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MDMA exposure alters cognitive and electrophysiological sensitivity to rapid tryptophan depletion in rhesus monkeys

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Abstract

Repeated treatment with (±)3,4-methylenedioxymethamphetamine (MDMA) produces lasting depletions in serotonin (5-HT) markers in the brains of New and Old World monkeys. We have previously shown that macaques treated with MDMA (4 days, 10 mg/kg im, b.i.d.), exhibit an immediate, ~50% reduction of the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) in cerebrospinal fluid (CSF) and 76–93% reductions in neocortical 5-HT content postmortem, but no lasting behavioral deficits under unchallenged conditions. Those monkeys were, however, more behaviorally sensitive to challenge with the 5-HT_{2C} agonist 1-(3-chlorophenyl)piperazine (mCPP) 1 year after the MDMA regimen. A rapid tryptophan-depletion protocol was employed to determine further if these MDMA-exposed monkeys are more behaviorally and electrophysiologically sensitive to perturbation of 5-HT neurotransmission. Acute intragastric administration of a tryptophan-deficient ($TRYP^-$) mixture of amino acids resulted in significant reductions in CSF 5-HIAA in both MDMA-exposed and control monkeys. The $TRYP^-$ mixture also reduced the brainstem auditory-evoked potential (BSAEP) P4 latency in MDMA-exposed monkeys, similar to an effect observed for 13 weeks post-MDMA. Spatial working memory performance was improved by the $TRYP^-$ mixture in the control group, but not the MDMA-exposed monkeys. Other behavioral capabilities [visual recognition memory, reaction time (RT), reinforcer efficacy and fine motor control] were not significantly affected by the $TRYP^-$ mixture in either group of monkeys. Thus, underlying alterations in brain function resulting from prior exposure to MDMA, that were not observed under normal conditions, may be revealed following perturbation of 5-HT signaling. The BSAEP response and spatial working memory appear particularly sensitive to lasting functional differences associated with MDMA exposure.

Keywords: Ecstasy; Memory; Tryptophan; Evoked potential; CANTAB

1. Introduction

Recreational use of (±)3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") has become increasingly popular in recent decades (Peroutka, 1987; Pope et al., 2001; Schuster et al., 1998) and it's use continued to accelerate through 2001 (Johnston et al., 2002b,c). A sharp reduction in use for U.S. teens in 2002 (Johnston et al., 2002a) is promising, but may be associated with a general drop in drug and alcohol use observed following the events of September 11, 2001. As the popularity of MDMA grew in the 1980s and 1990s, preclinical research demonstrated that MDMA exposure can result in persisting and possibly permanent alterations in serotonin (5-HT) neurons of the central nervous system of New and Old World monkeys as well as rodents (for a review, see McKenna and Peroutka, 1990; Ricaurte et al., 2000; Steele et al., 1994). Concern regarding the possible functional consequences of MDMA exposure has been supported by recent studies demonstrating neurocognitive performance deficits in frequent recreational users of MDMA (Bhattachary and Powell, 2001; Gouzoulis-Mayfrank et al., 2000; McCann et al., 1999; Morgan, 1999; Rodgers, 2000; Verkes et al., 2001; Zakzanis and Young, 2001), even after prolonged abstinence (Bhattachary and Powell, 2001; Gouzoulis-Mayfrank et al., 2000; McCann et al., 1999; Morgan, 1999). Since repeated treatment of rats or nonhuman primates with MDMA has consistently been demonstrated to produce lasting and selective depletions of brain 5-HT markers, an obvious working hypothesis is that the alterations of normal 5-HT transmission observed in abstinent human users may cause cognitive impairment. Such a hypothesis is supported

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by evidence that human MDMA users exhibit depletions in markers of central 5-HT systems (McCann et al., 1999, 1998; Reneman et al., 2000, 2001; Semple et al., 1999).

The MDMA-using human populations observed in behavioral studies have typically consisted of multidrug users with considerable prior and continuing exposure to cannabis and alcohol, among other substances, which may, themselves, produce lasting negative effects on cognition (e.g., Croft et al., 2001). Therefore, the evidence from human studies cannot definitively establish a direct relationship between MDMA-related alterations of serotonergic neurotransmission and cognitive impairment. Efforts to directly link MDMA-associated alterations of CNS 5-HT systems, therefore, require experimental studies conducted in nonhuman models. The wide behavioral repertoire of nonhuman primates is ideal for investigation of relationships between controlled brain insults and complex cognition. Studies conducted in three separate laboratories have now demonstrated that nonhuman primates exposed to short-course, high-dose repeated regimens of MDMA do not exhibit performance deficits on a number of behavioral assays under normal conditions (Frederick et al., 1998; Taffe et al., 2001; Winsauer et al., 2002). Behavior was essentially unaffected in all three studies despite evidence that 5-HT markers in several neocortical brain regions were decreased by 50–90% (Frederick et al., 1998; Taffe et al., in press; Winsauer et al., 2002).

The brains of monkeys treated with repeated MDMA regimens are not, however, functionally intact since we demonstrated in our original study that alterations in brainstem auditory-evoked potential (BSAEP) latencies persisted up to 13 weeks following MDMA exposure (Taffe et al., 2001). Furthermore, MDMA-exposed monkeys exhibit altered behavioral sensitivities upon systemic challenge with centrally acting serotonergic agents. Frederick et al. (1998) showed that MDMA-exposed rhesus monkeys were less sensitive to the behaviorally disrupting effects of challenges with acute doses of the 5-HT-releasing agents MDMA or *d*-fenfluramine and we have shown that MDMA-exposed rhesus are more sensitive to the behaviorally disrupting effects of challenge with the 5-HT_{2C} agonist 1-(3-chlorophenyl)piperazine dihydrochloride (mCPP) (Taffe et al., in press). These effects are far from universal, since not all behavioral measures were affected in like fashion in those studies. Furthermore, Winsauer et al. (2002) failed to find any altered impact of mCPP or racemic fenfluramine in squirrel monkeys performing a single operant learning task following repeated MDMA exposure. Finally, we failed to demonstrate any substantial alteration in the impact of the 5-$HT_{2A/2C}$ antagonist, ketanserin or the 5-HT_{1A} agonist, 8-hydroxy-DPAT on a range of behavioral assays in MDMA-exposed rhesus monkeys (Taffe et al., in press).

The present study was conducted to determine if the monkeys treated under a repeated high-dose MDMA regimen in our previous study (Taffe et al., 2001) exhibited increased behavioral or electrophysiological sensitivity to reductions in brain 5-HT levels produced by rapid tryptophan depletion (RTD). The present study was conducted prior to a series of acute 5-HT agonist/antagonist probes in these same monkeys which have been previously reported (Taffe et al., in press). In contrast with using acute drug challenges to probe underlying sensitivity, RTD offers two advantages. First, it permits study of the BSAEP electrophysiological response under normal and challenge conditions. Second, it constitutes a probe challenge of 5-HT neurotransmission that is more similar to the original MDMA-associated insult (i.e., a reduction in released 5-HT), in comparison with an antagonist drug challenge. RTD has been employed to temporarily deplete CNS 5-HT levels in a number of human and preclinical investigations (see (Carpenter et al., 1998; Moore et al., 2000) for review). In many human studies, RTD is employed in an attempt to determine if clinical symptoms presumed to result from 5-HT deficits can be exacerbated or elicited. In a typical RTD procedure, the subject is administered (per os or intragastric) a mixture of essential amino acids designed to mimic the proportions contained in human milk with tryptophan omitted. The procedure leads to a reduction of tryptophan concentrations in plasma (~ 85%) and cerebrospinal fluid (CSF, ~ 92%) in both human (Carpenter et al., 1998; Williams et al., 1999) and nonhuman (Palmour et al., 1998; Young et al., 1989) primates with a CSF tryptophan nadir being reached approximately 6–8 h after ingestion of the cocktail in humans (Carpenter et al., 1998; Williams et al., 1999). RTD also leads to a ~ 33% reduction in CSF concentrations of the major 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) in humans and nonhuman primates (Carpenter et al., 1998; Palmour et al., 1998; Williams et al., 1999; Young et al., 1989) and a PET study indicates that RTD results in a multifold reduction of 5-HT synthesis in human brain (Nishizawa et al., 1997). In sum, there is good evidence that RTD can elicit significant reductions in 5-HT neurotransmission, thus, this procedure may be employed to probe alterations in 5-HT mediated brain function.

Two hypotheses were under investigation in the present study, governed by the overriding assumption that despite exhibiting CSF 5-HIAA concentrations, which were statistically indistinguishable from control animals, the MDMA-exposed animals had remaining 5-HT perturbations in selected brain regions. This assumption was later supported by the finding upon postmortem evaluation of cortical 5-HT reductions in these MDMA-exposed monkeys in the absence of subcortical alterations (Taffe et al., in press). The working hypotheses were predicated on our previous findings and generally propose that MDMA-exposed monkeys would be more sensitive to RTD using measures affected by the original drug regimen and less sensitive to RTD using measures which were not affected by the original regimen. Thus, one major prediction of the present study was that the BSAEP P4 latency of MDMA-exposed monkeys would be reduced, following RTD, to a greater extent than in control monkeys. This would be consistent with the persistent latency reduction observed following the original MDMA regimen (Taffe et al., 2001). The second major prediction was that

MDMA-exposed monkeys would be less sensitive to effects of RTD on the behavioral measures utilized, particularly those which depend to a large degree on cortical mechanisms (e.g., self-ordered spatial search, SOSS; delayed nonmatching to sample, DNMS).

2. Methods

2.1. Subjects

Six adult male rhesus monkeys (*Macaca mulatta*) served as subjects. The monkeys were approximately 6 years of age and weighed an average of 8.1 kg (S.D. = 0.63) at the beginning of the present study. Animals were individually housed and fed in the home cage after completion of the daily testing session. The animals' normal diet (Lab Diet 5038, PMI Nutrition International) was supplemented with fruit or vegetables 4 days per week and water was available ad libitum in the home cage at all times. Principles of laboratory animal care (Clark et al., 1996) were followed, and all protocols were approved by the Institutional Animal Care and Use Committee of the Scripps Research Institute. The monkeys had extensive prior training on all components of the behavioral test battery.

2.2. MDMA exposure

Three of the monkeys were exposed to a repeated, high-dose regimen of MDMA HCl (4 days, 10 mg/kg im, b.i.d., dose expressed as the salt), 11 months prior to beginning of the present study as participants in a study which has been previously described (Taffe et al., 2001). The remaining three animals were treated with vehicle injections under the same schedule and served as control subjects. The MDMA regimen resulted in an initial reduction (~ 50%) in CSF concentrations of 5-HIAA compared with pretreatment values and control subjects, which persisted for up to 17 weeks. These animals were sacrificed approximately 5 (two MDMA-exposed; two control) or 8 months (one MDMA-exposed) after the present study, and postmortem analysis of brain tissue confirmed 76–93% reductions in neocortical and hippocampal 5-HT content in the MDMA-exposed animals, compared with the control subjects (Taffe et al., in press), as is illustrated in Fig. 1. Similar magnitude reductions were observed in one monkey (the remaining control subject) treated with the repeated high-dose regimen of MDMA, 8 months after the present study and sacrificed 2 weeks later. The experiments described here were conducted prior to the acute drug challenges reported by Taffe et al. (in press).

2.3. Rapid tryptophan depletion

Both groups of monkeys were administered 2 g/kg of a balanced (BAL) or TRYP $^-$ amino acid mixture (Bio-Serv, Frenchtown, NJ) in distilled water by orogastric intubation

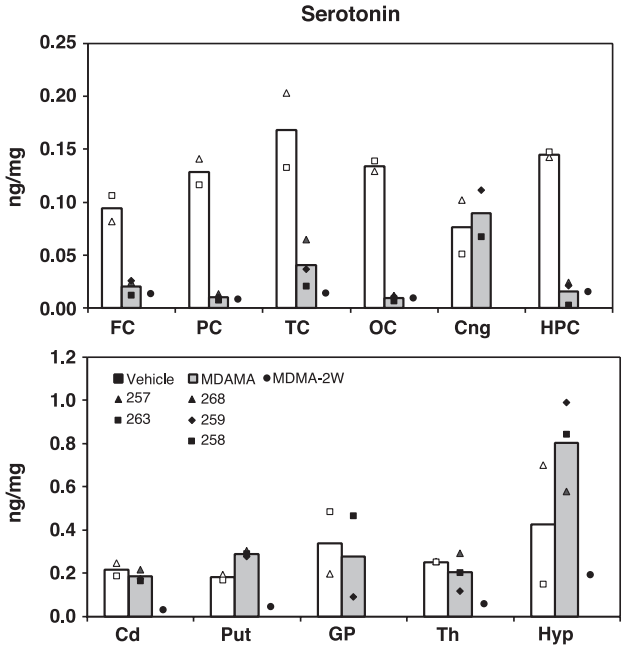

Fig. 1. Tissue concentrations of 5-HT. Mean and individual concentrations of 5-HT in several brain regions are presented for three animals exposed to the MDMA regimen 17 ($n = 2$) or 20 ($n = 1$) months prior to sacrifice, and two vehicle-treated control animals. Regional concentrations are also presented for one monkey exposed to the repeated MDMA regimen 2 weeks prior to sacrifice (MDMA-2W; data were not available for the anterior cingulate and globus pallidus of this animal). The MDMA treatment resulted in large, persistent reductions in tissue 5-HT content in all cortical regions except anterior cingulate. The cortical reductions were similar to those observed in the monkey sacrificed 2 weeks post-MDMA. Tissue concentrations of 5-HT in subcortical regions were not significantly altered 17–20 months following MDMA exposure. Frontal cortex, FC; parietal cortex, PC; temporal cortex, TC; occipital cortex, OC; anterior cingulate, Cng; hippocampus, HPC; caudate, Cd; putamen, Put; globus pallidum, GP; thalamus, Th; hypothalamus, Hyp. This figure was previously published in Taffe et al. (in press) and is reproduced here by permission of Nature Publishing Group.

(gavage) under light ketamine anesthesia (5 mg/kg im). The total volume administered in all conditions was 50 ml. The amino acid mixtures were tinted with blue food coloring to enhance detection of possible emesis, following recovery from immobilization, as regurgitation of the amino acid mixture has been observed, albeit very infrequently, in both human and nonhuman primate studies (Moeller et al., 1996; Palmour et al., 1998). The gavage procedures were conducted 5 h prior to the start of the behavioral testing sessions. The normal diet was not altered prior to or during the study. The amino acid proportions (see Table 1), the amount administered and the pretreatment interval were derived from a previous study which reported approximately 45% reductions in CSF 5-HIAA in vervet monkeys following TRYP $^-$ administration (Young et al., 1989). Each treatment condition (BAL, TRYP $^-$) was administered four times, twice for each type of behavioral testing session [i.e., DNMS/reaction time (RT)/bimanual motor skill or coordination (BMS); progressive ratio (PR)/SOSS/BMS, see below] in a pseudorandomized order with a minimum 3-day interval between challenges. The monkeys were trained/tested on the behav-

Table 1
Test mixtures

Amino acid	Balanced, % (w/w)	Tryptophan deficient, % (w/w)
L-Alanine	5.2	5.5
L-Arginine	4.7	4.8
L-Cysteine	2.6	2.6
Glycine	3.1	3.1
L-Histidine	3.1	3.1
L-Isoleucine	7.7	7.8
L-Leucine	12.9	13.2
L-Lysine	10.5	10.8
L-Methionine	2.9	2.9
L-Phenylalanine	5.5	5.6
L-Proline	11.7	11.9
L-Serine	6.6	6.8
L-Threonine	6.2	6.4
L-Tryptophan	2.2	0.0
L-Tyrosine	6.6	6.8
L-Valine	8.5	8.7

Animals were administered 2 g/kg i.g., of one of the amino acid mixtures 5 h prior to initiation of the behavioral testing sessions.

ioral tasks 5 days per week on a continuing basis, i.e., even when challenges were not performed. Electrophysiological recording and CSF sampling were conducted 6 h postgavage on one occasion each for the BAL and TRYP$^-$ conditions, at intervals of greater than 2 weeks. The RTD protocol was successful in that no episodes of emesis, following gavage, were observed in any monkeys during the course of this study; thus, the mixtures were well tolerated at this dose.

2.4. BSAEP recording

BSAEPs were recorded under ketamine anesthesia (20 mg/kg im) on one occasion each following BAL or TRYP$^-$ administration and after the conclusion of the behavioral testing sessions (i.e., 6 h postgavage). A comparison baseline recording was obtained 2 months prior to the present study (i.e., 9 months post-MDMA). For recording, electrodes were placed subcutaneously at the cranial vertex (active), over the greater alar cartilage (reference) and in the musculature of the neck (ground). Raw electroencephalographic signals (EEG) were amplified and filtered within a bandwidth of 30–3000 Hz. Binaural condensation stimulation, produced by clicks generated using 0.10 ms square waves and delivered at a 70 dB sound pressure level at a rate of 10 Hz, was used to generate BSAEPs. BSAEPs were characterized by averaging the first 10 ms of EEGs recorded for 1024 samples. Several component peaks are identified in the average waveform for individual BSAEPs including P1, P2a, P2b, P3, P4 and P5. The focus of the present investigation was on P4 latency because it was shown to be most consistently and persistently reduced by the repeated MDMA regimen in our prior study (Taffe et al., 2001). Ambient lights remained on throughout the recording and latencies were not corrected for the delay introduced due to the length of tubing connecting the speaker to the ear canals.

2.5. CSF evaluation

CSF (1–2 ml) was collected by percutaneous cisternal puncture using aseptic techniques under ketamine anesthesia (20 mg/kg). Samples were collected following electrophysiological recordings, i.e., once each following BAL and TRYP$^-$ administration as well as on timepoints 2 weeks and 3 months after the conclusion of the present study (e.g., 13 and 16 months post-MDMA). Data from these latter two samples were averaged to construct the baseline values. Samples were centrifuged to remove cellular contamination and stored at −70 °C. CSF concentrations of 5-HIAA were measured by high-performance liquid chromatography with electrochemical detection (HPLC-EC), as previously described (Taffe et al., 2001).

2.6. Behavioral testing

Cognitive and behavioral performance was evaluated using a battery of tests designed for neuropsychological testing of rhesus monkeys which has been described previously (Weed et al., 1999). The battery included tests of memory (SOSS, DNMS), reinforcer efficacy (PR), RT and BMS. The performance of macaques on tests in this battery has been shown to be differentially sensitive to acute challenge with psychoactive compounds (Taffe et al., 1999, 2002b,c; Weed and Gold, 1998). The individual tests are described briefly below whereas comprehensive descriptions of the training protocols have been detailed previously (Weed et al., 1999).

2.6.1. Apparatus

Animals were transferred to the testing room in transport cages similar to the subjects' home cages, but modified by the removal of several bar sections to allow the animals to easily reach out of the cage. The transport cage was placed in front of a computer monitor, fitted with a touch-sensitive screen, on which visual stimuli were presented. To obtain a food pellet reward, each animal was trained to reach out of the cage, to touch the location on the screen, at which visual stimuli were presented. Stimulus presentation and response detection were conducted with a microcomputer equipped with a version of the Cambridge Neuropsychological Test Automated Battery (CANTAB; Cambridge Cognition, Cambridge, UK) designed for use with nonhuman primates. A dispenser delivered 190-mg flavored Noyes precision pellets (Research Diets, New Brunswick, NJ) to a bin mounted on the front of the cage after correct responses. A white noise generator (~ 85 dB) located behind the touchscreen was turned on during each behavioral session.

2.6.2. Test battery

The test battery employed consists of five behavioral tasks. Four of the tasks are part of the nonhuman primate CANTAB and require monkeys to respond by touching the touch-sensitive computer screen and are reinforced with the

delivery of food pellet reinforcers. The fifth task requires subjects to extract raisins from holes in a transparent plastic board. Animals performed daily (M–F) in behavioral sessions lasting approximately 1 h with combinations of tasks being completed on alternating days. Thus, on 1 day, an animal would complete the PR, SOSS and BMS tasks. On the next test day, the animal would complete the DNMS, five-choice RT and BMS tasks. The effect of the treatment conditions were double-determined for DNMS, SOSS, RT and PR and (therefore) quadruple-determined for BMS.

2.6.3. Self-ordered spatial search

In each trial of the SOSS task, two, four, six or eight small colored rectangles (boxes) were displayed on the screen in positions randomly allocated from 16 possible locations. The animal was required to touch a box within 30 s of stimulus onset. After each successful touch, the color of the touched box was briefly (100 ms) changed and then the screen was blanked and a reinforcer was delivered. After a 2-s delay, the boxes were redisplayed and the animal was required to touch a box, which had not previously been touched during a given trial for the next reinforcer. The trial was completed when the animal had either touched all boxes without a repetition (correct), touched a box that had previously been selected in that trial (error) or failed to touch a box within 30 s of stimulus presentation (omission). Errors and omissions were followed by a tone and a 4-s screen blank. After an intertrial interval of 5 s, another trial was presented with stimuli in new (randomly allocated) positions. A session consisted of 40 trials grouped into eight blocks by trial type as follows: 5 (two boxes), 5 (four boxes), 5 (six boxes), 5 (eight boxes), 5 (four boxes), 5 (six boxes), 5 (eight boxes) and 5 (two boxes). Accuracy scores were calculated for each trial type by dividing the number of correctly completed trials by the number of trials in which there was at least one response (i.e., errors of omission were excluded from the calculation). The monkeys' accuracy on two- and four-box trials was essentially perfect; therefore, only performance on the six- and eight-box trials was included in the analysis.

2.6.4. Delayed nonmatching to sample

The DNMS task is a recognition memory task involving sets of visual discriminations. A sample stimulus is presented in the center of the screen and the animal must make an observing touch to its location within 30 s. After an observing touch, the screen is blanked and following a variable retention interval (0, 1, 2 or 3 min), two-choice stimuli are presented in the lower left and right of the screen. One stimulus is identical to the sample stimulus and the other is novel. A touch directed to the novel, or nonmatching stimulus is followed by reinforcer delivery. In addition to the four retention interval conditions, a simultaneous condition is included in which the sample stimulus remains present after the observing touch, and while the choice stimuli are presented. A session consisted of 10 trials at each retention interval and the simultaneous condition presented in randomly intermixed fashion for a total of 50 trials. Performance accuracy is measured as a proportion of correct responses to all responses (i.e., errors of omission are excluded from the accuracy calculation). The software utilizes 469 shapes and 7 colors to ensure that discriminations are unique for approximately 120,000 trials. Preliminary analysis of the data indicated that performance for the 1-, 2- and 3-min delay conditions did not differ to any observable degree; thus, accuracy scores were collapsed across these conditions and the data were statistically analyzed for the simultaneous and delayed (average of 1, 2 and 3 min) conditions.

2.6.5. Five-choice RT

For the RT task, a response lever (BRS/LVE, Laurel, MD) was mounted below the monitor and in front of the transport cage. For each trial, a grid of five circles, or possible target locations, connected by lines was presented in white on the dark screen. Subjects initiated a trial by holding down the lever. After a pseudorandomly variable delay lasting between 0.75 and 2.5 s, a yellow circle ("target") appeared within one of the five target locations for 20, 100 or 1000 ms Touching the appropriate circle within 2 s of target onset resulted in reinforcer delivery. The time required for the monkey to release the lever (release latency) as well as the time required to touch the circle (response latency) following target onset were recorded in milliseconds. The time required to move from lever to target (movement time) was calculated by subtracting the release from the response latency.

2.6.6. PR schedule of reinforcement

In the PR task, a large colored rectangle was presented in the center of the screen and the animal was required to touch the rectangle for reinforcer delivery. The response requirement started at one and incremented by arithmetic progression within blocks of eight reinforcers, and by geometric progression between blocks of eight (i.e., the first successive eight ratios increase by 1, the second successive eight increase by 2, the third successive eight increase by 4, etc.). The session was terminated after 10 min, or earlier, if 3 min had elapsed since the previous response. In either case, the number of reinforcers acquired in the session was recorded.

2.6.7. BMS task

A rectangular transparent plastic board drilled with 15 holes and filled with raisins was mounted perpendicular (long axis vertical) to the door of the transport cage. The hole diameter is such that for efficient retrieval of raisins, the animal must push the raisin partially out of the hole with one finger before retrieving it. With training, animals universally adopt a strategy of pushing the raisin with one hand while retrieving it with the other hand, thus, exhibiting bimanual dexterity. The time required to retrieve all 15 raisins was recorded by stopwatch.

2.7. Data analysis

Behavioral test battery measures were analyzed by repeated measures analysis of variance (ANOVA) with a between-subjects factor of MDMA-treatment condition (MDMA-exposed, control) and a within-subjects factor of tryptophan treatment (BAL, TRYP$^-$). An additional within-subjects factor of task difficulty was included for the SOSS (six- and eight-box) and DNMS (simultaneous, delayed) procedures. The CSF 5HIAA concentrations and the BSAEP P4 peak latencies were analyzed by repeated ANOVA with a between-subjects factor of MDMA-treatment condition (MDMA-exposed, control) and a within-subjects factor of tryptophan condition (baseline, BAL, TRYP$^-$). Post hoc exploration of significant main effects was conducted using the Tukey–Kramer procedure. All analyses were conducted using GB-STAT v7.0 for Windows (Dynamic Microsystems, Silver Spring MD). The criterion for significance in all tests was $P \leq .05$ and the criterion values were corrected for all possible comparisons in the post hoc tests. Given the limited sample size in the design, a number of steps were taken to verify the validity of the ANOVA procedure. A parametric approach to analysis provides additional statistical power over nonparametric techniques (Glantz, 2002; Motulsky, 1995) and nonhuman primate research is under particular requirement to reduce the number of subjects, where possible. Therefore, the selection of (appropriate) analysis techniques that offer maximum statistical power, combined with appropriate protection against Type I errors, is preferred. First, the underlying population of scores for each behavioral measure has been evaluated with Shapiro–Wilk test of normalcy. This population was estimated with a sample of baseline scores from 10 to 30 (depending on the behavioral measure) monkeys trained in the laboratory to levels of performance similar to the present group. In the vast majority of cases, the distributions of scores are normal; and where this is not the case, outliers tend to broaden the distribution. Thus, any violation in the assumption of normalcy in the populations of scores would tend to increase Type II but not Type I errors. Second, common tests for homogeneity of variance in the sample (Hartley's F_{max}, Cochran's C, Levene's F and Bartlett's chi-square) were employed for each analysis conducted for the present study, and in no case was the null hypothesis rejected. These steps confirm the appropriateness of the ANOVA procedure. Finally, nonparametric analyses were employed to evaluate the effect of a less powerful technique in cases where the result would appear obvious. Using Friedman's ANOVA, several comparisons, found to be significant with the parametric technique, failed to be confirmed (MDMA group: P4 latency, P value of .11; SOSS, P value of .063; control group: DNMS, P value of .06). The likelihood-ratio analysis of contingency tables, or information statistic, (Kullback, 1968; Robbins, 1977) is a nonparametric technique used in behavioral studies (Fray et al., 1980; Sahgal et al., 1975), which may offer enhanced statistical power. In comparison with Friedman's ANOVA, this technique was indeed slightly more powerful, confirming a significant difference in P4 latency for the MDMA group, but did not approach the power of the parametric approach.

3. Results

3.1. CSF 5-HIAA

Administration of the TRYP$^-$ mixture significantly reduced 5-HIAA by 23% (control group) or 22% (MDMA group) in CSF samples obtained 6 h postgavage Fig. 2, as confirmed by a significant main effect of tryptophan condition [$F(2,8) = 5.38$, $P < .05$] in the repeated measures ANOVA. Post hoc analysis of this main effect confirmed that the TRYP$^-$ mixture significantly lowered CSF 5-HIAA in comparison with the BAL mixture. The effect of the TRYP$^-$ mixture was equivalent between groups as there were no differences in 5-HIAA attributable to MDMA-exposure condition [$F(1,4) = 1.12$, P=.35], nor any interaction between the tryptophan and MDMA factors [$F(2,8) = 0.05$, P=.95].

3.2. Brainstem auditory-evoked potentials

Administration of the TRYP$^-$ mixture significantly reduced P4 latency in the MDMA group as is illustrated in Fig. 3. Analysis confirmed a significant main effect of tryptophan condition [$F(2,8) = 11.76$, $P < .05$], MDMA condition [$F(1,4) = 19.6$, $P < .05$] and the interaction between the two factors [$F(2,8) = 7.66$, $P < .05$]. Post hoc exploration of these effects confirmed that the P4 latency was significantly reduced in the MDMA-exposed group, following the TRYP$^-$ mixture relative to either the control group after

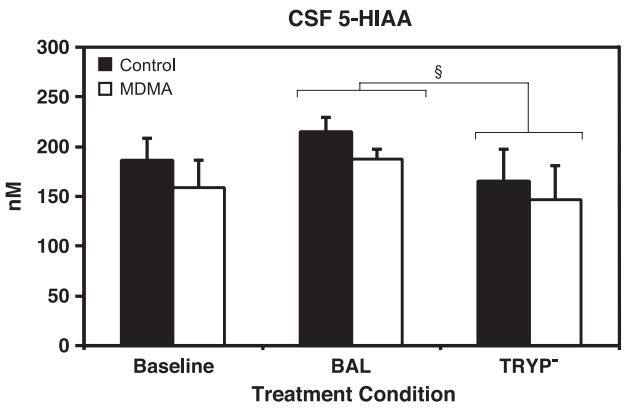

Fig. 2. CSF 5-HIAA. The mean (+S.E.M.) CSF concentrations of the 5-HT metabolite 5-HIAA are presented for MDMA-exposed ($n = 3$) and control ($n = 3$) monkeys under baseline conditions (see text) and following challenge with BAL and TRYP$^-$ mixtures. The TRYP$^-$ mixture significantly lowered 5-HIAA concentrations relative to the BAL mixture in both groups. §Significant difference from BAL across treatment group.

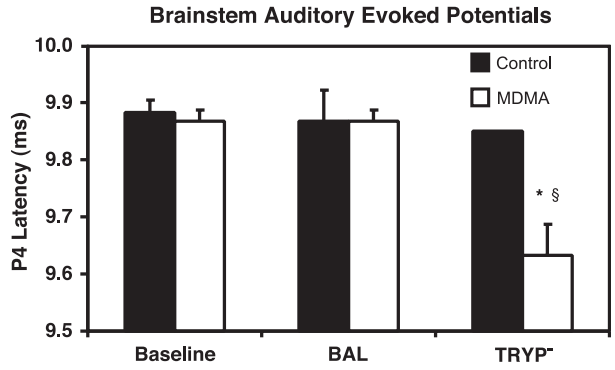

Fig. 3. BSAEPs. The mean (+S.E.M.) peak latencies for the P4 potentials are presented for MDMA-exposed ($n=3$) and control ($n=3$) monkeys under baseline conditions and following challenge with BAL and TRYP$^-$ mixtures. In the MDMA-exposed group P4 latencies were lower after the TRYP$^-$ mixture. * Significant difference from baseline within a treatment group (MDMA, control); §Significant difference between BAL and TRYP$^-$ conditions within a treatment group.

TRYP$^-$ administration or relative to their own P4 latencies recorded following BAL administration or under baseline conditions.

3.3. Self-ordered spatial search

There were no significant differences in SOSS accuracy between the treatment groups [$F(1,4)=0.02$, $P=.905$] confirmed by the three-way ANOVA; however, both trial difficulty [$F(1,4)=83.78$, $P<.05$] and tryptophan condition [$F(1,4)=14.34$, $P<.05$] significantly affected accuracy as is illustrated in Fig. 4. The tryptophan treatment affected the control group differentially from the MDMA-exposed group, as confirmed by a significant interaction between treatment group and tryptophan condition [$F(1,4)=10.77$, $P<.05$]. No additional interaction of the main factors produced a significant effect on SOSS accuracy, and post hoc exploration, including all possible comparisons, failed to confirm any pair-wise differences, potentially due to the limited statistical power associated with the sample sizes. To further explore this pattern of results, two-way repeated measures ANOVAs were conducted for each treatment group (MDMA-exposed and control). This analysis confirmed that performance was graded by trial difficulty, as both control [$F(1,2)=30.94$, $P<.05$] and MDMA-exposed animals [$F(1,2)=70.43$, $P<.05$] made fewer errors on the six-, compared with the eight-box trials. Only the control animals were significantly affected by the tryptophan condition, making significantly fewer errors following TRYP$^-$ administration [$F(1,2)=34.00$, $P<.05$] for both six- and eight-box trials. This was further evidenced by a lack of a significant interaction between trial difficulty and tryptophan condition [$F(1,2)=0.99$, $P=.424$]. The MDMA-exposed animals were not significantly affected by tryptophan condition [$F(1,2)=0.10$, $P=.781$], nor was there any significant interaction of tryptophan condition with trial difficulty [$F(1,2)=0.10$, $P=.781$].

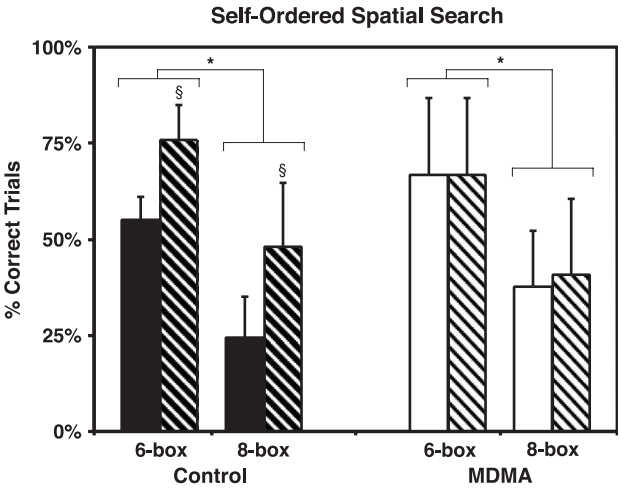

Fig. 4. SOSS task. The mean (+S.E.M.) percentage of correct trials on the 6- and 8-box trials of the SOSS task is presented for MDMA-exposed ($n=3$) and control ($n=3$) monkeys following challenge with BAL and TRYP$^-$ mixtures. The solid/open bars indicate BAL and the hatched bars indicate TRYP$^-$. Performance on this test of spatial working memory depended on trial difficulty for both groups and was improved in the control animals following TRYP$^-$ administration. * Significant difference between trial difficulty conditions; §Significant difference between BAL and TRYP$^-$ conditions.

3.4. Delayed nonmatching to sample

Choice accuracy in the DNMS procedure (Fig. 5) was poorer for delayed trials than for simultaneous trials, as confirmed by a significant main effect of retention interval in the ANOVA [$F(1,4)=7.48$, $P=.05$]. However, choice accuracy did not significantly differ between the treatment

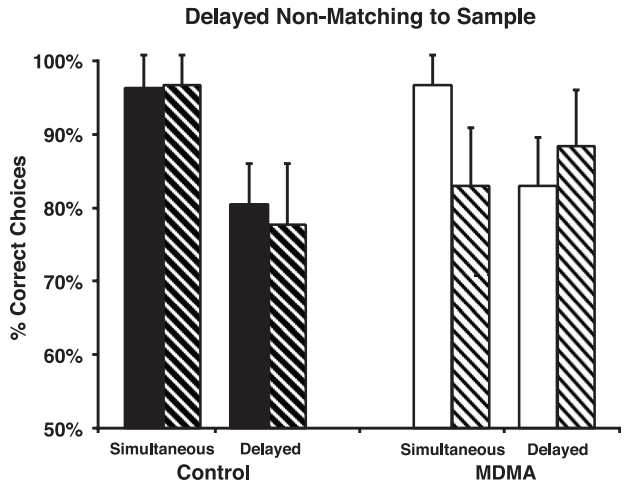

Fig. 5. DNMS task. The mean (+S.E.M.) percentage of correct choices on simultaneous and delayed trials of the DNMS task is presented for MDMA-exposed ($n=3$) and control ($n=3$) monkeys following challenge with BAL and TRYP$^-$ mixtures. The solid/open bars indicate BAL and the hatched bars indicate TRYP$^-$. Although monkeys made significantly fewer correct choices on delayed vs. simultaneous trials, TRYP$^-$ administration did not affect choice accuracy for either trial type.

Table 2
RT, PR and BMS tasks

	Control group		MDMA-exposed	
	BAL	TRYP $^-$	BAL	TRYP $^-$
RT: release latency (ms)	286.7 (30.2)	314.1 (31.0)	334.7 (32.3)	327.2 (25.7)
RT: movement time (ms)	377.9 (45.9)	342.7 (24.5)	414.6 (11.3)	390.1 (23.9)
PR (reinforcers acquired)	22.2 (4.3)	23.7 (3.19)	21.3 (8.6)	21.8 (7.8)
BMS (s)	18.0 (3.1)	19.8 (3.8)	16.5 (2.0)	16.8 (3.2)

Mean (S.E.M.) scores on the RT, PR (PR> and BMS) tasks are presented for the MDMA-exposed and control monkeys following administration of either the BAL or TRYP $^-$ amino acid mixtures.

groups [$F(1,4) = 0.00$, P=.995], nor was it affected significantly by tryptophan condition [$F(1,4) = 1.58$, P=.272]. There were no significant interactions between any of the factors.

3.5. Five-choice RT

The MDMA-exposed and control animals' release latencies in the RT task (see Table 2) did not differ [$F(1,4) = 0.41$, P=.559], nor was there any significant effect of tryptophan condition on performance [$F(1,4) = 2.71$, P=.175]. A trend for an interaction between treatment group and tryptophan condition on release latency was not statistically reliable [$F(1,4) = 6.87$, P=.059]. Similarly, movement times were similar between the treatment groups [$F(1,4) = 0.86$, P=.406] and a trend for reduced movement time associated with TRYP $^-$ administration was not statistically reliable [$F(1,4) = 5.65$, P=.076]. Finally, there was no interaction between the effects of tryptophan condition and treatment group [$F(1,4) = 0.20$, P=.679].

3.6. Progressive ratio

The number of reinforcers acquired in the PR test (see Table 2) was not significantly altered by tryptophan condition [$F(1,4) = 5.40$, P=.080]. There were no significant differences between the groups [$F(1,4) = 0.28$, P=.624], nor any interaction between the two factors.

3.7. Bimanual motor skill

The amount of time required to retrieve 15 raisins in the BMS task (see Table 2) was not significantly altered by tryptophan condition [$F(1,4) = 0.69$, P=.452]. There were no significant differences between the groups [$F(1,4) = 0.44$, P=.542], nor any interaction between the two main factors.

4. Discussion

This investigation was conducted to test the hypothesis that rhesus monkeys previously exposed to a short-course, high-dose, repeated regimen of MDMA would exhibit altered behavioral and electrophysiological sensitivity to reductions in central 5-HT achieved by RTD. The major goal of this study was to determine if experimental reductions in 5-HT revealed MDMA-associated alterations in brain function which were not apparent under normal (unchallenged) conditions to further characterize the persisting effects of prior high-dose MDMA exposure. The results demonstrate that monkeys exposed to MDMA 1 year previously, exhibit altered sensitivity to RTD, in comparison with control animals. Specifically, the BSAEP response of MDMA-exposed monkeys was more sensitive to RTD, and performance on a test of spatial working memory was less sensitive to RTD, in comparison with control monkeys. Other behavioral domains were not significantly affected by RTD. Thus, monkeys previously exposed to MDMA do exhibit selective and persistent alterations in brain function that are revealed when brain 5-HT levels are reduced.

The observation that CSF concentrations of 5-HIAA were reduced by 23% (control monkeys) or 22% (MDMA-exposed monkeys), following the TRYP $^-$ mixture (compared with the BAL mixture), supports the utility of the primary experimental manipulation in this study. While CSF 5-HIAA analysis probably underestimates brain tissue concentrations of 5-HT or 5-HIAA (Ricaurte et al., 1988; Taffe et al., in press), nevertheless, CSF 5-HIAA levels have been found to be positively correlated with tissue concentrations of 5-HT and 5-HIAA in multiple species (Ricaurte et al., 1988; Ruckebusch and Sutra, 1984; Wester et al., 1990). Therefore, it is possible to infer that CSF 5-HIAA reductions observed here likely reflect even greater reductions in tissue 5-HIAA and 5-HT content. Prior studies have reported that CSF 5-HIAA is reduced 31–33% from baseline in humans (Carpenter et al., 1998; Williams et al., 1999) and reduced 33%, compared with the balanced mixture in vervet monkeys (Young et al., 1989). The somewhat lesser effect of the TRYP $^-$ mixture on CSF 5-HIAA reported here, in comparison with the human studies, may be because the two human studies included repeated sampling up to 12 h or more postadministration (Carpenter et al., 1998; Williams et al., 1999). These data show that the CSF 5-HIAA nadir would be expected 8 h or more postgavage so our finding of ~ 10% lower reductions may be attributable to sampling only 6 h postgavage. This expectation is partially offset, however, by the fact that the human studies collected CSF from lumbar puncture, whereas, the present and previous monkey studies sampled CSF from the cisterna magna. Rostrocaudal gradients of CSF monoamine metabolites (Prell et al., 1988; Vaughn et al., 1988) would predict that RTD-related alterations in 5-HIAA in cisternal samples would be larger and observed earlier post-RTD, in comparison with lumbar samples. Nevertheless, one possible limitation of the present experimental design is that the maximum reduction of brain levels of 5-HT in rhesus monkeys might be predicted to occur at a time point later than 5–6 h postadministration. Thus, the mag-

nitude of the behavioral and electrophysiological changes may have been greater, or more comprehensive, if measured at a time more distant from the administration of the TRYP$^-$ mixture. The 5-HIAA findings also may reflect pharmacokinetic differences between vervet and rhesus monkeys. This alternative seemed unlikely at the outset, since the nadir of plasma tryptophan is reached approximately 5–6 h postadministration in both vervet monkeys and humans (Carpenter et al., 1998; Chamberlain et al., 1987; Williams et al., 1999), which suggests consistency across the primate order. In fact, such evidence was the primary reason for selecting the 5–6 h postgavage interval for the behavioral testing sessions in the present study, since only single-timepoint data were available regarding CSF 5-HIAA levels in monkeys (Young et al., 1989). Ultimately, however, the observed differences from the vervet monkey data may be attributable to experimental variability. Individual subject values from the Carpenter study indicate that large fluctuations (in either direction) of CSF 5-HIAA may be observed over intervals as short as 30–45 min (Carpenter et al., 1998; Williams et al., 1999). Thus, it may be difficult to observe anything other than a general trend when CSF is obtained at only a single timepoint post-RTD.

One major finding of the present investigation was that the TRYP$^-$ mixture reduced BSAEP P4 latency in the MDMA-exposed, but not in the control monkeys. This effect on the early processing of auditory stimulation was similar to a P4 latency decrease produced by the original MDMA exposure, an observation which persisted for 13 weeks post-MDMA before abating (Taffe et al., 2001). In that prior study, the P4 latency was decreased by approximately 0.4 ms compared with a 0.25 ms reduction observed here. Interestingly, CSF 5-HIAA was reduced by 50% following the original MDMA regimen and by 23% following RTD in this study. Therefore, these data together suggest the possibility that there is a linear relationship between P4 latency shifts and brain 5-HT alterations; although, clearly, the inability of RTD to affect P4 latency in the control group indicates that 5-HT levels must be suppressed below some threshold value. This result is of particular importance since the BSAEP latency alterations are the only functional measures that have been shown to be persistently altered (under unchallenged conditions), following repeated MDMA administration in monkeys. For example, previous work has shown that a large number of behavioral tasks designed to assay aspects of learning, memory, attention, motivation, motor function, etc are unaltered following repeated MDMA administration (Frederick et al., 1995, 1998; Taffe et al., 2001; Winsauer et al., 2002). Furthermore, this finding is consistent with a variety of evidence from multiple species which suggests that 5-HT modulation of the brainstem auditory nuclei involved in the BSAEP response is inhibitory (for a review, see Thompson et al., 1994). Therefore, the present result supports the conclusion that RTD inhibits 5-HT input to the brainstem of MDMA-exposed monkeys to a much greater extent than in control animals because of an underlying 5-HT deficit associated with prior MDMA exposure. The consequences for this effect on higher level aspects of auditory processing, such as stimulus discrimination or vocalization identification, is not well established.

A second important observation was that SOSS performance was improved in the control animals following the TRYP$^-$ mixture, whereas the MDMA monkeys' performance was unchanged, suggesting a decrease in sensitivity associated with MDMA exposure. This result is likely related to evidence that 5-HT neurotransmission can be detrimental to working memory performance (although a potential alternative explanation is that RTD improves focused attention; Coull et al., 1995; Schmitt et al., 2000). For example, tryptophan *loading* impairs performance of a prototypical working memory task (backward digit span) and a novel affective working memory task in humans (Luciana et al., 2001) and RTD was associated with a trend for fewer errors on the more difficult aspect (e.g., "between-search" errors) of the human version of this SOSS task (Park et al., 1994). Perhaps, more specifically for the present finding, we have shown that acute administration of the 5-HT$_{2C}$ agonist mCPP impaired performance on the SOSS task in these same animals (Taffe et al., in press), although in that study, MDMA-exposed monkeys were equally sensitive, in comparison with control monkeys. Analyzed across treatment groups, the effects of mCPP were greatest on the more difficult (six- and eight-box) trials (unpublished observations), thus indicating a specific effect on the working memory aspects of the task. Therefore, there is evidence that 5-HT activity is negatively correlated with performance accuracy on working memory tasks, and the SOSS task, in particular, even under unchallenged conditions. A plausible interpretation of the present finding is that the preexisting neocortical depletions of 5-HT in the MDMA-exposed animals meant that their performance was already free from 5-HT-related detrimental effects; thus, the TRYP$^-$ mixture was not able to improve performance by "relieving" their spatial working memory performance from 5-HT influence.

The effect of the TRYP$^-$ mixture on the SOSS task may be selective for spatial working memory, since there were no statistically reliable effects of RTD on DNMS performance. It is more likely, however, that working memory tasks are simply more sensitive to 5-HT alterations, since human studies suggest that it may require retention intervals on the order of 30 min or more to observe RTD-related impairment in visual recognition memory (Riedel et al., 1999; Rubinsztein et al., 2001; Schmitt et al., 2000). The trend for a reduction in performance on simultaneous trials by the MDMA-exposed monkeys, following the TRYP$^-$ mixture, is potentially of interest, however. In simultaneous trials, the sample stimulus is presented in the presence of the choice stimuli, so the animal must only compare the sample stimulus directly with the two-choice stimuli. It is not required to remember any information about the sample to

perform accurately. Assuming the animal is able to discriminate the patterns presented, the most plausible interpretation of errors on such trials is a failure of inhibitory control over impulsive responding. Evidence suggests that 5-HT signaling is involved in impulse control, since reductions of 5-HT neurotransmission have been shown to increase impulsivity in rodents (Harrison et al., 1997; Mobini et al., 2000; Soderpalm and Svensson, 1999) and markers of 5-HT are negatively correlated with impulsivity in monkeys (Fairbanks et al., 2001; Higley et al., 1996). Similarly, RTD increases impulsivity in nonalcoholic men with a multigenerational family history of alcohol abuse (LeMarquand et al., 1999), a population which typically exhibits low CSF 5-HIAA (Ballenger et al., 1979; Fils-Aime et al., 1996). Thus, it is possible that an increase in "impulsive" responding on the simultaneous trials of the DNMS task was produced by the $TRYP^-$ mixture in the MDMA-exposed monkeys only, and the failure to confirm this effect simply reflects insufficient statistical power in the study design. Interestingly, no evidence of "impulsive" responding on the SOSS task (i.e., paradoxically poor performance on the easiest trials, response perseveration, faster latency on error trials) was observed here (data not shown). Finally, the failure to find any significant effects of RTD on performance of the PR, RT or BMS tasks provides further confirmation of the selectivity of the effects of the present manipulation, either by itself or in context with the original MDMA-exposure. This lack of an effect is generally consistent with previous studies in humans, which have reported no effect of RTD on RT (Riedel et al., 1999; Schmitt et al., 2000) or motor speed (Luciana et al., 2001). The negative finding with the present BMS task is, however, at odds with a report that RTD improves fine motor coordination (Luciana et al., 2001). Ultimately, however, any conclusion of behavioral specificity here must be tentative, given that the sample size did not provide overwhelming statistical power.

In summary, the present study demonstrates that monkeys exposed 1 year previously to a short-course, high-dose repeated regimen of MDMA exhibit lasting alterations in electrophysiological and behavioral sensitivity to challenge of 5-HT neurotransmission. Effects on BSAEP P4 latency and performance on a working memory task were the most sensitive to a prior history of exposure to MDMA. This was the case despite the lack of observable lasting effects of MDMA treatment on these (or other) measures under unchallenged conditions; thus, this study provides additional evidence that exposure to MDMA results in lasting sensitivity of crucial brain functions to acute alterations in 5-HT signaling.

These findings have important implications for public health, in that they point to the possibility that human MDMA users who do not appear to exhibit lasting detrimental behavioral effects associated with their drug exposure may suffer critical lapses of brain function under conditions which stress normal serotonergic signaling. For example, the consumption of alcohol lowers plasma 5-HT and induces a diurnal pattern consistent with depression (Pietraszek et al., 1991). Chronic low environmental illumination suppresses central 5-HT function in individuals with seasonal affective disorder (Schwartz et al., 1997), and those with either Parkinson's or Alzheimer's disease (Tohgi et al., 1992, 1993), exhibit low 5-HT in CSF. Finally, those with traitlike differences in central 5-HT function (Chotai and Adolfsson, 2002; George et al., 2001; Kaye et al., 1998; Mann and Arango, 1992; van Praag, 1998) may also be at particular risk for MDMA-related behavioral problems. That is, MDMA-associated reductions of serotonergic neurotransmission in those at risk for depression, bulimia nervosa, alcoholism, etc. may push the individual over a threshold for psychopathology.

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