Pergamon

Pharmacology Biochemistry and Behavior, Vol. 49, No. 3, pp. 599–607, 1994

0091-3057/94 $6.00 + .00

0091-3057(94)00184-7

Methylenedioxyamphetamine: A Selective Effect on Cortical Content and Turnover of 5-HT

ANTHONY G. ROMANO,[1] WEI DU AND JOHN A. HARVEY

Division of Behavioral Neurobiology, Department of Pharmacology, Medical College of Pennsylvania, 3200 Henry Avenue, Philadelphia, PA 19129

Received 14 January 1994

ROMANO, A. G., W. DU AND J. A. HARVEY. *Methylenedioxyamphetamine: A selective effect on cortical content and turnover of 5-HT.* PHARMACOL BIOCHEM BEHAV **49**(3) 599–607, 1994.—This study examined the effects of the hallucinogen, MDA, on brain content of monoamines and their metabolites in the rabbit. A single 1.8 mg/kg dose of MDA produced 30 to 64% increases in the 5-HT content of frontal cortex from 30 to 120 min after injection and a decrease in 5-HT turnover from 30 min to 8 h, but had no effect in hippocampus, caudate nucleus, or hypothalamus. A single 3.6 mg/kg dose of MDA also reduced the turnover of 5-HT in frontal cortex, but this was accompanied by a decrease in 5-HIAA with no increase in 5-HT. The 1.8 and 3.6 mg/kg doses of MDA had no significant or consistent effects on the contents of DA, DOPAC, HVA, and NE in any brain area examined. Chronic administration of MDA (3.6 mg/kg/day for 4 days) failed to produce any evidence of a neurotoxic action on 5-HT neurons. Higher doses could not be employed because the LD_{50} of MDA was approximately 5 mg/kg. This study has demonstrated that behaviorally effective and nonneurotoxic doses of MDA produce increases in the content and decreases in turnover of 5-HT in frontal cortex that resemble those of other hallucinogens such as LSD and DOM.

Methylenedioxyamphetamine Serotonin Catecholamines Hallucinogens Rabbit Frontal cortex Hippocampus

METHYLENEDIOXYAMPHETAMINE (MDA) is a psychedelic drug with effects that resemble those of other phenethylamine hallucinogens such as *d,l*-2,5-dimethoxy-4-methylamphetamine (DOM), as well as indoleamine hallucinogens such as *d*-lysergic acid diethylamide (LSD). Drug discrimination studies in rats have shown that the stimulus properties of racemic MDA resemble those of both DOM and LSD (7,8). In the rabbit, MDA, DOM, and LSD share the unique ability to enhance the acquisition of conditioned responses (CRs) during classical conditioning of the nictitating membrane (NM) response (11,22,23,26). The similarities in the behavioral effects of MDA, DOM and LSD may be due to actions at a common receptor. Evidence from a variety of sources strongly suggests that the effects of both indoleamine and phenethylamine hallucinogens may be due to actions at 5-HT_2 and/or 5-HT_{1C} receptors (6). Using the newly proposed nomenclature (15) these receptors are now classified as 5-HT_{2A} and 5-HT_{2C}, respectively. Most recently, it has been confirmed that hallucinogenic drugs, including MDA, LSD, and DOM, are direct agonists at the 5-HT_{2C} receptor, and that their potency at this receptor is highly correlated with their potency in eliciting behavioral effects in animals and humans (24).

The systemic injection of LSD or DOM has been shown to decrease the turnover of 5-HT in brain as measured by an increase in the content of 5-HT and a decrease in its metabolite, 5-HIAA (4,5,9,16,19), an effect that is due to the stimulation of 5-HT receptors (1). In contrast, a single, acute dose of MDA (10 mg/kg) has been reported to produce decreases in both 5-HT and 5-HIAA at 3 h (27), and this effect was still present at 14 days after injection (20). The decreases in 5-HT and 5-HIAA were more prominent after chronic administration and occurred in cortex, hippocampus, neostriatum (caudate), and hypothalamus of the rat (2,20,27). However, the doses of MDA employed (10 to 40 mg/kg) also produced clear evidence of neurotoxicity in the rat as measured by the loss of 5-HT terminals in forebrain (2,18,20) and brain stem (10).

[1] To whom requests for reprints should be addressed.

The acute doses of MDA (10 mg/kg) that have been employed to demonstrate decreases in cerebral content of 5-HT and 5-HIAA are well above those that produce detectable, behavioral effects. For example, in the drug discrimination studies cited above, the training dose of MDA in the rat is typically 1.5 mg/kg (7,8). In the monkey, operant responding is almost totally disrupted by MDA at doses of 1.7 mg/kg (28). In the rabbit, MDA produces a significant enhancement of learning at doses of 0.54 to 1.8 mg/kg (22), an effect that is lost at a higher dose of 3.6 mg/kg (23). It is possible, therefore, that these lower, behaviorally effective and presumably nonneurotoxic doses of MDA might have effects on brain content of 5-HT and 5-HIAA that would more closely resemble those of other hallucinogens such as DOM and LSD. The present study examined these possibilities.

We first determined the lethality of MDA in the rabbit as measured by 24-h survival times, to employ sublethal doses in subsequent experiments. We next determined whether the highest sublethal dose of MDA (3.6 mg/kg), given once a day for 4 days, would have a neurotoxic effect on 5-HT neurons as determined by immunocytochemical procedures. Finally, we examined the effects of MDA (1.8 mg/kg) on the turnover of 5-HT as measured by changes in the brain content of 5-HT and 5-HIAA in frontal cortex, hippocampus, caudate nucleus, and hypothalamus, as well as the time course and dose dependency of these effects in frontal cortex and hippocampus. Contents of the catecholamines NE and DA and the DA metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), were also measured in each experiment.

METHOD

Subjects

New Zealand White rabbits of both sexes, weighing 2–3 kg, were obtained from either Ace Animals Inc. (Boyertown, PA) or Hazleton Research Animals (Denver, PA). Rabbits were individually housed and had free access to food and water. The colony room was illuminated according to a 12 L : 12 D cycle.

Drugs

MDA (*d,l*-methylenedioxyamphetamine hydrochloride), obtained from the National Institute on Drug Abuse, was dissolved in sterile physiological saline just prior to use. MDA and its vehicle were injected SC in a volume of 1 ml/kg at doses of 1.8 to 10.8 mg/kg as the base.

Lethality of MDA in the Rabbit

Separate groups of rabbits were injected with a single SC injection of MDA at doses of 1.8 (n = 12), 3.6 (n = 12), 5.4 (n = 8), or 10.8 (n = 7) mg/kg and their 24-h survival was recorded. Four rabbits were injected with MDA, twice a day, for 4 days at a dose of 2.7 mg/kg (5.4 mg/kg/day) and their survival time was recorded.

Effect of Chronic Injection of MDA on 5-HT Neurons as Measured by Immunocytochemistry

Six rabbits were divided into two groups of three rabbits each. One group was injected with saline vehicle and the other with MDA at a dose of 3.6 mg/kg given once a day for 4 consecutive days. Fourteen days after the last injection, rabbits were perfused transcardially, under deep sodium pentobarbital anesthesia (75 mg/kg, IV, Sigma Chemical Co., St. Louis, MO), with ice-cold phosphate buffered saline (PBS; pH 7.4) followed by ice-cold 4% paraformaldehyde dissolved in a 0.15 M phosphate buffer (PB; pH 7.4). The brains were removed and placed sequentially in ice-cold solutions for the times indicated: 48 h in the 4% paraformaldehyde dissolved in PB; 12 h in 10% (w/v) sucrose dissolved in PB; and 24 h in 20% (w/v) sucrose dissolved in PB. Brains were then frozen and 30-μm thick coronal sections were cut in a cryostat and placed in ice-cold PBS. The free-floating sections were then incubated for 24 h on a rotating platform at room temperature with an anti-5-HT antisera obtained from Incstar Corporation (Stillwater, MN). The antibody was diluted 1:10000 in PBS containing 1% normal goat serum and 0.3% Triton X-100. 5-HT-like immunoreactivity was visualized with the avidin-biotin-peroxidase complex (ABC) kit supplied by Vector Laboratories, Inc. (Burlingame, CA) which is based on a method described elsewhere (14). Sections were then mounted on chrome alum-gelatin coated slides. The average optical density of the immunoreactivity was measured in various brain regions by means of a Bioquant Image Analysis System (R & M Biometrics, Inc., Nashville, TN) and expressed as average optical density as previously described (29).

Effect of a Single Injection of MDA on Monoamines and Their Metabolites

Three separate experiments were carried out. In Experiment 1, 12 rabbits were divided into two groups of 6 rabbits each. One group was injected with saline vehicle and the other with MDA (1.8 mg/kg). One-half hour later, all rabbits received a lethal dose of sodium pentobarbital, the brain of each rabbit was rapidly removed from the skull, the frontal cortex, hippocampus, hypothalamus, and caudate nucleus were frozen in crushed dry ice within 9 min of the pentobarbital injection, and stored at −70°C until the time of assay. The frontal cortex was obtained by making a coronal cut that passed just rostral to the head of the caudate nucleus and the cortical gray matter was then removed for assay. The hypothalamus was dissected from the base of the brain, after which the hippocampus and caudate nuclei were obtained following a midsagittal cut through the brain.

In Experiment 2, 18 animals were evenly distributed among six groups. Rabbits were injected with saline vehicle or MDA (1.8 mg/kg) followed by a lethal dose of sodium pentobarbital either 0.5, 1, 2, 4, or 8 h later. Rabbits receiving the saline vehicle served as the 0 h time point. The brains were removed

TABLE 1

24-HOUR SURVIVAL TIME AFTER SC INJECTION OF MDA

MDA Dose (mg/kg)	Survival Ratio*	Percentage Surviving
1.8	12/12	100
3.6	12/12	100
5.4	3/8	38
5.4 (2 × 2.7)†	1/4	25
10.8	1/7	14

*Number surviving/number tested.

†Animals were given two doses of 2.7 mg/kg spaced 12 h apart. Only one animal was alive 12 h after the second injection (24 h after the first injection).

and the frontal cortex and hippocampus of each animal were frozen and stored as described above.

Finally, in Experiment 3, nine animals were evenly distributed among three groups and injected with saline vehicle or MDA at doses of 1.8 or 3.6 mg/kg. Two hours later, rabbits received a lethal dose of sodium pentobarbital, the brains were removed, and the frontal cortex and hippocampus of each animal were frozen and stored as described above.

The amines 5-HT, DA, and NE, and their major metabolites, 5-HIAA, HVA, and DOPAC, were analyzed according to a modification of a procedure published elsewhere (33). For Experiments 2 and 3, approximately 50 mg of tissue was sonicated in 200 μl of 0.1 M perchloric acid containing 0.3 mM Na_2EDTA and 0.5 mM sodium meta-bisulfite. Isoproterenol (30 ng in 30 μl) was added as an internal standard to each tube prior to sonication. Tissue was sonicated on ice for 15 s using a Fisher sonic dismembrator at 50% relative output. Homogenates were centrifuged at 4°C at 48,000 × g for 15 min. Supernatants were placed in sample vials in a refrigerated autosampler. Samples were injected into a high performance

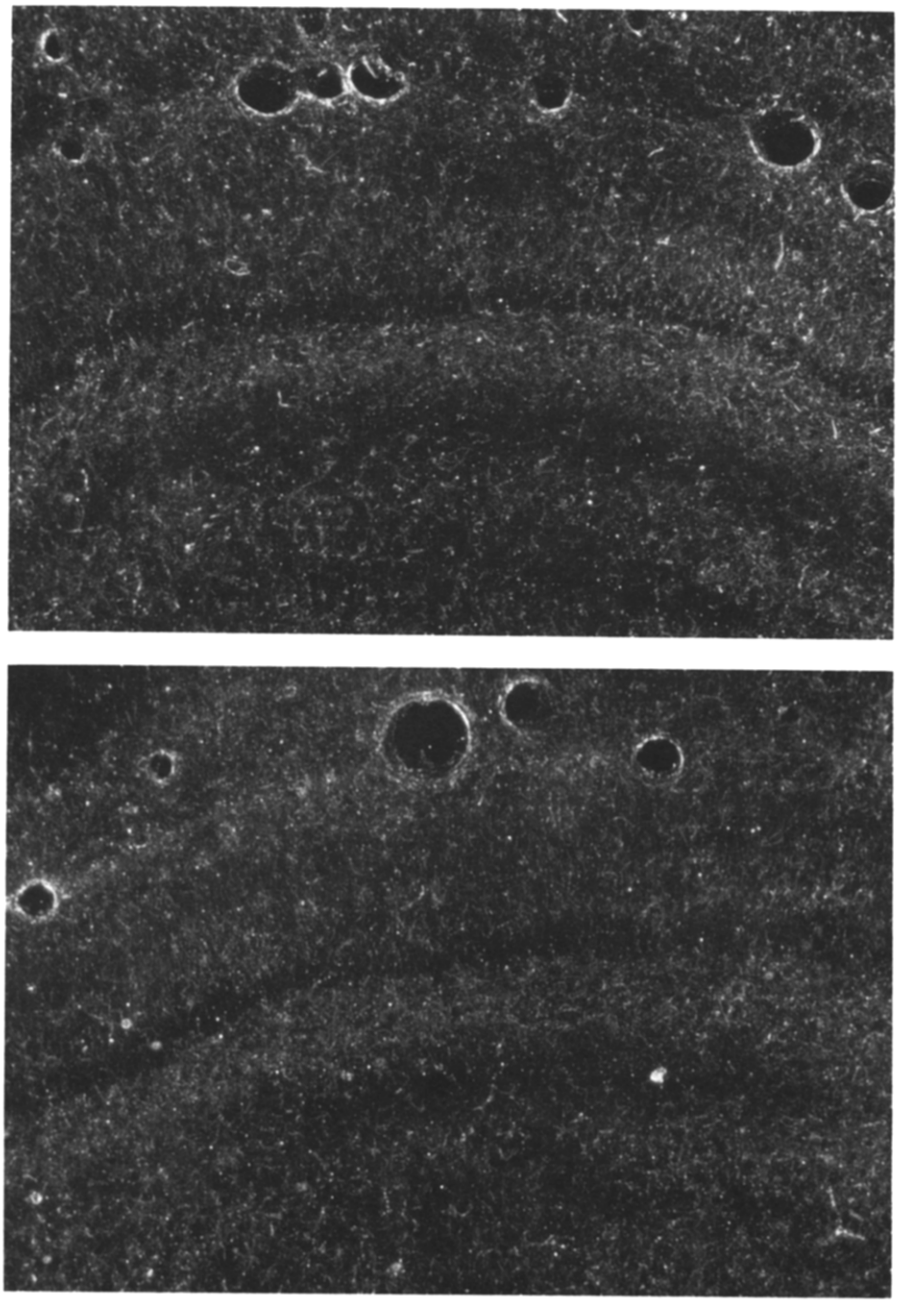

FIG. 1. Dark-field photomicrographs (35.5×) of 5-HT-like immunoreactive neurons in the hippocampus of two rabbits given either saline vehicle (top panel) or MDA (3.6 mg/kg) once a day for 4 days (bottom panel). Survival time was 14 days.

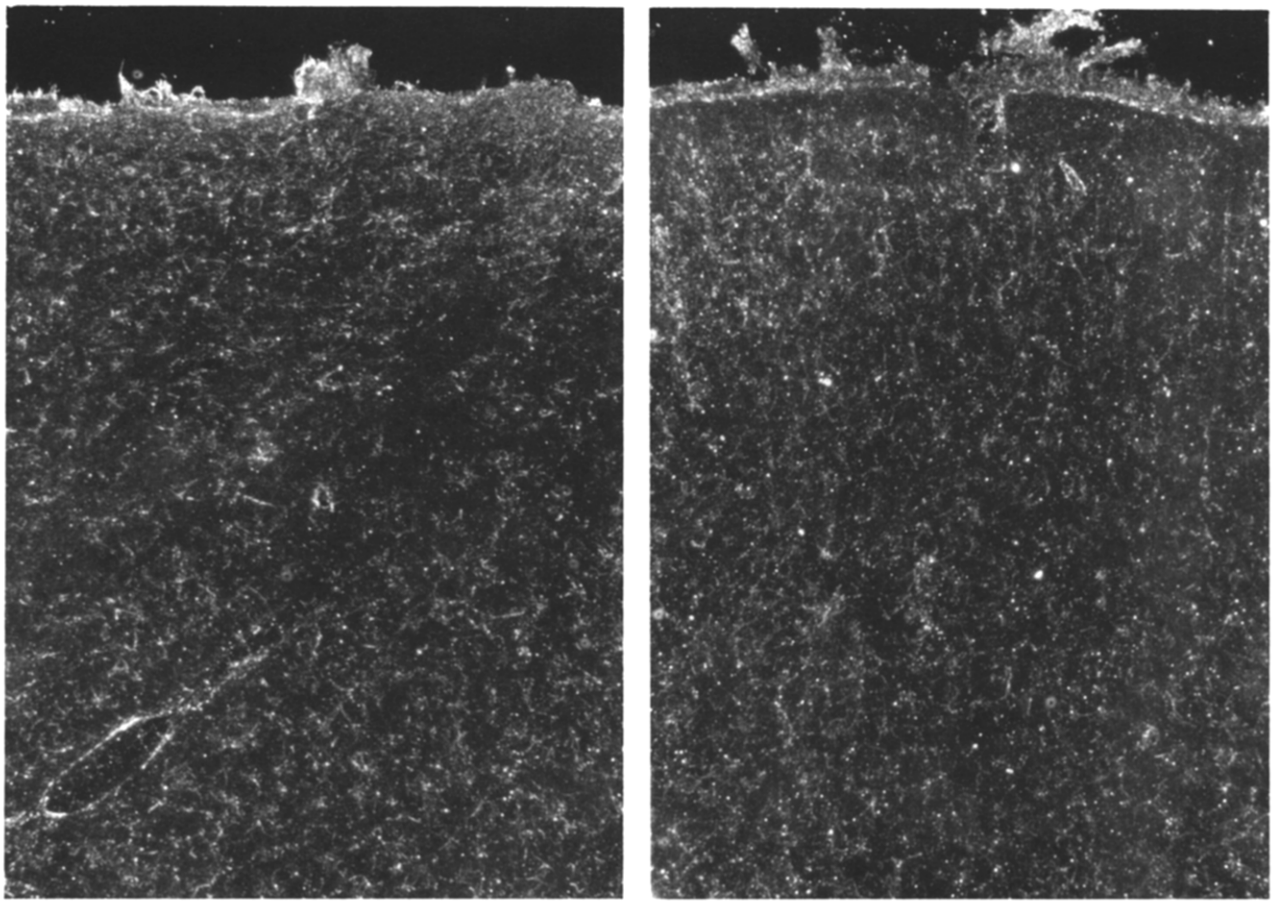

FIG. 2. Dark-field photomicrographs (35.5×) of 5-HT-like immunoreactive neurons in the frontal cortex of two rabbits given either saline vehicle (left panel) or MDA (3.6 mg/kg) once a day for 4 days (right panel). Survival time was 14 days.

liquid chromatograph, separated by a C18 ODS, 250 × 4.6 mm, 5 μm column (Biophase, Bioanalytical Systems) and measured amperometrically by an electrochemical detector (LC-4B, Bioanalytical Systems) set at +0.8 V. The mobile phase consisted of 100 mM citrate buffer including 0.3 mM Na_2EDTA, 5% (v/v) acetonitrile and 0.334 mM octylsulfate at a pH of 2.35. The flow rate was 1.2 ml/min. The same procedures were employed for Experiment 1 except that dihydroxybenzylamine (10 ng/10 μl in 0.01 N HCl) served as the internal standard, and the mobile phase consisted of 50 mM Na acetate, 20 mM citric acid, 0.5 mM Na_2EDTA, 1.1 mM octylsulfate, 1 mM di-n-butylamine, 15% (v/v) methanol at pH 3.8. The peak-area ratios of the amine or metabolite to internal standard in each sample were compared to the ratios of the standard to internal standard at known concentrations. Quantitations were done by linear regression analysis. Results were expressed as nmol/g of tissue, fresh weight.

Data Analysis

One-way analyses of variance were carried out on the various measures using the SYSTAT statistical package, version 5.0 (35). Follow-up tests were performed with the method of Dunnett to allow comparison with vehicle controls (36). Significance for all statistical comparisons was set at $p \leq 0.05$, two-tailed test.

RESULTS

Lethality of MDA in the Rabbit

The lethal effects of MDA are presented in Table 1. All animals given a single dose of 1.8 or 3.6 mg/kg survived over the next 24 h and were normal in appearance and in general patterns of behavior. However, a single dose of 5.4 mg/kg produced convulsive behavior and death in five of eight animals within 24 h after injection. An injection of 10.8 mg/kg produced convulsion and death within 24 h in six of seven animals. Three of four animals receiving MDA doses of 2.7 mg/kg spaced 12 h apart (i.e., 5.4 mg/kg/day) also died within 24 h (within 12 h of the second injection). The fourth animal survived the 4 days of MDA injections (Table 1). These data indicate that the LD_{50} of MDA in the rabbit is approximately 5 mg/kg. Based on these findings, the following studies did not employ doses greater than 3.6 mg/kg.

Effect of Chronic Administration of MDA on 5-HT Neurons as Revealed by Immunocytochemistry

All three rabbits injected with MDA at a dose of 3.6 mg/kg, given once a day for 4 consecutive days, survived and appeared healthy throughout the experiment. Brains obtained 14 days after the last injection of saline or MDA failed to reveal any consistent or significant differences in the density,

distribution, or appearance of the 5-HT-like immunoreactive neurons. Representative photomicrographs of sections taken through hippocampus and frontal cortex are presented in Figs. 1 and 2, respectively. The lack of any evidence of a neurotoxic effect of MDA on 5-HT neurons in the rabbit brain was supported by optical density measurements in the regions depicted in Figs. 1 and 2. There were no significant differences in average optical densities in either frontal cortex, $F(1, 4) = 1.21$, or hippocampus, $F(1, 4) < 1$. The actual values in frontal cortex were: saline vehicle, 56.7 ± 1.0; and MDA, 58.2 ± 0.8. For hippocampus, the values were: saline vehicle, 60.8 ± 0.9; and MDA, 59.7 ± 1.5.

Effect of a Single Injection of MDA on Brain Content of Monoamines and Their Metabolites

In Experiment 1, MDA (1.8 mg/kg) produced a significant ($p < 0.05$) increase of 30% in the 5-HT content of frontal cortex at 30 min after injection but had no effect on the 5-HT content of hippocampus, hypothalamus, or caudate nucleus (Fig. 3). There was no significant effect of MDA on the content of 5-HIAA, NE, or DA and its metabolites, DOPAC and HVA (Fig. 3).

In Experiment 2, MDA (1.8 mg/kg) again produced a significant ($p < 0.025$) increase in the 5-HT content of frontal cortex (Fig. 4, upper left panel). This increase ranged from 35 to 47% above control values over the first 2 h after MDA injection and then declined to control levels. Follow-up tests using Dunnett's t indicated that 5-HT was significantly elevated in frontal cortex only at 0.5 and 2 h after injection of MDA. In agreement with Experiment 1, there was no significant effect of MDA on the 5-HT content of hippocampus or on the contents of 5-HIAA in either frontal cortex or hippocampus. Also in agreement with Experiment 1, MDA had no effect on the content of DA or its metabolites, DOPAC and HVA, in either frontal cortex or hippocampus. By contrast, the content of NE in frontal cortex increased over time, reached a peak at 2 h, and declined thereafter, $F(5, 12) = 4.33$, $p < 0.025$. Follow-up tests indicated that only the increase in NE content at 2 h after injection was significantly different from control values. There was no effect of MDA on the NE content of hippocampus.

In Experiment 3, the 1.8 mg/kg dose of MDA produced a 64% increase in the 5-HT content of frontal cortex at 2 h after injection ($p < 0.05$), with no significant change in 5-HIAA (Fig. 5, top panel). In contrast, the 3.6 mg/kg dose of MDA

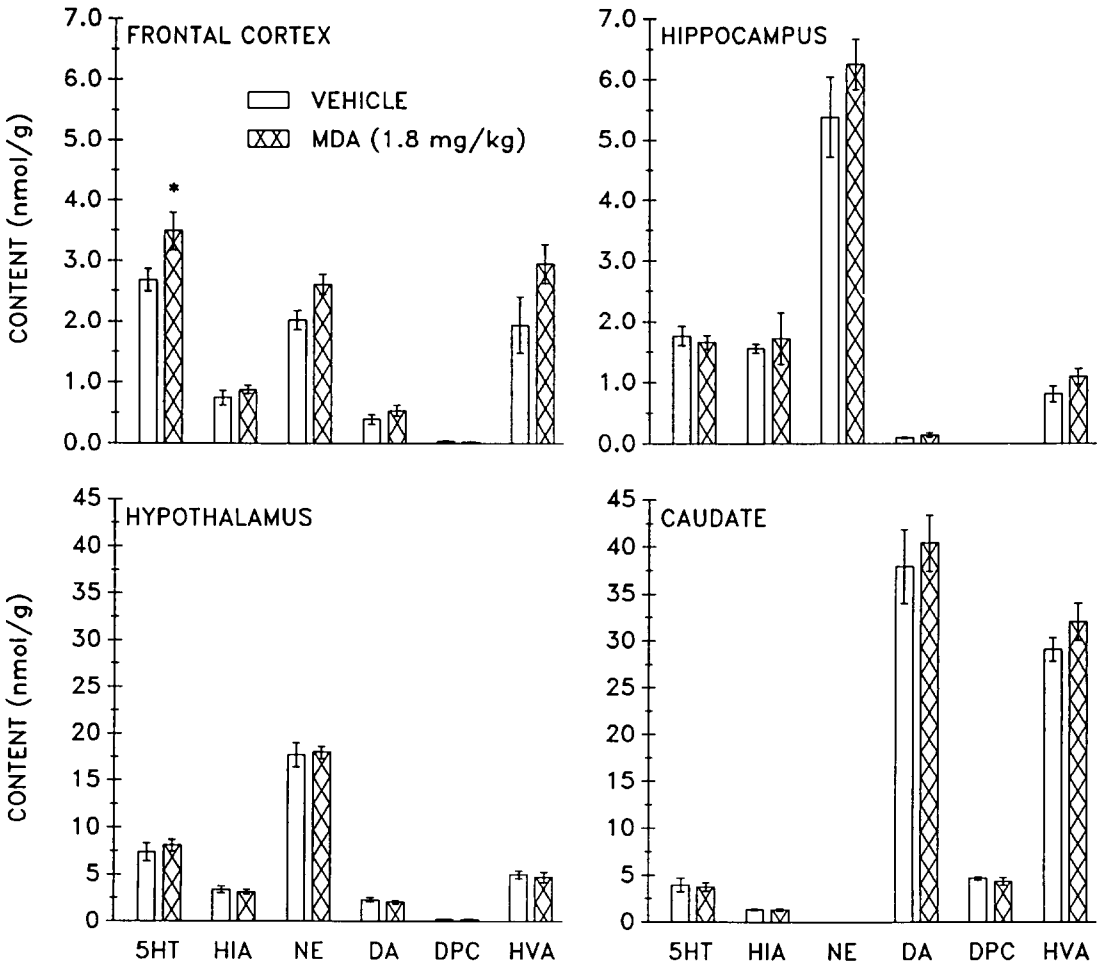

FIG. 3. Effect of MDA (1.8 mg/kg) on monoamines and their metabolites in frontal cortex, hippocampus, caudate nucleus, and hypothalamus at 30 min after injection. Data are expressed as nmol/g of brain tissue, fresh weight. Vertical bars represent 1 SEM. Asterisk indicates a mean value significantly different from vehicle control at $p \leq 0.05$, two tailed.

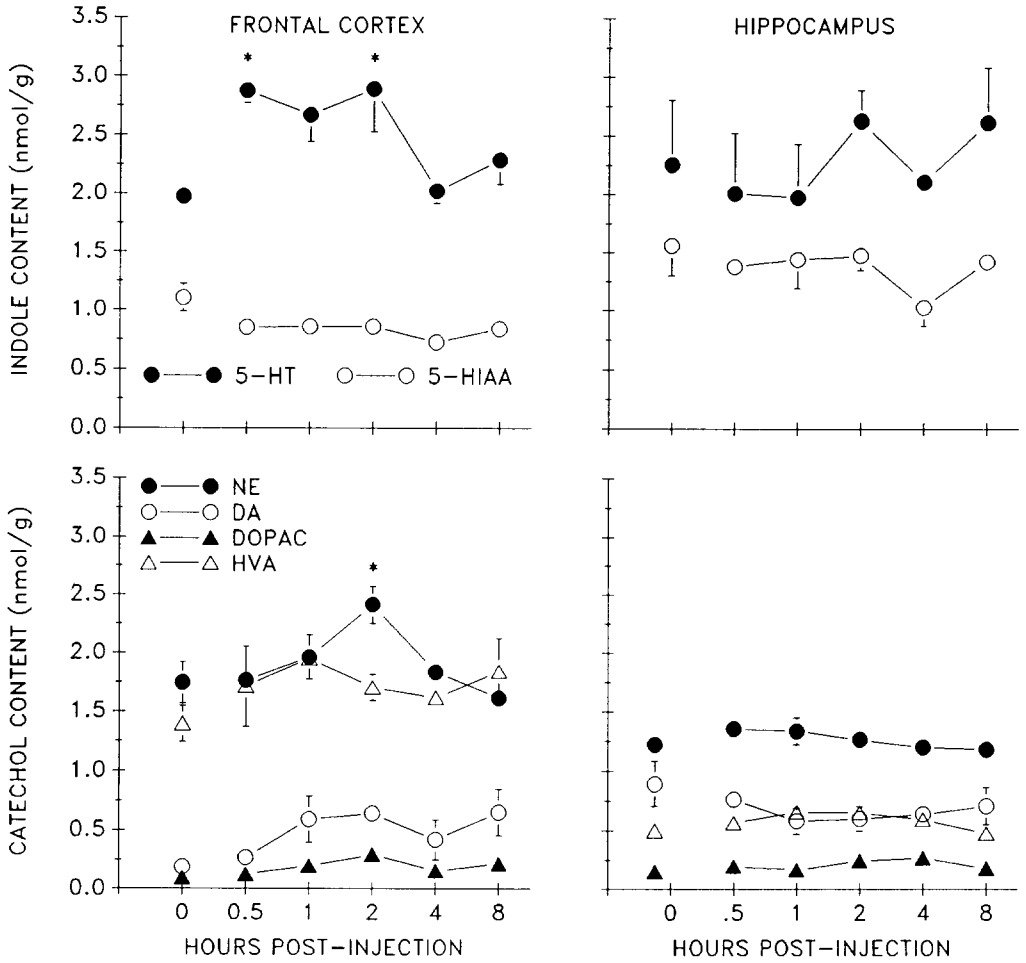

FIG. 4. Time course for the effects of MDA on monoamines and their metabolites in frontal cortex and hippocampus from 0.5 to 8 h after a single injection of 1.8 mg/kg. All symbols as in Fig. 1.

produced only a small (17%) and nonsignificant increase in the 5-HT content of frontal cortex along with a large (37%) and significant decrease in its content of 5-HIAA ($p < 0.025$). There was no significant effect of either dose of MDA on the 5-HT content of hippocampus; however, its 5-HIAA content was significantly ($p < 0.05$) reduced by 27% after the 3.6 mg/kg dose. Finally, neither dose of MDA had any significant effect on NE, DA, DOPAC, and HVA of either frontal cortex or hippocampus.

To measure the possible effects of MDA on 5-HT and DA turnover, we calculated for each animal the ratio of amine to its primary metabolite, i.e., the ratios 5-HIAA/5-HT and HVA/DA, respectively. There was no significant effect of MDA on the HVA/DA ratio in any of the experiments in any brain region examined, nor was there any effect of MDA on the 5-HIAA/5-HT ratio of hippocampus, hypothalamus, and caudate. However, MDA (1.8 mg/kg) did produce significant reductions in the ratio of 5-HIAA/5-HT of frontal cortex in two of the three experiments (Fig. 6). Thus, there was no significant effect of MDA (1.8 mg/kg) on 5-HIAA/5-HT ratios, obtained at 30 min after drug injection, in any of the four brain regions examined (Fig.6, top panel). However, in Experiment 2, MDA (1.8 mg/kg) produced a significant decrease in the 5-HIAA/5-HT ratio of frontal cortex at 30 min, and this effect slowly declined but was still significant at 8 h after injection (Fig. 6, middle panel). There was no significant or consistent effect of MDA on the 5-HIAA/5-HT ratio in hippocampus. Again, in Experiment 3, the 1.8 and 3.6 mg/kg dose of MDA produced a significant decrease in the 5-HIAA/5-HT ratio in frontal cortex but not in hippocampus.

DISCUSSION

The present study has demonstrated that a single, 1.8 mg/kg dose of MDA produced a significant and consistent increase in the 5-HT content of the rabbit's frontal cortex that ranged from 30 to 64% in three separate experiments and occurred between 30 and 120 min after injection of MDA. The content of 5-HIAA in frontal cortex tended to be decreased, but this was never significant. However, there was a decrease in the turnover of 5-HT in frontal cortex, as measured by the 5-HIAA/5-HT ratio. The decrease in turnover produced by MDA had a variable onset, in that it was observed to occur at 30 min in only one of two experiments, but persisted for up to 8 h after injection. The MDA-induced increase in content and decrease in turnover of 5-HT observed in frontal cortex was highly selective. For example, there were no changes in 5-HT content or turnover in three other brain regions (caudate nu-

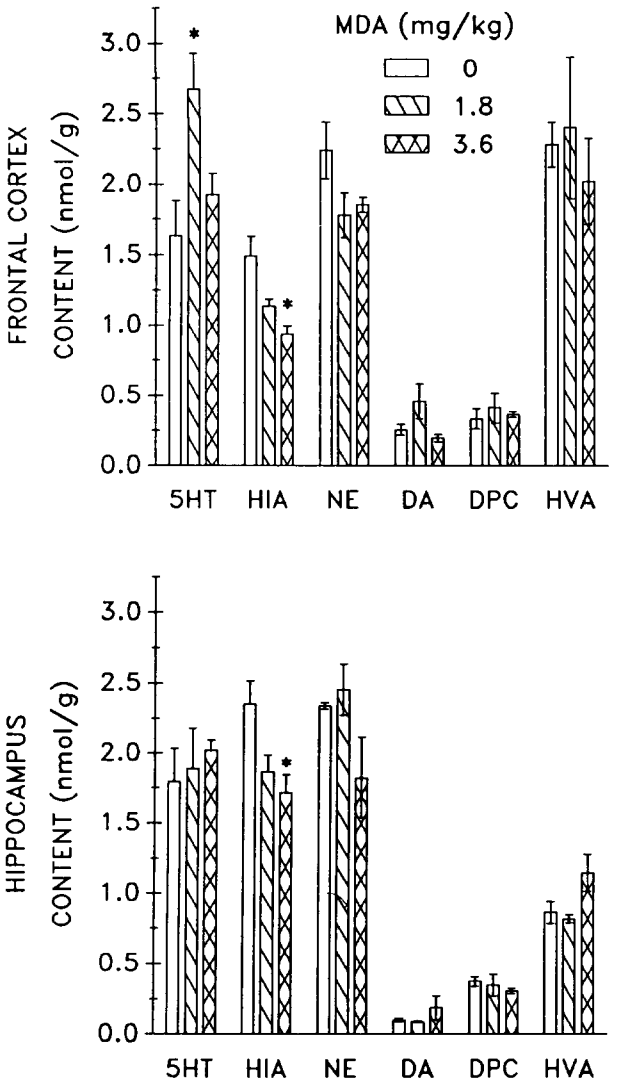

FIG. 5. Dose-dependent effect of MDA on monoamines and their metabolites in frontal cortex and hippocampus. Monoamine values were obtained 2 h after a single dose of 1.8 or 3.6 mg/kg.

cleus, hippocampus, or hypothalamus), and there were no changes in the content of DA, HVA, or DOPAC and no consistent changes in content of NE in any brain region examined.

Increases in brain content and decreases in the turnover of 5-HT are produced by hallucinogens such as LSD and DOM as well as by nonhallucinogenic drugs such as lisuride and 8-OH-DPAT. Hallucinogens are presumed to act postsynaptically on $5\text{-HT}_{2A/2C}$ receptors located on nonserotonergic neurons in forebrain (6,24). Activation of these receptors has been suggested to produce an inhibition of neuronal release of 5-HT and, hence, a decrease in its turnover (9). In agreement with this hypothesis, direct application of LSD to raphe neurons does not produce a decrease in 5-HT turnover in forebrain (19), and lesions of ascending 5-HT pathways do not block the ability of LSD to reduce 5-HT turnover (9).

In contrast to hallucinogens, lisuride and 8-OH-DPAT are thought to produce decreases in 5-HT neuronal release by

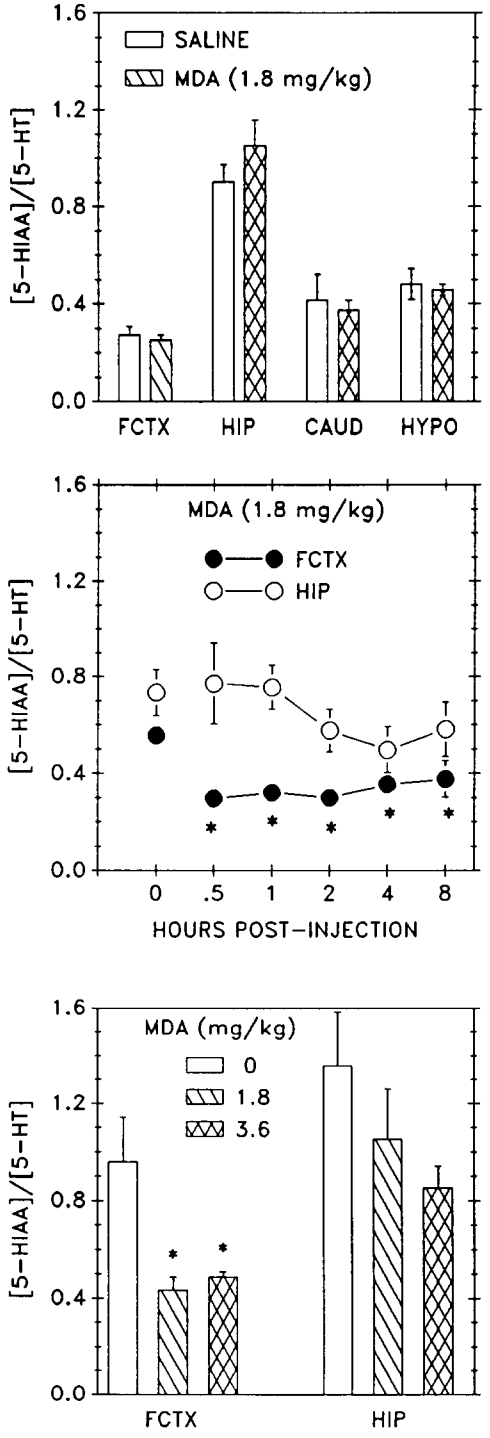

FIG. 6. Effect of MDA on the turnover of 5-HT as measured by the ratio of 5-HIAA/5-HT. Upper panel, effect of MDA (1.8 mg/kg) on 5-HT turnover in frontal cortex, hippocampus, hypothalamus, and caudate at 30 min after injection. Middle panel, time course for the effects of MDA (1.8 mg/kg) on the turnover of 5-HT in frontal cortex and hippocampus. Bottom panel, dose-dependent effects of MDA on turnover of 5-HT in frontal cortex and hippocampus at 2 h after injection of 1.8 or 3.6 mg/kg. All symbols as in Fig. 1.

activating somatodendritic 5-HT_{1A} receptors located on raphe neurons. This hypothesis is supported by reports that the decrease in turnover of 5-HT in forebrain could be produced by the direct application of lisuride and 8-OH-DPAT to the raphe nuclei and could be blocked by lesions that interrupted the ascending serotonergic projections (12,19). Because 5-HT_{1A} receptor agonists such as lisuride and 8-OH-DPAT act to inhibit the firing of raphe neurons, they decrease the release of 5-HT in all areas of the brain innervated by midbrain raphe nuclei, e.g., cortex, limbic forebrain, hippocampus, striatum, and hypothalamus (12,13,19). Thus, our finding that MDA only affected 5-HT turnover in frontal cortex is more consistent with a direct action on 5-$HT_{2A/2C}$ receptors in frontal cortex in a manner similar to that of other hallucinogenic/psychedelic drugs, rather than on 5-HT_{1A} receptors in midbrain raphe nuclei. The results of the present study also indicate that the effects of MDA are much more selective than those of other hallucinogens such as LSD and DOM because its actions were only seen in frontal cortex and unlike LSD or DOM, it had no effect on catecholamines.

The effects of MDA on 5-HT of frontal cortex were dose dependent. Both the 1.8 and 3.6 mg/kg doses of MDA produced a decrease in turnover in frontal cortex. However, this decrease was associated with a significant increase in 5-HT for the lower dose but with only a decrease in 5-HIAA at the higher dose. A decrease in 5-HIAA with no change in 5-HT was observed in rats 14 days after the administration of neurotoxic doses of MDA (2), but this has not been a consistent finding (20,27). The more consistent finding in the rat has been that neurotoxic doses of MDA produce a large decrease in both 5-HT and 5-HIAA (2,20,27). These latter findings and those of the present study suggest that there are two dose-dependent effects of MDA on monoamines. Low, behaviorally active doses of MDA such as those employed in the present study (1.8 mg/kg) produce a selective increase in content of 5-HT, due to a decrease in 5-HT turnover that is limited to cortex, persists for 2 h, and is not accompanied by any effect on catecholamines. Higher, neurotoxic doses of MDA (10 mg/kg or more) produce a significant decrease in 5-HT and 5-HIAA throughout the brain that is present at 3 h after injection and is accompanied by a significant decrease in the turnover of DA as reflected by increases in DA content and decreases in DOPAC (27). Thus, we would predict that a low, behaviorally active dose of MDA in the rat (e.g., 1 to 2 mg/kg) would also produce an increase in 5-HT that was restricted to forebrain areas such as the frontal cortex.

Our findings indicate that the rabbit is far more sensitive to the lethal effects of MDA (LD_{50}, 5 mg/kg) than the rat, which can survive doses of 40 mg/kg given twice a day for 4 days (20). However, the rabbit was not found to be more sensitive to the neurotoxic effects of MDA because the sublethal dose of 3.6 mg/kg/day given for 4 days failed to produce any evidence of degenerative changes in serotonergic neurons. The behaviorally active doses of MDA in the rabbit (1–2 mg/kg) are within the range of 1–3 mg/kg reported to produce psychedelic effects in humans (3,17,25) and just below the lethal dose for both the rabbit (5 mg/kg) and human (7.5 mg/kg) (21).

In conclusion, the results of this study demonstrate that behaviorally effective and nonneurotoxic doses of MDA produce an increase in 5-HT and decrease in its turnover that resemble the effects of other hallucinogens. It is not clear, however, whether the decreased release of 5-HT is simply a compensatory response to stimulation of 5-$HT_{2A/2C}$ receptors and unrelated to any behavioral effects as suggested by some (19), or whether the decrease in 5-HT release contributes to some of the behavioral effects of MDA and other hallucinogens.

ACKNOWLEDGEMENTS

This research was supported by United States Public Health Service Grant DA04944 and MH16841. We thank the National Institute on Drug Abuse for providing samples of MDA.

REFERENCES

1. Andén, N.-E.; Corrodi, H.; Fuxe, K.; Meek, J. L. Hallucinogenic phenylethylamines: Interactions with serotonin turnover and receptors. Eur. J. Pharmacol. 25:176–184; 1974.
2. Battaglia, G.; Yeh, S. Y.; O'Hearn, E.; Molliver, M. E.; Kuhar, M. J.; De Souza, E. B. 3,4-Methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine destroy serotonin terminals in rat brain: Quantification of neurodegeneration by measurement of [^{3}H]paroxetine-labeled serotonin uptake sites. J. Pharmacol. Exp. Ther. 242:911–916; 1987.
3. Braun, U.; Shulgin, A. T.; Braun, G. Centrally active n-substituted analogs of 3,4-methylenedioxyphenylisopropylamine (3,4-methylene-dioxyamphetamine). J. Pharmaceut. Sci. 69:192–195; 1980.
4. Freedman, D. X. Effects of LSD-25 on brain serotonin. J. Pharmacol. Exp. Ther. 134:160–166; 1961.
5. Freedman, D. X.; Gottlieb, R.; Lovell, R. A. Psychotomimetic drugs and brain 5-hydroxytryptamine metabolism. Biochem. Pharmacol. 19:1181–1188; 1970.
6. Glennon, R. A. Do classical hallucinogens act as 5-HT_2 agonists or antagonists? Neuropsychopharmacology 3:509–517; 1990.
7. Glennon, R. A.; Young, R. MDA: A psychoactive agent with dual stimulus effects. Life Sci. 34:379–383; 1984.
8. Glennon, R. A.; Young, R. MDA: An agent that produces stimulus effects similar to those of 3,4-DMA, LSD and cocaine. Eur. J. Pharmacol. 99:249–250; 1984.
9. Halaris, A. E.; Rosenthal, M.; DeMet, E. M.; Freedman, D. X. The raphe neuronal system and serotonergic effects of LSD. Neuropharmacology 21:811–816; 1982.
10. Harvey, J. A.; McMaster, S. E.; Romano, A. G. Methylenedioxyamphetamine: Neurotoxic effects on serotonergic projections to brain stem nuclei in the rat. Brain Res. 619:1–14; 1993.
11. Harvey, J. A.; Gormezano, I.; Cool, V. A. Effects of *d*-lysergic acid diethylamide, *d*-2-bromolysergic acid diethylamide, *dl*-2,5-dimethoxy-4-methylamphetamine and *d*-amphetamine on classical conditioning of the rabbit nictitating membrane response. J. Pharmacol. Exp. Ther. 221:289–294; 1982.
12. Hjorth, S.; Magnusson, T. The 5-HT_{1A} receptor agonist, 8-OH-DPAT, preferentially activates cell body 5-HT autoreceptors in rat brain in vivo. Arch. Pharmacol. 338:463–471; 1988.
13. Hjorth, S.; Sharp, T. Effect of the 5-HT_{1A} receptor agonist 8-OH-DPAT on the release of 5-HT in dorsal and median raphe-innervated rat brain regions as measured by in vivo microdialysis. Life Sci. 48:1779–1786; 1991.
14. Hsu, S. M.; Raine, L.; Fanger, H. The use of avidin-biotin peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabeled antibody (PAP) procedures, J. Histochem. Cytochem. 29:577–580; 1981.
15. Humphrey, P. P. A.; Hartig, P.; Hoyer, D. A proposed new nomenclature for 5-HT receptors. Trends Pharmacol. Sci. 14: 233–236; 1993.
16. Leonard, B. E. Some effects of the hallucinogenic drug 2,5-dimethoxy-4-methyl amphetamine on the metabolism of bio-

genic amines in the rat brain. Psychopharmacologia 32:33–49; 1973.
17. Marquardt, G. M.; DiStefano, V.; Ling, L. L. Pharmacological effects of (±)-, (S)-, and (R)-MDA. In: Stillman, R. C.; Willette, R. E., eds. Psychopharmacology of hallucinogens. New York: Pergamon Press; 1978:84–104.
18. O'Hearn, E.; Battaglia, G.; De Souza, E. B.; Kuhar, M. J.; Molliver, M. E. Methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA) cause selective ablation of serotonergic axon terminals in forebrain: Immunocytochemical evidence for neurotoxicity. J. Neurosci. 8:2788–2803; 1988.
19. Pieri, L.; Keller, H. H.; Burkard, W.; Da Prada, M. Effects of lisuride and LSD on cerebral monoamine systems and hallucinosis. Nature 272:278–280; 1978.
20. Ricaurte, G.; Bryan, G.; Strauss, L.; Seiden, L.; Schuster, C. Hallucinogenic amphetamine selectively destroys brain serotonin nerve terminals. Science 229:986–988; 1985.
21. Richards, K. C.; Borgstedt, H. H. Near fatal reaction to ingestion of the hallucinogenic drug MDA. JAMA 218:1826–1827; 1971.
22. Romano, A. G.; Bormann, N. M.; Harvey, J.,A. A unique enhancement of associative learning produced by methylenedioxyamphetamine. Behav. Pharmacol. 2:225–231; 1991.
23. Romano, A. G.; Harvey, J. A. Enhanced learning following a single, acute dose of MDA. Pharmacol. Biochem. Behav. 44:965–969; 1993.
24. Sanders-Bush, E.; Breeding, M. Choroid plexus epithelial cells in primary culture: A model of 5-HT_{1C} receptor activation by hallucinogenic drugs. Psychopharmacology (Berlin) 105:340–346; 1991.
25. Shulgin, A. T. Psychotomimetic drugs: Structure-activity relationships. In: Iversen, L. L.; Iversen, S. D.; Snyder, S. H., eds. Handbook of psychopharmacology, vol 11, Stimulants. New York: Plenum; 1978:243–333.
26. Siegel, S.; Freedman, D. X. Effects of LSD-25 on classical trace conditioning. Pharmacol. Biochem. Behav. 30:427–431; 1988.
27. Stone, D. M.; Stahl, D. C.; Hanson, G. R.; Gibb, J. W. The effects of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxy-amphetamine (MDA) on monoaminergic systems in the rat brain. Eur. J. Pharmacol. 128:41–48; 1986.
28. Thompson, D. M.; Moerschbaecher, J. M. Differential effects of phencyclidine and MDA on complex operant behavior in monkeys. Pharmacol. Biochem. Behav. 21:453–457; 1984.
29. Wang, S.-D.; Goldberger, M. E.; Murray, M. Plasticity of spinal systems after unilateral lumbosacral dorsal rhizotomy in the adult rat. J. Comp. Neurol. 304:555–568; 1991.
30. Wester, P.; Gottfries, J.; Johansson, K.; Klintebäck, F.; Winblad, B. Simultaneous liquid chromatographic determination of seventeen of the major monoamine neurotransmitters, precursors and metabolites. I. Optimization of the mobile phase using factorial designs and a computer program to predict chromatograms. J. Chromatogr. 415:261–274; 1987.
31. Wilkinson, L. SYSTAT: The System for Statistics. Evanston, IL: SYSTAT Inc.; 1990.
32. Winer, B. J. Statistical principles in experimental design. New York: McGraw-Hill Book Company; 1971.