = 2000/30 ms, 2048 spectral points, 2500 Hz spectral bandwidth, 64 averages. Automated linear shims were used to optimize the B0 field variation over the selected volume. Higher order shims were also utilized when available in conjunction with the linear shims to further optimize B0 field variation for specified volumes encompassing each sampled voxel. Unsuppressed water was acquired immediately prior to metabolite acquisition and used for spectral quantification (Webb and Macovski, 1991; Webb et al., 1994). Voxels of interest, 2×2 cm^2 in plane by 0.9 cm, were sequentially placed using a priori rules in the anterior cingulum, dorsolateral (DLPFW) and ventrolateral prefrontal white matter (VLPFW), and the control region, the primary visual cortex.

2.2.2. Placement of voxels

2.2.2.1. Anterior cingulum. The cingulum voxel (Fig. 1) 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 rich striatal connections.

2.2.2.2. Ventrolateral prefrontal white matter. This voxel was sampled at the same superior–inferior position as the anterior cingulum voxel. The voxel was placed on the right side of cortex sampling primarily white matter. The placement avoided striatum, cingulum, and frontal gray matter.

2.2.2.3. Primary visual cortex. This voxel (Fig. 1) was sampled 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 in order to ensure exclusion of signal from posterior scalp

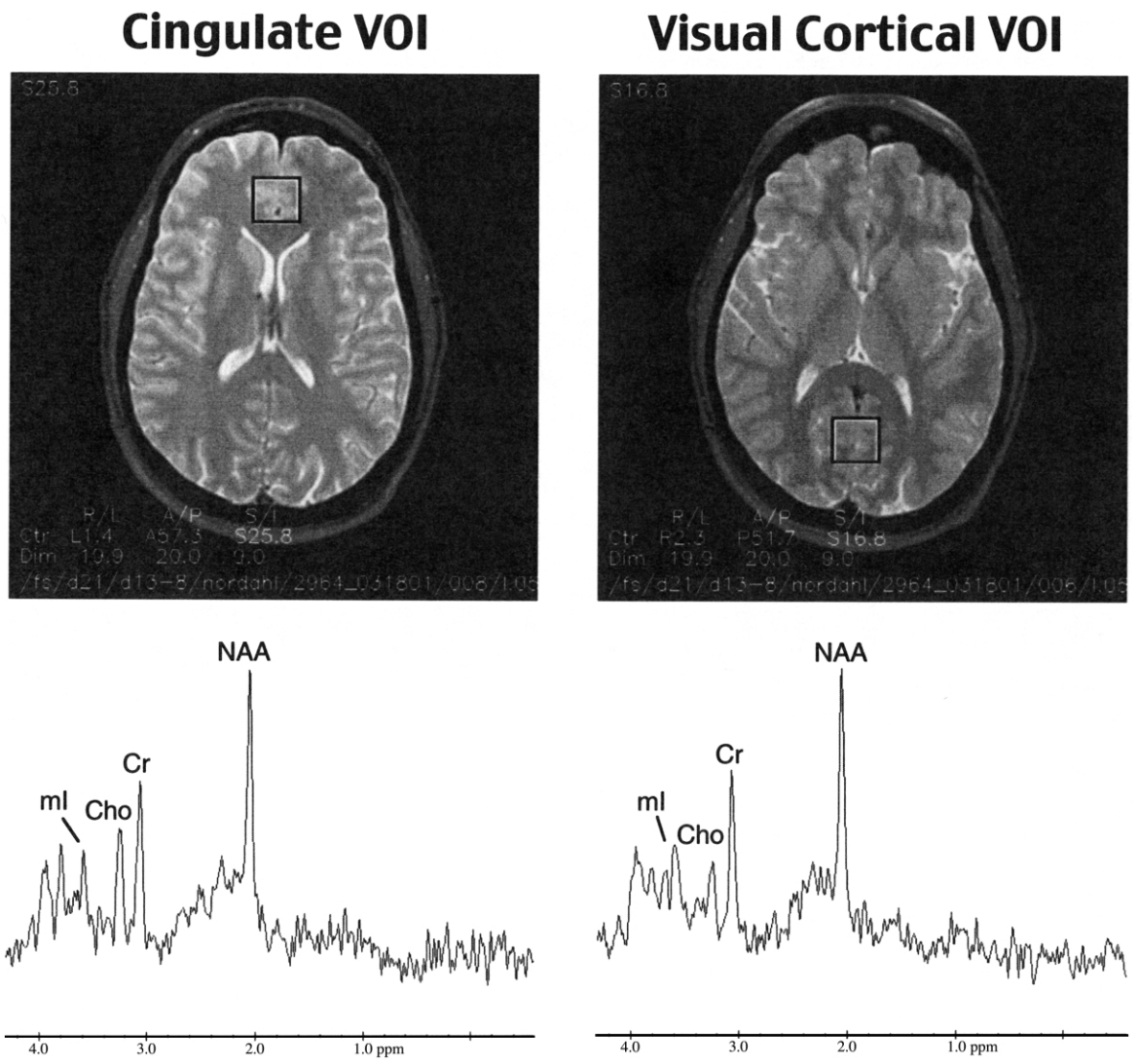

Fig. 1. VOI's, displayed on the middle slice of the three slices subtending the volume, and spectra for the cingula (left) and visual cortical (right) regions.

lipids. This voxel was acquired at a level sufficiently inferior so that the superior portion of the voxel did not sample parietal cortex.

2.2.2.4. Dorsolateral prefrontal white matter. This voxel was sampled 9–12 mm above the VLPFW. The sample emphasizes white matter and avoids sampling the striatum, cingulum, and cortical gray. The superior portion of the voxel is below the frontal curvature.

2.3. MRS analysis

Metabolite signal intensities (mI, Cho, Cr and NAA) were computed using SAGE/IDL (GE Medical Systems and Research Systems Inc.). The SAGE frequency domain fitting algorithms performed an FFT on the FID, and determined frequency and phase from the water spectrum which was used to phase correct the metabolite spectra. Eddy current and baseline corrections were

applied, a Lorentzian to Gaussian transformation was performed, and Marquardt–Levenberg non-linear optimization peak fitting was performed. Results were presented primarily as metabolite ratios relative to Cr. We first observe that there are no absolute Cr differences (in machine units) prior to forming ratios with respect to Cr. The common practice of normalization by Cr removes the across-subject variability, which arises from technical factors, such as coil loading, that affect the signal strength.

2.4. Statistical analysis

Only MRS data with adequate quality of shim (main field homogeneity) were included for statistical analysis, and so some regions of interest had different numbers of samples than other regions. Values of mI were less reliable due to their proximity to the water peak, and so we do not present those data. We utilized the conservative non-parametric Mann–Whitney test for group comparisons and similarly the non-parametric Spearman correlations for correlational analyses. All values presented are two-tailed unless otherwise specified. For the group comparisons, we first examined the absolute Cr values for the primarily gray regions, the anterior cingulum (ACC) and (control) primary visual cortex, and the primarily white matter regions, DLPFW and VLPFW, as these absolute Cr values were to be utilized in the normalization process. Next, we examined NAA/Cr and Cho/Cr values in the ACC, primary visual cortex, DLPFW, and VLPFW. Subsequently correlational analyses tested the relationship for MD subjects between VLPFW, as well as DLPFW, NAA/Cr values and age and length of MA use.

3. Results

3.1. Group differences in regional metabolic values

The MD and control groups did not differ significantly in any region of interest in absolute Cr values, which served as the denominator for the ratios tested (anterior cingulum, $z=-0.174$, $P=0.86$; occipital cortex, $z=-0.529$, $P=0.596$; VLPFW, $z=-0.116$, $P=0.908$; DLPFW, $z=-0.694$, $P=0.488$).

Abnormally low NAA/Cr was noted for the anterior cingulum ($z=-2.55$, $P=0.01$) of the MD subjects but not for the control region, the primary visual cortex ($z=+0.63$, $P=0.52$). The group differences in anterior cingulate NAA/Cr ratios persisted even when group differences in premorbid intelligence and years of education were controlled for in analyses of covariance (NART IQ: $F(1, 10)=7.092$, $P=0.024$; education: $F(1, 11)=8.520$, $P=0.0140$). Relative to the control subjects, the MD subjects had no evidence of a significant NAA/Cr difference for either frontal white matter region (VLPFW: $z=-1.389$; $P=0.165$; DLPFW: $z=-0.694$; $P=0.49$) (Table 2).

Anterior cingulate Cho/Cr was higher in the MD subjects than in the controls ($z=+1.678$, $P=0.0465$, one-tailed). Surprisingly, the primary visual cortex, the control region, showed a trend toward an abnormally *low* Cho/Cr value for the MD subjects ($z=-1.868$, $P=0.0618$). The MD subjects showed no evidence of abnormal Cho/Cr value in either of the two white matter regions: (VLPFW: $z=+1.469$; $P=0.14$; DLPFW: $z=-0.199$; $P=0.85$) (Table 2).

3.2. Metabolic correlations with age and years of MA use

We examined the relationship between age, years of use, VLPFW NAA/Cr values and DLPFW NAA/Cr values in the MD group. The results revealed a pattern of inverse correlations across variables. The VLPFW NAA/Cr value correlated significantly with age ($\rho=-0.738$, $z=-1.953$, $P=0.0508$) but not with years of usage ($\rho=-0.238$, $z=-0.630$, $P=0.52$). This pattern is in contrast to that of the controls who showed no notable correlation between NAA/Cr ratio and age. For the MD subjects there is a significant correlation between age and years of usage ($\rho=0.783$, $z=2.216$, $P=0.0267$). A factor in the significant correlation between age and metabolite ratio for the MD subjects is the increased usage with age.

Table 2
Group means ± S.E.M. in metabolite ratios for each VOI

	MD			Control			z	P (two-tailed)
	Mean	S.E.M.	n	Mean	S.E.M.	n		
NAA/Cr								
White matter								
DLPFW	1.67	0.08	7	1.79	0.12	8	−0.69	0.490
VLPFW	1.57	0.09	8	1.79	0.22	7	−1.39	0.165
Gray matter								
Anterior cingulate	1.30	0.03	7	1.46	0.03	8	−2.55	0.011
Occipital	1.69	0.11	8	1.64	0.06	8	0.63	0.529
Cho/Cr								
White matter								
DLPFW	1.11	0.03	7	1.16	0.15	7	−0.19	0.848
VLPFW	1.03	0.05	7	0.86	0.09	7	1.47	0.141
Gray matter								
Anterior cingulate	0.99	0.06	6	0.86	0.04	8	1.68	0.093
Occipital	0.51	0.05	5	0.62	0.03	7	−1.87	0.060

4. Discussion

In support of our main hypothesis, abnormally low NAA/Cr was present in the anterior cingulum, but not in the primary visual cortex, which served as the control region. The anterior cingulate NAA/Cr finding is similar to that noted by Taylor et al. (2000) in MD subjects. Ernst et al. (2000) did not sample the anterior cingulum. The Cho/Cr values for the anterior cingulum were elevated in MD subjects compared with controls. Of relevance to these MRS findings, Bartzokis et al. (2000) reported no evidence of abnormally low frontal white or gray matter volumes in MD subjects of roughly the same age range as our group. On clinical radiological reading, our subjects did not evidence atrophy in the regions sampled.

No evidence of abnormal NAA/Cr values was noted for the primary visual cortex, the control region. However, contrary to our predictions, there was a trend toward an abnormally low Cho/Cr value for the primary visual cortex of the MD subjects. We selected the primary visual cortex as our control region because this region has not been implicated in other functional neuroimaging studies of MD subjects (Kapur et al., 1994; Mann et al., 1996; Gouzoulis-Mayfrank et al., 1999; Iyo et al., 1997) and is a region with relatively little DA innervation. Furthermore, Molliver et al. (1990) noted significantly less axonal sprouting, a frequent concomitant of neural damage, in the occipital cortex compared with frontal brain regions following animal exposure to amphetamine analogues.

The frontal white matter regions, VLPFW and DLPFW, did not evidence abnormal NAA/Cr levels in the MD group. Consistent with our findings, Ernst et al. (2000) also failed to observe a significant difference in frontal white matter absolute NAA values in their MD subjects, although they did not examine ratios. They did, however, report an inverse correlation between their sampling of frontal white matter (probably closer to our VLPFW sampling) NAA and years of usage in their MD subjects. Although we did not observe a significant correlation between years of usage and VLPFW NAA/Cr, we did observe a significant inverse correlation between age and VLPFW NAA/Cr levels. As we found that years-of-usage correlated directly with age, this suggests that we may have found a significant correlation between usage and VLPFW NAA/Cr in a larger sample size.

The proton spectroscopic pattern of findings of low NAA and high Cho has been observed in a number of degenerative conditions, including

Alzheimer's disease (Pfefferbaum et al., 1999a,b) and alcoholism (Fein et al., 1994; Seitz et al., 1999). Our finding of abnormally low anterior cingulate NAA/Cr and high anterior cingulate Cho/Cr in the MD subjects is consistent with the pattern associated with neuronal loss or damage. The finding of discrete neuronal metabolite alterations, consistent with damage in the anterior cingulum, may help explain some of the cognitive deficits observed in the same MD subjects imaged in this study (Salo et al., 2002). Related cognitive findings have also been reported in other studies of MD subjects (Simon et al., 2000; Taylor et al., 2000; Volkow et al., 2001a). This is the case especially for deficits in selective attention and response inhibition, which rely on anterior cingulate integrity (for reviews, Cabeza and Nyberg, 1997).

A natural question to ask is whether these metabolite changes represents lasting damage or a temporary change given evidence of reversal of transporter abnormalities in MD-abstinent subjects (Volkow et al., 2001b). To address this, longitudinal studies of prolonged abstinence in MD patients are needed but have not yet been conducted.

This study has limitations, including lack of a pre-MA baseline. All MD subjects were men and so we cannot comment upon potential brain effects in MD women. To minimize the possibility that group differences were due to pre-existing abnormalities in the MD subjects, we excluded those who had non-drug-related Axis I disorders. Nonetheless, history of drug abuse other than MA, 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 MA and whose alcohol abuse/dependence, for example, was greater than 8 years prior to time of study. We sought subjects who, in addition to meeting the criteria for MD, had also abused MA sufficiently to have psychotic symptoms during MA use. In conclusion, these results are consistent with the presence of selective damage in the anterior cingulum, but not the primary visual cortex, of the MD subjects.

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