Cerebral $^1$H MRS Alterations in Recreational 3,4-Methylenedioxymethamphetamine (MDMA, “Ecstasy”) Users

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3,4-Methylenedioxymethamphetamine (MDMA) is an illicit drug that has been associated with serotonergic axonal degeneration in animals. This study evaluates neurochemical abnormalities in recreational MDMA users. Twenty-two MDMA users and 37 normal subjects were evaluated with magnetic resonance imaging (MRI) and proton magnetic resonance spectroscopy ($^1$H MRS) in the mid-frontal, mid-occipital, and parietal brain regions. $^1$H MRS showed normal N-acetyl (NA) compounds in all brain regions. The myo-inositol (MI) concentration ($+16.3\%, P = 0.04$) and the MI to creatine (CR) ratio ($+14.1\%, P = 0.01$) were increased in the parietal white matter of MDMA users. The cumulative lifetime MDMA dose showed significant effects on MI in the parietal white matter and the occipital cortex. The normal NA concentration suggests a lack of significant neuronal injury in recreational MDMA users. However, the usage-related increase in MI suggests that exposure to MDMA, even at recreational doses, may cause increased glial content. J. Magn. Reson. Imaging 1999;10:521–526. © 1999 Wiley-Liss, Inc.

Index terms: 3,4-methylenedioxymethamphetamine (MDMA or “Ecstasy”); proton magnetic resonance spectroscopy; N-acetyl-L-aspartate; myo-inositol; human; brain

3,4-Methylenedioxymethamphetamine (MDMA), also known as “Ecstasy” or “Adam,” has been used illicitly by several million individuals; however, little is known about its neurobiological effects. MDMA is a methamphetamine derivative with structural similarities to the hallucinogenic mescaline. First synthesized prior to World War I in Germany, it remained of little interest to investigators until approximately two decades ago, when it was experimented with as an adjunct to psychotherapy (1). Psychotherapists found it to be a short-acting drug that enhanced the capacity for introspection, empathy, and intimacy and also reduced depression and anxiety (2). Since the mid-1980s, however, MDMA has become a popular recreational drug, particularly among young people and in the rapidly growing “rave” scene as the preferred “dance drug” of choice (3). Prevalence of MDMA use dramatically escalated: 24.3% (4) to 39% (5) of college students in the United States, and 500,000 young “ravers” used MDMA every weekend in Great Britain (6). There exists a pressing need to investigate how the drug might affect the brain in MDMA users.

In rodents, MDMA has been shown to affect serotonergic (5-HT) neurons after single or multiple doses (7–10); the neurotoxicity appears to be limited to axon terminals (11,12). Studies in monkeys showed profound reductions in 5-HT, 5-hydroxyindole acetic acid (5-HIAA), and 5-HT uptake sites at 2 weeks and 18 months after MDMA administration; however, recovery appears to occur in some regions such as the thalamus and the hypothalamus (13). Since it is believed that humans are generally more sensitive than monkeys to the toxic effects of drugs (9), humans who use MDMA might be at risk from its neurotoxic effects. However, biological data gathered on the effects of MDMA in humans are limited (14). A few studies have evaluated the cerebrospinal fluid 5-HIAA concentrations in MDMA users and found either normal (5) or decreased levels (15,16). The later study also found diminished aggression and impulsivity (15) and long-term decrease of stage II sleep and sleep time (17). Case reports suggest that MDMA may lead to life-threatening cardiovascular (18), cerebrovascular (19), or hyperthermic (20) reactions, as well as adverse psychiatric sequelae (21).

Proton magnetic resonance spectroscopy ($^1$H MRS) is a sensitive and non-invasive technique that can measure concentrations of N-acetylaspartate [NA], a neuronal marker, and myo-inositol [MI], a tentative glial marker. Since MDMA may cause glial activation (22) and down-regulation of 5-HT neurons (11), we hypothesized that $^1$H MRS may be able to detect long-term neurochemical effects associated with changes in neurons and glial cells in abstinent recreational MDMA users.
MATERIALS AND METHODS

Subjects

Twenty-one subjects with a history of MDMA use (15 men and 6 women, median age 43.0 ± 14.6, range 19–75 years) and 37 normal control subjects (22 men and 15 women, median age 38.0 ± 14.7 years; range 22–80 years) were consecutively recruited from the local community and studied with MRI and localized 1H MRS. MDMA subjects were included in the study if they had used MDMA on a regular basis recreationally (6 times or more a year) for at least 1 year and had been abstinent for at least 2 weeks prior to the study. Each MDMA subject underwent a psychiatric examination, a medical history, and a physical examination to rule out any chronic psychiatric or medical illnesses. MDMA usage and history of other drug use were also recorded. In addition, either MDMA or the control subjects were excluded: a) if they were on medications for any chronic psychiatric or medical illnesses; b) if they had a history of alcohol abuse or substance dependence (except for MDMA) according to the DSM IV diagnostic criteria; c) if they had a history of head trauma with loss of consciousness for more than 30 minutes; d) if they were pregnant (females only); and e) if they had any metallic objects in their body. Prior to the study, each subject was verbally informed of the protocol and signed an informed consent approved by the Human Subjects Institutional Review Board at Harbor-UCLA Research and Education Institute.

MRI and Localized 1H MRS

MRI and 1H MRS were performed on a 1.5 T scanner (General Electric, Signa 5.4, Milwaukee, WI) using a quadrature head resonator. The examination began with the acquisition of a sagittal T1-weighted localizer (TE/TR 11/500 msec, 4 mm slice thickness, 1 mm gap, 24 cm field of view [FOV]), followed by a coronal fast double spin-echo sequence (TE1/TE2/TR 17/102/4000 msec, 5 mm slice thickness, no gap, 24 cm FOV). Finally, an axial fast inversion recovery scan (TE/TI/TR 32/120/4000 msec, 3.5 mm slice thickness, no gap, 24 cm FOV) was performed.

1H MRS was performed in three brain regions: the mid-occipital gray matter, the mid-frontal gray matter, and the right parietal white matter (Fig. 1). Two of the MDMA subjects were claustrophobic and could not complete the studies for all three voxels. Voxel sizes varied (from 3 to 5 cc) due to the different anatomy of the subjects: voxel sizes and location were carefully chosen to ensure that each voxel contained primarily gray matter or white matter. Data were acquired using a double spin-echo sequence, point resolved spectroscopy (PRESS) (23), with TR/TE 3000/30 msec, 128 averages, 2 K acquisition size, and 2.5 kHz bandwidth. The PRESS sequence was optimized for the chosen echo times and locations (24). The spectroscopic data were preprocessed by digital low-frequency filtering to suppress the residual water signal (25). Next, the data were baseline corrected using a DC correction only, zero-filled to 8 K, apodized with an exponential decay of 1 Hz, Fourier transformed, and phase-corrected manually (zero-order only).

The areas of NA, creatine (CR), choline compounds (CHO), MI, and glutamate/glutamine (GLX) were obtained as described previously (26,27). Metabolite concentrations were determined using a slightly modified protocol described previously (26,27). Briefly, the T2 decay of the unsuppressed water signal from the PRESS experiment was measured at 10 different echo times. These T2 measurements were then used to determine the water signals of brain parenchyma and cerebrospinal fluid (CSF) by fitting a double-exponential decay curve to the measured data (26). The water signal from brain parenchyma was used as a concentration reference and to determine loading. The data were processed on a SPARC 2 workstation using the SA/GE platform (General Electric Medical Systems) and a semi-auto-

Figure 1. The three voxel locations for the localized 1H MRS studies: mid-occipital gray matter (left), right parietal white matter (middle), and mid-frontal gray matter (right), superimposed on an axial inversion recovery scan.
matic program (26,27). This procedure yielded metabolite concentrations in “institutional units,” which were converted into millimolar concentrations using the published normal values in occipital cortex and parietal white matter (27). For comparison with the existing literature on MRS of other brain diseases, we also determined metabolite ratios using CR as an internal standard.

Statistical analyses were performed using Statview (Abacus, Berkeley, CA). Descriptive statistics were performed on the age, education level, and MDMA usage (number or times used, cumulative lifetime dose of MDMA, duration of usage, and time since last use); the median and range for each of these variables are reported. Comparisons between MDMA users and normal subjects for each brain metabolite in each brain region were performed using two-tailed unpaired Student’s t-tests. Analysis of covariance (ANCOVA) was used to assess the influence of age on differences in cerebral metabolite concentrations between MDMA users and control subjects. Regression analyses of metabolite concentrations on the MDMA usage were performed using a linear regression model. All P values < 0.05 were considered significant.

RESULTS
The MDMA users had a median education level of 15.8 (range 12–20) years; all of the subjects were employed or in school and used MDMA only recreationally. None of the subjects had a criminal record. These MDMA subjects reported they had last used MDMA 4.0 (range 0.5–26) months previously. Duration of use was 10.0 (range 1–17) years, and estimated total number of times of MDMA use was 75 (range 6–1,500) times. The cumulative lifetime exposure to MDMA, based on the total number of times used and the average dose per use (125–225 mg), was 13.1 g (range 0.5–263 grams). Some of the subjects had experimented with several other substances in the past, usually at low recreational doses, and were never dependent on them. These other substances included lysergic acid diethylamide-25 (71% of the subjects), marijuana (83% of the subjects), mushrooms (46% of the subjects), and other amphetamines (29% of the subjects). All subjects reported little or no interest in cocaine; only three subjects admitted to having used cocaine up to 3 times in their lifetime but were never dependent on cocaine. MDMA was reportedly the primary drug of choice for all the subjects.

MRI
All images in both the MDMA users and the normal control subjects appeared normal with no significant brain atrophy or any white matter lesions.

1H MRS
In the parietal white matter, both the MI concentration (+16.3%, P = 0.04) and MI/CR (+14.1%, P = 0.01) were elevated in the MDMA users (Table 1 and Fig. 2). This finding of increased [MI] remained statistically significant (P = 0.04) when an ANCOVA, with age as a covariate, was performed to correct for slight differences in the subjects’ age (see also Fig. 3). In addition, CHO/CR was elevated (P = 0.05) in the occipital gray matter region (Table 1); this was due primarily to a lower [CR] (−5.7%) in the MDMA users. The metabolite concentrations of NA, CR, CHO, and in all three brain regions were comparable between MDMA users and normal subjects. No lactate or excess lipids were observed in any of the spectra.

A significant effect of the logarithm of the cumulative lifetime MDMA dose on [MI] was found in the parietal white matter (r = 0.48, P = 0.04) and in the occipital cortex (r = 0.68, P = 0.002) (Fig. 4). For example, the subject who had used MDMA the most number of times (1500 times) showed elevated [MI] in both the parietal white matter and occipital region compared with the normal subjects in his age group (Fig. 2). No significant effect of the log of cumulative lifetime MDMA dose was found on the other brain metabolite concentrations in the three brain regions, except for a trend for significance on [CHO] in the occipital cortex (r = 0.41, P = 0.08). In particular, [NA] in all three brain regions was not affected by the cumulative MDMA dose.

The duration of MDMA use was also related to [MI] in the parietal white matter (r = 0.53, P = 0.02) and in the frontal cortex (r = 0.56, P = 0.009), with a trend for significance in the occipital cortex (r = 0.44, P = 0.07). The duration of MDMA use showed a trend for significant relationship to [CHO] in the parietal white matter (r = 0.4, P = 0.06). There was no significant relationship between recent timing of MDMA use and concentration of any of the metabolites.

DISCUSSION
Our group of recreational MDMA users showed elevated [MI] and MI/CR in the parietal white matter, but no other changes in the metabolite concentrations on 1H MRS. In particular, we found normal [NA] in the MDMA users in all three brain regions examined. NA is considered a neuronal marker that is very sensitive to death or damage of neurons (28,29). Reduced [NA] has been reported in many conditions, including hypoxia (30), cerebral infarction (31), closed head trauma (26), brain tumors (32,33), dementias (34,35), human immunodeficiency virus (HIV) brain diseases (36), and epilepsy (37). Among our subjects, normal [NA] was found even in the two subjects who had used MDMA 750 times and 1500 times over 10 and 15 years, respectively. Although the lack of a change in [NA] is consistent with preclinical data that 5-HT neurons are down-regulated at the axon terminals rather than damaged with MDMA exposure (11,12), it is possible that neuronal recovery or re-sprouting of previously damaged 5-HT axons might have occurred in the MDMA users. It is also possible that, at the given sample size, 1H MRS may not be sensitive enough to detect small changes in [NA] associated with low recreational doses of MDMA or to detect changes associated only with damage to 5-HT terminals. In contrast, regular use of methamphetamine, a compound with similar actions to MDMA, has been shown to be associated with significantly lower [NA] (38).
The MDMA users showed no abnormalities in the glutamate/glutamine region or the presence of lactate. The normal [GLX] is noteworthy in view of numerous studies demonstrating that elevated extracellular concentrations of glutamate are toxic to neurons (39). Specifically, preclinical studies indicate that glutamate is involved in MDMA-induced neurotoxicity since various N-methyl-D-aspartate antagonists can inhibit or attenuate the neurotoxic effects (40). Studies in rats also indicate that MDMA-induced 5-HT neurotoxicity may in part be due to an increase in dopamine neurotransmission (41–43). This increase in dopamine, if also present in the human brain, could potentially lead to acute vasoconstriction and ischemia or infarcts. However, the lack of observable (excess) lactate, along with the normal-appearing MRI scans, indicates that no cerebral ischemia or infarcts occurred in these recreational users.

The glial marker [MI] was elevated (+16.4%) in the parietal white matter of MDMA users. In addition, [MI] and MI/CR in both the parietal white matter and the occipital gray matter all showed positive correlation with MDMA usage, which suggests that long-term use of MDMA increases [MI], or glial content. Elevated [MI] has been reported in abstinent cocaine abusers (44), degenerative brain diseases such as Alzheimer disease (35,45) and frontotemporal dementia (34), demyelinating conditions such as multiple sclerosis (46,47), and viral infections such as human immunodeficiency virus (36,48,49) and JC virus (50). In all of these conditions, as well as in our MDMA users, elevated [MI] most likely reflects increased glial hypertrophy or proliferation, in association with insults to the brain, and possibly ongoing repair processes. A recent report by Poble et al. and Azmitia showed that glycogen phosphorylase, an enzyme that co-localizes with the astroglia-specific marker (glial fibrillary acidic protein), can be activated by both enzymes that co-localize with the astroglia-specific marker (glial fibrillary acidic protein), can be activated by both.

Table 1
Comparisons of Metabolite Concentrations (mmol/kg; mean ± S.D.) and Ratios From 1H MRS in MDMA Users and Normal Subjects

<table>
<thead>
<tr>
<th>Voxel location</th>
<th>MDMA users</th>
<th>Normal subjects</th>
<th>% difference</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mid-Occipital Gray Matter</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NA</td>
<td>9.16 ± 0.72</td>
<td>9.31 ± 0.71</td>
<td>(-1.6%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>CR</td>
<td>7.79 ± 1.21</td>
<td>8.26 ± 0.67</td>
<td>(-5.7%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>CHO</td>
<td>1.48 ± 0.30</td>
<td>1.46 ± 0.28</td>
<td>(+1.4%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>MI</td>
<td>7.66 ± 1.37</td>
<td>7.35 ± 1.79</td>
<td>(+4.2%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>GLX</td>
<td>16.03 ± 3.06</td>
<td>15.00 ± 3.15</td>
<td>(+6.9%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>NA/CR</td>
<td>1.63 ± 0.11</td>
<td>1.63 ± 0.14</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>CHO/CR</td>
<td>0.57 ± 0.07</td>
<td>0.53 ± 0.06</td>
<td>P = 0.05</td>
<td></td>
</tr>
<tr>
<td>MI/CR</td>
<td>0.68 ± 0.07</td>
<td>0.65 ± 0.09</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>GLX/CR</td>
<td>0.51 ± 0.10</td>
<td>0.48 ± 0.10</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>% CSF</td>
<td>7.8 ± 3.9</td>
<td>7.9 ± 2.6</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td><strong>Temporoparietal White Matter</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>NA</td>
<td>8.60 ± 0.81</td>
<td>8.48 ± 0.82</td>
<td>(+1.4%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>CR</td>
<td>6.34 ± 0.68</td>
<td>6.27 ± 0.69</td>
<td>(+1.1%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>CHO</td>
<td>1.62 ± 0.19</td>
<td>1.62 ± 0.24</td>
<td>(-0%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>MI</td>
<td>7.91 ± 1.39</td>
<td>6.80 ± 1.46</td>
<td>(+16.4%)</td>
<td>P = 0.04</td>
</tr>
<tr>
<td>GLX</td>
<td>15.45 ± 1.75</td>
<td>15.00 ± 2.59</td>
<td>(+0.7%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>NA/CR</td>
<td>1.77 ± 0.12</td>
<td>1.73 ± 0.14</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>CHO/CR</td>
<td>0.98 ± 0.10</td>
<td>0.97 ± 0.13</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>MI/CR</td>
<td>0.81 ± 0.08</td>
<td>0.71 ± 0.14</td>
<td>P = 0.01</td>
<td></td>
</tr>
<tr>
<td>GLX/CR</td>
<td>0.44 ± 0.07</td>
<td>0.43 ± 0.07</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>% CSF</td>
<td>3.1 ± 2.3</td>
<td>3.2 ± 1.4</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td><strong>Mid-Frontal Gray Matter</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NA</td>
<td>8.97 ± 0.62</td>
<td>9.03 ± 1.01</td>
<td>(-0.7%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>CR</td>
<td>8.20 ± 0.67</td>
<td>8.10 ± 1.47</td>
<td>(+1.2%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>CHO</td>
<td>2.21 ± 0.30</td>
<td>2.24 ± 0.42</td>
<td>(-1.3%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>MI</td>
<td>8.55 ± 1.05</td>
<td>8.74 ± 1.77</td>
<td>(-2.2%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>GLX</td>
<td>14.04 ± 1.76</td>
<td>15.00 ± 1.61</td>
<td>(-6.4%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>NA/CR</td>
<td>1.46 ± 0.09</td>
<td>1.48 ± 0.13</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>CHO/CR</td>
<td>0.83 ± 0.08</td>
<td>0.81 ± 0.08</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>MI/CR</td>
<td>0.75 ± 0.06</td>
<td>0.74 ± 0.06</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>GLX/CR</td>
<td>0.54 ± 0.07</td>
<td>0.58 ± 0.06</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>% CSF</td>
<td>1.4 ± 3.6</td>
<td>1.8 ± 2.6</td>
<td>n.s.</td>
<td></td>
</tr>
</tbody>
</table>
persistent neuronal damage or ischemic lesions occurred in the MDMA users. It is possible that at recreational dosages of MDMA (ie, 1.5–3.0 mg/kg), 5-HT neurotoxicity is minimal, or that neuronal recovery may have occurred. Future longitudinal studies are needed to determine the possible relationship between acute and subacute MDMA use, including dose-response relationships and cerebral metabolite concentrations.

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REFERENCES