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MDMA-induced impairment in primates: antagonism by a selective norepinephrine or serotonin, but not by a dopamine/norepinephrine transport inhibitor

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Abstract

Human MDMA (*R,S*-3,4-methylenedioxymethamphetamine) users display selective cognitive deficits after acute MDMA exposure, frequently attributed to serotonin deficits. We postulated that MDMA will compromize executive function in primates and that an inhibitor of the serotonin transporter (SERT) and the norepinephrine transporter (NET) but not the dopamine (DAT) transporter, will prevent impairment. The potencies of DAT/NET, NET and SERT inhibitors to block transport of [^{3}H]MDMA and [^{3}H]monoamines were compared *in vitro*. Subsequently, cynomolgus monkeys (*Macaca fasicularis*) were trained to stable performance in a reversal learning task. Effects of once-weekly oral or i.m. dose of MDMA (1.5 mg/kg, $n = 4$) on performance were monitored, alone or after pretreatment with inhibitors of the SERT, DAT or NET (prior to i.m. MDMA). 1) Drug potencies for blocking [^{3}H]MDMA or [^{3}H]monoamine transport were not consistent; 2) Oral MDMA increased error rates in a cognitive task for up to three days following exposure, whereas intramuscular MDMA prevented subjects from performing the cognitive task on the day of administration, but not on subsequent days; 3) The SERT inhibitor citalopram and the NET inhibitor desipramine, but not the DAT/NET inhibitor methylphenidate, reversed the effects of MDMA on task performance and mandibular movements induced by i.m. MDMA and 4) MDMA altered sleep latency. Oral MDMA impairs executive function in monkeys for several days, a finding of potential relevance to MDMA consumption by humans. Reversal of impaired executive function by a NET inhibitor implicates the NET and norepinephrine in MDMA-induced cognitive impairment and may be relevant to therapeutic strategies.

Keywords

MDMA, rhesus monkey, monoamine transporter, cognition, aversion, inhibitor, impairment

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Introduction

The amphetamine derivative MDMA (*R,S*-3,4-methylenedioxymethamphetamine) acutely elicits a range of subjective effects and symptoms of sympathetic stimulation (Peroutka *et al.*, 1988; Parrott and Lasky, 1998; Vollenweider *et al.*, 1998; Liechti *et al.*, 2000; Verheyden *et al.*, 2003; Farre *et al.*, 2004). Accumulating evidence also implicates acute or repeated doses of MDMA in compromizing cognition. An acute dose of MDMA impairs performance in cognitive tests of complex attention, decision-making and executive function (Cami *et al.*, 2000; Farre *et al.*, 2004; Kuypers *et al.*, 2005; Vollenweider *et al.*, 2005). Impaired function and dysphoria reportedly persists for several days or emerges several days after a 'Saturday night ingestion' (Curran and Travill, 1997; Parrott and Lasky, 1998; but see Downing, 1986), but has not been viewed prospectively. Impaired cognitive deficits are also detectable in long-term abstinent MDMA users, along with other adverse psychological and affective consequences, apparently related with extent of drug exposure (McCann and Ricaurte, 1991; Parrott, 2001; Verheyden *et al.*, 2003; Halpern *et al.*, 2004; Gouzoulis-Mayfrank *et al.*, 2005; Morgan *et al.*, 2005). The neurochemical mechanisms that precipitate acute cognitive dysfunction are poorly understood and whether they portend the long-term cognitive deficits are unknown. No medications have been tested to counteract the cognitive deficits in the acute phase, and there is sparse literature describing assessment of medications to reverse the reported cognitive impairment after long-term MDMA use.

There is ample evidence, however, that MDMA is a substrate for the serotonin (SERT), dopamine (DAT) and norepinephrine (NET) transporters (Verrico *et al.*, 2007) and promotes release of the corresponding monoamines from neurons (Green *et al.*, 2003). Its behavioral effects are directly or indirectly attributed to sequestration by monoamine neurons via the DAT, NET and SERT. As prolonged MDMA use by humans is associated with the loss of markers for brain serotonin neurons, SERT, expressed by serotonin neurons, is strongly implicated in mediating both the positive and the adverse consequences associated with MDMA use (McCann *et al.*, 1999; Reneman *et al.*, 2001). Several reports have monitored whether SERT inhibitors impede the acute subjective or biochemical effects of MDMA (Liechti and Vollenweider, 2000; Liechti *et al.*, 2000; Pacifici *et al.*, 2004) and a clinical study tested whether a SERT inhibitor reversed impaired cognition associated with long-term MDMA exposure (McCann and Ricaurte, 1991). Whether SERT, DAT or NET transport inhibitors attenuate the MDMA-induced acute cognitive impairment remains unexplored.

We postulated that cognitive deficits induced acutely by MDMA could be modeled in nonhuman primates, with the obvious advantage of avoiding confounding variables frequently encountered in clinical studies, such as polysubstance abuse, impurities in illicit MDMA and psychiatric co-morbidity. We further hypothesized that a SERT inhibitor (citalopram) and possibly a NET inhibitor (desipramine) would attenuate MDMA-induced cognitive impairment. We included a NET inhibitor because [^{3}H]MDMA affinity for *human* NET was higher than for SERT or DAT (Verrico *et al.*, 2007). Results were compared with the DAT/NET inhibitor methylphenidate. To predict whether transport inhibitors would effectively block MDMA transport *in vivo*, we initially compared the relative potencies of transport inhibitors on [^{3}H]MDMA and [^{3}H]monoamine transport *in vitro*.

Cognitive testing and MDMA effects on task performance were conducted in cynomolgus monkeys trained in a well-characterized reversal learning task that monitors a component of executive function (Miyake *et al.*, 2000; von Geusau *et al.*, 2004; Dafters, 2005). The three-choice object discrimination task assesses acquisition (stimulus-reward learning), reversal (capacity to shift to a new set of reward parameters) and perseveration defined as inhibition of a previously learned reward-related behavior (Taylor *et al.*, 1990; Jentsch *et al.*, 2002). Using a protocol that mirrored human patterns, oral MDMA was given at a dose and dosing regimen (once weekly) comparable to the 70–150 mg commonly used in clinical research or by MDMA users (Liechti *et al.*, 2000; Green *et al.*, 2003). Subsequently, weekly i.m. MDMA doses (e.g., Frederick *et al.*, 1997; Taffe *et al.*, 2001) replaced oral doses. After stable testing performance was achieved, subjects were pretreated with inhibitors of the SERT, DAT or NET prior to MDMA.

Intriguingly, route of administration was a critical factor in the persistence of MDMA effects: an oral dose of MDMA increased error rates for three days, whereas i.m. MDMA impaired function only on the day of administration. Of potential clinical relevance, NET and SERT inhibitors were equally effective in attenuating i.m. MDMA-induced impairment of task performance, and mandibular movements. The functional and therapeutic implications of these discoveries warrant further investigation.

Methods

In vitro assays

(±)MDMA (Specific activity: ~0.4 Ci/mmol) was kindly provided by the National Institute on Drug Abuse (NIDA, Bethesda, MD, USA). [^{3}H]DA, (Specific activity: 23.5 Ci/mmol) was purchased from Perkin Elmer Life Sciences (Boston, MA, USA). [^{3}H]5-HT (Specific activity: 133 Ci/mmol) was purchased from Amersham Biosciences (Buckinghamshire, UK). Methylphenidate HCl was obtained from Sigma-RBI (St. Louis, MI, USA), citalopram HBr was a gift of Lundbeck, Inc. (Kobenhavn-Valby, Denmark) and desipramine HCl was obtained from Sigma (St. Louis, MI, USA). All others drugs were obtained from Sigma Aldrich (St. Louis, MO, USA). Cell lines were generated in our laboratory.

Stable expression of monoamine transporters in HEK-293 cells

HEK-293 cells were stably transfected with the human DAT, NET or SERT as previously described (Verrico *et al.*, 2007). Cells were grown in 145 mm untreated tissue culture dishes (Greiner America, Inc., Lake Mary, FL, USA) in Dulbecco's modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/mL penicillin, 100 μg streptomycin and 0.1 mM nonessential amino acids (all from Life Technologies, Rockville, MD, USA), at 5% CO_2 in a 37°C water-jacketed incubator.

Drug potencies for inhibiting [3H]DA, [3H]NE, [3H]5-HT and [3H]MDMA transport

Test tubes containing 200 μL of the cell suspension and 200 μL of various concentrations of test compounds were preincubated for 15 min preincubation at 37°C. Next, 200 μL of [3H]DA, [3H]NE, [3H]5-HT (10 nM or 10 nM plus 90 nM of the unlabeled monoamine) or [3H](±)MDMA (100 nM) were added to initiate transport and incubated for another 10 min. Following incubation, experiments were terminated by placing the test tubes in ice. The cells were harvested by centrifuging test tubes at 16595 g units for 15 min (~0°C) and aspirating the supernatant. Cells were lysed with 100 μL sodium dodecyl sulfate (SDS, 0.1% for ~24 h at room temperature) and counted, in 4 mL Readysafe® scintillation fluid for 5 min on an LS6000IC Beckman (Fullerton, CA, USA) scintillation spectrophotometer. Nonspecific transport, as measured by transport in the presence of 10 μM mazindol (DAT assay), 10 μM fluoxetine (SERT), 10 μM desipramine (NET) was subtracted from the total disintegrations per minute (dpm) to yield specific accumulation of [^{3}H]DA, [^{3}H]NE, [^{3}H]5-HT or [^{3}H](±)MDMA, as described previously (Verrico *et al.*, 2007).

Data analysis

All points were assayed in triplicate and each experiment was independently repeated at least three times (except were noted) with different cell preparations. Data analyses were performed as previously described (Verrico *et al.*, 2007) using GraphPad Prism software (San Diego, CA, USA). In uptake experiments, K_i values were calculated according to the formula: $K_i = IC_{50}/(1 + L/K_m)$, where L is the concentration of the radiolabeled substrate ([^{3}H]DA, [^{3}H]NE, [^{3}H]5-HT or [^{3}H]MDMA).

Object discrimination test

Subjects Five male drug-naïve cynomolgus monkeys (*Macaca fasicularis*), weighing on average 9.5 ± 1.2 kg and ranging in age from 4 to 6 years (corresponding to human age 18–28 years) were individually housed in a colony room with a 12 h light/dark cycle (lights on at 6:30 am). Animals had unrestricted access to water at all times and were maintained on monkey chow (Teklad Monkey Diet, Harlan Teklad, Madison, WI, USA) supplemented with fresh fruit after daily testing, which concluded by 12:00 pm. The use of highly palatable food rewards (e.g., peanuts, mini-marshmallows, M & M's, cereal, grapes or raisins) minimized the need for dietary restrictions. Four of the five subjects had been trained two years before in an entirely different test of manual dexterity. All procedures were performed with the approval and under the supervision of the Harvard University Institutional animal Care and Use Committee. Monkeys were cared for according to the *Guide for Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, 1996).

The reversal learning test was designed and custom-built to attach to a monkey's home cage, thereby minimizing the stress of changing environments during test procedures. The apparatus consisted of a tray with three food wells. An opaque screen between the monkey and the tray, that could be raised or lowered, enabled the experimenter to covertly change the position of the retrievable test objects and reward. Elevation of the screen allowed for unobstructed access to the testing platform/food wells/objects.

The overall objective of the test was to monitor the number of errors made by monkeys trained to find a positive stimulus/object associated with a food reward in a three-choice paradigm for 10 trials, and determine their accuracy in finding and subsequently choosing a novel positive stimulus/object after the stimulus-reward association was altered for 20 trials. Procedures were similar to those previously published (Jentsch *et al.*, 2002).

Acquisition

Each monkey was initially habituated to the apparatus and trainer. To facilitate familiarizing the monkeys with the apparatus, a reward was placed in the food wells on the tray and the screen was raised. This continued for one week, until the animals consistently reached for the reward in each of three wells. Following this initiation, a novel set of visually distinct objects was used on each day to cover the food wells, requiring the subjects to learn a new stimulus-reward association daily. Although similar in size, the three objects utilized on any given day were distinguishable by shape and color. In an effort to minimize object preferences or aversions, the rewarded stimulus was balanced across all subjects. At the beginning of each testing session, with the opaque screen lowered, a reward was concealed under the designated positive stimulus. The screen was raised and the monkeys were allowed to search the objects for the reward on consecutive trials, making one selection per trial. Between trials, the screen was lowered, and the food well re-baited. During the pretraining phase, the location of the objects was not altered between trials. Once the positive stimulus was identified, there were nine additional trials with the same reward-stimulus association (10 total trials). The number of errors after choosing the positive stimulus was the first of three dependent measures and designated as *acquisition errors* (see Table 1).

Reversal and perseverative errors

Following the acquisition training period, a reversal component was introduced. Each session began with acquisition phase, but after 10 acquisition trials, the reward was re-located beneath a previously nonreinforced stimulus. After the monkey selected the new positive stimulus there were nineteen additional trials, for a total of 20. This second dependent measure was designated *reversal errors* and computed based on the total number of errors committed out of 20 reversal trials. The third dependent measure, designated *perseverative errors* (selection of the object from a previously reinforced position), was based on the errors committed during the initial search after acquisition and combined with the perseverative errors during the 20 trials under the reversed contingency. Pretraining continued until a stable level of performance on all three parameters was maintained for five consecutive days (Figure 1: First segment). Subsequently, monkeys were trained under these same conditions with the noted exception that after each trial, the position of the objects was randomly altered or switched

Table 1 Comparison of the affinities of the SERT uptake inhibitors fluoxetine and citalopram, the DAT uptake inhibitors mazindol and methylphenidate and the NET uptake inhibitor desipramine for inhibiting the transport of 100 nM [^{3}H](±)MDMA and endogenous substrates

	SERT					
Compound	100 nM [^{3}H](±)MDMA			100 nM [^{3}H]5-HT		
	IC_{50} (nM)	K_i (nM)	*n*	IC_{50} (nM)	K_i (nM)	*n*
Fluoxetine	336 ± 65	303	6	17.3 ± 3.1	13	6
Citalopram	38.9 ± 9.6	35	6	5.76 ± 0.4	4.3	3
	DAT					
	100 nM [^{3}H](±)MDMA			100 nM [^{3}H]DA		
	IC_{50} (nM)	K_i (nM)	*n*	IC_{50} (nM)	K_i (nM)	*n*
Mazindol	141 ± 60	189	5	110 ± 16	99	6
Methylphenidate	16.9 ± 4.0	15.8	5	172 ± 9.4	155	3
	NET					
	100 nM [^{3}H](±)MDMA			100 nM [^{3}H]NE		
	IC_{50} (nM)	K_i (nM)	*n*	IC_{50} (nM)	K_i (nM)	*n*
Desipramine	500 ± 192	395	8	1.69 ± 0.2	1.50	5

(Figure 1, second segment). The position of the rewards in daily sessions was determined by a random number generator (www.random.org).

Stable performance

Monkeys were trained to perform object discriminations and reversals over several weeks to ensure no pre-existing differences in performance. Animals were tested 4–6 days a week with 1–2 days separating each weekly testing session. This continued for one month until a stable level of performance was maintained for five consecutive days (Figure 1: second segment).

MDMA administration

MDMA was orally ingested after embedding the powder in favored foods. Controls received the identical food without the drug admixed. Alternatively, MDMA was dissolved in grape juice and injected into grapes. The trainer handed the food to the monkey and they self-administered the drug mixtures prior to behavioral testing. The p.o. doses were completely consumed and food residue was not observable.

MDMA effects on acquisition and reversal

Four monkeys were evaluated after acute oral ingestion of MDMA (1.5 mg/kg, p.o.) 60 min prior to testing. This dose of MDMA closely corresponds to an average dose consumed with an illicit 'ecstasy' tablet and can be considered lower than a human dose because no allosteric adjustment was made for differences in surface area/body mass between monkeys and humans. Performance was evaluated on the day of (Day 0), and for four days following MDMA exposure. One week after the first MDMA exposure, monkeys were challenged again with MDMA and performance assessed 1 h after exposure and at the same time daily for four subsequent days. This protocol continued for three weeks. After three weeks of oral MDMA, MDMA was injected i.m. for all subsequent experiments. After a five-week period of weekly MDMA exposure and a four-week period of drug combination studies, animals were left untreated for two months. When testing was reintroduced, all subjects quickly re-acquired baseline performances, which did not differ from their performances prior to the first exposure to MDMA. MDMA (i.m.) was reintroduced for additional studies.

Methylphenidate effects alone on acquisition and reversal As methylphenidate is a psychostimulant drug, we tested whether it would disrupt performance prior to introduction of MDMA in this study. Methylphenidate (0.1–0.3 mg/kg) was administered i.m. and orally 60 min prior to testing.

Methylphenidate pretreatment

To determine whether methylphenidate prevents performance deficits induced by MDMA, monkeys were pretreated at 60 min

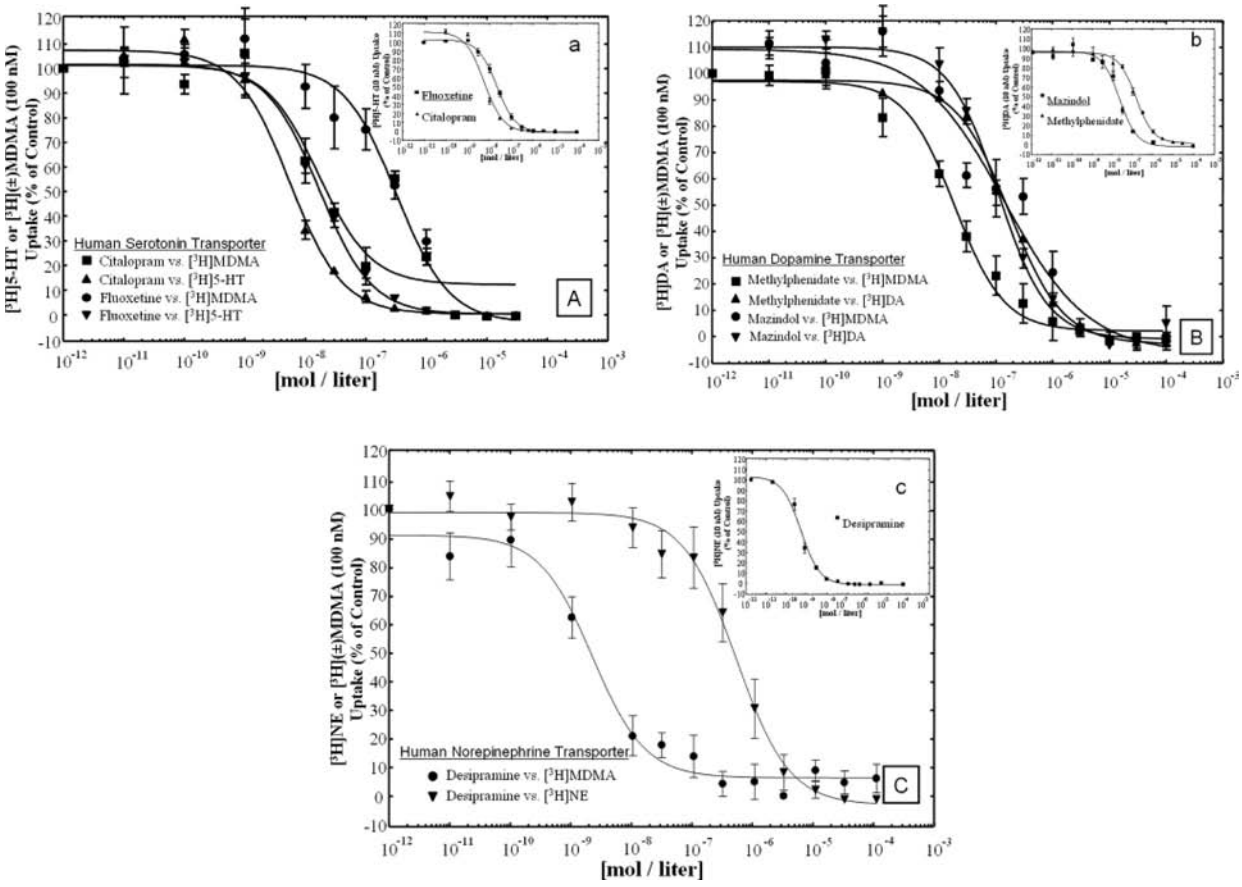

Figure 1 Potencies of monoamine transport inhibitors to block the transport of [^{3}H]serotonin (100 nM, 10 nM) or [^{3}H]MDMA (100 nM) by the human serotonin (SERT), [^{3}H]dopamine (100 nM, 10 nM) or [^{3}H]MDMA (100 nM) by the (DAT) or [^{3}H]norepinephrine (100 nM, 10 nM) or [^{3}H]MDMA (100 nM) by the (NET) transporters. a) Top panel: Citalopram and fluoxetine inhibition of 100 nM [^{3}H]serotonin or [^{3}H]MDMA transport by the SERT. Both compounds were more potent in blocking [^{3}H]serotonin than [^{3}H]MDMA transport. The insert displays parallel data using 10 nM [^{3}H]serotonin. b) Middle panel: Methylphenidate and mazindol inhibition of 100 nM [^{3}H]dopamine or [^{3}H]MDMA transport by the DAT. Methylphenidate was more potent in blocking [^{3}H]MDMA than [^{3}H]dopamine transport. The insert displays parallel data using 10 nM [^{3}H]dopamine. c) Bottom panel: Desipramine inhibition of 100 nM [^{3}H]norepinephrine or [^{3}H]MDMA transport by the NET. Desipramine was considerably more potent in blocking [^{3}H]norepinephrine than [^{3}H]MDMA transport. The insert displays parallel data using 10 nM [^{3}H]norepinephrine. Each independent experiment was conducted 3–6 times, in triplicate. Corresponding IC_{50} and K_i values are given in Table 1.

prior to MDMA, with an oral dose of methylphenidate, comparable to an average human therapeutic dose (0.3 mg/kg). This dose had produced no effects when administered alone. The component protocol was tested once weekly for three weeks following six weekly doses of i.m. MDMA (1.5 mg/kg).

Citalopram pretreatment

To determine whether a serotonin transport inhibitor could attenuate the cognitive deficits induced by MDMA, monkeys received citalopram dissolved in sterile saline, at a dose corresponding to a human therapeutic dose (0.55 mg/kg). The drug was administered i.m. 240 min (4 h) prior to MDMA (1.5 mg/kg, i.m.), a pretreatment time calculated on the basis of pharmacokinetic properties of citalopram in humans. Test sessions were continued daily for two subsequent days and the experiment was repeated.

Desipramine pretreatment To determine whether the antidepressant desipramine, a NET-selective inhibitor, attenuated the cognitive deficits induced by MDMA, monkeys received desipramine dissolved in sterile saline, at a dose corresponding to a human therapeutic dose (1.5 mg/kg, i.m.). The drug was administered 90 min prior to MDMA (1.5 mg/kg, i.m.), a pretreatment time calculated on the basis of the pharmacokinetic

properties of desipramine in humans. Cognitive testing was performed on the day of the drug study and continued daily for four days, after which the experiment was repeated.

Video ratings Spontaneous behavior of subjects was rated from videotapes, using a rating scale developed specifically for this purpose. Subjects were filmed periodically in their home cages for 2 h during the baseline period and following drug administration. Cameras, placed four feet from the cages, were left undisturbed during the filming. Selected behaviors were recorded for 2 min, every 5 min, for the duration of a 2 h tape, using a rating scale described in Table 2. For each parameter, each subject received a score based on the presence, severity and duration of a behavior. Each of the activities measured in three animals were analysed from approximately 420 data points for baseline activity, 107 data points for oral MDMA, 425 data points for i.m. MDMA, 130 data points for desipramine response, 67 data points each for citalopram or methylphenidate.

Statistical analysis

Data were analysed with repeated measure analysis of variance followed by Tukey's post-hoc test when ANOVA revealed main effects or interactions.

Sleep latency

Occasionally after the end of a test session, monkeys were anesthetized (ketamine 1 mg/kg i.m.) to place collars containing activity monitors on, for recording of day/night activity (Mini Mitter Company Inc. Actiwatch®; Bend, Oregon, USA). Sleep latency was assessed with these omnidirectional accelerometers (Actiwatch aw4-64K, Mini-Mitter Co., Inc., Sunriver, OR, USA) as previously described (Goulet and Madras, 2000).

Results

MDMA and monoamine transporter inhibitors

Initially, we investigated whether the drugs used in the present study were effective blockers of [^{3}H](±)MDMA transport by measuring the potencies of methylphenidate, citalopram and desipramine for inhibiting [^{3}H](±)MDMA transport into HEK-293 cells stably transfected with the DAT, NET and SERT. We previously reported that [^{3}H](±)MDMA is a substrate for the human DAT, NET and SERT (Verrico *et al.*, 2007). Reliable detection of [^{3}H](±)MDMA transport required the use of 100 nM MDMA, as the specific activity of [^{3}H](±)MDMA was low (see methods, (Verrico *et al.*, 2007). The potencies of drugs for inhibiting [^{3}H](±)MDMA and [^{3}H]neurotransmitter transport differed (Table 1, Figure 1). At the human SERT, citalopram and fluoxetine blocked serotonin transport ([^{3}H]5-HT) in the low nanomolar range (<20 nM). In sharp contrast, citalopram was a relatively potent inhibitor of [^{3}H](±)MDMA transport (K_i: 35 nM) whereas fluoxetine was relatively weak (K_i: 303 nM). Methylphenidate was 10 times more potent at inhibiting transport of [^{3}H](±)MDMA (K_i: 15.8 nM) than [^{3}H]DA (K_i: 155 nM), whereas mazindol values were relatively consistent for both substrates (Figure 1, Table 1). Intriguingly, desipramine was 260 times more potent at blocking NET transport of [^{3}H]NE (K_i: 1.5 nM) than of [^{3}H](±)MDMA (K_i: 395 nM), (Figure 1, Table 1).

Table 2 Rating scale for MDMA associated spontaneous behaviors

Score	Designation	Frequency
Vigilance/surveillance of other animals		
0	Normal	0–15 s during 2 min observation
1	Slight	15–60 s during 2 min observation
2	Severe	60–120 s during 2 min observation
Locomotion involving two full steps or repetitive locomotion		
0	Normal	0–2 locomotor movements during 2 min observation
1	Slight	3–5 locomotor movements during 2 min observation
2	Severe	5+ locomotor movements during 2 min observation
General activity		
0	Sedated	Asleep, sedated posture for 2 min
1	Slightly sedated	Awake active for less than 2 min
2	Active	Awake, active for 2 min
Mandibular motion		
0	Normal	0–2 times during 2 min observation
1	Slight	3–5 times during 2 min observation
2	Abnormal	5+ times during 2 min observation

Object discrimination Test

Baseline performance During the pretraining period in which the cue was in a fixed position, monkeys performed well in tests of acquisition, reversal and perseveration (Figure 2, left). During the training period, the subjects required one day to consistently find the object in acquisition and nine days to perform consistently during reversal, during which the position of the object was switched. Thereafter, a stable baseline level of performance was established for all the monkeys (Figure 2, center, right) and monkeys were tested four–six days each week (except for two holiday weeks) for 32 weeks. The stability of their performance is exemplified by comparing three separate trial periods, consisting of five-consecutive days of testing. Repeated monitoring over months established that performance did not deteriorate over holiday periods (> two days) during which we did not perform testing, and here were no differences in acquisition, reversal learning or perseverative error rates during three weeks of nonsequential testing. The stability of these baseline responses was confirmed by stable test scores in the same cohort of monkeys, following a two month hiatus during which the animals were exposed neither to the testing apparatus nor drugs.

Oral MDMA

To determine whether environmental disturbances or other factors may have contributed to MDMA effects, we initially used a vehicle control group (Figure 3a, top panel, $n = 2$) for comparative purposes. Task performance was stable in subjects receiving food vehicle alone once weekly for three weeks. We then focused on intra-subject comparisons. Four subjects were administered an oral dose of MDMA (1.5 mg/kg, p.o.) once weekly for three weeks and MDMA effects were compared with their baseline levels of performance. One MDMA monkey did not perform 1 h after exposure in week-1 and week-2, and was excluded from data analysis on those days. A different monkey did not perform the day of MDMA

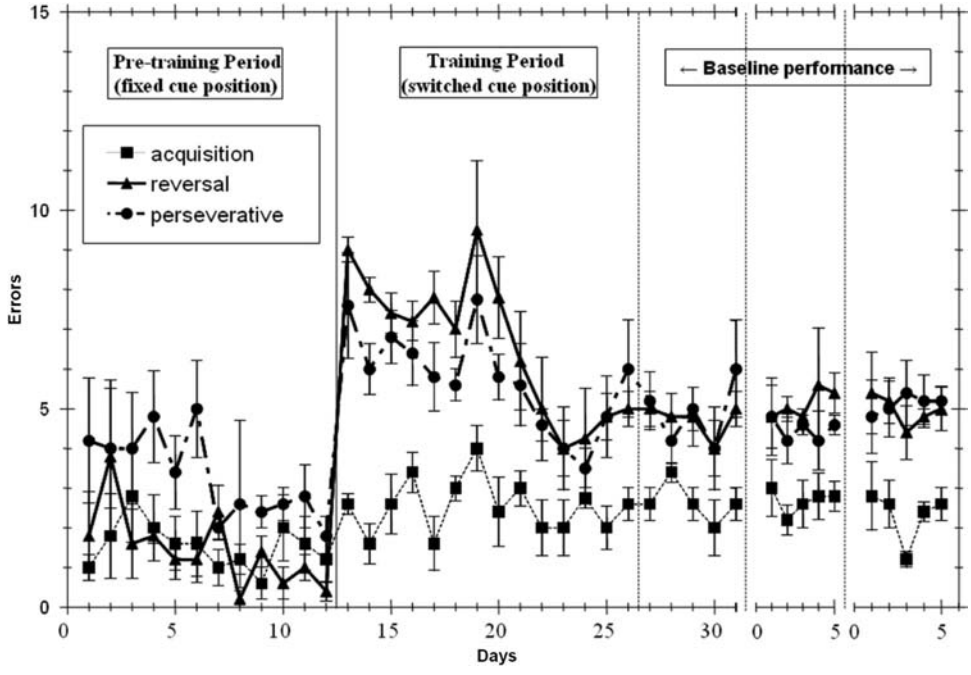

Figure 2 Protocol for training of cynomolgus monkeys in a test of cognitive function Monkeys were first habituated to the apparatus and trainer. For the first twelve days, designated 'Pretraining period', monkeys were trained to find a hidden reward with the reward cue and the location of the objects was not altered between and during trials (10 trials). The number of errors after choosing the positive stimulus was the first of three dependent measures and designated as *acquisition errors* (Table 2). Following the acquisition only 10 trials, a reversal component was introduced, by re-locating the reward to a new location. After the monkey selected the new positive stimulus, nineteen additional trials followed, to measure *reversal errors*. During the 'Training period', the position of the objects was randomly altered or switched (2nd segment). Monkeys were trained to perform object discriminations and reversals over several weeks to ensure no pre-existing differences in performance. Animals were tested 4–6 days a week with 1–2 days separating each weekly testing session. This continued for one month until a stable level of performance was maintained for five consecutive days. Note the stable error rates from Day 23 onwards. An additional 20 days of training was performed after methylphenidate testing to ensure stable, consistent 'Baseline performance' (3rd segment). Three nonsequential weeks of five consecutive days of stable performance are shown in the 3rd segment. Seven days of stable task performance preceded MDMA exposure.

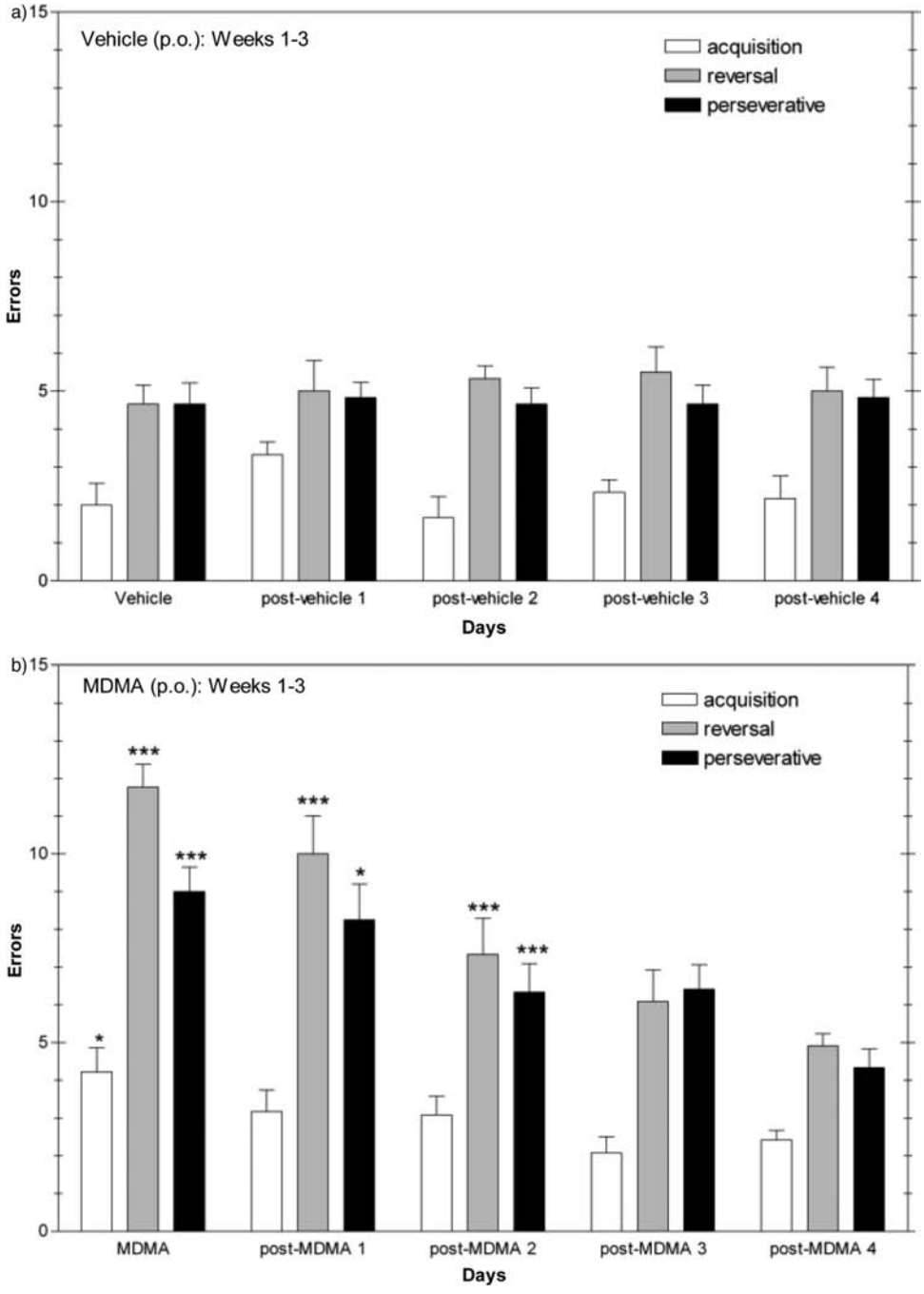

Figure 3 Error rates in task performance of monkeys treated with vehicle or with oral MDMA, as a function of time. a) (Top panel): Control cynomolgus monkeys ($n = 2$) were treated with vehicle food once weekly for three weeks and performance was measured daily, as described in methods. Note the relatively stable error rates, with low acquisition errors (approximately 2–3), and reversal errors. Error rates are averaged from three weeks of testing in two animals and expressed as means $\pm$ SEM. b) (Bottom panel): Monkeys ($n = 4$) received food mixed with MDMA (1.5 mg/kg). Error rates are averaged from five weeks of testing and expressed as means $\pm$ SEM, and compared with stable baseline error rates of the same subjects determined for five days prior to MDMA exposure. Acquisition, reversal and perseverative error rates increased the day of MDMA exposure (MDMA), with reversal and perseverative errors persisting in post-MDMA days 1 and 2.
$^{*}P < 0.05$; $^{***}P < 0.001$.

exposure during week 3 and on that day this data set was excluded. Accordingly, three of four subjects performed consistently on the day they received MDMA. Importantly, the time required to complete the tasks following oral MDMA was the same as during acquisition of baseline data. Each subject served as its own control and baseline data were compared with MDMA effects ($n = 4$, within group comparisons of MDMA versus baseline).

Acquisition During the acquisition search period, animals were required to identify the positive stimulus, and once identified, tested for nine additional trials (Table 3). An acquisition error was recorded when the subject chose one of the two nonreinforced stimuli during these nine trials. Of the three outcome measures, acquisition was least affected by oral MDMA (Figure 3b, white bars). We compared acquisition performance after p.o. MDMA to baseline acquisition performance (Figure 2: right, square symbols). Comparison of daily task performance after MDMA administration to baseline performance levels of the same animals revealed a significant main effect of treatment on acquisition errors on the day of exposure ($F_{(3,17)} = 4.149$, $P = 0.022$), with no other day significantly affected.

Reversal In the reversal phase, one of the remaining two positions in the apparatus (stimuli) not reinforced during the acquisition phase, assumes the role of a positive stimulus. A second search is initiated to identify this new positive stimulus for food reward. Once identified, 19 more trials followed. A reversal error was recorded when the subject errs, by selecting the reinforced stimulus from an incorrect position, either the stimulus from the acquisition phase or the remaining stimulus that was not reinforced during any trial. Oral MDMA induced significant reversal errors (Figure 3b, grey bars), reflecting a compromized ability to alter behavioral responses when the position of the reward changes (rules of the task change). In comparison with baseline performance, performance on the day of MDMA ingestion was significantly compromized, with a $184 \pm 15.5\%$ increase in reversal errors relative with baseline ($P < 0.001$) which persisted on post-MDMA 1 ($89.5 \pm 52.1\%$ increase; $P < 0.001$) and post-MDMA 2 ($96 \pm 31.1\%$ increase; $P < 0.001$) compared with baseline performance. No other days were significantly affected. Reversal performance following MDMA was compromized each week in parallel, with performance deficits significantly increased on the day of, and for two days after ingestion.

Perseverative errors A perseverative error is a variant of the reversal error (Table 2). It is calculated from data acquired during the second search period (the reversal phase), by computing only errors of persistence, that is the subject persists in selecting the previously reinforced stimulus of the acquisition phase. Accordingly, the values of these errors are lower than total reversal errors, but constitute a majority of reversal errors. MDMA increased perseverative error rates compared with baseline performance levels (Figure 3b, black bars). There was a significant main effect of oral MDMA on perseverative error rates on the day of exposure ($P < 0.001$), post-MDMA 1 ($P < 0.05$) and post-MDMA 2 ($P < 0.001$). No other days were significantly affected. Accordingly, in a comparison of MDMA effects on daily performance on a week-by-week basis, performance was consistently impaired on the day of and for two days following MDMA. The perseverative error data precisely paralleled the reversal data, suggesting causality between reversal errors and perseverative behavior.

Methylphenidate alone Prior to initiating this phase of the study, we quelled a concern that the psychostimulant methylphenidate could, by itself affect task performance. If administered alone ($n = 3$), various doses of i.m. methylphenidate (0.1, 0.2, 0.3 mg/kg, i.m.) were without significant effect on any parameter of task performance (Figure 4). In contrast, an oral dose (0.3 mg/kg) of methylphenidate reduced acquisition errors modestly.

i.m. MDMA

We also performed i.m. MDMA studies, and tested whether methylphenidate and other monoamine transport inhibitors in combination with MDMA would affect. As monkeys did not perform on the day of i.m. MDMA, task completion on Day 0 was considered to reflect antagonism of MDMA effects on task performance.

Table 3 Explanation of terms used to define performance on cognitive task

Dependent measures	Parameters defined
Acquisition errors	There is an initial search period to identify the positive stimulus. Once the subject identifies the positive stimulus, there are nine more trials (10 total trials). An acquisition error is recorded when the subject chooses one of the two nonreinforced stimuli during these nine trials.
Reversal errors	One of the two stimuli not reinforced in the acquisition phase becomes the positive stimulus. There is a second search period to identify this new positive stimulus. Once identified, 19 more trials follow (20 total trials). A reversal error is recorded when the subject chooses the previously reinforced stimulus from the acquisition phase (perseverative error; see below) or the one remaining stimulus that was never reinforced during any trial.
Perseverative errors	A perseverative error is recorded when, during the second search period and the reversal phase, the subject chooses the previously reinforced stimulus from the acquisition phase.

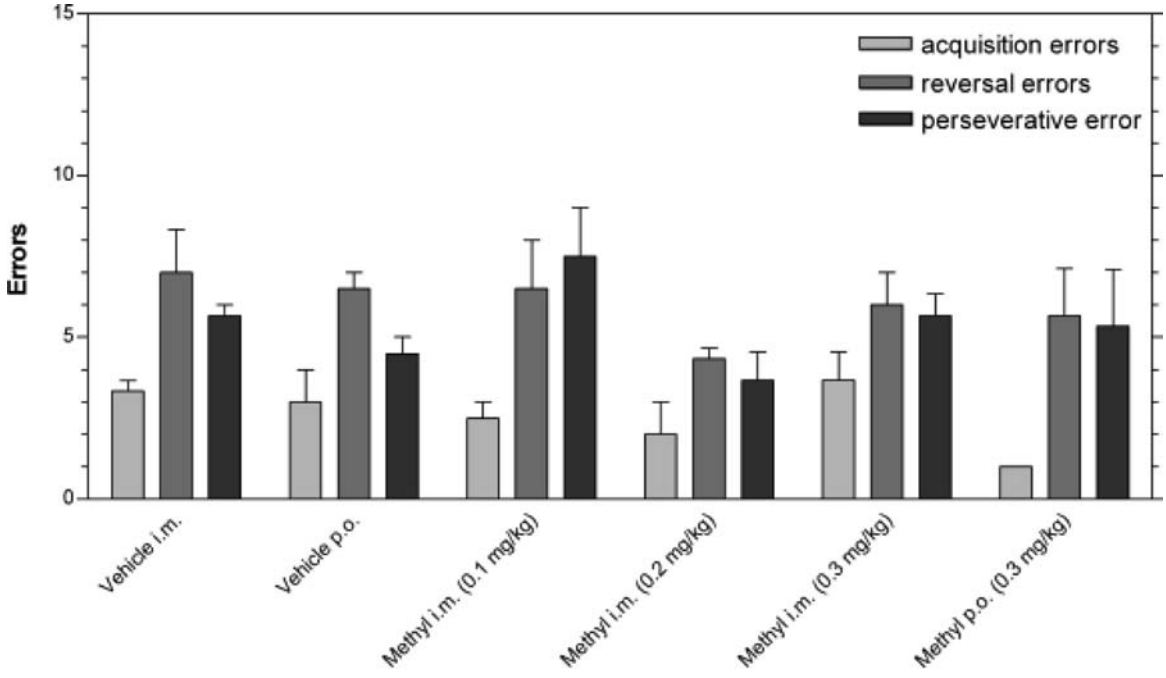

Figure 4 Effects of methylphenidate on cognitive task performance (acquisition, reversal, perseverative behavior) by trained monkeys. Acquisition, reversal and perseverative error rates for vehicle (i.m., p.o.) and for i.m. methylphenidate (0.1, 0.2 and 0.3 mg/kg), or oral methylphenidate (0.3 mg/kg) are shown.

Acquisition errors The second phase of the study was conducted with i.m. MDMA, because the monkeys refused oral MDMA even when hidden in food. This cohort had previously been treated with i.m. saline injections and these injections did not alter task performance. MDMA (i.m.) alone was administered for five weeks, and during this phase, we compared performance levels of oral and i.m. MDMA. In contrast to monkeys treated with oral MDMA, i.m.-treated monkeys failed to initiate or complete tasks during the normal period of testing after MDMA administration (Figure 5a, bars set arbitrarily at 15). Accordingly, performance errors could not be computed. MDMA effects dissipated by the following day and were restored to baseline levels in week one. We took advantage of this quantal change in performance as an opportunity to evaluate monoamine transporter inhibitors as candidate therapeutics. It is important to point out that on the post-MDMA days, the rate at which the animals performed the task was the same as during the control testing period (time to complete trials).

MDMA and methylphenidate (DAT inhibitor) Methylphenidate was administered 1 h prior to MDMA, orally in the first week and i.m. (0.3 mg/kg) in the second week of testing. The effects of pretreatment with oral or i.m. methylphenidate did not differ and data were grouped together. Pretreatment for 1 h with methylphenidate did not affect MDMA-mediated performance deficits on acquisition on Day 0 (Figure 5b), but acquisition errors increased $166 \pm 28.9\%$ ($P < 0.01$) two days after exposure (post-MDMA 2) in the pretreated group in week 2. There were no differences in reversal errors or perseverative errors in animals treated with MDMA alone or pretreated with methylphenidate followed by i.m. MDMA during weeks 1–4.

MDMA and citalopram (SERT inhibitor)

The effects of citalopram alone were not assessed because of its long half-life (35 h) and the possibility that repeated dosing with citalopram could induce neuroanatomical changes and alter MDMA-induced effects. Based on onset times reported for humans, citalopram was administered i.m. 4 h prior to a single dose of i.m. MDMA and animals were tested daily for five days, with the experiment repeated again after two weeks. In contrast to i.m. MDMA alone, which prevented subjects from performing or completing the task, animals performed and completed the task during regular test times on the day they received citalopram and MDMA. Citalopram (0.55 mg/kg) significantly attenuated MDMA effects on acquisition, reversal and perseverative errors on the day of MDMA administration. (Figure 5c). These results stand in apparent contrast to the data obtained with methylphenidate, which failed to attenuate MDMA inhibition of performance. In this segment, animals were monitored for two days post-MDMA and it was not feasible to make comparisons based on five days of testing. In comparison with baseline performance levels in the absence of any drug, the combination of citalopram and MDMA did not affect

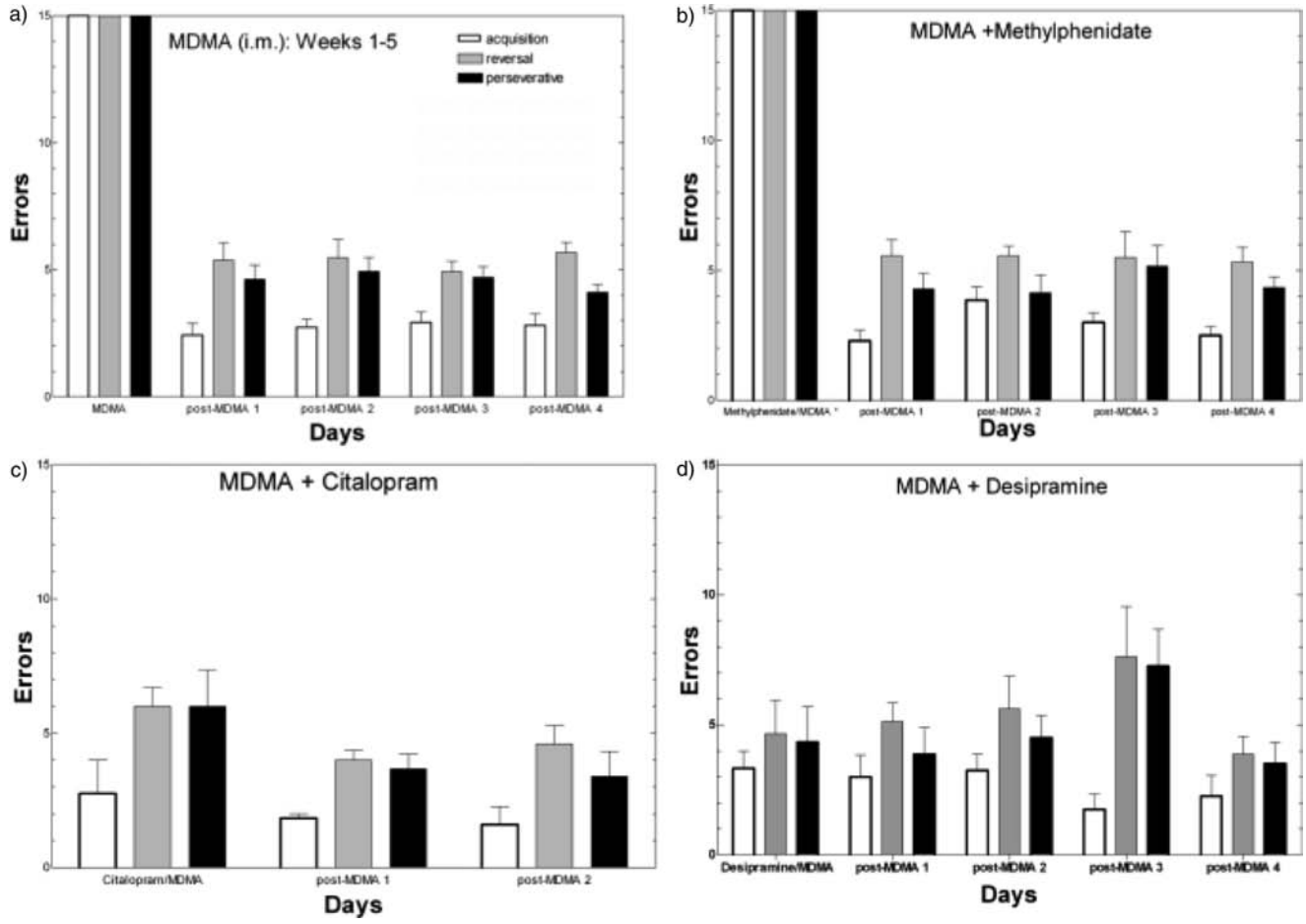

Figure 5 MDMA (i.m.) was administered for five weeks. The effects of methylphenidate (DAT), desipramine (NET) and citalopram (SERT) transport inhibitors on cognitive deficits induced by i.m. MDMA were assessed. Monkeys received MDMA (1.5 mg/kg, i.m.) once weekly and were tested with a DAT (b), SERT (c), or a NET (d) inhibitor. Citalopram (0.55 mg/kg), methylphenidate (0.3 mg/kg) and desipramine (1.5 mg/kg) were administered prior to i.m. MDMA (1.5 mg/kg) and tested twice, in two weekly sessions. a): Monkeys ($n = 4$) received i.m. MDMA (1.5 mg/kg), and failed to perform or complete each task on the day of exposure. Error rates on the day of exposure are arbitrarily set to 15 for illustrative purposes. b) The DAT inhibitor methylphenidate (p.o. or i.m., which had the same effects) did not attenuate the impaired task performance induced by MDMA. c) The SERT inhibitor citalopram (i.m.) fully attenuated the impaired task performance induced by MDMA. d) The NET inhibitor desipramine mitigated impaired task performance elicited by MDMA.

acquisition, reversal or perseverative behavior performance on the day of administration, nor were performance levels altered on the two subsequent days, indicating that citalopram attenuated compromized performance mediated by MDMA. Comparing individual weekly acquisition performances to citalopram-MDMA the combination of drugs revealed no significant treatment effect, with no increases in acquisition, reversal or perseverative errors, when analysed with repeated measures ANOVA.

MDMA and desipramine (NET inhibitor) After a hiatus of eight weeks with no testing, subjects were retrained and rapidly achieved stable task performance for 15 days. Baseline performance for the two different testing periods yielded no statistically significant differences. Subjects were again treated with i.m. MDMA once a week for four weeks. During this period, one of the four subjects completed the task on the day of i.m. MDMA day in week 2 and week 3, but not week 1 or 4. None of the MDMA-treated monkeys performed on the day of MDMA treatment in week 4, prior to the week of i.m. MDMA following pretreatment with desipramine. In week 5 and week 6, subjects were administered desipramine (1.5 mg/kg) 1.5 h prior to MDMA and 3 of 4 desipramine-treated monkeys displayed no statistically significant performance deficits. Parallel data were obtained when the experiment was repeated the following week (Figure 5d).

Spontaneous behavior: MDMA and monoamine transport inhibitors

To determine whether transport inhibitors altered spontaneous behaviors mediated by MDMA in parallel with their effects on task performance, three subjects were videotaped to monitor spontaneous activity prior to oral or i.m. MDMA exposure and after i.m. MDMA following pretreatment with three transport inhibitors. Videotapes were rated on a scale described in Table 3 and in the methods, and combined scores of three animals were averaged (Figure 6). Oral or i.m. MDMA significantly increased general activity ($P < 0.05$), and none of the monoamine transport inhibitors attenuated this enhanced wakefulness (Figure 6a). MDMA significantly increased vigilance, with i.m. MDMA being slightly more pronounced than oral MDMA (Figure 6b). Intriguingly, methylphenidate, but not citalopram or desipramine, exacerbated MDMA-induced vigilance, whereas the other drugs failed to attenuate this response. Oral MDMA significantly increased locomotor activity ($P < 0.05$, Figure 6c), whereas i.m. MDMA attenuated locomotion compared with baseline levels of activity. Citalopram, methylphenidate or desipramine did not mitigate this effect of i.m. MDMA (Figure 6c). Oral or i.m. MDMA also significantly enhanced mandibular movement (Figure 6d). Methylphenidate failed to attenuate this behavior whereas citalopram and desipramine attenuated MDMA-induced jaw activity ($P < 0.05$). We also observed that MDMA altered latency to sleep three animals following i.m. exposure, all of which showed highly individualized responses as measured by Actiwatch (Figure 7).

Discussion

The present study reports three unanticipated discoveries. A low oral dose of MDMA compromized executive function for as long as three days. If the findings generalize to human users, conceivably, a single exposure to MDMA on the weekend may decrease and compromize cognitive function several days after ingestion. Second, this is the first report to document differences in duration of cognitive deficits as a function of oral MDMA (common human route) compared with i.m. MDMA (common monkey route). An order effect may contribute to these differences, but the findings are supported by differences in the pharmacokinetics and neurotoxicity or oral and subcutaneous MDMA (Ricaurte *et al.*, 1988; Mechan *et al.*, 2005). Accordingly, it is necessary to exercise caution when extrapolating preclinical findings with i.m. MDMA to human MDMA oral use. Third, MDMA impairment of task of cognitive function was blocked by a NET, as well as a SERT inhibitor, signifying that MDMA effects on the NET and norepinephrine neurons, as well as the SERT, may contribute to compromized task performance.

Monkeys were administered MDMA orally or i.m. once weekly, at a dose (1.5 mg/kg) and dosing regimen corresponding to human patterns of MDMA consumption (Liechti and Vollenweider, 2000; Green *et al.*, 2003; Verheyden *et al.*, 2003). This dosing regimen stands in apparent contrast to much higher (scaled) and more frequent doses of MDMA doses, that generally are administered several times daily for several sequential days in primate MDMA research (e.g., Frederick and Paule, 1997; Taffe *et al.*, 2001; Mechan *et al.*, 2005). Notwithstanding important conclusions drawn from these protocols, the present study attempted to replicate a human dose and human patterns of MDMA use (a single dose, once weekly). After oral MDMA ingestion, error rates remained above baseline values for three days in a behavioral task that quantified a component of executive function designated 'shifting' (Miyake *et al.*, 2000). Increased reversal and perseverative errors reflected a compromized ability to suppress conditioned, irrelevant information and shift to a new response, in the face of a novel set of rewarding contingencies (Miyake *et al.*, 2000; Jentsch *et al.*, 2002). Our findings insinuate that an acute oral dose of MDMA may compromize cognitive function in inexperienced (or heavy MDMA users?) for as long as 72 h after a single oral dose. Prolonged error rates may result from a secondary rise in blood levels of MDMA due to release from peripheral stores, from production of an active metabolite (Green *et al.*, 2003; Mechan *et al.*, 2005) or from protracted monoamine depletion in brain. These findings and the altered latency to sleep following an oral MDMA dose may reflect lingering psychological and physiological impairment in humans several days after MDMA exposure (Curran and Travill, 1997; Vollenweider *et al.*, 1998; Parrott and Lasky, 1998; Parrott, 2001; Liechti *et al.*, 2001).

Our subjects did not voluntarily ingest MDMA after four weekly exposures, conceivably due to taste aversion to the MDMA powder embedded in small quantities of palatable foods, although they did maintain bodyweight throughout the course of the entire study. This interpretation is supported by the absence of adverse behavioral reactions of subjects to i.m. MDMA and reports that MDMA is self-administered by monkeys initially trained to self-administer cocaine (Lile *et al.*, 2005). In contrast to oral MDMA, i.m. MDMA prevented animals from performing or completing the task within several hours of exposure but performance was restored to near baseline levels by the following day (but not reversal errors). All parameters were at baseline levels on subsequent days. Rhesus monkeys treated acutely with i.m. MDMA also perform poorly in a different battery of cognitive tests, but the duration of effects on the next or subsequent days was not measured (Frederick and Paule, 1997; Jentsch *et al.*, 2002).

Notwithstanding the caveats of self-reports of MDMA usage, heavy users of MDMA display lowered neurocognitive performance as a function of extent of exposure (Parrott and Lasky, 1998; McCann *et al.*, 1999; Dafters, 2005; Gouzoulis-Mayfrank *et al.*, 2005). Inaccurate retrospective self-reports, polysubstance abuse, impure preparations, strenuous physical exertion at high temperatures, unreliable self-reporting, pre-existing psychiatric disorders and sleep deprivation have been invoked as contributory factors. Performed in MDMA-naïve subjects, the present study clearly indicates that a weekly acute oral dose of pure MDMA consistently disrupts cognitive function for three days following exposure. The contrasting magnitude and duration of effects of oral and i.m. MDMA indicate the necessity of including oral doses when investigating the behavioral effects of MDMA in preclinical models.

The next phase investigated the relative contributions of SERT, NET and DAT to the acute behavioral effects of MDMA by

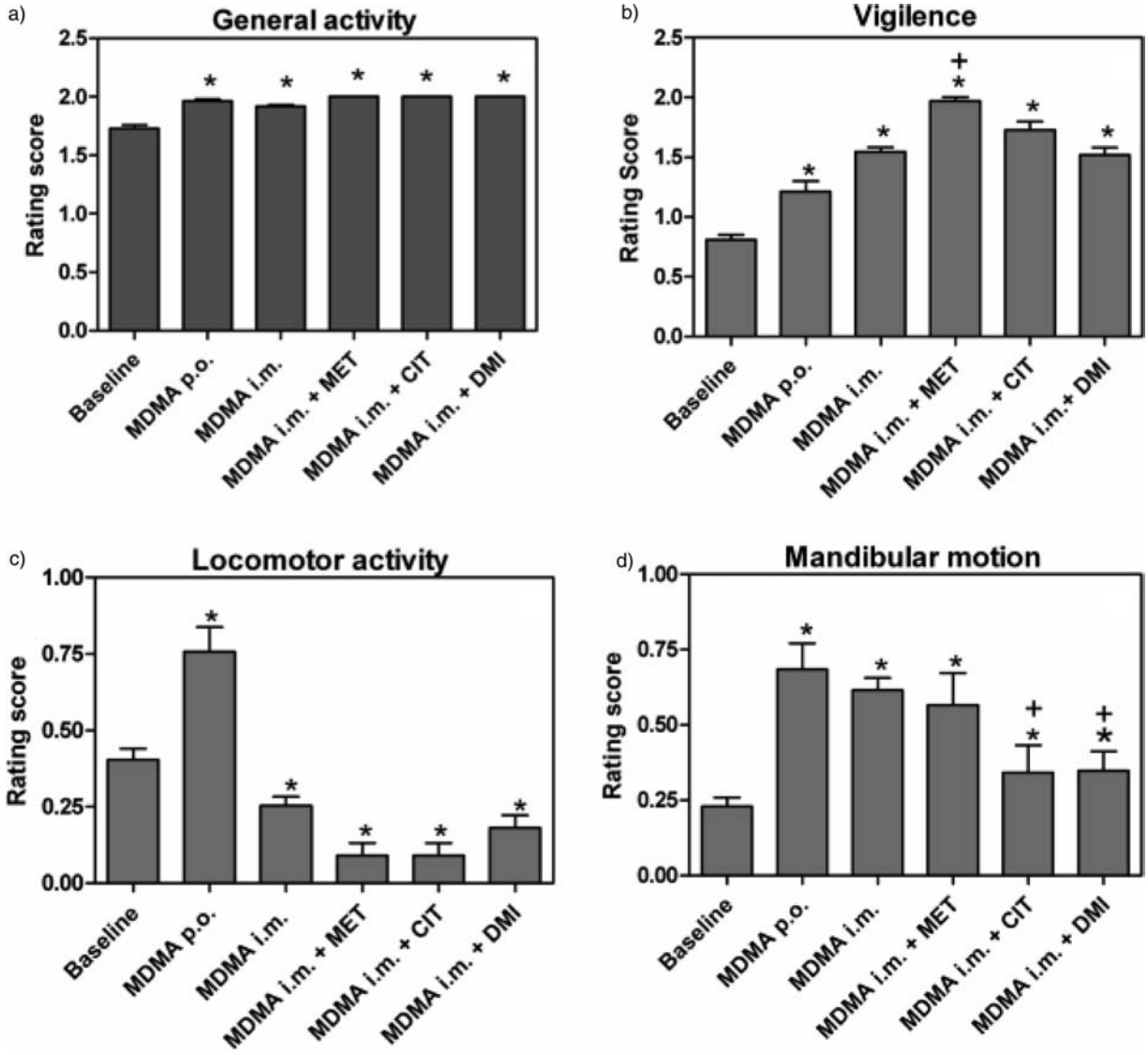

Figure 6 Spontaneous, unconditioned behaviors of monkeys at baseline, 2 h following treatment with oral or i.m. MDMA (1.5 mg/kg), or with i.m. MDMA (1.5 mg/kg) after pretreatment with the DAT inhibitor methylphenidate (MET), SERT inhibitor citalopram (CIT) or the NET inhibitor desipramine (DMI). a) Effects of MDMA alone (oral or i.m.) or i.m. MDMA combined with a DAT, NET or SERT inhibitor on general activity. Oral or i.m. MDMA increased general activity and transport inhibitors did not attenuate this response. b) Effects of MDMA alone (oral or i.m.) or i.m. MDMA combined with a DAT, NET or SERT inhibitor on vigilance (defined in Table 3). Oral or i.m. MDMA increased vigilance and this response was augmented by methylphenidate, but not by citalopram or desipramine. c) Effects of MDMA alone (oral or i.m.) or i.m. MDMA combined with a DAT, NET or SERT inhibitor on locomotor activity, Oral MDMA increased locomotor activity but i.m. MDMA reduced locomotor activity either alone or combined with transport inhibitors. d) Effects of MDMA alone (oral or i.m.) or i.m. MDMA combined with a DAT, NET or SERT inhibitor on mandible motion (Table 3). Oral or i.m. MDMA increased mandibular motion and citalopram or desipramine attenuated this response, whereas methylphenidate did not. Scores from three monkeys are averaged from 1–6 observation days.
*$P < 0.05$ versus. baseline; +, *$P < 0.05$ versus in same graph as well as baseline.

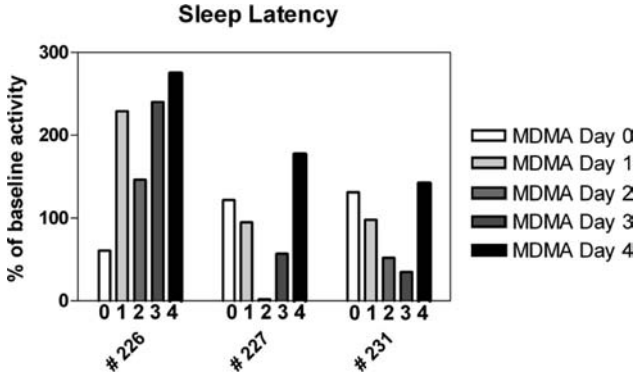

Figure 7 Sleep latency in three monkeys treated with i.m. MDMA (1.5 mg/kg). In two of three monkeys, sleep latency appeared to decrease on days 1–3 after i.m. MDMA exposure, but rose significantly on day 4 above baseline levels. In one subject, MDMA increased sleep latency for four days. For the three subjects, sleep latency on day 4 after i.m. MDMA increased.

monitoring the effects of transport antagonists on MDMA. Conceivably, transporter antagonists may reveal which monoamine transporters contribute critically to MDMA pharmacology and provide leads for reversing compromized cognition and other adverse consequences of MDMA. The transporter substrate MDMA is sequestered into monoamine neurons via DAT, NET and SERT, releases monoamines via DAT, NET, SERT and behaves as an indirect agonist or direct agonist at monoamine or trace amine receptors (Battaglia *et al.*, 1988; Hansen *et al.*, 2002; Green *et al.*, 2003; Miller *et al.*, 2005; Sulzer *et al.*, 2005; Verrico, 2007). Transport inhibitors to alleviate MDMA-induced cognitive impairment have rarely been tested (McCann and Ricaurte, 1991), although SERT inhibitors reverse acute MDMA-induced subjective, cardiovascular and immune system effects (Liechti and Vollenweider, 2000; Liechti *et al.*, 2000; Pacifici *et al.*, 2004). In the present study, inhibitors of the SERT and NET, but not the DAT/NET, reversed MDMA-induced incapacitation of monkeys and enabled task execution to proceed at baseline levels and error rates. We selected citalopram, as serotonin signaling is implicated in MDMA-induced cognitive deficits (McCann *et al.*, 1999; Green *et al.*, 2003). Methylphenidate was included to study, as MDMA elicits selective dopamine-mediated pharmacological effects (Green *et al.*, 2003), although it is a potent NET inhibitor (Madras *et al.*, 2006). We postulated that desipramine (NET inhibitor) may antagonize MDMA-induced cognitive deficits, as MDMA affinity for the human NET is higher than DAT or SERT and norepinephrine is implicated in executive function (Madras *et al.*, 2005; Verrico, 2007). *In vitro*, we discovered that K_i values for transport inhibitors were not necessarily the same for endogenous substrate blockade as for MDMA blockade. These intriguing differences in drug potencies for inhibiting the transport of [^{3}H]MDMA, in comparison with their corresponding potencies for blocking [^{3}H]NE, [^{3}H]5-HT and [^{3}H]DA may reflect differences in the binding domains of endogenous substrates compared with chemically different MDMA or differences in transporter-mediated extrusion rates of MDMA or other contrasting regulatory mechanisms (Madras *et al.*, 2005; Sulzer *et al.*, 2005). On the basis of *in vitro* data, transport inhibitors may attenuate MDMA-induced error rates by two mechanisms: citalopram may directly block MDMA entry into serotonin neurons, but desipramine, which potently blocks [^{3}H]NE but not [^{3}H]MDMA transport, conceivably attenuates MDMA entry into norepinephrine neurons by increasing competition between extracellular norepinephrine and MDMA for transport. In the absence of information on relative inhibitor occupancies of DAT, NET and SERT at doses used in this study, these postulates are considered preliminary.

Desipramine and citalopram were equally effective in enabling monkeys to perform and restoring errors rates to baseline levels, implicating the NET, as well as the SERT, as a contributor to MDMA-induced performance deficits. The NET may offer an additional therapeutic target, as citalopram attenuates, but does not eliminate, the psychoactive and adverse consequences of MDMA in human users (Liechti *et al.*, 2000; Liechti and Vollenweider, 2000). It is conceivable that citalopram and desipramine may have altered cognitive function in the absence of MDMA, but they were not tested alone. Methylphenidate, a nonselective DAT/NET inhibitor was ineffective in blocking the acute impairment induced by MDMA. Higher doses may have enhanced its pharmacological effects, but we cannot predict whether exacerbation of the psychostimulant properties of MDMA or NET-mediated attenuation of MDMA effects would ensue. Conceivably, methylphenidate ineffectiveness may be attributable to its psychostimulant synergism with MDMA. Methylphenidate also uniquely affected spontaneous behavior differently than citalopram and desipramine. It exacerbated vigilance and increased, rather than decreased mandibular movements, implicating DA/DAT in promoting both these behaviors.

The findings also have therapeutic implications. The rapid surge and depletion of serotonin or norepinephrine may contribute to the acute cognitive impairment produced by MDMA in a clinical setting or in long-term users (Cami *et al.*, 2000; Farre *et al.*, 2004; Vollenweider *et al.*, 2005). SERT blockers antagonize some, but not all of the psychoactive and cardiovascular effects of MDMA in human subjects (Liechti *et al.*, 2000; Liechti and Vollenweider, 2000). However, NET or SERT blockers have not been investigated in clinical studies to determine whether they antagonize the cognitive deficits of acute MDMA. The blockade of MDMA-induced impairment by desipramine should nevertheless be viewed with caution because of potential synergism between desipramine and MDMA on cardiovascular function (Liechti and Vollenweider, 2000). As MDMA is also a direct agonist at several receptors, it is not known whether combined SERT and NET transport inhibitors will effectively attenuate the full spectrum of pharmacological effects of MDMA.

Caveats associated with study design require consideration. It is unlikely that food reward as a motivator for task performance was compromized by MDMA, as time-to-completion of each task was not altered by MDMA, if the subjects performed. Single drug doses may reflect a narrow range of responses. The within-subject study design of i.m. MDMA administration could have caused order

effects to occur. Finally, as the majority of data were derived from male cynomolgus monkeys, our conclusions may not generalize to other primate species or to females. Human studies with both genders indicate quantifiable male-female differences in psychological, behavioral and neurochemical responses to MDMA (McCann *et al.*, 1994; Liechti *et al.*, 2001; Buchert *et al.*, 2004).

Unresolved questions include the underlying mechanisms for the persistence of cognitive deficits with acute oral but apparently not i.m. MDMA, whether differences are relevant to human users and whether extending the study will result in enduring, irreversible cognitive deficits along with monoamine neurotoxicity. The novel discovery that noradrenergic signaling is a potential contributor to MDMA-induced behavioral effects requires exploration. Will NET and/or SERT blockers alleviate the cognitive or neurochemical impairment associated with acute or prolonged MDMA (or methamphetamine) exposure in human subjects? Conceivably, this clinically relevant, experimental protocol designed for primates will facilitate documentation of MDMA consequences to cognition and pursuit of candidate medications to treat these consequences.

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