Published in final edited form as:

Psychiatry Res. 2007 August 15; 155(3): 179–188.

Occipital cortical proton MRS at 4 Tesla in human moderate MDMA polydrug users

Ronald L. Cowan^{a,*}, Nicolas R. Bolo^{b,d}, Mary Dietrich^a, Erica Haga^{b,d}, Scott E. Lukas^{c,d}, and Perry F. Renshaw^{b,d}

a Department of Psychiatry, Vanderbilt University Medical Center, Nashville, TN 37212

b Brain Imaging Center, McLean Hospital, 115 Mill Street, Belmont MA 02478

c Behavioral Psychopharmacology Research Laboratory, McLean Hospital, 115 Mill Street, Belmont MA 02478

d Department of Psychiatry, Harvard Medical School, Boston, MA 02155

Abstract

The recreational drug MDMA (3,4, methylenedioxymethamphetamine; sold under the street name of *Ecstasy*) is toxic to serotonergic axons in some animal models of MDMA administration. In humans, MDMA use is associated with alterations in markers of brain function that are pronounced in occipital cortex. Among neuroimaging methods, magnetic resonance spectroscopy (MRS) studies of brain metabolites N-acetylaspartate (NAA) and myoinositol (MI) at a field strength of 1.5 Tesla (T) reveal inconsistent results in MDMA users. Because higher field strength proton MRS has theoretical advantages over lower field strengths, we used proton MRS at 4.0 T to study absolute concentrations of occipital cortical NAA and MI in a cohort of moderate MDMA users ($n = 9$) versus non-MDMA using ($n = 7$) controls. Mean NAA in non-MDMA users was 10.47 mM (± 2.51), versus 9.83 mM (± 1.94) in MDMA users. Mean MI in non-MDMA users was 7.43 mM (± 1.68), versus 6.57 mM (± 1.59) in MDMA users. There were no statistical differences in absolute metabolite levels for NAA and MI in occipital cortex of MDMA users and controls. These findings are not supportive of MDMA-induced alterations in NAA or MI levels in this small sample of moderate MDMA users. Limitations to this study suggest caution in the interpretation of these results.

Keywords

Ecstasy; neuroimaging; drug abuse; spectroscopy; neurotoxicity

1. INTRODUCTION

The recreational drug MDMA (3,4, methylenedioxymethamphetamine), sold under the street name of “Ecstasy”, has enjoyed renewed popularity in the last decade. The names Ecstasy and MDMA are often used synonymously in the world’s literature. However, not all preparations sold as MDMA actually contain MDMA and these preparations may also contain other potentially neurotoxic components. MDMA appears to exert stimulant and euphoriant effects

*Address for correspondence: Ronald L. Cowan, M.D., Ph.D. Psychiatric Neuroimaging Program, Department of Psychiatry, Vanderbilt University Medical Center, 601 23rd Avenue South, Suite 3057, Nashville, TN 37212, Phone: 615-322-2303, Fax: 615-936-3563, Email: Ronald.L.Cowan@Vanderbilt.Edu, URL: www.cowanlab.com.

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via blockade of serotonergic, and to a lesser extent dopaminergic, axon terminal reuptake transporters (reviewed in Bankson and Cunningham, 2001). Studies in rodents and non-human primates have revealed that MDMA exposure at sufficiently large or sufficiently frequent dosages produces acute and chronic alterations in central nervous system (CNS) serotonergic markers, including a potential long-lasting distal axotomy of cortical and subcortical fine-diameter serotonin axons (reviewed in Kish 2002; Green et al., 2003; Lyles and Cadet 2003). However, it is unclear to what degree these changes in serotonergic markers represent structural axotomy *per se* or functional or structural changes in surrogate indicators of axon integrity (Fantegrossi et al., 2004; Wang et al., 2004; Wang et al., 2005).

Consistent with predictions of serotonergic axon damage derived from rodent and non-human primate models, neuroimaging studies in human MDMA users have revealed evidence for altered central nervous system (CNS) serotonin function (e.g. McCann et al., 1998; Semple et al., 1999; Reneman et al., 2000; Reneman et al., 2000a; Buchert et al., 2004). Among brain regions potentially affected by MDMA exposure, the occipital cortex has been consistently implicated. For example, several studies using a variety of methods, including positron emission tomography (PET) (McCann et al., 1998; Buchert et al., 2004; McCann et al. 2005), single photon emission tomography (SPECT) (Semple et al. 1999; Reneman et al., 2000; Reneman et al., 2000a), voxel-based morphometry (VBM) (Cowan et al., 2003), magnetic resonance spectroscopy (MRS) (Chang et al., 1999) have revealed associations between MDMA exposure and potential structural differences in occipital cortex. The physiology of visual occipital cortex in human MDMA users may also be altered by MDMA exposure as evidenced by lowered phosphene threshold using transcranial magnetic stimulation (TMS) (Oliveri and Calvo, 2003) and MDMA-associated increases in spatial extent of fMRI BOLD-assayed occipital cortical activation (Cowan et al., 2006).

Among studies investigating structural alterations in occipital cortex, proton MRS studies of CNS metabolites associated with structural brain integrity, including the neuronal marker N-acetylaspartate (NAA) and the putative glia-associated marker myoinositol (MI) in MDMA users have revealed mixed findings. In proton MRS investigations in human MDMA users at 1.5 Tesla (T), MDMA users were found to have no differences in occipital cortical NAA (Chang et al., 1999; Reneman et al., 2002b) versus controls. However, Chang et al. (1999) reported increased MI in MDMA users (relative to controls) in parietal white matter (WM) and a positive correlation between MI levels and prior MDMA exposure in both parietal white matter and occipital gray matter, whereas Reneman et al. (2002b) reported no differences in MI levels in their MDMA cohort (versus controls).

Given the numerous sources of potential variability in human MDMA research (including pill purity, polydrug effects, variations in brain drug level, and degree of hyperthermia) (Cowan, 2007), it is not surprising that similar studies in separate cohorts produce divergent findings. In addition, the ability of proton MRS to detect MDMA-induced metabolite alterations in human users (if present) is not known. One method to potentially improve upon the ability of proton MRS to detect subtle alterations in brain metabolites is to conduct proton MRS studies using scanners with field strengths greater than 1.5 T. Increasing the scanner field strength offers important theoretical advantages, including increased signal to noise ratio and more specific metabolite identification (Hetherington et al., 1997). As part of a larger investigation into structural and functional effects of MDMA exposure in human subjects (Cowan et al., 2003; Cowan et al., 2006), we conducted an investigation of occipital cortical NAA and MI using proton MRS with absolute metabolite quantification at 4 T.

2. METHODS

2.1 Subjects

Subjects for this study enrolled as part of a broader study examining structural, functional, and spectroscopic neuroimaging measures in MDMA users. Some subjects providing data to the current report were also participants in structural (Cowan et al., 2003) and functional MRI studies (Cowan et al., 2006). We recruited subjects via advertisements in local college newspapers advertising a brain imaging study for 18–35 year olds who had used drugs. Because studies of MDMA users worldwide have indicated that they are almost universally users of marijuana, and because their rate of use of other drugs is also higher, we attempted to recruit age and sex-matched controls that also had used a variety of drugs. We used two different advertisements to recruit the MDMA and comparison groups. The ads were identical in their description of the study, reimbursement, etc. but differed in that one advertisement mentioned a study in individuals who had used “drugs” and the other mentioned having used “MDMA (Ecstasy)” or other drugs. We screened the subjects initially by telephone and those who met provisional inclusion criteria were screened in person by a psychiatrist using a semi-structured interview to obtain personal, medical, and psychiatric history. To permit the assessment of potential cumulative drug toxicity, subjects also completed drug use history questionnaires assessing the frequency of drug use as lifetime “episodes” of drug use. Responses on the questionnaires were reviewed with the subjects by a psychiatrist. Subjects provided written informed consent as approved by the McLean Hospital Human Subjects committee and conforming to the World Health Organization’s Declaration of Helsinki (1996). Subjects were compensated for their participation.

Inclusion criteria were: 1) age 18–35, 2) at least 5 episodes of lifetime MDMA use (MDMA users group), 3) no lifetime MDMA use (non-MDMA users group), 4) negative urine drug screen on the study day (Triage Drugs of Abuse Panel, Biosite Diagnostics, San Diego, CA), 5) negative alcohol breath test on the study day (Alco Sensor III, Intoximeters, St. Louis, MO). Exclusion criteria were: 1) contraindications to MRI scanning, 2) general medical illness, 3) pregnancy (by self-report or urine test result on the study day; QuPid One Step Pregnancy Test; Stanbio Laboratory, Inc. San Antonio, TX), 4) history of substance dependence (excluding nicotine or caffeine dependence), 5) current Axis I psychiatric illness (except substance abuse disorder), 6) MDMA use within 3 weeks of the study day.

2.2 Drug use assessment

We assessed cumulative MDMA exposure using self-report of MDMA and other drug use obtained as lifetime *episodes* of use. Episodes were defined as the 24-hour period following MDMA ingestion. Subjects were instructed to estimate their lifetime prior use of recreational drugs and nicotine in categories of 1–4, 5–10, 11–39, and 40 or more episodes of drug use (categories 1–4 and 5–10 were subsequently aggregated for reporting due to low incidence in those categories). The additional drugs surveyed were non-MDMA hallucinogens (as lysergic acid diethylamide (LSD), mescaline, peyote, or psilocybin), cocaine, cannabis, sedative hypnotics (benzodiazepine or barbiturates), non-heroin opiates, alcohol, heroin, amphetamines (excluding MDMA), and phencyclidine (PCP). To minimize deception in drug use reporting, subjects were not aware of inclusion/exclusion criteria, or of the 3-week exclusion window. Subjects were aware that a negative urine drug screen was required for entry into the study.

2.3 Localized Proton MRS

Proton MRS data were acquired on a 4 T, whole body MR system (Varian, Inc., Palo Alto, CA) operating at 170.3 MHz for protons. Proton MR signals from the human brain were recorded by using a 27 cm inner diameter TEMTM design radiofrequency head coil (MR Instruments, Inc., Minneapolis, MI). Localized proton MR spectroscopy data were acquired

by using the point-resolved spectroscopy (PRESS) sequence. The acquisition parameters were as follows: spectral width, 2000 Hz; size of data set, 2048 complex points; repetition time, 3000 ms; echo time, 30 ms; number of scans averaged, 64; number of non recorded pre-scans (for attaining steady state), 4; total recording time, 3.4 minutes; acquisition volume, $1.5 \times 1.5 \times 2.0 \text{ cm}^3$ (4.5 ml). The chemical shift selective (CHESS) sequence was used for water suppression. For each subject, a single volume for spectral recordings was obtained. The volume was localized in the occipital lobe (according to the method of Chang et al., 1999) at a medial position containing mostly gray matter from the calcarine sulcus (primary visual cortex, Brodmann area 17). Localization was determined by graphical overlay of the borders of the acquisition voxel on the high contrast, high-resolution images oriented in the sagittal plane, and in an axial-oblique plane aligned along the anterior commissure-posterior commissure (AC-PC) axis passing through the visual cortex (Figure 1).

2.4 Spectral processing of Proton MRS data

Final processing of spectral data was performed using the software analysis method LCModel (Version 6.0–1) (Provencher, 1993) running on a SGI Origin workstation. The LCModel method analyzes a spectrum *in vivo* as a linear combination of model spectra of metabolite solutions *in vitro*. Absolute concentrations of MI and NAA in mM were computed after all spectra were scaled by the total gain at the time of acquisition taking into account the resonance area of the unsuppressed reference water signal (Provencher S, LCModel & LCMgui User's Manual, July 2003). The basis set of model spectra *in vitro* used to analyze the spectra *in vivo* were recorded on the same 4 T MR system using the same spectroscopic recording conditions as the spectra *in vivo*. Basis set metabolite solutions were prepared in-house according to the LCModel specifications.

2.5 Statistical analysis

Statistical analysis was performed with SPSS 15.0.1 (Statistical Package for the Social Sciences, Version 15) for Windows. Comparisons between the groups (MDMA user and non-MDMA user) of distributions of nominally-scaled variables (e.g., gender) were conducted using Chi-square tests of independence. Several of the distributions of continuously-scaled variables (e.g., years of age, NAA) were severely positively skewed. Because normal distributions could not be assumed and due to the limited sample size, nonparametric methods were used to test for statistical significance. Comparisons between the MDMA usage groups of continuously scaled variables (e.g., years of age, Absolute metabolite concentrations of NAA and MI) were conducted using Mann-Whitney U tests. These tests were also used to compare ordinal drug use frequencies between the groups. To enhance comparability with previous studies reporting NAA to creatine ratios (NAA/Cr) and MI to creatine ratios (MI/Cr) we also analyzed NAA/Cr and MI/Cr alongside the absolute NAA and MI concentrations. Spearman's rho correlations were used to assess the association of age and ordered drug exposure with absolute metabolite concentrations for NAA and MI and with NAA/Cr and MI/Cr. Mann-Whitney tests were used to assess possible differences in metabolite concentrations by gender. A maximum alpha level of 0.05 was used as the threshold for statistical significance.

3. RESULTS

3.1. Subject characteristics

Seven non-MDMA users and 9 MDMA users had high quality spectra suitable for analysis (spectra from two subjects were excluded for standard deviations above a predetermined cutoff value of 25%). There was only one female (14.3%) in the control group compared to 5 females (55.6%) in the MDMA user group ($X^2_{(16, df=1)} = 2.861, P = 0.091$). MDMA users were significantly younger ($U = 1.50, Z = 3.20, P < 0.001$) than non-users with a mean age of 26.7

years ($\pm$ SD = 4.1, min. = 24, max. = 29) for non-MDMA users and a mean age of 20.9 ($\pm$ SD = 1.9, min. = 19, max. = 23) for MDMA users.

Other drug exposure was statistically significantly higher among the MDMA users than non-users for lifetime usage of amphetamines, cannabis, cocaine, hallucinogens, and opiates (Table 1). No subjects in either group reported heroin use. Within the MDMA group, most subjects were moderate users of MDMA, with only one subject reporting 40 or more MDMA exposures.

3.2. Proton MRS

Figure 1 shows voxel placement and a representative spectrum obtained from an MDMA user. There were no statistically significant differences in absolute mean levels of MI and NAA between the non-MDMA users and MDMA user groups (Table 2). Neither were there statistically significant differences between the groups in NAA and MI to creatine ratios (Table 2). As is evident from the scatterplots of metabolite concentrations in each group, (Figure 2), there is clear overlap between non-MDMA and MDMA user groups. Of note, the MDMA users demonstrated slightly more variability in MI (Coefficient of Variation (C.V.)=0.24) than the non-MDMA users, however the reverse pattern was seen for concentrations of NAA (MDMA: C.V.=0.20; non-MDMA: C.V.=0.24) (Figure 2).

There was a statistically significant association of age with MI ($r_s=0.537$, $P=0.032$) but not with NAA ($r_s=0.187$, $P=0.448$) nor with either of the relative metabolite ratios (not shown). Statistically significantly higher levels of NAA were found for females ($M=11.43$, $SD=2.76$, min=8.33, max=15.39) than for males ($M=9.32$, $SD=1.28$, min=7.74, max=11.94) ($U=11.00$, $Z=2.06$, $P=0.039$). However, differences between gender in levels of MI were not statistically significant (Females: $M=7.86$, $SD=1.51$, min=6.42, max=9.95; Males: $M=6.40$, $SD=1.52$, min=3.90, max=9.60) ($U=14.00$, $Z=1.74$, $P=0.083$). Neither were gender differences statistically significant for the relative metabolite ratios (not shown).

Because the MDMA group had significant exposure to other drugs of abuse that could potentially influence the mean metabolite levels for NAA and MI we next considered examining the specific correlation between MDMA exposure and metabolite levels. However, this analysis was precluded by the fact that the low and high MDMA user groups had only one subject at each of these exposure levels, suggesting any correlation would be spurious.

4. DISCUSSION

In an initial study using proton MRS at 4 T, we did not detect differences in absolute levels of MI or NAA in the midline occipital cortex in a sample of primarily moderate MDMA users versus non-MDMA users. MDMA users overall had higher levels of polydrug exposure than did non-MDMA users, suggesting a potential impact of polydrug effects on the outcome variables.

4.1. Myoinositol and N-Acetylaspartate

MI is an important compound in cell membranes and second messenger cascades and has been implicated in psychiatric disorders such as bipolar disorder (Stoll et al., 2000). Increased MI and choline (Cho) due to glial proliferation have been shown in demyelinating disorders (Bitsch et al., 1999), and may serve as osmolytes in glial cell volume regulation (Ross BD et al., 1996). MI levels could potentially be altered in MDMA users due to direct drug effects or secondary influences on glial function or metabolic processes. While NAA is often cited as a neuronal specific marker (Guimaraes et al., 1995), NAA is mitochondrially synthesized (Patel and Clark, 1979; Bates et al., 1996) developmentally detectable in some glial cells, and may be present in adult glia as well (Sager et al., 1999; Bhakoo and Pearce, 2000). Decreased NAA

may serve as a reversible marker of neuronal injury (e.g. Kalra et al., 1998; De Stefano et al., 1995) and defense against cellular edema during osmotic stress (Taylor et al., 1995). Levels of NAA have been demonstrated to correlate with overall neuropsychological performance, suggesting that NAA has a global role in neuronal function (Jung et al., 1999). Like MI levels, NAA levels in MDMA users could theoretically be altered due to direct MDMA effects, or other glial, neuronal, or metabolic effects.

The mean absolute metabolite levels measured in MDMA users and MDMA non-users in the current study are generally in line with those reported by others in control populations (Savic et al., 2000; Mader et al., 2002; Auer et al., 2001).

4.2. CNS Serotonin

CNS serotonin has at least three important roles: 1) classical neurotransmitter actions in cell-cell signaling (e.g. Hoyer et al., 2002), 2) vasoconstrictive effects via innervation of intracranial blood vessels (Marco et al., 1999), and 3) cytoskeletal effects, including positive coupling to the activity of growth factors (brain derived nerve growth factor (BDNF) and S100B) implicated in maintaining neuronal integrity (Whitaker-Azmitia, 2001). Thus, MDMA use could potentially result in absolute or relative reductions in brain neuronal or glial size or volume, and therefore brain NAA or MI concentrations via transmitter-mediated toxicity, vascular ischemia or hemorrhage, or loss of neurotrophic effects. With regard to BDNF effects, recent findings indicate that MDMA exposure may increase or decrease BDNF messenger ribonucleic acid (mRNA) expression in a region-dependent manner in rats (Martinez-Turrillas et al., 2006).

4.3. MR Spectroscopy

To our knowledge, this pilot study is the first report of brain absolute metabolite concentration analysis using 4T proton MRS in human MDMA polydrug users. Proton MRS at high field strengths potentially offers advantages over lower field strength studies. These advantages include improved resolution, improved spectral editing selectivity, and improved signal to noise ratios (Hetherington et al., 1997). Use of LCModel to analyze absolute metabolite concentrations avoids assumptions regarding the influence of MDMA or other drug use on Cr or Cho levels that are necessary to express metabolites of interest relative to Cr and Cho.

The interpretation of spectroscopic studies in MDMA users is complicated by different drug use patterns among groups in the separate studies, different experimental methods and different brain regions investigated by the different groups. Overall, findings from the current literature do not suggest a consistent pattern of regional brain metabolite changes in MDMA users. Chang et al. (1999), in a 1.5 T proton MRS study examining mid-frontal gray, mid-parietal white, and mid-occipital gray matter, reported that MDMA users had elevated parietal white matter MI to Cr ratios and that MI in occipital cortex gray matter correlated positively with extent of prior MDMA use. NAA to Cr ratios did not differ from the control group in either tested region. Reneman et al., (2002b) also used proton MRS at 1.5 T to examine midfrontal gray, midoccipital gray, and right parietal white matter NAA and MI ratios in MDMA users and polydrug using controls. This study found reduced NAA/Creatine (Cr) and NAA/Choline ratios in the frontal cortex of MDMA users but no midfrontal MI/Cr differences between the two groups. There were no differences in NAA/Cr, NAA/Cho, or MI/Cr in mid-occipital cortex or in parietal white matter. Reneman et al. (2002b) further demonstrated a significant correlation between the log of MDMA exposure level (an analysis including the zero MDMA exposure level of the control group) and reduced midfrontal NAA/Cho, NAA/Cr. In a study using proton magnetic resonance spectroscopic imaging (MRSI) (Obergruesser et al. 2001), a small sample (n=5) of MDMA users did not differ from controls on measures of hippocampal NAA, Cr, Cho or NAA/(Cr+Cho) ratios. More recently Daumann et al. (2004) used proton

MRS to investigate neocortical and hippocampal NAA at 1.5 T as NAA/Cr ratio in a group of MDMA users (having an estimated mean lifetime MDMA usage of 325 tablets) and controls partially matched for cannabis exposure. Spectra were obtained from hippocampus, mid-frontal neocortex, and mid-occipital neocortex. Overall, NAA/Cr ratios in MDMA users did not differ significantly from the control group for any region studied.

Although not directly comparable due to subject characteristics and methodological differences in regions of interest, field-strength, and spectroscopic methods, our present findings are generally in line with those of Reneman et al., (2002b) and Obergriesser et al., (2001) with regard to cortical MI and with Chang et al. (1999), Obergriesser et al., (2001), and Daumann et al. (2004) regarding cortical NAA. The mean MDMA exposure was considerably greater in the above referenced spectroscopic studies when compared to the present report. Of note, of the studies discussed above, the Reneman et al., 2004 study reported the highest mean MDMA exposure levels but did not find evidence for reduced NAA ratios in occipital cortex of MDMA users.

4.5. Occipital Cortex

Converging evidence from animal and human studies of MDMA effects suggests that occipital cortex is altered by systemically administered MDMA. Some layer IV and other visual cortical serotonin axons appear to be permanently lost in animal models of MDMA toxicity (e.g. Hatzidimitriou et al., 1999). PET and SPECT studies using serotonin transporter ligand binding analyses have generally found reduced levels of serotonin transporter binding in the occipital cortex (among other regions) of MDMA users (McCann et al., 1998; McCann et al. 2005; Semple et al. 1999; Reneman et al., 2000; Reneman et al., 2000a; Buchert et al., 2004) with some evidence for normalization of ligand binding with increased duration of MDMA abstinence. The serotonin 2A receptor subtype (5-HT_{2A}) receptors are up-regulated in the occipital cortex of human MDMA users, a finding potentially reflecting a response to loss of serotonergic neurotransmission (Reneman et al., 2002b). As reviewed above, Chang and colleagues, (1999) reported an increase in MI in the occipital cortex of MDMA users, a finding potentially suggesting regional gliosis in response to axon death (Chang et al., 1999). Using the voxel-based morphometry (VBM) method, we reported that MDMA polydrug users have lower gray matter concentrations bilaterally in occipital cortical Brodmann area 18 (lateral to the midline voxel employed in the current MRS study) when compared to controls (Cowan et al., 2003). Oliveri and Calvo (2003) used single pulse transcranial magnetic stimulation (TMS) and found that MDMA users had lower phosphene thresholds that were potentially associated with MDMA-induced visual hallucinations. And, the extent of MDMA use appears to positively correlate with the spatial extent of regional brain BOLD signal change in response to 8 Hz color visual stimulation (Cowan et al., 2006). As such, converging data from a variety of investigators and methods suggests that visual cortical structure and function might be affected by MDMA use. Further study seems warranted before the significance of these findings can be fully integrated into the MDMA neuroimaging literature.

4.6. Limitations

Several limitations to the study constrain our ability to generalize and interpret the current findings. The study was conducted on a small sample of moderate MDMA users and non-MDMA users. Therefore, it is not possible to conclusively determine whether or not these levels of MDMA exposure influence MI or NAA levels. A larger sample size may be necessary to demonstrate effects if moderate levels of MDMA use produce subtle neuronal or glial alterations or subtle effects on metabolite levels. If there is an actual effect of MDMA on metabolite levels, given our detected effect size of 0.3 for NAA levels, a sample size of 180 subjects per group would be needed to detect a statistical difference using a two-tailed test at a 0.05 significance level with 80% power. Subject drug use history was assessed solely by self

report. As such, we have no laboratory evidence that subjects were actually exposed to MDMA. This problem is a limitation of most studies in substance abuse research. While hair sampling for MDMA or metabolites can be used to verify abstinence for up to 90 days, hair sampling has lower utility in verifying exposure to MDMA because a positive hair sample confirms only recent exposure and the effects of MDMA usage are potentially additive over a lifetime of use. Also, we collected drug use data categorically, thereby limiting our ability to examine continuous levels of drug exposure versus outcome relationships. We did not assess subjects for MDMA dosage stacking within a single episode of use, a behavior that likely correlates with higher serum MDMA levels than similar total MDMA taken across a longer time frame. Similarly, the duration of MDMA use may potentially influence MDMA toxicity. We required a minimum 3-week abstinence period from MDMA use and it is possible that a longer abstinence period may have revealed different findings. We used a mixed sex sample of MDMA and non-MDMA users. While we found no evidence for sex-specific effects, it is possible that a larger sample size might have revealed a sex effect on outcome variables. The region of interest that we chose for this study did not overlap with occipital areas that showed lower gray matter concentration in our prior VBM study (Cowan et al., 2003); therefore it is possible that a voxel placed to overlap with the region of BA 18 having lower gray matter in the VBM study might have demonstrated differences in the current cohort. NAA in particular might be predicted to be lower in regions having lower brain gray matter concentrations if the reduced brain gray matter concentrations are due to neuronal volume loss. Like many reports of MDMA users worldwide, the present study cohort had polydrug exposure. Thus, it is also possible that other drugs of abuse may have influenced our outcome variables in directions opposite to that of MDMA use, thereby obscuring a net change in metabolite concentrations.

4.7. Significance

A great deal of evidence from animal models and human subjects exposed to MDMA suggests that MDMA may be selectively toxic to serotonin axon terminals. Serotonin has numerous roles in CNS cellular function and has important influences on a variety of cognitive and emotional processes. Therefore, the examination of MDMA's long-lasting brain effects in human users is an important mental health and public health issue. Because MI and NAA reflect important aspects of brain cellular structure and function, and because earlier studies had suggested variability of these metabolites in MDMA users, we used high-field MRS at 4T to investigate absolute levels of these metabolites in human subjects previously self-exposed to moderate dosages of MDMA. Overall, the absence of differences between MDMA users and control subjects in this study suggests that MDMA use at the reported moderate exposure levels does not substantially alter these metabolites or that study limitations prevented the demonstration of MDMA effects. Lack of consensus in the scientific literature regarding MDMA use and brain metabolites suggest that additional studies are needed. Larger retrospective and prospective studies addressing the numerous confounding variables that influence human MDMA research seem warranted.

Acknowledgements

This work was supported by NIDA Grants DA03994 (SEL), DA00343 (SEL), DA09448 (PFR), DA15116 (PFR), DA14178 (PFR), DA14013 (BBF), DA00366 (RLC), DA015137 (RLC), the American Psychiatric Institute for Research and Education (RLC), and the Shervert Frazier Foundation (RLC).

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Figure 1. Voxel placement

Sagittal image depicting occipital lobe voxel placement. Voxel placement was obtained by identifying the calcarine sulcus in sagittal images and placing the voxel so that volume above and below the calcarine sulcus was approximately equal. Posterior edge of the voxel was adjusted using axial scan to place the voxel as posteriorly as possible within brain tissue. Voxels were adjusted to have about 50% volume in each hemisphere. All voxels were placed blind to subject status.

Figure 2. Scatter plot of absolute metabolite concentrations

Scatter plot of absolute metabolite concentrations (in mM) by non-MDMA user (n = 7) or MDMA user (n = 9) status. NAA = N-acetylaspartate; MI=Myoinositol. Note scale differences in the NAA and MI graphs.

Table 1

Drug use episode frequencies in Non-MDMA users and MDMA users

Table 1 depicts lifetime self-report of episodes of drug use in a given category. Categories are None = 0 episodes use of a drug, Low = 1–10 episodes use of a drug, Med = 11–39 episodes use of a drug, High = 40 or more episodes use of a drug. PCP = phencyclidine, MDMA = 3, 4, methylenedioxymethamphetamine.

Drug	None*	Non-MDMA Users (n = 7)			MDMA Users (n = 9)			** z	P
		Low	Med	High	None	Low	Med	High	
Alcohol	0	1	0	6	0	0	0	9	1.134
Amphetamines	7	0	0	0	4	4	1	0	2.277
Cannabis	3	2	1	1	0	0	2	7	2.848
Cocaine	6	1	0	0	1	5	3	0	2.853
Hallucinogens	6	1	0	0	1	6	1	1	2.838
Heroin	7	0	0	0	9	0	0	0	0.000
MDMA	7	0	0	0	0	1	7	1	3.648
Opiates	7	0	0	0	3	4	2	0	2.582
PCP	7	0	0	0	7	2	0	0	1.291
Sedatives	6	1	0	0	4	3	2	0	1.721
									0.085

* Drug use categories correspond to lifetime episodes of use. None = 0 uses of the drug; Low = 1–10 uses of the drug; Med = 11–39 uses of the drug; High = 40 or more uses of the drug.

** Drug use frequencies were compared between Non-MDMA Users and MDMA Users using Mann Whitney U test for non-parametric comparisons to generate Z score and corresponding p value. No subjects in either group reported heroin use.

Table 2

Metabolite	Non-MDMA Users M±S.D. (Min, Max)	MDMA Users M±S.D.(Min, Max)	Z*	P*
Myoinositol (MI)	7.43±1.68(5.70,9.95)	6.57±1.59(3.90,9.43)	0.900	0.368
N-Acetylaspartate (NAA)	10.47±2.51(7.89,15.39)	9.83±1.94(7.74,14.28)	0.370	0.711
MI/Creatine	1.04±0.11(0.91,1.18)	1.05±0.10(0.86,1.19)	0.159	0.874
NAA/Creatine	1.47±0.17(1.26,1.78)	1.55±0.16(1.27,1.78)	1.111	0.266

* Results based on Mann-Whitney tests of differences.