Prefrontal *N*-Acetylaspartate is Strongly Associated with Memory Performance in (Abstinent) Ecstasy Users: Preliminary Report

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Background: *3,4-methylenedioxymethamphetamine (MDMA or "Ecstasy") is known to damage brain serotonin neurons in animals and possibly humans. Because serotonergic damage may adversely affect memory, we compared verbal memory function between MDMA users and MDMA-naïve control subjects and evaluated the relationship between verbal memory function and neuronal dysfunction in the MDMA users.*

Methods: *An auditory verbal memory task (Rey Auditory Verbal Learning Test) was used to study eight abstinent MDMA users and seven control subjects. In addition ^{1}H-MRS was used in different brain regions of all MDMA users to measure* N*-acetylaspartate/creatine (NAA/Cr) ratios, a marker for neuronal viability.*

Results: *The MDMA users recalled significantly fewer words than control subjects on delayed ($p = .03$) but not immediate recall ($p = .08$). In MDMA users, delayed memory function was strongly associated with NAA/Cr only in the prefrontal cortex ($R^2 = .76$, $p = .01$).*

Conclusions: *Greater decrements in memory function predicted lower NAA/Cr levels—and by inference greater neuronal dysfunction—in the prefrontal cortex of MDMA users.* Biol Psychiatry 2001;50:550–554 © *2001 Society of Biological Psychiatry*

Key Words: MDMA, 5-HT, neurotoxicity, memory function, proton magnetic resonance spectroscopy, ^{1}H-MRS

Introduction

Although generally perceived by the public as safe, it has become increasingly apparent that the popular recreational drug 3,4-methylenedioxymethamphetamine (MDMA or "Ecstasy") can lead to toxic effects on brain serotonin (5-HT) neurons in animals (Boot et al 2000) at doses that approach or overlap those used by humans. Recent studies suggest that MDMA may also be neurotoxic to the human brain. For instance, recent positron emission tomography (PET) studies have shown decreases in a structural component of 5-HT neurons similar to those observed in MDMA-treated monkeys (McCann et al 1998; Scheffel et al 1998).

5-HT neurotoxic damage induced by MDMA may lead to impairment of functions in which 5-HT is involved (such as memory and other cognitive functions); however, few functional consequences of MDMA-induced neurotoxicity have been identified. Memory function is of particular interest because several studies have found that recreational MDMA users display significant memory impairments (Bolla et al 1998) and because the hippocampal formation may be particularly vulnerable to MDMA's neurotoxic effects (Hatzidimitriou et al 1999).

The reduction of the amino acid *N*-acetylaspartate (NAA) detected by proton magnetic resonance spectroscopy (^{1}H-MRS) represents a robust but unspecific marker for neuronal loss or dysfunction (Urenjak et al 1993). In addition to NAA, creatine/phosphocreatine (Cr) can be assessed. Determining NAA changes in relation to Cr is commonly employed and apparently valid because Cr remains stable in a variety of brain diseases.

The purpose of our study was to determine whether memory deficits exist in MDMA users and, if they do, whether memory deficits correlate with regionally specific NAA/Cr ratios. We obtained NAA/Cr ratios in the prefrontal cortex, occipital gray matter, and temporo-parietal white matter because PET studies have shown that the bilateral prefrontal cortex is the site of maximal increase in cerebral blood flow during execution of an auditory verbal memory tasks (Grasby et al 1993), such as those used in our study. Furthermore, although MDMA is known to induce 5-HT neurotoxicity in cortical gray matter, cortical white matter remains relatively unaffected (Scheffel et al 1998).

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With this in mind, we hypothesized that MDMA users would perform worse on a memory test than MDMA-naïve subjects and that memory impairments in MDMA users would only predict NAA/Cr ratios in brain regions known to be affected by MDMA and involved in auditory verbal memory tasks. Thus, we expected that memory deficits in MDMA users would only be associated with decrements in NAA/Cr of the prefrontal cortex.

Methods and Materials

Subjects

Eight male subjects with a history of MDMA use participated in the study. Subjects were recruited with flyers distributed at venues associated with the "rave scene" in Amsterdam. For a comparison group, we used our Rey Auditory Verbal Learning Test (RAVLT) database of seven male MDMA-naïve control subjects matched for age, gender, and IQ. The same inclusion and exclusion criteria were applied to all control subjects. A detailed drug history questionnaire was obtained from all subjects. All participants agreed to abstain from use of psychoactive drugs for at least 1 week before the study and were asked to undergo urine drug screening with an enzyme-multiplied immunoassay before enrollment. Exclusion criteria were a positive drug screen and a lifetime psychiatric disorder (obtained with Composite International Diagnostic Interview, version 2.1). The institutional medical ethics committee approved the study. After a complete description of the study to the subjects, written informed consent was obtained from all.

Assessments

Brain ^{1}H spectroscopy was performed on a 1.5 T Signa Echo Speed (General Electric Medical Systems, Milwaukee, WI). Multislice coronal fast spin-echo T2-weighted images (TE/TR, 97/4000 m/sec; 5-mm slice thickness; 23-cm field of view; 256 × 256 matrix) were obtained. Voxel size was 4.5 cm^3 and chosen carefully to ensure that each voxel primarily contained gray or white matter and placed consistently across subjects. Data were acquired using a fully automated execution of PROBE (Proton Brain examination), developed to automatically and reliably acquire and process single-voxel proton spectra, as described elsewhere (Webb et al 1994). The PRESS sequence was optimized for the chosen echotimes and locations (TE/TR, 35/3000 msec, 128 averages). We computed NAA/Cr ratios from the fitted peak integrals.

Memory was assessed in the MDMA users within 1 day from the ^{1}H-MRS study using the RAVLT, a verbal memory test. It comprised immediate and delayed recall. In addition, the Dutch adaptation of the National Adult Reading Test (DART) was administered as an estimate of verbal intelligence (Schmand et al 1992).

Statistical Analysis

Differences between both groups with regard to demographic variables and other drug exposure were analyzed using Student *t* test. Differences in RAVLT scores were analyzed using ANCOVA, with one between-group factor (Group) and two covariants (age and DART-IQ). To examine the relation between NAA/Cr ratios and memory performance, we used linear regression analysis with both immediate and delayed recall as independent variables and NAA/Cr ratios obtained in the three different brain regions as dependent variables. In addition, the relation between RAVLT scores and extent of previous MDMA and cannabis use was assessed using linear regression. To correct for multiple comparisons in the multiple regression analysis, $p <$ .017 was taken to be significant (.05/3:3 brain regions studied).

Results

The two groups were similar in age. The level of education was significantly lower in MDMA users; however, verbal intelligence (DART-IQ) was similar between MDMA users and control subjects in (Table 1). The use of drugs other than MDMA was higher in MDMA users compared with control subjects, although not statistically different (Table 1). Three MDMA users reported incidental use of cocaine and psilocybin in the 3 months before this study.

The MDMA users recalled a significantly lower number of words compared with the MDMA-naïve control subjects on the delayed (10.6 [SD: 2.0] vs. 12.8 [1.9], respectively; $F = 6.67$, $df = 1$, $p = .03$) but not on the immediate recall (45.8 [SD: 9.3] vs. 53.8 [6.6], respectively; $F = 3.71$, $df = 1$, $p = .08$).

Within the group of MDMA users, NAA/Cr ratios in the prefrontal cortex were highly associated with delayed (B = .16, SE = .04, $R^2 = .76$, $p = .013$; Figure 1) but not with immediate recall (B = −.01, SE = .009, $R^2 = .76$, $p = .237$). In contrast, no associations were observed between RAVLT scores and NAA/Cr ratios in midoccipital gray matter (delayed recall: B = .08, SE = .06, $R^2 = .25$, $p = .238$; immediate recall: B = −.01, SE = .013, $R^2 = .25$, $p = .573$), or parietal white matter (delayed recall: B = .02, SE = .06, $R^2 = .30$, $p = .748$; immediate recall: B = .01, SE = .012, $R^2 = .30$, $p = .402$).

Extent of previous MDMA use was significantly associated with delayed (B = −.01, SE = .001, $p = .047$), but not immediate recall (B = −.01, SE = .003, $p = .208$). In contrast, the extent of previous cannabis use was not significantly associated with either immediate or delayed verbal memory function (B = −.01, SE = .017, $p = .343$; B = −.01, SE = .004, $p = .149$, respectively).

Discussion

We found significant differences in delayed recall between MDMA users and MDMA-naïve control subjects. In

Table 1. Demographics and Characteristics of MDMA Use and Other Recreational Drug Exposure in Control Subjects[a] and MDMA Users[b]

	Control subjects $n = 7$	MDMA users $n = 8$
Age	29.3 (6.9)	28.3 (7.0)
Education (years)	15.0 (1.1)	11.5 (2.1)[c]
DART-IQ	92.4 (4.8)	92.6 (5.6)
MDMA		
Duration of use (years)	–	6.6 (3.3)
Usual dose (no. tablets)	–	2.6 (0.7)
Lifetime exposure (no. tablets)	–	902.0 (801.2)
Time since last dose (months)	–	7.1 (11.1)
Last 3 months use of		
Alcohol (no. consumptions)	158.6 (140.7)	210.0 (165.1)
Tobacco (no. cigarettes)	516.1 (517.1)	1044.6 (1312.0)
Cannabis (no. joints)	1.3 (1.3)	138.5 (174.8)
Amphetamine (exposure g)	–	4.4 (8.2)

DART-IQ, National Adult Reading Test; MDMA, 3,4-methylenedioxymethamphetamine.
[a]Control subjects were selected from a Rey Auditory Verbal Learning Test database.
[b]Data are expressed in mean ± SD values.
[c]Significantly different from control subjects ($p < .01$; Student t test).

addition, a strong association was observed between impaired memory function in MDMA users and neuronal pathology of the prefrontal cortex. This relationship was regionally specific, involving only the prefrontal cortex.

In line with our findings, a number of studies have reported verbal memory impairments in MDMA users (Bolla et al 1998), whereas their performance on other cognitive tests is generally normal. Similarly, it has been shown that 5-HT appears to play a role in mnemonic function and that MDMA severely damages 5-HT axons in brain regions implicated in learning and memory in MDMA-treated animals (Hatzidimitriou et al 1999).

We observed that poorer performance on a verbal memory test predicted lower prefrontal NAA/Cr ratios—and by inference greater neuronal loss or dysfunction—in MDMA users. This finding was in agreement with our hypothesis and confirms earlier studies that detected an association between indicators of MDMA-induced 5-HT neuronal damage and memory impairment (Bolla et al 1998; Reneman 2000). Taken in conjunction with reports of reduced CSF 5-HIAA (McCann et al 1994) and density of 5-HT transporters (McCann et al 1998) in recreational MDMA users and similar observations in animals with documented neurotoxic lesions, our findings suggest that the association between memory function and prefrontal NAA/Cr ratios may be attributed to MDMA-induced neuronal pathology or dysfunction.

In view of the small brain mass occupied by 5-HT axon terminals in the prefrontal cortex (e.g., much less than 1%), it is not likely that the presently observed association between reduced NAA levels and poor memory function can be fully ascribed to MDMA-induced gross loss of 5-HT neurons in the prefrontal cortex. It could also be hypothesized that our findings reflect a low abundance of 5-HT axon terminals from other regions (e.g., the thalamus). In line with this, it has been shown that neonatal mesial-temporal limbic lesions can induce NAA deficits in the dorsolateral prefrontal cortex (Bertolino et al 1997), perhaps reflecting a loss of inputs from the lesioned areas.

Frontal lobe lesions, although not generally recognized as causing memory deficits, are reported to impair the free recall of word lists similar to the one used in this study. Furthermore, PET measurements of regional cerebral blood flow show that the prefrontal cortex is involved in auditory–verbal long-term memory (Grasby et al 1993). It has been suggested that the free recall of words requires extensive use of retrieval strategies and planning. A neuropsychologic explanation for the observed association may relate to the retrieval strategies necessary for dealing with an amount of information that exceeds the span of delayed verbal memory such as that present in the RAVLT.

Because most of the MDMA users had experimented with other recreational drugs, primarily cannabis, we cannot completely rule out the possibility that our findings are related to other drugs of abuse; however, no significant differences in the use of recreational drugs other than MDMA were observed between both groups. Also, we did not observe an association between RAVLT scores and extent of previous cannabis use, whereas the extent of previous MDMA use was significantly associated with delayed memory performance. Because recreational cannabis use, as was reported by our subjects, has not been shown to produce neurotoxic effects in animals (Scallet 1991) and drug tests indicated that no participant used

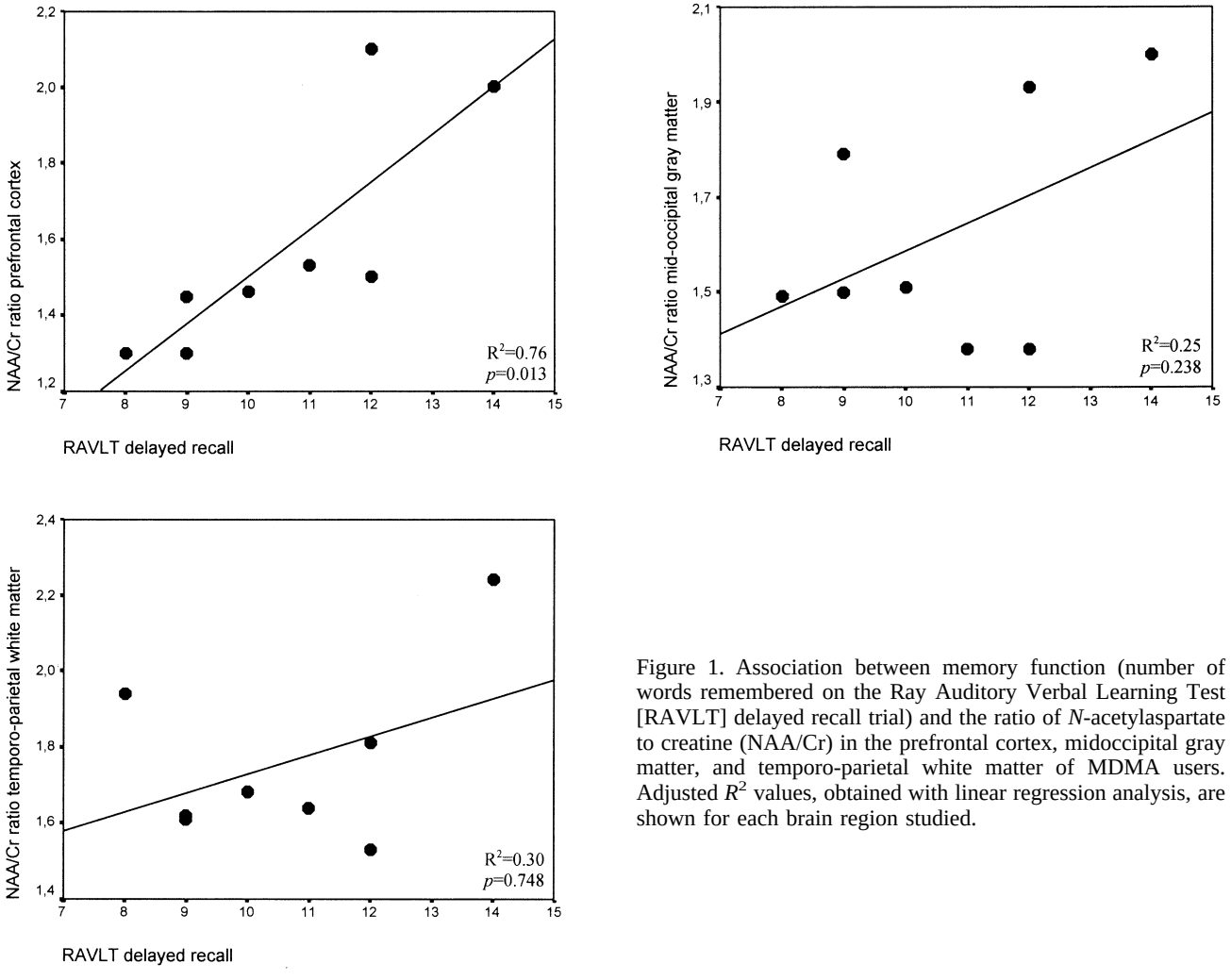

Figure 1. Association between memory function (number of words remembered on the Ray Auditory Verbal Learning Test [RAVLT] delayed recall trial) and the ratio of *N*-acetylaspartate to creatine (NAA/Cr) in the prefrontal cortex, midoccipital gray matter, and temporo-parietal white matter of MDMA users. Adjusted R^2 values, obtained with linear regression analysis, are shown for each brain region studied.

cannabis in the week before the study, it is unlikely that the findings of our study could be attributed to substances other than MDMA. In addition, the adverse effects of long-term cannabis use on cognitive skills compared with MDMA use have not been clearly demonstrated in the literature. For instance, Gouzoulis-Mayfrank et al (2000) did not observe differences in cognitive performance between cannabis users, ecstasy users, and control subjects, whereas Rodgers (2000) did. As with all retrospective studies, there is the possibility that individuals engaged in the "rave" scene have preexisting risk factors (e.g., a familial history or psychiatric disorder) for impaired memory function and reduced prefrontal NAA; however, by excluding MDMA users with a lifetime psychiatric disorder, this was not likely to account for changes in NAA and memory performance. Clearly, to establish definitively whether the presently observed relationship between neuronal function and performance in the prefrontal cortex is caused by MDMA-induced neuronal loss or dysfunction, a prospective study design would be needed; however, because studies of MDMA in humans are subject to ethical and methodologic constraints it is difficult, if not impossible, to perform such a study. Another limitation of our study is that other than self-report, we have no confirmation that the MDMA-using subjects did in fact take MDMA. A recent survey in the Netherlands investigated the validity of the drug history questionnaire that was used in this study. In 93% of the cases (n = 594), the reported use of MDMA was in agreement with the drug urine test (van de Wijngaart et al 1997). In future studies, hair sample analysis would be a useful way to ascertain previous use of MDMA.

In sum, our preliminary data, which must be confirmed in a larger number of subjects, suggest that verbal memory impairment is associated with prefrontal cortex neuronal loss or dysfunction (as indicated by low NAA measures) in MDMA users. Our study shows a potentially unique

(regionally specific) relationship between function of cortical neurons and cognitive performance.

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