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ORIGINAL INVESTIGATION

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Effects of fenfluramine, m-CPP and triazolam on repeated-acquisition in squirrel monkeys before and after neurotoxic MDMA administration

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Abstract *Rationale:* Establishing functional deficits as a result of neurotoxic dosing regimens of MDMA has been difficult. However, moderate success has been achieved when sensitive animal models and drug challenge have been used together. *Objective:* The present study used a repeated-acquisition technique and dose-effect determinations before, during and after neurotoxic MDMA exposure to characterize the effects of serotonergic drugs on learning, and to determine if MDMA-induced serotonin (5-HT) neurotoxicity is associated with learning deficits as measured by changes in response rate or the percentage of errors. *Method:* The effects of various serotonergic drugs were characterized in six squirrel monkeys responding under a repeated-acquisition procedure before and after neurotoxic dose regimens of MDMA. Specifically, cumulative dose-effect curves for m-CPP (0.032–1 mg/kg), fenfluramine (0.1–3.2 mg/kg) and triazolam (0.0032–0.1 mg/kg) were obtained prior to MDMA administration, with the latter drug serving as a non-5-HT control. *Results:* In general, all of the drugs tested decreased overall response rate as the cumulative dose increased, whereas only triazolam markedly increased the percentage of errors. MDMA treatment produced significant (80–99%) decreases in brain 5-HT and 5-HIAA axonal markers, but did not lead to changes in either dependent measure of responding or shifts in the dose-effect curves obtained during pharmacological challenges with m-CPP, fenfluramine or triazolam. *Conclusions:* Taken together, these results demonstrate that serotonergic drugs can disrupt learning in monkeys, but indicate that MDMA-induced 5-HT neurotoxicity does not lead to disruptions in this particular type of serial learning task.

Keywords Fenfluramine · m-CPP · Triazolam · MDMA · Squirrel monkey · Repeated acquisition

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Introduction

Increasing evidence suggests that 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") can produce disruptions in learning or memory in humans (Bolla et al. 1998; Parrott et al. 1998; McCann et al. 1999b; Morgan 1999; Gouzoulis-Mayfrank et al. 2000). The pharmacological basis for these cognitive disruptions is unknown, but presumably involves MDMA-induced 5-HT neurotoxicity. However, as noted in many of the studies above, procedural aspects of clinical studies often confound data obtained from recreational MDMA users. Potential confounding variables include retrospective reporting of drug use, possible impurities or contaminants of MDMA samples, poly-drug abuse, and potential differences in intelligence, socioeconomic status and even nutrition. These factors, along with the unethical nature of conducting prospective studies of MDMA neurotoxicity in humans, make controlled preclinical laboratory studies essential for characterizing the effects of MDMA neurotoxicity on cognitive function.

Identifying preclinical research techniques that can be used in humans is a significant challenge, particularly for relatively complex cognitive abilities that require learning or memory. With cognitive studies, the problem is exacerbated by the possibility that there are different forms of learning (e.g. associative versus non-associative) and memory (e.g. semantic versus episodic), which may or may not involve different neural circuits. Consequently, there is a distinct need to use tasks that can be used with both humans and animals, and to use tasks ca-

pable of repeatedly assaying the effects of a neurotoxic insult over time (cf. Winsauer and Mele 1993). One such task is a repeated-acquisition procedure (see Thompson 1973; Thompson et al. 1987), and it is particularly well suited for studying the effects of drugs on learning because it is: 1) valid and generalizable in humans, nonhuman primates, and rodents (Desjardins et al. 1982; Moerschbaecher et al. 1987; Bickel et al. 1990; Winsauer et al. 1995), 2) able to assess both the quantity and quality of behavior (Cohn et al. 1992), 3) sensitive to numerous drugs and drug classes (Thompson and Winsauer 1985, 1986; Thompson et al. 1987), 4) capable of detecting the chronic effects of a drug (Thompson 1974; Cohn et al. 1993), and 5) useful for assessing the effects of pharmacological challenges in animals with neurotoxic lesions (Cohn and Cory-Slechta 1993).

Several investigators have recently identified a number of functional changes in MDMA-treated animals, which heretofore have shown little by way of lasting functional deficits following a neurotoxic regimen of MDMA. This has been accomplished using specific pharmacologic challenges in carefully selected neurobehavioral tests. For example, toxic regimens of MDMA have been shown to lead to abnormal neuroendocrine responses to fenfluramine (Poland et al. 1997), disruption of the discriminative stimulus effects of MDMA (Virden and Baker 1999) and suppression of behavioral, thermal and neurochemical responses (Shankaran and Gudelsky 1999). In humans, recreational MDMA use has been shown to alter various neuroendocrine and behavioral responses to *m*-chlorophenylpiperazine (m-CPP) and fenfluramine (Gerra et al. 1998; McCann et al. 1999a), drugs that interact with 5-HT in the central nervous system (Pettibone and Williams 1984; Murphy et al. 1989; Kahn and Wetzler 1991; Baumann et al. 1993; Baxter et al. 1995; Eriksson et al. 1999). Collectively, these reports suggest that the combination of an appropriately sensitive task with selective pharmacological challenge may increase the likelihood of detecting the functional consequences of seemingly silent damage or lesions (Reuhl 1991).

In the present studies, m-CPP, fenfluramine and triazolam were used to evaluate the functional consequences of MDMA neurotoxicity in primates responding in a repeated-acquisition procedure. m-CPP is a metabolite of the antidepressant trazodone that has been shown to act as an agonist at 5-HT_{2C} receptors (Kennett and Curzon 1988; Baxter et al. 1995) and to release 5-HT in vivo (Pettibone and Williams 1984). m-CPP has also been used extensively as a probe of 5-HT function in psychiatry (Murphy et al. 1989), and neurotoxin-induced deficits in the 5-HT system have been shown to alter the neuroendocrine and behavioral responses produced by m-CPP (Quattrone et al. 1981; Lucki et al. 1989; Berendsen et al. 1991). A second serotonergic drug, fenfluramine, was selected for testing because it is known to release 5-HT from presynaptic nerve terminals (Berger et al. 1992) and has been reported to alter neuroendocrine responses in male MDMA users (Gerra et al. 1998). Triazolam was used as a non-serotonergic challenge drug because its effects on repeated-acquisition in both Old-World (Brockelhurst et al. 1987) and New-World monkeys (Savage and Moerschbaecher 1996) have been characterized, and therefore, its effects in the present experiment would systematically replicate those reported previously and serve as an appropriate comparison for the effects of m-CPP and fenfluramine. After comparing the effects of cumulative doses of m-CPP, fenfluramine and triazolam in squirrel monkeys responding under a repeated-acquisition task, these effects were redetermined following two different neurotoxic regimens of MDMA. Upon completion of the behavioral studies, the neurotoxic effects of MDMA were quantified by measuring several 5-HT axonal markers 61 days after the last exposure to MDMA.

Materials and methods

Subjects

Six adult squirrel monkeys (*Saimiri sciureus*), ranging in age from approximately 7–15 years of age, were used in the behavioral studies. Three monkeys were treated with MDMA, as detailed below, and later killed for neurochemical analysis when behavioral testing was complete. Three different monkeys served as controls for the neurochemical studies. All six monkeys in the behavioral studies had an extensive history of behavioral testing under a variety of operant procedures. Each subject was maintained at approximately 85% of its free-feeding body weight on a diet consisting of banana-flavored food pellets (P.J. Noyes Co. Inc., Lancaster, N.H., USA), monkey chow, Zu/Preem Marmoset or Primate Diet, fresh fruit, peanuts and vitamins. The 190-mg banana-flavored pellets were received during the experimental sessions, whereas the remainder of the diet was fed to the subjects after each daily session. The mean weight in grams for each monkey in the behavioral study was as follows: 645 for AL, 650 for BR, 620 for CA, 650 for GE, 645 for GI and 670 for RO. All subjects were housed individually in a temperature- and humidity-controlled room that was maintained on a 12-h light-dark cycle (7 a.m.–7 p.m.). Water was available in the home cages and during the experimental sessions. In all situations, the "Guide for the Care and Use of Laboratory Animals" (National Research Council, National Academy of Sciences, Washington, D.C., 1996) was followed in conducting these experiments.

Apparatus

During each session, monkeys were seated in Plexiglas chairs (STC-300, BRS/LVE, Inc. Laurel, Md., USA), and responded on aluminum panels in one of four identical sound-attenuating test chambers. The details of these chambers and their respective response panels have been described previously (Pakarinen et al. 1995). Each experimental chamber was connected via an interface to a computer programmed in MED-PC/MEDSTATE NOTATION software (MED Associates, Inc. and Thomas A. Tatham, St Albans, Vt., USA) and to cumulative recorders (Gerbrands Corp., Arlington, Mass., USA) located in an adjacent room.

Procedure

A repeated acquisition of behavioral chains procedure served as the behavioral baseline (Thompson 1973). Briefly, in this procedure, the subject's task was to respond (key press) on a particular key in the presence of each sequentially illuminated set of colors

(e.g. keys white – center (C) correct, keys green – left (L) correct, keys red – center (C) correct, keys amber – right (R) correct). Completion of the four-response sequence turned off the keylights, illuminated the food-pellet aperture for 0.5 s and reset the sequence. The same sequence (in this example, center-left-center-right or CLCR) was repeated throughout a given session and responding on this sequence was maintained by food presentation under a second-order fixed-ratio (FR) 7 schedule; that is, every seventh completion of the sequence produced a food pellet. When the subject pressed an incorrect key, the response (error) produced a 5-s timeout during which the keylights were off and responses had no programmed consequence. To establish a steady state of repeated acquisition (learning), the four-response sequence was changed from session to session. An example of a typical set of six sequences was as follows: Left-Right-Center-Right (LRCR), CLRL, LRLC, RCRL, CLCR and RCLC, with the order of the color presentations always white, green, red and amber (reinforcement).

There were four sessions on each experimental day with a 15-min intersession interval. Each session terminated after 20 reinforcements or 20 min, whichever occurred first. When responding stabilized (30–79 days), as indicated by consistent levels of within-session error reduction, overall response rate and accuracy from session to session or day to day in each of the subjects, cumulative dose-effect curves were obtained for fenfluramine (0.1–3.2 mg/kg), triazolam (0.0032–0.1 mg/kg), and m-CPP (0.032–1 mg/kg). After the dose-effect curves for each drug had been determined at least twice, MDMA (2.5 mg/kg) was administered to three of the subjects twice per day (7 a.m. and 7 p.m.) for 4 days. Following this regimen with MDMA and several days of responding under baseline conditions, the cumulative dose-effect curves for fenfluramine, m-CPP and triazolam were redetermined (in that order). Specifically, the effects of fenfluramine were redetermined on post-treatment days 4 (RO and CA) or 6 (BR), the effects of m-CPP were redetermined on post-treatment days 8 (RO and CA) or 10 (BR), and the effects of triazolam were redetermined on post-treatment days 12 (RO), 13 (CA) or 14 (BR). Saline or vehicle was always given as a control on the day preceding drug.

Eighteen days after the regimen of MDMA and 5 days after redetermining the cumulative dose-effect curves for triazolam, a higher dose of MDMA (5 mg/kg) was administered to the same three subjects twice per day for four days at 7 a.m. and 7 p.m. Following this second dosing regimen of MDMA, cumulative dose-effect curves for each of the three drugs were redetermined on at least two occasions; for example, fenfluramine was administered to subjects RO and BR on post-treatment days 5 and 39, m-CPP was administered on post-treatment days 10 and 23, and triazolam was redetermined on days 19 and 27. With subject CA, fenfluramine was administered on post-treatment day 38, m-CPP on post-treatment day 25, and triazolam on post-treatment day 29. Control injections were generally administered prior to each administration of drug and on additional occasions after the second regimen of MDMA. Sixty-one days after the second regimen of MDMA, the three subjects were killed for neurochemical analyses. Regional brain levels of 5-HT axonal markers in the MDMA-treated animals were compared to those in three different monkeys without a history of MDMA exposure, with the tissues from the two groups of monkeys (n=3, per group) always assayed in tandem.

5-HT and 5-HIAA level determinations

Regional brain levels of 5-HT and 5-HIAA were measured by means of high performance liquid chromatography coupled with electrochemical detection (HPLC-EC), as described previously (Ricaurte et al. 1992).

5-HT transporter measurements and quantitative autoradiography

[^{3}H] Paroxetine was used to label and quantify 5-HT transporters as described previously (Ricaurte et al. 1992), whereas [^{3}H]citalopram was used in the autoradiographic studies to label 5-HT transporters (SERTs) (Fischer et al. 1995).

Drugs

Fenfluramine hydrochloride (fenfluramine) was purchased from A.H. Robbins Co. (Richmond, Va., USA), whereas m-CPP dihydrochloride (1-(3-chlorophenyl)piperazine; m-CPP) and triazolam were purchased from RBI/Sigma (Natick, Mass., USA). MDMA was obtained from the National Institute on Drug Abuse (Research Technical Branch, Rockville, Md., USA). Fenfluramine and MDMA were dissolved in saline (0.9%), m-CPP was dissolved in sterile water, and triazolam was dissolved in vehicle containing 60% propylene glycol and 40% sterile water. All of the drugs and drug vehicles were administered intramuscularly (IM) in the gluteus muscle in a volume of 0.5 ml/kg body weight.

Data analysis

The behavioral data for each session were analyzed in terms of overall response rate (total responses/min, excluding timeouts) and overall accuracy, expressed as the percentage of errors [(incorrect responses/total responses)×100]. The mean data for each subject were grouped and analyzed statistically for an effect of drug treatment using a one-way ANOVA with repeated measures (SigmaStat Statistical Software, SPSS Inc.). Mean data were excluded from the analyses for percent errors when the response rate was fewer than five responses/min because of the small number of correct and/or incorrect responses involved. Dunnett's tests were used to compare drug sessions with control (saline or vehicle) sessions. Within-session changes in responding were monitored by a cumulative recorder and the computer. For example, acquisition of a response sequence was generally indicated by a reduction in within-session errors; i.e. a decrease in the number of errors between food presentations as the session progressed.

5-HT axonal markers were analyzed by one-way ANOVA, followed by Duncan's multiple range post-hoc comparisons. Data analysis was done using the Statistical Program for the Social Sciences (SPSS for Windows, Release 6). Results from all statistical analyses were considered significant when P was <0.05 using a two-tailed test.

Results

Figure 1 shows the effects of increasing cumulative doses of triazolam, fenfluramine and m-CPP in all six subjects. For clarity, the data from each of the four sessions conducted on each day were collapsed and combined with data from other days in which either vehicle or saline was administered prior to the start of each session. As indicated by the graph in the upper panels, significant effects of treatment on response rate were obtained for triazolam [$F(4,20)=16.24$, $P<0.001$], fenfluramine [$F(4,20)=16.03$, $P<0.001$], and m-CPP [$F(4,20)=13.28$, $P<0.001$]. As indicated by the graph in the lower panel, significant effects of treatment on the percentage of errors were only obtained for triazolam [$F(2,10)=19.20$, $P<0.001$).

The effects of all three drugs on the within-session pattern of responding are shown in the cumulative records in Fig. 2. The record in the top row shows an experimental day in which vehicle was administered IM prior to the start of each session. During each of these sessions, a small number of errors occurred early in the

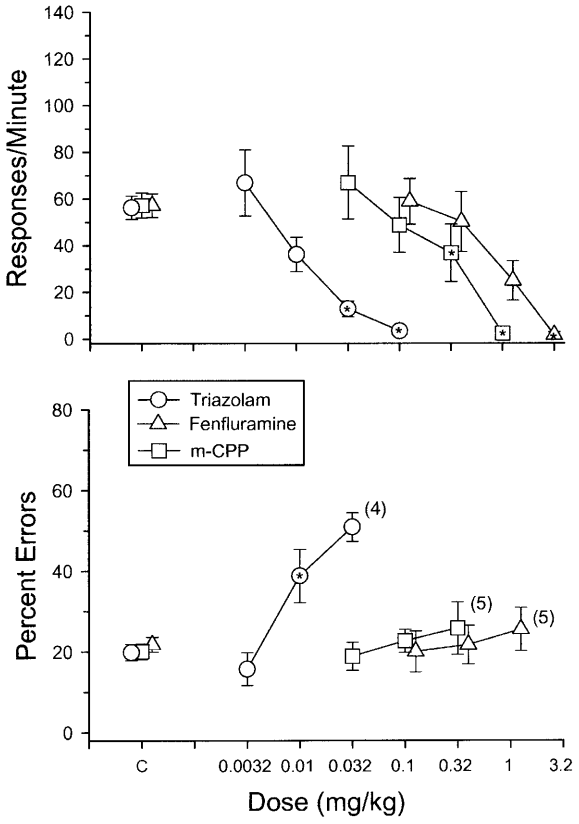

Fig. 1 Effects of triazolam, fenfluramine and m-CPP on the overall response rate and percentage of errors in six squirrel monkeys responding under a repeated-acquisition procedure. Data points and vertical lines above *C* indicate the mean and SEM for control sessions in which either saline or vehicle was administered, whereas data points and vertical lines in the dose-effect curves indicate the mean and SEM for sessions in which increasing cumulative doses of drug were administered. Any points without vertical lines (*error bars*) indicate instances in which the SEM is encompassed by the data point. *Asterisks* within the data points indicate the dosages that produced effects that were significantly different from saline or vehicle, and the values in parentheses adjacent to the data points indicate the number of subjects represented by that data point when it differed from n=6

Table 1 Regional brain 5-HT, 5-HIAA and [^{3}H]paroxetine binding in MDMA-treated squirrel monkeys and controls. Monkeys were treated with 2.5 and 5 mg/kg MDMA, as detailed in Materials and methods. Control animals were treated with saline. The animals were killed 61 days after the last MDMA treatment. Values for 5-HT and 5-HIAA are expressed in pg/mg; values for [^{3}H]paroxetine binding are expressed in DPM/mg tissue

	Control	MDMA	% Control
5-HT (pg/mg tissue)			
Frontal cortex	168±2	7±4*	4.3
Parietal cortex	103±6	1±0*	1.3
Temporal cortex	255±12	7±4*	2.9
Occipital cortex	187±5	1±0*	0.7
Hippocampus	138±7	5±1*	4.0
5-HIAA (pg/mg tissue)			
Frontal cortex	170±21	67±13*	39.2
Parietal cortex	183±2	18±4*	9.7
Temporal cortex	232±66	45±9*	9.3
Occipital cortex	192±37	26±1*	13.7
Hippocampus	185±18	36±2*	19.5
SERT (DPM/mg tissue)			
Frontal cortex	509±59	170±24*	33.4
Parietal cortex	320±38	144±23*	45.2
Temporal cortex	394±19	157±28*	39.9
Occipital cortex	412±12	176±40*	42.7

* Indicates a significant difference from controls, P<0.05, ANOVA followed by Duncan's test

session during the acquisition of each response sequence, and was followed by a high, steady rate of consecutive correct responses as the session progressed. In addition, both the overall response rate and the percentage of errors were consistent across each of the four sessions. When increasing cumulative doses of triazolam preceded four experimental sessions, however, the lowest dose was ineffective (i.e. the pattern of responding was similar to that seen in the vehicle sessions), whereas higher doses produced a large decrease in correct responding and a substantial increase in errors. At the highest cumulative dose administered (0.1 mg/kg), for example, the sequence for this session was only completed 10 times during the 20-min session and errors occurred throughout this session.

In contrast, both fenfluramine and m-CPP decreased the overall number of sequence completions as the session progressed, but these drugs did not produce the same increase in errors and acquisition was still evident after many of the higher doses. This was particularly true for fenfluramine after a cumulative dose of 1 mg/kg, which substantially decreased the number of correct responses emitted during the session, but did not increase errors substantially above control levels. Although this effect on the within-session pattern of responding was less apparent, and less graded with m-CPP, there was little or no indication of a large increase in errors similar to that observed with triazolam.

As indicated by the data in both the top and bottom panels of Fig. 3, there was little or no change in either the rate-decreasing or error-increasing effects of each drug after the two regimens of MDMA (i.e. after 4 consecutive days of twice-daily treatment with 2.5 mg/kg MDMA and after 4 consecutive days of twice-daily treatment with 5 mg/kg MDMA). In almost all cases, the dose-effect curves obtained with each drug after the two regimens of MDMA overlapped with those obtained prior to MDMA administration (suggesting little or no change in the sensitivity of the organism to the effects of these drugs). An inspection of individual subject data revealed a profile of effects that was entirely consistent with the grouped data and indicated that there were no systematic changes in either dependent measure following each dosing regimen with MDMA.

Analyses of 5-HT axonal markers 61 days after the second MDMA regimen with 5 mg/kg, however, revealed marked reductions in the frontal cortex, parietal

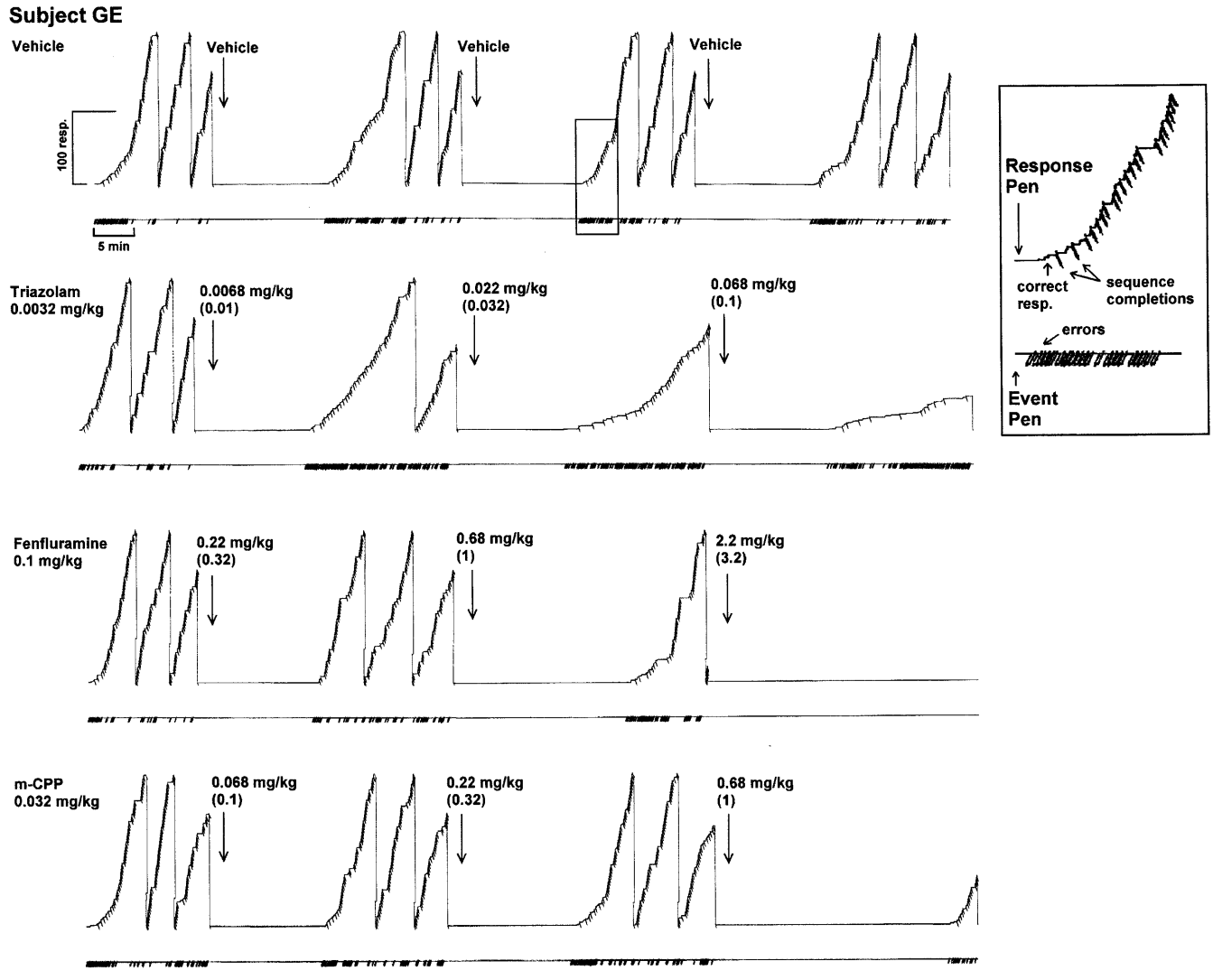

Fig. 2 Effects of triazolam, fenfluramine and m-CPP on the within-session pattern of responding during four consecutive sessions; both the dose administered and the cumulative dose are indicated for each session. In each record, as exemplified by the inset in the upper right-hand corner of the figure, the response pen stepped upward with each correct response and was deflected downward each time the four-response sequence was completed. Errors are indicated by the event pen (below each record), which was held down for 5 s during each timeout. The stepping pen reset either at the top of the page or at the end of each session

cortex, temporal cortex and occipital cortex of each of these three experimental squirrel monkeys when compared to levels established in the three monkeys that served as control subjects. These reductions are evident in the values presented in Table 1 where mean levels of 5-HT, 5-HIAA (in ng/mg) and SERT (in DPM/mg tissue) were substantially decreased in each area of the brain examined. The MDMA-induced deficits in SERT were also clearly revealed by autoradiography (see Fig 4).

Discussion

In the present study, triazolam, fenfluramine and m-CPP produced comparable dose-dependent rate-decreasing effects in squirrel monkeys responding under a repeated-acquisition procedure. Triazolam, but not fenfluramine or m-CPP, dose-dependently decreased accuracy of responding with increasing doses of drug. Neurotoxic MDMA regimens that led to profound decrements in brain 5-HT axonal markers did not change either baseline response rate or accuracy. Further, the effects of triazolam, fenfluramine and m-CPP in MDMA-lesioned monkeys were similar to those observed prior to MDMA administration. These data support the fact that serotonergic drugs can disrupt learning by decreasing the quantity (rate) of behavior, but indicate that certain types of learning and/or complex behavioral responses may not be particularly sensitive to rather profound changes in central serotonergic mechanisms.

The effects of triazolam, fenfluramine and m-CPP obtained in monkeys prior to MDMA administration were consistent across the range of doses tested for each drug,

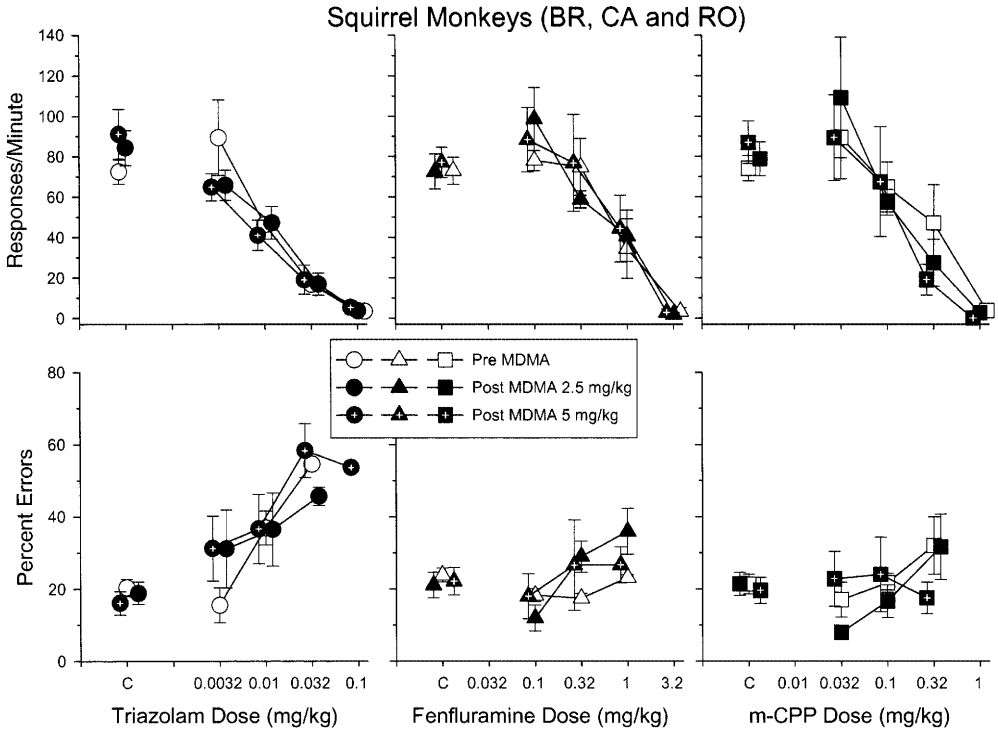

Fig. 3 Effects of triazolam, fenfluramine and m-CPP on overall response rate and percentage of errors in squirrel monkeys BR, CA and RO after the administration of two different dosage regimens of MDMA. For other details, see legend for Fig. 1

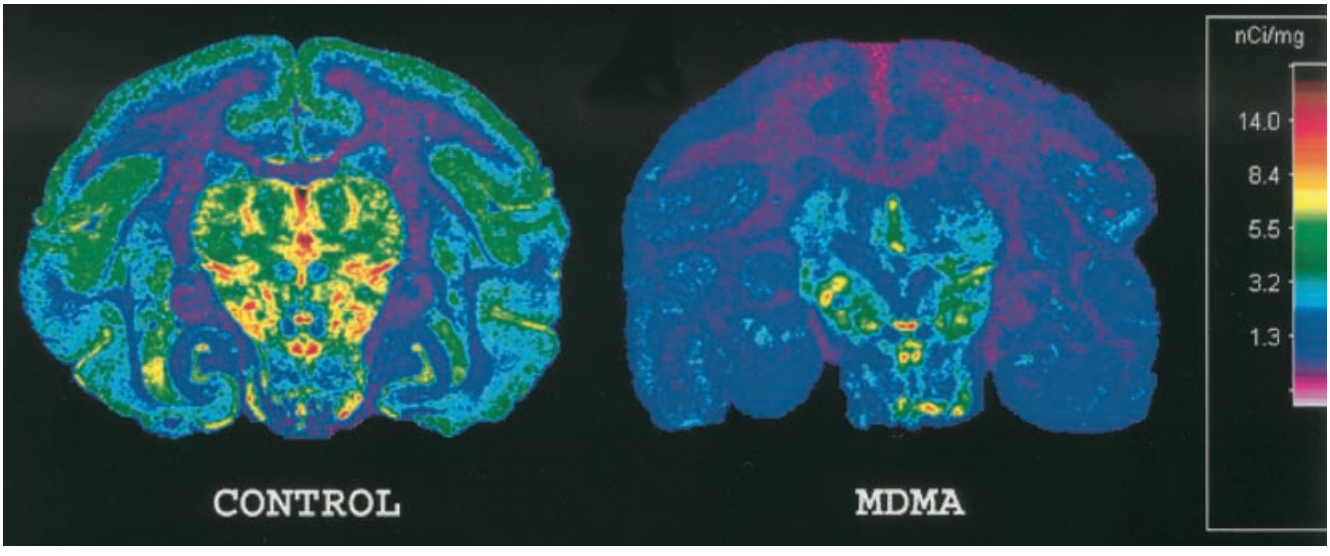

Fig. 4 Representative autoradiogram showing the distribution of [^{3}H]citalopram-labeled SERT in a coronal section of an untreated (*lefthand section*) and an MDMA-treated (*righthand section*) squirrel monkey brain. Visible in these sections are the frontal/temporal cortex, basal ganglia and hippocampal formation. In the control animal, there is intense ligand binding in the hippocampus and basal ganglia, with less binding in the neocortex. Note that MDMA administration produced marked reductions in SERT ligand binding in all represented brain structures

and with a small amount of data in several previous reports on the effects of these drugs on operant behavior in squirrel monkeys. There are surprisingly few reports describing the effects of fenfluramine on schedule-controlled behavior in squirrel monkeys, and even fewer (if any) describing their effects on complex operant behavior involving the acquisition of behavioral response chains. In rats, for example, Sabol et al. (1995) have shown that fenfluramine can disrupt inter-response intervals in rats responding under a DRL 36-s schedule and it has been shown to produce dose-dependent rate-decreasing effects on responding under a fixed-ratio (FR) schedule of food presentation (Taylor 1973) and in a FR-discrimination task (Zhang et al. 1995). Notably, in the FR-discrimination task where reinforcement of a left or right lever response depended upon the completion of a FR8 or FR16 on the center lever, fenfluramine had no effect on accuracy. In one of the few studies involving nonhuman primates, Frederick et al. (Frederick et al.

1998) reported that *d*-fenfluramine dose-dependently decreased response rate in an incremental repeated-acquisition task that was part of an operant battery of tests. Although accuracy data were not presented, the authors stated that the acute disruptive effects of *d*-fenfluramine were concomitant with dramatic decreases in response rates.

Unlike fenfluramine, the effects of m-CPP have been examined in squirrel monkeys responding under operant schedules of reinforcement, fixed-interval schedules in particular (Brady and Barrett 1985; McKearney 1989, 1990). Brady and Barret (1985), for example, found that m-CPP, TFMPP (trifluoromethylphenylpiperazine), MK-212 (6-chloro-2(1-piperazinyl)pyrazine) and *l*-5-HTP (*l*-5-hydroxytryptophan) decreased rates of responding under two different fixed-interval (FI) schedules, one maintained by shock presentation and one maintained by food presentation. Although a 7-fold larger dose was necessary to reduce shock-maintained responding to 50% of control than to produce an equivalent reduction in food-maintained responding, m-CPP decreased FI responding across a similar dose range to that used in the present study (i.e. 0.03–10 mg/kg).

The effects of triazolam on responding were comparable to those previously obtained in squirrel monkeys (Savage and Moerschbaecher 1996), Old-World monkeys (Moerschbaecher et al. 1987) and humans (Bickel et al. 1990) responding under repeated-acquisition procedures. Savage, for example, looked at the effects of triazolam under both chain strained-ratio and tandem strained-ratio schedules of repeated acquisition and found that triazolam produced rate-decreasing and error-increasing effects under both learning conditions. In patas monkeys responding under a multiple schedule of acquisition and performance of conditional discriminations, triazolam produced dose-related decreases in response rates in both components of the multiple schedule, and disrupted accuracy in the learning component at doses lower than those required to disrupt accuracy in the performance component (Moerschbaecher et al. 1987). Given these data across several species of monkey and in humans responding in similar learning tasks, it has been proposed that the addition of the triazolo-ring to the benzodiazepine structure produces compounds (e.g. triazolam and alprazolam) with greater ability to disrupt learning than other benzodiazepines, perhaps rendering them high efficacy allosteric modulators at either specific $GABA_A$ receptor subtypes or specific benzodiazepine receptors on the $GABA_A$ complex (Bradley and Nicholson 1984; Thompson et al. 1995; Winsauer et al. 1996; Auta et al. 2000). Certainly, in this study, triazolam was more disruptive to the accuracy of responding than either m-CPP or fenfluramine.

The absence of a clear shift in the dose-effect curves in MDMA-lesioned monkeys for either fenfluramine or m-CPP was somewhat surprising, and deserves comment. Given that both m-CPP and fenfluramine exert many of their effects by acting on 5-HT systems, changes in the behavioral effects of these two drugs were anticipated, either as a result of postsynaptic upregulation of 5-HT receptors (e.g. Nelson et al. 1978) or the 5-HT denervation frequently produced by MDMA (e.g. Ricaurte et al. 1988). The fact that this was not observed suggests several possibilities, including the possibility that the observed effects on response rate were not mediated by the 5-HT system. Another possibility is that even a small fraction of residual 5-HT axon terminals is sufficient to mediate the behavioral effects of m-CPP and fenfluramine. Alternatively, some type of compensation occurred, both within the 5-HT system and/or other neural systems (Kalivas et al. 1998). There is also the possibility that the pharmacologic effects of these drugs may have been altered, but responding under this behavioral baseline was sufficiently conditioned as to overcome these changes (i.e. a form of behavioral tolerance). Additional studies are needed to determine which, if any, of these possible factors may be relevant. Notably, previous studies that have used operant procedures (Meneses and Hong 1997; Kant et al. 1998), including similar repeated-acquisition procedures (Winsauer et al. 1996, 1999), have shown that the effects of specific agonists for the various 5-HT receptor subtypes can vary substantially in terms of their ability to disrupt learning and memory processes. These factors alone could obscure possible explanations along with the possibilities that 1) only a few of the 5-HT receptor subtypes have the ability to play more than a modulatory role in the learning processes of animals, 2) learning tasks that require only working memory may be inherently less susceptible to disruption than tasks requiring further consolidation and storage of memory, 3) well-rehearsed strategies for learning may be difficult to disrupt (even subtly), and 4) the loss of 5-HT activity resulting from destruction of 5-HT axonal terminals may not uniformly affect responses mediated by postsynaptic receptors.

Demonstrating functional deficits as a result of a neurotoxic regimen of MDMA has been difficult, and in the preponderance of animal studies that have been conducted, few deficits have been reported either across behavioral tasks or experimental species (see Ricaurte et al. 1993). In rhesus monkeys performing an operant test battery that included a incremental repeated-acquisition task, Frederick et al. (1998) found that baseline responding (non-drug or vehicle) was not significantly altered after short-course high-dose administration to MDMA, and that residual tolerance to the acute effects of MDMA was evident for up to 6 months after high-dose administration. In another study involving rhesus monkeys where increasing doses of MDMA were each administered twice a day for 14 consecutive days (Frederick et al. 1995), this same group of investigators reported that responding on each of the tasks in an operant test battery did not significantly change after treatment was stopped and that varying degrees of behavioral tolerance occurred in all subsequent tests of the acute effects of MDMA. Indeed, to a large extent, those studies that have reported functional deficits following neurotoxic regimens of MDMA have involved rats (e.g. Battaglia and

De Souza 1989; Li et al. 1989; Byrne et al. 2000). However, even in rats, some investigators have reported finding little or no functional deficits following a neurotoxic regimen of MDMA (Ricaurte et al. 1993; Robinson et al. 1993) or 5,7-DHT (Soubrie et al. 1986).

In summary, although the present study demonstrated differences in the rate-decreasing and error-increasing effects of two serotonergic drugs (m-CPP, fenfluramine) and a nonserotonergic drug (triazolam) in squirrel monkeys responding under a repeated-acquisition task, it failed to demonstrate any functional deficits in learning after neurotoxic serotonergic injury by MDMA. Furthermore, pharmacological challenge with fenfluramine, m-CPP or triazolam in MDMA-lesioned monkeys did not unmask learning deficits that were not apparent in baseline measures of responding. These data are in keeping with other animal studies involving complex operant behavior where few or no functional deficits were seen following MDMA alone, and some animal studies utilizing drug challenges after serotonergic depletion. Together, these studies indicate that there may be differential disruptions of behavior following large depletions of 5-HT that probably depend on several variables including the type of cognitive function involved and specific 5-HT receptor subtypes. Clearly, further work is necessary to understand the functional consequences of toxic MDMA administration in both animals and humans.

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