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Dissociation of the Neurochemical and Behavioral Toxicology of MDMA ('Ecstasy') by Citalopram

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High or repeated doses of the recreational drug 3,4-methylenedioxymethamphetamine (MDMA, or 'Ecstasy') produce long-lasting deficits in several markers of serotonin (5-HT) system integrity and also alter behavioral function. However, it is not yet clear whether MDMA-induced serotonergic neurotoxicity is responsible for these behavioral changes or whether other mechanisms are involved. The present experiment tested the hypothesis that blocking serotonergic neurotoxicity by pretreatment with the selective 5-HT reuptake inhibitor citalopram will also prevent the behavioral and physiological consequences of an MDMA binge administration. Male, Sprague–Dawley rats ($N = 67$) received MDMA (4×10 mg/kg) with or without citalopram (10 mg/kg) pretreatment. Core temperature, ejaculatory response, and body weight were monitored during and immediately following drug treatments. A battery of tests assessing motor, cognitive, exploratory, anxiety, and social behaviors was completed during a 10-week period following MDMA administration. Brain tissue was collected at 1 and 10 weeks after drug treatments for measurement of regional 5-HT transporter binding and (for the 1-week samples) 5-HT and 5-HIAA concentrations. Citalopram pretreatment blocked MDMA-related reductions in aggressive and exploratory behavior measured in the social interaction and hole-board tests respectively. Such pretreatment also had the expected protective effect against MDMA-induced 5-HT neurotoxicity at 1 week following the binge. In contrast, citalopram did not prevent most of the acute effects of MDMA (eg hyperthermia and weight loss), nor did it block the decreased motor activity seen in the binge-treated animals 1 day after dosing. These results suggest that some of the behavioral and physiological consequences of a high-dose MDMA regimen in rats are mediated by mechanisms other than the drug's effects on the serotonergic system. Elucidation of these mechanisms requires further study of the influence of MDMA on other neurotransmitter systems.
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INTRODUCTION

Over 20 years of biochemical and histological research has established that high or repeated doses of 3,4-methylenedioxymethamphetamine (MDMA) cause enduring deficits in the serotonergic system of several animal species including rats and monkeys. MDMA reduces brain levels of serotonin (5-HT), the 5-HT metabolite 5-HIAA, and 5-HT transporter (SERT) binding (reviewed in Green *et al*, 2003). Immunohistochemical studies using antibodies against 5-HT or SERT have also found decreased immunoreactive fiber density in several forebrain areas of animals treated with MDMA (O'Hearn *et al*, 1988; Wilson *et al*, 1993; Xie *et al*, 2006). Taken together, these findings have generally been interpreted to reflect a pruning of serotonergic fibers (sometimes referred to as a distal axotomy) in the affected areas (Green *et al*, 2003; although also see Wang *et al*, 2004, 2005).

Transporter availability during the period shortly after drug administration is believed to be necessary for substituted amphetamine neurotoxicity (McCann and Ricaurte, 2004). In the case of MDMA, this hypothesis proposes that SERT carries the drug or a toxic by-product from the extracellular fluid into serotonergic neurons, where the toxic agent exerts its damaging effects. Support for the transport hypothesis comes from studies showing that blockade of SERT by pretreatment with the selective 5-HT reuptake inhibitor (SSRI) fluoxetine completely blocked MDMA-induced serotonergic deficits assessed shortly after drug treatments without modifying the hyperthermic response to MDMA (Malberg *et al*, 1996; Sanchez *et al*, 2001; Schmidt, 1987). However, interpretation of these

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findings is confounded by the fact that fluoxetine is a potent inhibitor of cytochrome P450 (CYP) isozymes that are important for MDMA metabolism (de la Torre *et al*, 2004; Hemeryck and Belpaire, 2002). Indeed, it is interesting to note that pretreatment with citalopram, the currently available SSRI that is most selective for SERT and that has the least effect on CYP activity (Hemeryck and Belpaire, 2002) attenuated but did not completely prevent MDMA neurotoxicity (Battaglia *et al*, 1988).

Neurotoxic dosing regimens of MDMA can also cause both short- and long-term behavioral changes. For example, we and others have shown reduced activity in MDMA-treated rats that begins the day after dosing and that persists for at least several more days thereafter (Piper *et al*, 2006; Timár *et al*, 2003; Wallace *et al*, 2001). Longer-term effects associated with MDMA neurotoxicity have been studied extensively by McGregor and his colleagues. These investigators have identified a constellation of changes, which they refer to as the MDMA syndrome, that includes a reduction in cognitive function in the object recognition test, increased anxiety-like behavior in the emergence test, and decreased social behavior in the social interaction test (Gurtman *et al*, 2002; McGregor *et al*, 2003a, b; Morley *et al*, 2001, 2004). The same group also determined that depressive-like behaviors were augmented following MDMA (McGregor *et al*, 2003b; Thompson *et al*, 2004). Other measures of complex behavior have yielded variable results with respect to the influence of MDMA treatment. For example, some, but not all, studies have found that anxiety-like behavior in the elevated plus-maze is sensitive to MDMA (Ho *et al*, 2004; Mechan *et al*, 2002; Piper and Meyer, 2004; Piper *et al*, 2005; Sumnall *et al*, 2004).

Because MDMA is often described as a selective 5-HT neurotoxin (Cole and Sumnall, 2003; McCann and Ricaurte, 2004) that also produces long-lasting behavioral changes, investigators have commonly assumed that the 5-HT toxicity mediates such behavioral alterations. This hypothesis was tested in the present study. Our objectives were to determine whether (1) access to SERT is necessary for MDMA-induced neurotoxicity using a more selective SSRI than in most previous studies, (2) the acute physiological responses to MDMA are inhibited by SSRI pretreatment, and (3) later behavioral alterations produced by MDMA are similarly prevented by SERT blockade. These objectives were accomplished by testing the effects of pretreatment with citalopram, an SSRI with a limited inhibitory effect on liver cytochrome P450 enzymes (Hemeryck and Belpaire, 2002), on the physiological, behavioral, and neurochemical responses of MDMA-exposed rats measured over a 10-week period.

MATERIALS AND METHODS

Animals and Drug Treatments

Young adult, male Sprague–Dawley rats, 307.7 ± 3.4 g (Charles River Laboratories, Wilmington, MA) were pair-housed in plastic tubs on a 10:14 reversed light–dark cycle (lights on 2000 h and off at 0600 h), with all experimental procedures being conducted during the dark phase of the cycle. The colony room temperature was maintained at $23 \pm 1^{\circ}$C. Solutions of R,S-citalopram HBr (Sigma, St Louis, MO) and (±)MDMA HCl (Research Triangle Institute Research Triangle Park, NC) were prepared fresh in sterile 0.9% NaCl in a volume of 10 mg/ml (based on the weight of the salt) and injected s.c. One hour following pretreatment with citalopram (10 mg/kg) or saline, four doses of MDMA (10 mg/kg per dose) or saline were administered at hourly intervals. These pretreatment and treatment conditions resulted in four groups: SAL/SAL, CITAL/SAL, SAL/MDMA, and CITAL/MDMA. The selected dose of citalopram completely blocks MDMA-induced tail-flicks in rats (Millan and Colpaert, 1991) and is also within the reported range of ED_{50} values for the drug's efficacy in the forced-swim test and in potentiation of 5-hydroxytryptophan-induced behaviors (Sánchez *et al*, 2003). Based on these functional considerations as well as the drug's affinity for SERT, we expected the citalopram pretreatment to produce complete or near-complete SERT blockade. The MDMA treatment regimen used in the present study is well known to produce severe, long-term depletions in serotonergic markers such as 5-HT levels and SERT binding (Green *et al*, 2003). However, it is important to note that these high doses of MDMA also cause an acute release of dopamine (DA) and norepinephrine (Green *et al*, 2003; Kankaanpaa *et al*, 1998). The location of the injection site was varied to limit MDMA-related irritation, although skin lesions and alopecia still occurred in some animals.

The total number of animals ($N = 67$ or 15–18/group) was obtained in three replications with 20–24 rats per replication. All rats provided data on body weight changes and seminal discharge in response to the drug treatments. A subset of the total ($N = 23$ or 5–6/group) was used in a short-term study in which the rats were tested for post-treatment locomotor activity and were killed 1 week following dosing for tissue collection and neurochemical analyses. The remaining rats ($N = 44$ or 10–12/group) took part in a long-term study in which the animals were subjected to behavioral testing over a 10-week period after which they were also killed for tissue collection. This interval was selected based on the observation that although MDMA-treated rats exhibit measurable recovery of serotonergic markers during this period (Battaglia *et al*, 1988), some behavioral changes appear to be more persistent (McGregor *et al*, 2003b; Morley *et al*, 2001). All animal procedures were approved by the University of Massachusetts-Amherst Animal Care and Use Committee and were consistent with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

Physiological Measures

Short-term changes in core temperature, penile responses, and body weight were monitored to determine if citalopram pretreatment modified these responses to MDMA.

Core temperature. Colonic temperature during dosing was monitored by means of a digital thermometer (Thermalert TH-5, Physitemp Instruments, Clifton, NJ) connected to a rectal probe (RET-2, Physitemp) that was warmed to body temperature before insertion and lubricated with mineral oil. The rat was lightly restrained and the probe was inserted 4.5 cm for approximately 3 s to obtain a stable reading. Initial temperatures were recorded 30 min before the

citalopram pretreatment to habituate the animals to this procedure. Readings were also obtained immediately before the pretreatment (time −60 min), before the first MDMA treatment (time 0), and at 30, 60, 90, 120, 150, 180, 210, 240, and 270 min thereafter. Owing to an equipment malfunction, data from half of the subjects in one replication were unavailable, thereby reducing the *N*/group to 13–15. Subjects that became hyperthermic and had core temperatures that exceeded 40.5°C were cooled to prevent lethality (for details see Piper *et al*, 2006). As we previously found using the same procedures, this intervention does not have a significant effect on the serotonergic neurotoxicity produced by MDMA binge treatment (Piper *et al*, 2006).

Ejaculation. Because MDMA administration induces spontaneous ejaculation in rats (Bilsky *et al*, 1991; Piper *et al*, 2005, 2006), whereas citalopram and other SSRIs inhibit sexual responses (Rosen *et al*, 1999), the genitalia of the animals were inspected every 30 min following dosing and the presence of seminal discharge was noted. We determined the proportion of animals in each treatment group that ejaculated and, within this subset, we calculated the latency from first MDMA dose to the production of a seminal plug.

Weight. Drug-related weight changes were anticipated due to the inhibitory effects of both MDMA and citalopram on food intake (Frith *et al*, 1987; Grignaschi *et al*, 1998) as well as MDMA stimulation of urination (Bilsky *et al*, 1991). Therefore, all groups were matched for body weight before dosing, and weight was monitored 2 h after drug treatments and every other day thereafter for 6 days. Data are expressed as the change in weight after drug administration at each interval.

Behavioral Measures

Beginning on the day after dosing, animals were tested on a battery of functional assessments to determine the effects of MDMA with and without citalopram pretreatment on motor activity (activity chambers), anxiety-like behavior (emergence test, open field, and elevated plus-maze), working memory (object-recognition test), exploratory behavior (hole-board), and social behavior (social-interaction test). Each animal completed only one behavioral test on a given day. The testing room was illuminated by standard fluorescent lighting (two 48-inch, 34-W bulbs) and was equipped with a white-noise generator to mask ambient sounds. The number of days between dosing and behavioral testing is shown in parentheses for each test. Finally, the sequence of tests was similar to that used in previous work in our laboratory (Piper and Meyer, 2004; Piper *et al*, 2005) and was chosen to both maximize the detection of treatment effects and minimize carryover effects from one test to another.

Motor activity (1 or 56–58 days). Human Ecstasy users report that they experience 'mid week blues' characterized by a loss of energy and fatigue after weekend use (Parrott, 2002). We and others have shown a similar reduction in activity in rats tested 1 or 3 days after a neurotoxic dosing regimen of MDMA (Piper *et al*, 2006; Timár *et al*, 2003). To determine whether citalopram pretreatment would prevent this effect of MDMA, animals in the short-term study were tested for their activity 1 day following treatment. Testing was carried out in four ENV-510 activity chambers with internal dimensions of 27.5 × 27.5 × 20.5 cm (Med Associates, St Albans, VT). Each chamber was illuminated by a 28-V bulb and had a fan running during testing to limit background noise. Two sets of photobeam strips located at floor level determined horizontal activity, and a third set was elevated 13.5 cm above the floor to record vertical activity. Dependent measures were the distance traveled as well as rearing frequency and duration during a 10-min period. For the animals in the long-term study, motor activity was assessed on days 56–58. Note that here and in all instances below where a range of days is given, each animal was tested just once within that period. The rats remained in the activity chambers for 1 h to facilitate habituation to this environment as these chambers were also used for the hole-board test described below. However, only the first 10 min are reported to provide a comparison with the data obtained from the day-1 test.

Emergence test (7–9 days). This test was used to determine the effects of MDMA with and without citalopram on anxiety-like behavior (McGregor *et al*, 2003b; Piper *et al*, 2005). The apparatus consisted of a wooden hide box (24 × 40 × 15 cm), painted flat black with a hinged lid. An 8-cm opening at the end of the hide box allowed the rat to exit or enter the box freely. The hide box was placed in the corner of an open-field (described below). Animals were videotaped and the latency until first hide box emergence, frequency of emergences, and duration in the hide box during a 5-min test were scored from the tapes. Videotapes were coded by a rater who was unaware of the drug treatment of each rat.

Open-field (9–11 days). The open-field floor measured 60 × 60 cm and was constructed of wood and painted black. The floor was divided into nine 20 × 20 cm squares by 2-cm-thick lines. The rat was gently placed along the perimeter of the open-field and allowed to ambulate for 10 min while it was videotaped. An observer later recorded the number of entries into the peripheral or center squares, with center entries interpreted as an index of less anxiety (Prutt and Belzung, 2003). An entry was defined as placement of at least two paws into the designated square.

Object-recognition test (15–17 and 17–19 days). These tests were conducted in the aforementioned open-field to ensure habituation to the testing environment (Piper *et al*, 2005). The object-recognition test of working memory was conducted with two levels of difficulty. A relatively short (15-min) interval separated the sample and test periods when the test was conducted at 15–17 days after drug treatments, whereas a longer (60-min) interval was employed on days 17–19. For the initial object sampling period, two identical objects were situated in the corners of the open-field, 10 cm from the nearest walls. The rat was placed into the arena facing the center of the east wall and allowed to explore the objects for 3 min. At the end of the

sampling period, the rat was removed from the open-field and placed in an empty cage containing fresh pine shavings in a dark, quiet room. After the specified interval, the rat was returned to the open-field for a 3-min test period during which the open-field contained a third copy of the previously encountered familiar object as well as a novel object. A glass microscope slide holder ($9.7 \times 7.2 \times 4.5$ cm high) and a 355 ml soda can (Cherry Coke®) served as experimental objects for the 15-min interval. A yellow plastic electrical relay ($6.3 \times 3.0 \times 3.0$ cm; Knight Relays, Chicago, IL) and a round red plastic ashtray (4.3 high with a 10.1 cm diameter) served as objects for the 60-min interval. The novel object position and identity were counterbalanced across all subjects. Exploration was coded from videotapes to the nearest 0.1 s when the animal's nose was within 2 cm of the object. If the sample object exploration was <5.0 s, then that animal's data were excluded based on a lack of adequate experience for memory formation. This criterion resulted in the exclusion of two CITAL/SAL cases for the 15-min test and two additional cases (one CITAL/SAL and one SAL/SAL) for the 60-min test. Memory of the familiar object was indexed by the discrimination ratio, which is defined as the ratio of duration of exploration of the novel object divided by the total exploration duration of both objects during the test period. A discrimination ratio significantly greater than 0.5 is interpreted to indicate memory of the original (familiar) object. Attentional behavior in this test may also be reflected in the total duration of object exploration (Piper *et al*, 2005). A more complete description of the object-recognition test, including the habituation to the test environment, can be found in Piper and Meyer (2004).

Social-interaction test (26–30 days). This assessment was carried out under white light in a familiar setting for the rats (the open-field) to produce an intermediate baseline level of anxiety (File and Hyde, 1978). Two rats that were from the same treatment condition, but were not cagemates were placed together into the open-field. Social interactions were videotaped during a 5-min test session and were later scored for the duration of sniffing, grooming, adjacent lying, following, crawling over/under, and other behaviors in which the animals were within 3 cm of each other. Aggressive interactions were also of interest due to the depleting effects of MDMA on forebrain 5-HT levels and the generally inverse relationship between serotonergic activity and aggression (Renfrew, 1996). Consequently, we determined the proportion of animals in each group that exhibited aggression, the latency to first aggressive behavior, and the duration and frequency of aggressive behaviors. Aggression was defined as biting, boxing or wrestling. Pairs were closely matched for body weight to minimize the influence of this factor on social and aggressive behaviors. As both members of the pair provide data in the social-interaction paradigm, each rat was tested twice on two consecutive days with different partners. The average score of the two tests was used in all analyses (see Thompson *et al*, 2004).

Hole-board test (59 days). For this test, a steel floor insert containing 16 holes (each 2.3 cm diameter and 2.5 cm deep) arranged in a 4×4 pattern was added to the previously described Med Associates activity chambers. To increase the spatial distinctiveness of the hole-board environment, images of geometric figures were placed on the outside of the Plexiglas walls of the chambers. Animals were tested for 10 min, during which the Med Associates software automatically determined three measures of behavior: novel hole entries out of a possible total of 16 (a measure of exploratory efficiency), re-entries into previously entered holes, and total hole entries.

Elevated plus-maze (66 days). The apparatus and testing environment were the same as described in Piper and Meyer (2004). Briefly, the plus-maze was constructed of gray plastic and consisted of four $10 \times 50 \times 50$ cm arms with a 10×10 cm center area, elevated 50 cm from the floor. Animals were videotaped for 10 min and the tapes were later scored for the duration and frequency of open- and closed-arm entries, and the latency until first open-arm entry.

Neurochemical Measures (7 or 67 days)

The hippocampus and parietal cortex were collected at either 7 or 67 days after drug treatments for neurochemical analysis. For simplicity, these times will subsequently be referred to as 1 and 10 weeks. Tissues were frozen on dry ice and stored at -70°C until assay. Samples from both the 1- and 10-week cohorts were analyzed for [^{3}H]citalopram binding to SERT according to the methods of Piper *et al* (2005). Briefly, samples were homogenized in 40 vols of ice-cold buffer, and washed membrane fractions were prepared by repeated centrifugation and resuspension. Tissue homogenates were placed in a dry bath at 30°C for 20 min between the second and third centrifugation to dissociate any 5-HT or residual citalopram from the SERT. Membranes were assayed in triplicate using a 1.0 nM concentration of [^{3}H]citalopram (84.2 Ci/mmol, New England Nuclear) with 10 μM unlabeled fluoxetine to determine nonspecific binding. For the 1-week cohort, samples of cortex and hippocampus collected from the other cerebral hemisphere were analyzed for 5-HT and 5-HIAA concentrations by high-performance liquid chromatography with electrochemical detection (Ali *et al*, 1994).

Data Analysis

Statistical analyses were conducted using Systat, version 10.2 (Systat Software, Richmond, CA). The total thermal response to drug treatments was quantified by determining the area under the curve (AUC) of colonic temperature values collected 0–270 min after the first MDMA dose. AUC values were calculated by PKCALC version 1.0 software (Shumaker, 1986) using the linear-trapezoid rule. Differences between conditions on ratio-level variables (ie motor activity, memory, and neurochemistry) were determined with 2×2 analyses of variance (ANOVA) with citalopram pretreatment and MDMA treatment as between-group variables. Mixed ANOVAs were conducted on repeated-measure variables. To account for inter-assay variation in the 1- *vs* 10-week cohorts, SERT binding data were

expressed and analyzed as a percent of the appropriate SAL/SAL control group. Outlier cases, identified by Systat based on excessive studentized residuals, were removed from all parametric analyses. Mean, SEM, and α levels ($p<0.05$, 0.01, or 0.001) are listed in all tables; however, due to the large number of behavioral tests performed, we only present detailed statistical information (ie F, t, and df values) for those findings most relevant to the experimental objectives. Pearson product–moment correlations were used to determine the degree of association between regional SERT binding and 5-HT or 5-HIAA concentrations. For the aggressive behavior results, both mean and median values are presented because the results were not normally distributed. Consequently, the latency and duration results for aggression were analyzed by means of Mann–Whitney *U*-tests comparing the different treatment conditions. Finally, nominal-level variables (ie presence or absence of ejaculation or fighting) were analyzed with a χ^2 test.

RESULTS

Physiological Measures

Core temperature. MDMA administration induced a hyperthermic response independent of citalopram pretreatment. A mixed ($2\times2\times10$) ANOVA with MDMA treatment and citalopram pretreatment as between-group factors and time from 0 to 270 min as a repeated measure yielded significant main effects of MDMA ($F(1,50)=71.49$, $p<0.001$) and time ($F(9,450)=23.90$, $p<0.001$) as well as an MDMA $\times$ time interaction ($F(9,450)=17.73$, $p<0.001$). Simple effect ANOVAs conducted at each time interval revealed a significant MDMA effect at all intervals from 60 to 270 min (Figure 1a). AUC values (expressed as °C h) for the SAL/SAL, CITAL/SAL, SAL/MDMA, and CITAL/MDMA groups were 171.88 ± 0.27, 171.27 ± 0.31, 177.08 ± 0.73, and 177.41 ± 0.76 (mean $\pm$ SEM) respectively. A 2×2 ANOVA on these data showed a significant main effect of MDMA administration ($F(1,50)=94.98$, $p<0.001$) but no effect of

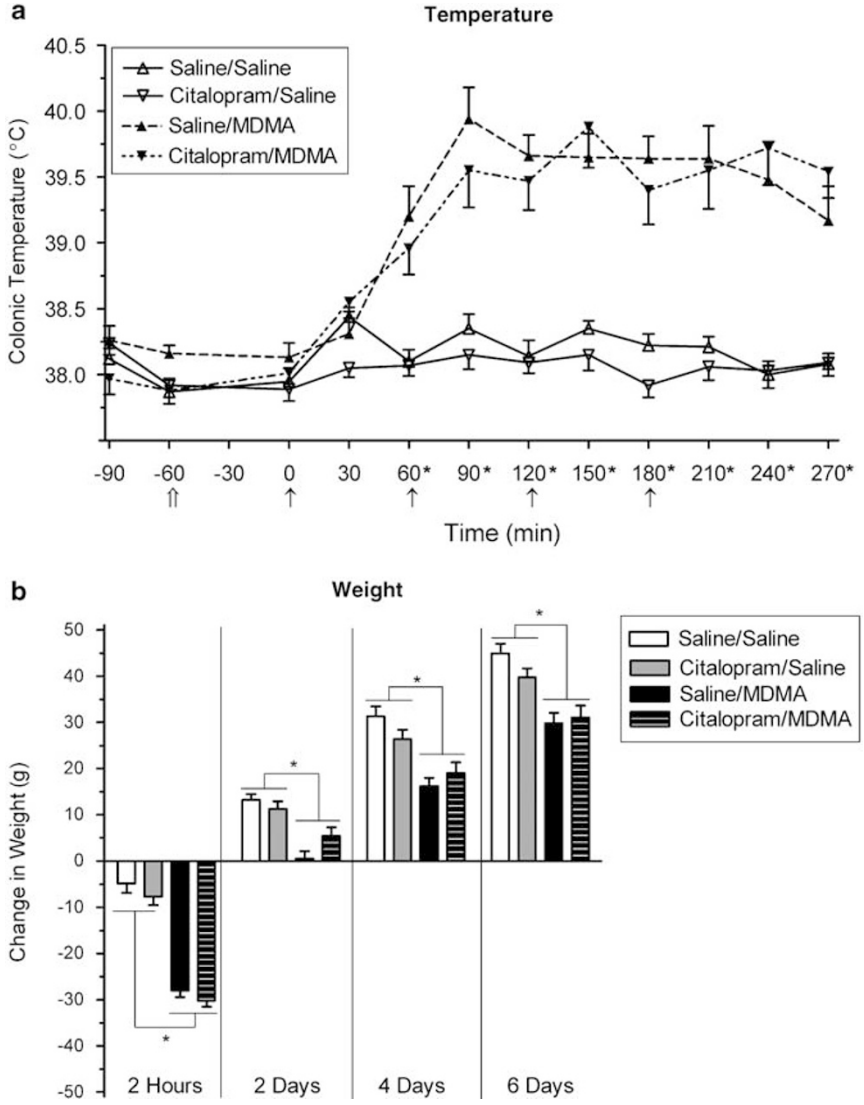

Figure 1 Acute and short-term physiological responses following MDMA with or without citalopram pretreatment. (a) Colonic temperature (°C) over time. Wide arrow designates time of citalopram (10 mg/kg) pretreatment and narrow arrows designate times of MDMA (10 mg/kg $\times$ 4) treatments. (b) Change in body weight (in g) 2 h or 2, 4, or 6 days after drug administration. *$p<0.001$ ANOVA main effect of MDMA.

citalopram pretreatment. A similar percentage of the SAL/MDMA and CITAL/MDMA groups required cooling due to excessive hyperthermia (65.3 *vs* 53.3% respectively).

Ejaculation. Seminal discharge was observed in 66.7% (12/18) of animals in the SAL/MDMA group, 44.4% (8/18) of animals in the CITAL/MDMA group, but none of the animals (0/31) that did not receive MDMA (SAL/SAL and CITAL/SAL groups). χ^2 tests showed that MDMA treatment increased the incidence of seminal discharge ($\chi^2(1) = 24.55$, $p < 0.001$) but that citalopram pretreatment did not significantly alter this effect of MDMA ($\chi^2(1) = 1.80$, $p = 0.18$). However, citalopram did significantly delay the mean latency from the first MDMA injection to ejaculation (152.4 ± 26.2 *vs* 240.0 ± 21.3 min; mean $\pm$ SEM for the SAL/MDMA and CITAL/MDMA groups respectively) ($t(18) = 2.37$, $p < 0.05$).

Body weight. Figure 1b presents body weight changes at 2 h, 2, 4, and 6 days post-treatment as a consequence of MDMA administration with and without citalopram pretreatment. A mixed ($2 \times 2 \times 4$) ANOVA revealed significant main effects of MDMA ($F(1,63) = 84.01$, $p < 0.001$) and time ($F(3,189) = 1109.0$, $p < 0.001$), and a trend for the MDMA $\times$ citalopram interaction ($F(1,63) = 3.05$, $p = 0.086$). ANOVAs conducted at each time point identified a significant MDMA effect at all points indicating that MDMA caused a persistent weight reduction that was not significantly affected by citalopram.

Behavioral Measures

Motor activity. Measures of horizontal and vertical activity at 1 day after drug treatments (short-term study) are shown in Figure 2d (distance traveled) and Table 1 (rearing duration and frequency). At this time point, MDMA-treated rats displayed substantial hypoactivity as reflected by substantial reductions in all three measures of activity. These effects were not altered by citalopram pretreatment. In contrast, there was relatively little influence of MDMA exposure on motor activity at the 56–58-day time point (long-term study) except for a modest trend towards a reduction in rearing frequency (Table 1).

Emergence test. There were no effects of either MDMA or citalopram on any measures of anxiety in the emergence test conducted on days 7–9 (data not shown).

Open field. In the open-field testing carried out on days 8–10, there was a significant reduction in overall number of grid crossings over time as the animals habituated to the test environment. This habituation was unaffected by either MDMA or citalopram administration. Drug treatments similarly had no effect on the total number of grid crossings (activity) summed across the entire 10-min test period. However, the CITAL/SAL group made more entries into the center area relative to the SAL/SAL or CITAL/MDMA groups (data not shown), suggesting less anxiety under the present test conditions.

Object-recognition test. Behavior in the object-recognition test was assessed in two ways. We analyzed the total duration of object exploration, to determine if attention was altered by the drug treatments. This measure has previously been shown to be sensitive to MDMA exposure in adolescent rats (Piper *et al*, 2005). In the present study, MDMA treatment significantly reduced the total duration of object exploration in the 60-min test ($F(1,36) = 4.17$, $p < 0.05$, Figure 2e) but not the 15-min test ($F(1,37) = 2.29$, NS). Citalopram pretreatment did not prevent the effect of MDMA at the longer delay period. The second analysis examined the discrimination ratios to determine if working memory was impaired by MDMA. There were no significant differences in total discrimination ratios over all 3 min after

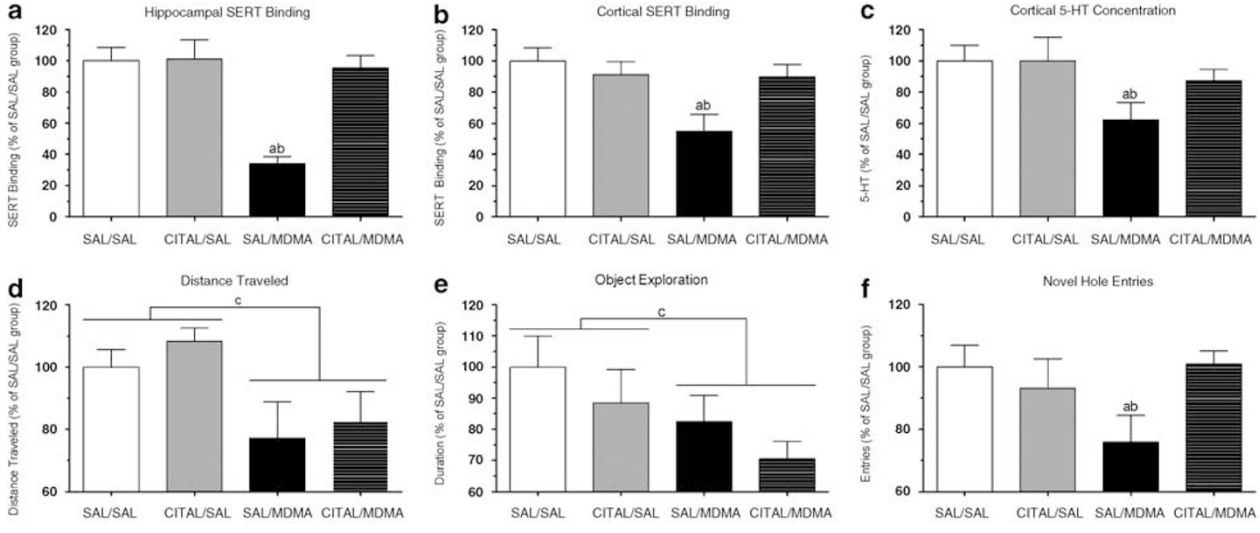

Figure 2 Neurochemical and behavioral effects of MDMA with or without citalopram pretreatment. (a) SERT binding in the hippocampus [7], (b) SERT binding in the parietal cortex [7], (c) cortical 5-HT concentrations [7], (d) distance traveled in the activity chamber [1], (e) total duration of object exploration during the object-recognition test with a 60-min delay [19], and (f) novel hole entries in the hole-board test [58]. Numbers in brackets represent the number of days between drug treatment and data collection. All data are expressed as a percentage of the SAL/SAL group. $^{a}p < 0.05$ *vs* SAL/SAL, $^{b}p < 0.05$ *vs* CITAL/MDMA, $^{c}p < 0.05$ ANOVA main effect of MDMA.

Table 1 Behavioral Assessments Conducted at Varying Time Intervals Following MDMA Administration with or without Citalopram Pretreatment

	Group				ANOVA *p*-value[#]		
	SAL/SAL	**CITAL/SAL**	**SAL/MDMA**	**CITAL/MDMA**	**MDMA**	**Citalopram**	**MDMA × Citalopram**
Motor activity (1)							
Rearing duration (s)	183.7 (15.1)	178.7 (8.7)	100.0 (14.0)[aa]	142.4 (19.8)	$p<0.01$		
Rearing frequency	72.2 (6.0)	65.2 (3.1)	53.3 (10.0)	53.3 (7.7)	$p=0.054$		
Motor activity (56–58)							
Distance traveled (cm)	2896.7 (228.2)	2930.6 (372.0)	3047.8 (209.3)	2694.3 (178.5)			
Rearing duration (s)	199.3 (14.0)	182.6 (15.1)	204.3 (12.5)	200.4 (16.9)			
Rearing frequency	85.9 (7.8)	81.9 (7.0)	71.8 (5.9)	72.3 (5.1)			
Object-recognition, 15-min interval (17)							
Object exploration (s)							
Sample	39.7 (4.1)	46.2 (6.0)	38.4 (2.9)	36.2 (2.7)			
Test	49.5 (2.2)	43.9 (3.4)	41.0 (5.1)	44.2 (5.4)			
Total	89.1 (5.0)	90.1 (7.8)	79.4 (7.1)	79.5 (6.3)			
Discrimination ratio							
1st minute	0.68 (0.07)	0.65 (0.06)	0.76 (0.06)	0.70 (0.05)			
2nd minute	0.65 (0.07)	0.53 (0.05)	0.50 (0.05)	0.60 (0.06)			
3rd minute	0.51 (0.08)	0.58 (0.08)	0.56 (0.07)	0.51 (0.08)			
Total	0.65 (0.06)	0.61 (0.05)	0.63 (0.04)	0.63 (0.02)			
Object-recognition, 60-min interval (19)							
Object exploration (s)							
Sample	41.1 (5.6)	35.5 (5.0)	33.5 (4.0)	27.2 (2.8)[a]			
Test	41.7 (3.8)	36.0 (3.7)	33.1 (3.9)	29.7 (3.8)[a]			
Total	80.7 (8.0)	71.4 (8.6)	66.6 (6.8)	57.0 (4.4)[a]	$p<0.05$		
Discrimination ratio							
1st minute	0.69 (0.06)	0.68 (0.16)	0.74 (0.05)	0.77 (0.03)			
2nd minute	0.71 (0.06)	0.55 (0.08)	0.48 (0.09)[a]	0.48 (0.09)	$p<0.05$*		
3rd minute	0.61 (0.11)	0.58 (0.09)	0.48 (0.06)	0.60 (0.10)			
Total	0.69 (0.05)	0.59 (0.04)	0.59 (0.04)	0.65 (0.04)			
Social-interaction (28–30)							
SI duration total (s)	176.4 (8.6)	160.7 (11.0)	186.3 (4.7)	178.2 (8.2)			
SI duration minus aggression	153.0 (12.1)	142.4 (12.5)	184.6 (5.6)[a,bb]	155.6 (6.9)	$p<0.05$	$p<0.05$	
Hole-board (57–59)							
Total hole entries	27.5 (4.1)	17.1 (3.3)[b]	17.1 (3.4)[b]	29.5 (3.0)			$p<0.01$
Repeat hole entries	15.9 (3.3)	7.9 (2.2)[b]	9.0 (3.1)[b]	18.0 (2.8)			$p<0.01$

Data shown represent the mean ± SEM for each condition. The number(s) in parentheses after the name of each behavioral test is the number of days between the drug treatments and that test. $N=10$–12/group except for motor activity (1), which had an $N=5$–6/group.
[a]$p<0.05$.
[aa]$p<0.01$ respectively *vs* SAL/SAL.
[b]$p<0.05$.
[bb]$p<0.01$ respectively *vs* CITAL/MDMA.
[#]A blank in these columns indicates $p>0.06$.
* Repeated measures ANOVA.

Table 2 Aggressive Behavior during the Social-Interaction Test as a Function of Drug Treatments

	SAL/SAL	CITAL/SAL	SAL/MDMA	CITAL/MDMA
Percentage of pairs showing aggression	40.0	40.0	16.6*	66.6
Aggression duration (s; mean/median)	23.5 (0)	18.3 (0)	2.9* (0)	22.5 (9.5)
Latency until aggression (s; mean/median)	201.8 (300)	232.9 (300)	261.0* (300)	204.8 (222.6)
Aggression frequency (mean/median)	1.9 (0)	1.0 (0)	0.7* (0)	1.4 (1.0)

*$p<0.05$ *versus* CITAL/MDMA.

either the 15 or 60-min delay (Table 1). However, additional information was obtained by examining the discrimination ratios across each minute of the test period. First, a mixed ($2\times2\times2$) ANOVA revealed a main effect of time for both the 15-min ($F(1,37)=14.81$, $p<0.001$) and 60-min ($F(1,37)=10.53$, $p<0.01$) delay tests. This effect consisted of an overall decrease in discrimination ratio across time, presumably occurring as the new object lost its novelty to the animals. Importantly, however, there was also a significant MDMA × time interaction ($F(1,37)=5.07$, $p<0.05$) under the 60-min delay condition indicating that the discrimination ratios were similar between the groups in the first minute, but that MDMA treatment reduced the second minute discrimination ratio compared to the non-MDMA-exposed animals. Taken together with the object exploration results, these findings indicate that MDMA produced subtle alterations in attention and memory performance that were not prevented by citalopram pretreatment.

Social-interaction test. The total duration of social interaction over the 5-min session was not altered by MDMA (Table 1). However, aggressive behavior during the social-interaction tests varied as a function of previous drug treatment. Forty percent (4/10) of both the SAL/SAL and CITAL/SAL pairs exhibited aggression; therefore, these two groups were collapsed into a single control group for statistical analysis. A χ^2 analysis examining the occurrence of fighting among the resulting three groups (control, SAL/MDMA, and CITAL/MDMA) revealed a significant group difference ($\chi^2(2)=6.22$, $p<0.05$). Table 2 shows the percent pairs exhibiting aggression as well as other indicators of agonistic behavior including the latency to first aggressive encounter and the overall frequency and duration of fighting. The results reveal that the SAL/MDMA animals exhibited reduced aggressive behavior compared to both the control and the CITAL/MDMA animals, although only the comparisons between the SAL/MDMA and the CITAL/MDMA groups reached statistical significance (but note that for aggression duration, the difference between the SAL/MDMA group and the control was extremely close to significance (Mann–Whitney $U=157.0$, $p=0.057$)). Thus, prior MDMA exposure generally decreased aggressive behavior in the social interaction test, and citalopram pretreatment protected against this decrease. Owing to these group differences in aggressive behavior, the total duration of social interaction was recalculated with the duration of aggression removed. This analysis of the total duration of non-aggressive social interaction revealed main effects of MDMA and of citalopram pretreatment, but no MDMA × citalopram interaction (Table 1).

Hole-board test. Behaviors in the hole-board test are shown in Table 1 (total and repeat hole entries) and Figure 2f (novel hole entries). A 2×2 ANOVA on these indices of exploratory behavior identified a significant MDMA × citalopram interaction for all three measures. In the case of novel hole entries, the SAL/MDMA group made significantly fewer entries than the SAL/SAL group, and this effect was blocked by citalopram pretreatment. For both total and repeat hole entries, an unusual pattern emerged in which both the SAL/MDMA and CITAL/SAL groups made fewer entries than either of the other two treatment groups.

Elevated plus-maze. There were no statistically significant group differences in any behaviors in the elevated plus-maze (data not shown).

Neurochemical Measures

SERT binding and 5-HT levels at 1 week. Table 3 shows that the SAL/MDMA animals exhibited significantly less cortical and hippocampal SERT binding as well as lower 5-HT and 5-HIAA levels than the SAL/SAL group. Moreover, citalopram pretreatment provided the anticipated protection against these neurotoxic effects of MDMA (see also Figure 2a–c). Pearson product–moment correlations between SERT, 5-HT, and 5-HIAA were conducted to determine the degree of similarity between pairs of neurochemical measures both within and across brain areas (Table 4). The binding of [^{3}H]citalopram in the cortex was strongly related to hippocampal binding ($p<0.001$). Also, as expected, 5-HT and 5-HIAA associations were quite high ($r=0.70$–0.84, $p<0.001$). SERT and 5-HT quantities showed significant correlations in both regions ($r=0.49$–0.60, $p<0.05$), and SERT was also positively related to 5-HIAA levels in the hippocampus ($p<0.05$).

SERT binding at 10 weeks. MDMA, independent of citalopram pretreatment, caused an enduring depletion in SERT binding at 10 weeks after drug treatments (Table 3). The SAL/MDMA group exhibited significantly less SERT binding in the hippocampus than the SAL/SAL group. Unexpectedly, SERT binding in this area was also significantly reduced in the CITAL/MDMA group relative to the SAL/SAL controls. Finally, cortical SERT binding was reduced in the CITAL/SAL and the CITAL/MDMA animals compared to the controls.

Table 3 Regional SERT, 5-HT, and 5-HIAA Values Expressed as a Percentage of the SAL/SAL Control Group at 1 or 10 Weeks Following MDMA Administration with or without Citalopram Pretreatment

	GROUP			
	SAL/SAL	**CITAL/SAL**	**SAL/MDMA**	**CITAL/MDMA**
1 week (N = 5–6/group)				
Hippocampus				
SERT	100.0 (8.5)	101.1 (12.5)	34.1 (4.3)[a,b]	95.4 (7.8)
5-HT	100.0 (9.7)	81.6 (12.0)	63.7 (5.0)[a,b]	87.0 (10.6)
5-HIAA	100.0 (9.1)	87.6 (9.5)	66.8 (3.2)[a]	65.9 (15.8)
Cortex				
SERT	100.0 (8.4)	90.9 (8.6)	55.0 (10.7)[a,b]	89.9 (7.9)
5-HT	100.0 (9.9)	99.8 (15.1)	61.9 (10.8)[a,b]	87.4 (7.5)
5-HIAA	100.0 (7.1)	86.0 (12.3)	65.0 (6.7)[a,b]	81.8 (5.5)
10 weeks (N = 10–12/group)				
Hippocampus				
SERT	100.0 (9.2)	101.2 (5.7)	68.0 (7.2)[a]	71.2 (8.6)[a]
Cortex				
SERT	100.0 (7.6)	80.2 (5.2)[a]	80.4 (11.1)	76.2 (8.4)[a]

Data are expressed as mean ± SEM. The mean control (SAL/SAL) values for SERT, 5-HT, and 5-HIAA at the 1-week time point were 186.0 fmol/mg protein, 16.6 ng/mg tissue, and 15.3 ng/mg tissue respectively for the hippocampus, and 219.2 fmol/mg protein, 19.0 ng/mg tissue, 16.4 ng/mg tissue respectively for the cortex. The mean SERT values at the 10-week time point were 311.0 and 298.3 fmol/mg protein in the hippocampus and cortex respectively.
[a]$p \leq 0.05$ *vs* SAL/SAL.
[b]$p < 0.05$ *vs* CITAL/MDMA.

Table 4 Correlation Matrix Relating the Neurochemical Measures Obtained 1 Week Following MDMA Administration with or without Citalopram Pretreatment

	Hippocampus			Cortex		
	SERT	**5-HT**	**5-HIAA**	**SERT**	**5-HT**	**5-HIAA**
Hippocampus						
SERT	1.00					
5-HT	0.60**	1.00				
5-HIAA	0.44*	0.70***	1.00			
Cortex						
SERT	0.77***	0.65**	0.44*	1.00		
5-HT	0.57**	0.33	0.37	0.49*	1.00	
5-HIAA	0.45*	0.35*	0.50*	0.38	0.84***	1.00

*$p < 0.05$, **$p < 0.01$, ***$p \leq 0.001$.

Table 5 Summary of MDMA Effects and the Influence of Citalopram Pretreatment

	SAL/MDMA	**CITAL/MDMA**
Short-term neurotoxicity		
SERT binding	Reduced	No change
5-HT levels	Reduced	No change
Acute effects		
Core temperature	Hyperthermia	Hyperthermia
Ejaculation	Present	Present/delayed
Body weight	Reduced	Reduced
Behavior		
Hangover	Present	Present
Object exploration	Reduced	Reduced
Aggression	Reduced	No Change
Hole-board exploration	Reduced	No change

The outcome measure for each group (SAL/MDMA and CITAL/MDMA) is compared against the SAL/SAL control condition.

DISCUSSION

The main objective of this study was to determine whether protection against MDMA-induced serotonergic neurotoxicity by pretreatment with the SSRI citalopram would prevent either the acute or long-term behavioral and physiological effects of MDMA. As summarized in Table 5, some effects of MDMA were blocked by citalopram while others were not. We will first discuss the neurochemical results followed by the physiological and behavioral findings.

In the brain samples obtained 1 week after drug administration, we measured both SERT binding and 5-HT and 5-HIAA concentrations in the hippocampus and parietal cortex. Correlational analyses performed on that entire dataset (ie collapsed across all treatment groups) showed a reasonable agreement among these different indices of serotonergic system integrity. As expected, MDMA treatment caused significant reductions in all neurochemical measures in both brain areas at the 1-week time point, and pretreatment with citalopram almost completely prevented these reductions. SERT levels showed some degree of recovery between 1 and 10 weeks after dosing, which is consistent with the previous findings of Battaglia *et al* (1988). However, in contrast to the 1-week results, the CITAL/MDMA group unexpectedly did not differ from the SAL/MDMA group with respect to SERT binding measured at 10 weeks post-treatment. Furthermore, compared to the SAL/SAL controls, the CITAL/SAL group showed a similar reduction in SERT binding as the two MDMA-treated groups at the 10-week time point. A reduction in radioligand binding to SERT following high-dose MDMA administration is commonly interpreted to be a sign of damage to serotonergic axons. This interpretation is based, in part, on immunohistochemical studies showing long-lasting MDMA-induced decreases in serotonergic fiber density in both rats and monkeys (see Introduction). Chronic antidepressant treatments can also result in decreased SERT binding, but this reduction is thought to reflect a downregulation of SERT expression (Benmansour

et al, 1999; Hirano *et al*, 2005). It is important to acknowledge that debate continues in the literature regarding the interpretation of MDMA-induced changes in SERT (measured by radioligand binding *vs* immunoblotting (see Wang *et al*, 2004, 2005; Xie *et al*, 2006)) as well as other putative measures of serotonergic neurotoxicity such as reduced levels of 5-HT and 5-HIAA (O'Callaghan and Miller, 1993). In the present study, the significant difference in SERT binding between the CITAL/SAL and the SAL/SAL animals complicates interpretation of the 10-week neurochemical data. Nevertheless, if we focus on the 1-week data instead, citalopram pretreatment did protect the animals against MDMA-induced serotonergic deficits (whether interpreted as axonal damage or not), and this finding allows us to draw some important inferences from the accompanying functional results.

Citalopram pretreatment failed to alter either MDMA-induced hyperthermia or the loss of body weight measured 2 h post-treatment in the SAL/MDMA group. The lack of influence of citalopram on MDMA's thermic effects has been reported previously for other SSRIs (Malberg *et al*, 1996; Sanchez *et al*, 2001) and indicates that non-serotonergic mechanisms, most likely dopaminergic (Green *et al*, 2004), play a key role in mediating MDMA elicited pyrexia. The rapid weight loss, which represents approximately 10% of total body weight, presumably results from increased urination and defecation (Bilsky *et al*, 1991) and, to a lesser extent, evaporative water loss resulting from increased respiration (Green *et al*, 2003). Although 5-HT is known to be involved in regulating both the urinary and gastrointestinal tracts, the failure of citalopram pretreatment to prevent acute MDMA-induced weight loss suggests that this effect does not require SERT availability.

In contrast to the absence of effects on temperature regulation and body weight, citalopram modified the ejaculatory response to MDMA. The inhibitory activity of citalopram on sexual function is well recognized and is thought to be an important element in human noncompliance with antidepressant treatments (Rosen *et al*, 1999). Bilsky *et al* (1991) first documented seminal plug production following MDMA treatment of isolated rats, and spontaneous (ie without copulation) ejaculation in response to MDMA has also been investigated previously in our laboratory (Piper *et al*, 2005, 2006). However, the present study is the first to demonstrate that pretreatment with an SSRI delays the latency to ejaculation in MDMA-treated animals. Yonezawa *et al* (2005) have investigated the mechanisms underlying the ejaculatory response to another 5-HT-releasing compound, *p*-chloroamphetamine (PCA). Their findings suggest that 5-HT is involved in this response, although the site of action may be outside of the CNS. Importantly, Yonezawa *et al* (2005) reported that PCA-induced ejaculation was completely prevented by pretreatment with 10 mg/kg of citalopram, whereas in the present study citalopram only increased the latency to ejaculation and had a slight (nonsignificant) effect of reducing the percentage of animals that ejaculated. Consequently, it is possible that unlike PCA, MDMA elicits ejaculation by a mechanism that includes serotonergic action but that does not require 5-HT as an essential factor.

Many Ecstasy users exhibit feelings of depressed mood, loss of energy, muscle cramping, fatigue, nausea, and in severe cases, compromised mental status. Such symptoms exist several hours after Ecstasy consumption and can persist for days. These after-effects of Ecstasy use are referred to as either the 'mid-week blues' (Parrott, 2002) or as the 'ecstasy hangover' (Traub *et al*, 2002), although it should be noted that Traub *et al* (2002) used the term 'hangover' in a limited sense to denote a hyponatremia-related state of delirium. In the present study, MDMA-induced hypoactivity was investigated by testing animals approximately 24 h after the last drug treatment. Consistent with previous findings (Piper *et al*, 2006; Timár *et al*, 2003), a neurotoxic dosing regimen of MDMA led to significant reductions in both horizontal and vertical activity measured at this time point. These decreases are unlikely to be a consequence of weight loss, as food restriction has been shown to cause hyperactivity rather than hypoactivity (Bronstein, 1972). Moreover, citalopram pretreatment had no effect in this animal model of the Ecstasy hangover. We previously hypothesized that the hangover effect of MDMA in rats was due to a short-term depletion in 5-HT (Piper *et al*, 2006). However, this hypothesis must be re-evaluated in light of the present results. Given the well-known role of DA in modulating activity, it is possible that the reduced ambulation and rearing observed in the MDMA-treated animals was due to abnormalities in the dopaminergic system. In addition to the previously mentioned ability of high MDMA doses to acutely release catecholamines, longer lasting alterations in catecholaminergic function have been reported not only in mice (which are known to exhibit dopamine neurotoxicity following MDMA), but also in rats, monkeys, and humans exposed to this compound (Commins *et al*, 1987; Gerra *et al*, 2002, 2003; Mayerhofer *et al*, 2001; McCann *et al*, 1994; Ricaurte *et al*, 1992). Moreover, we recently found that the same MDMA binge regimen used in the present study (ie with a 1-h interdose interval) significantly reduced DA transporter binding in the striatum (Piper *et al*, 2006). In accordance with a potential role for DA in the Ecstasy hangover, a number of studies have demonstrated hypoactivity during withdrawal from either acute or chronic amphetamine treatment (Paulson *et al*, 1991; White *et al*, 2004; White and White, 2006). It will be interesting in future studies to determine whether MDMA-induced hypoactivity can be blocked by pretreatment with a DA instead of a 5-HT reuptake inhibitor.

In addition to studying the ability of citalopram to block some of the acute and short-term effects of MDMA, we also sought to determine whether citalopram pretreatment would alter the long-term behavioral consequences of MDMA. As shown in Table 5, citalopram significantly modified some, but not all, parameters of behavioral function that were influenced by MDMA. We will first consider results from the hole-board test, which is typically used as an index of exploratory behavior (Makanjuola *et al*, 1977), particularly with respect to entries into holes not previously sampled. Behavior in the hole-board test was reported previously to be altered by several compounds acting on the dopaminergic and noradrenergic systems (Makanjuola *et al*, 1977; Sara *et al*, 1995). Serotonin has also been implicated in exploratory behavior, as shown by reduced head dipping behavior in rats treated chronically with the 5-HT reuptake inhibitor clomipramine (García-Marquez *et al*, 1987) as well as impaired habituation of this

response in animals given prior administration of the serotonergic neurotoxin 5,7-DHT (Mogensen *et al*, 2003). In the present study, MDMA caused a significant reduction in novel hole entries that was blocked by citalopram pretreatment, suggesting that this decreased exploratory behavior was related to MDMA's effects on the serotonergic system.

Performance in the object recognition test was subtly altered in the MDMA-exposed animals. Previous studies have found that certain MDMA dosing regimens significantly decrease the discrimination ratio in this test (Morley *et al*, 2001; Piper and Meyer, 2004), which is generally interpreted as a memory deficit. In the present study, the discrimination ratio calculated over all 3 min of the test was not significantly affected by MDMA administration (with or without citalopram). However, when the discrimination ratio for each minute was examined, all groups had equivalent performance for the first, but not the second, minute of the test in which the MDMA-treated animals showed a reduced discrimination ratio. This pattern was particularly pronounced for the most challenging (60-min) version of the task. Because the discrimination ratio in the first minute was equivalent across drug treatments, memory does not appear to have been influenced by this MDMA treatment regimen. Instead, the treatment may have induced a different kind of behavioral deficit such as an abnormally rapid rate of habituation to novelty. Enhanced habituation by the MDMA-treated animals was also suggested by a reduced duration of object exploration in the 60-min test. The process of habituation in rodents is known to be regulated in a complex manner by several different neurotransmitters, including 5-HT, DA, acetylcholine, and glutamate (Leussis and Bolivar, 2006). The mechanism by which MDMA may have affected habituation in the object recognition test is not yet clear, although it does not appear to involve 5-HT given that the effect was not prevented by citalopram pretreatment.

Many Ecstasy users value the drug for its reported prosocial effects (Parrott, 2001). Curran and her colleagues have found that these effects are manifested not only by enhanced sociality while under the influence of the drug, but also by decreased aggressiveness (Curran *et al*, 2004; Verheyden *et al*, 2002). However, the same studies showed that aggressive mood was elevated during the mid-week period following a weekend of Ecstasy use, a phenomenon that could be related to MDMA-induced depletion of 5-HT (Krakowski, 2003; Miczek *et al*, 2002). In accordance with the human literature, rats given relatively low doses of MDMA showed increased social interaction and decreased aggressive behavior while the drug is still 'on board' (Morley and McGregor, 2000), whereas 5-HT-depleting doses of MDMA produced later increases in aggressiveness in the social interaction test (Ando *et al*, 2006), though not in the resident–intruder paradigm (Kirilly *et al*, 2005). When the social interaction test was carried out in the present study, many of the control (SAL/SAL) rats fought when confronted with an unfamiliar male, whereas the animals in the SAL/MDMA group unexpectedly showed significantly less of this behavior. As there was no change in overall levels of social interaction, this result of the MDMA binge was specific to aggressive behavior. It is noteworthy that citalopram pretreatment completely blocked the effect of MDMA, suggesting that SERT availability at the time of MDMA administration (and, presumably, subsequent alterations in serotonergic function) does play a critical role in mediating the influence of this compound on later agonistic behavior.

Investigators have frequently proposed that the long-term functional effects of high doses of MDMA are due to the resulting serotonergic deficits measured typically as decreased brain 5-HT and 5-HIAA levels (eg Gurtman *et al*, 2002; Sprague *et al*, 2003). In many cases, this hypothesis has been based more on supposition than on supporting data. Nevertheless, Aguirre *et al* (1998) demonstrated that fluoxetine pretreatment prevented MDMA-induced enhancement of the hypothermic response to a subsequent challenge with 8-hydroxy-2-(di-*n*-propylamino)tetralin. Moreover, a recent study by Thompson *et al* (2004) found that chronic fluoxetine treatment following a neurotoxic dosing regimen of MDMA ameliorated many (though not all) of the behavioral changes observed in the MDMA-treated rats. In conjunction with the present results, these findings are consistent with the view that at least some of the short- and long-term functional consequences of MDMA administration are indeed mediated by altered serotonergic activity. On the other hand, other effects may not be related to the serotonergic system, as supported by the findings of Fone *et al* (2002) in which adolescent rats given a dosing regimen of MDMA that did not induce significant serotonergic neurotoxicity nevertheless displayed later alterations in social interaction and in cocaine reward. Furthermore, there is considerable evidence that MDMA can produce long-term changes in other neurotransmitter systems besides 5-HT. For example, in addition to the catecholaminergic effects cited earlier, Wotherspoon *et al* (1994) found a significant reduction in preprocholecystokinin mRNA in the substantia nigra of rats at 2 weeks following a neurotoxic regimen of MDMA. Finally, the neurotoxic effects of MDMA may not be limited to serotonergic neurons. Schmued (2003) found that high doses of MDMA led to cellular damage in the rat forebrain (where no serotonergic somata are present) as indicated by staining with Fluoro-Jade B, a marker for neurodegeneration that has been validated with several known neurotoxins (Schmued and Hopkins, 2000).

There are a few limitations to the present study. First, although the assessments of cognitive function and some measures of anxiety-like behavior were similar to those employed by McGregor's group (eg Gurtman *et al*, 2002; McGregor *et al*, 2003a, b; Thompson *et al*, 2004), the profile of MDMA-induced functional alterations was not identical. These discrepancies in outcome are likely due to a combination of factors, including differences in MDMA dosing regimen, rat strain, and possibly the intervals between drug administration and behavioral tests. Because of the fact that recovery from the initial serotonergic insult occurs over time, a second limitation of the present study is that various behavioral tests were conducted at different time points following MDMA administration. Additional experiments comparing the time course of serotonergic recovery with behavioral effects on specific tests at different post-treatment intervals would help to clarify when functional deficits emerge and provide additional insight into the relationships between the serotonergic and behavioral toxicity of MDMA.

In conclusion, the present study found that citalopram pretreatment, which protected animals from the serotonergic neurotoxic consequences of a high-dose MDMA regimen, failed to prevent most of the acute or short-term effects of this regimen. A few of the long-term effects observed in the MDMA-treated animals were sensitive to citalopram, whereas others were not. This pattern of findings suggests that some changes in behavioral function may not be the result of MDMA-induced serotonergic deficits. We further argue that such a conclusion has potential clinical relevance for the treatment of Ecstasy users who are suffering from cognitive, mood, or anxiety disorders that may be related to their substance use. Although the overall single-day dose of 40 mg/kg of MDMA used in the present study is clearly much greater than recreational human doses, SERT imaging studies of users raise the possibility that cumulative exposure involving many smaller Ecstasy doses could, over time, produce the kind of serotonergic neurotoxicity seen in animal binge studies (Cowan, 2007). Finally, some websites aimed at the general public suggest that taking an SSRI with Ecstasy can prevent the adverse side effects of MDMA, which may be a fallacious assumption based in part on the present results. Studies to identify additional neurotransmitter systems besides 5-HT (eg DA, norepinephrine, or others) that may contribute to the enduring behavioral consequences of MDMA exposure would greatly enhance our understanding of the long-term risks of recreational Ecstasy use.

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DISCLOSURE/CONFLICT OF INTEREST

We declare that, except for income received from our respective primary employers, no financial support or compensation has been received from any individual or corporate entity over the past 3 years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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