Brain Research, 543 (1991) 56–60
Elsevier

BRES 16393

The action of (±)-MDMA on medial prefrontal cortical neurons is mediated through the serotonergic system

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(Accepted 2 October 1990)

Key words: 3,4-Methylenedioxymethamphetamine; 5-Hydroxytryptamine; Medial prefrontal cortex; Single unit recording

The mechanism of action of systemically administered (±)-MDMA (3,4-methylenedioxymethamphetamine) on spontaneously active neurons in the medial prefrontal cortex (mPFc) of chloral hydrate anesthetized rats was examined using standard single unit extracellular recording techniques. Intravenously administered MDMA dose-dependently decreased the firing rates of the majority of mPFc neurons in control rats. In contrast, in rats that were pretreated with *p*-chlorophenylalanine (PCPA), which depletes the brain serotonin (5-hydroxytryptamine, 5-HT) content by inhibiting tryptophan hydroxylase, the rate-limiting enzyme in the synthesis of 5-HT, MDMA was largely ineffective in inhibiting the firing of mPFc cells. In PCPA-treated animals, the administration of 5-hydroxytryptophan (5-HTP), which presumably restored the brain 5-HT content, but not L-DOPA, reinstated MDMA's inhibitory action in PCPA-treated rats. In rats that were pretreated with α-methyl-*p*-tyrosine (AMPT), which depletes the brain dopamine (DA) content by inhibiting tyrosine hydroxylase, the rate-limiting enzyme in the synthesis of DA, MDMA inhibited the firing of all of the mPFc cells. MDMA's effect on mPFc neurons was reversed by 5-HT receptor antagonists such as granisetron and metergoline. These results strongly suggest that MDMA exerts its action on mPFc cells indirectly by releasing endogenous 5-HT.

INTRODUCTION

The designer drug MDMA (3,4-methylenedioxymethamphetamine; 'Ecstasy') reportedly enhances emotion and cognition but is non-hallucinogenic[13,18]. It has been shown to potently release and block the reuptake of serotonin (5-hydroxytryptamine, 5-HT) in the frontal cortex, striatum and hippocampus[15–17,19,20] and to a lesser extent dopamine (DA) in the striatum and nucleus accumbens[7,11,17,19,26].

Electrophysiological studies have demonstrated that MDMA modulates the firing activity of neurons in brain regions which contain 5-HT and DA cell bodies. For example, in a brain slice preparation, superfused MDMA releases 5-HT and inhibits the firing of dorsal raphe 5-HT neurons[21]. However, it is unlikely that the MDMA-induced inhibitory effect on dorsal raphe neurons could account for the unique pharmacological profile of MDMA, since this is a common action shared by all indolealkylamine hallucinogens. The systemic administration of high doses of MDMA inhibits the firing of spontaneously active DA neurons in the substantia nigra[8,12]. This effect is thought to be indirectly mediated by feedback pathways from the forebrain regions[8]. Thus, it appears that the interaction between the released neurotransmitters and postsynaptic receptors in 5-HT and perhaps DA projection areas rather than the effect of MDMA on 5-HT and DA-containing neurons could best account for the unique behavioral and psychological effects of MDMA.

The mPFc is densely innervated by serotonergic and dopaminergic neurons and plays a significant role in emotion and cognition[5]. Hence, using standard extracellular single unit recording techniques[25], we decided to examine the mode of action of (±)-MDMA on mPFc cells.

MATERIALS AND METHODS

Animals and surgery

Male Sprague–Dawley rats (200–250 g, Taconic Farms, NY), naive or drug treated, were anesthetized with chloral hydrate (400 mg/kg, i.p., Sigma) and placed in a stereotaxic frame. The tail vein was then cannulated with a 25 gauge needle for the administration of additional anesthetic or drugs. A burr hole was drilled above the mPFc (0.3–1.0 mm lateral, 11.0–11.5 mm anterior and 0.5–3.0 mm ventral to Lambda) according to the atlas of Paxinos and Watson[14] to allow the lowering of a recording electrode.

Electrophysiological methods and histology

The standard extracellular recording techniques used have been

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described in detail elsewhere[24,25]. Single barrel glass pipettes prefilled with a few strands of fiberglass were pulled by a vertical microelectrode puller (Narishige) and the tips were broken to a tip diameter of about 1 μm with the aid of a light microscope. The electrode was then filled with a solution of 2 M NaCl saturated with 1% Fast green dye. The *in vitro* impedance of the electrode was between 2 and 4 MΩ.

The typical mPFc neurons studied had positive-negative waveforms and signal to noise ratios of greater than 5. They were primarily located in layers I-III and have been shown previously to be unresponsive to electrical stimulation of the medial dorsal nucleus of the thalamus[1].

MDMA was given according to a regimen in which each dose doubled the previous cumulative dose until the cell was completely inhibited or the end of the dose-response regimen was reached (final cumulative dose: 25.6 mg/kg). To obviate residual drug effects, only one cell was studied in each rat. At the end of each experiment, Fast green was ejected by passing −30 μA of current through the electrode for 20 min. This resulted in the deposit of Fast green around the electrode tip. The rats were then perfused transcardially with 10% buffered formalin (Fisher). Recording sites were verified on 50 μm coronal sections stained with Cresyl violet and counterstained with Neutral red.

Drug treatment

The relative contribution of 5-HT and DA release in mediating MDMA's action on medial prefrontal cortical neurons was assessed by studying the effect of depleting and replenishing these two neurotransmitters. To deplete 5-HT, the rats were pretreated with *p*-chlorophenylalanine (PCPA, Regis, 400 mg/kg, i.p.) 24 h before the electrophysiological experiments were conducted[9,23,24]. To deplete DA, the rats were given two injections of α-methyl-*p*-tyrosine (AMPT, Sigma and RBI, 250 mg/kg, i.p., each injection) 4 and 2 h before the electrophysiological experiments[8]. To replenish 5-HT and DA, their respective immediate precursors L-5-HTP (25–200 mg/kg, in the form of the methyl ester HCl salt, i.v., Calbiochem) and L-DOPA (25–100 mg/kg, in the form of the methyl ester HCl salt, i.v., Sigma)[22] were administered in the presence of a low dose of benserazide (Ro4–4602, 20 mg/kg, i.v., Hoffmann-La Roche)[23,24] which inhibits peripheral aromatic amino acid decarboxylase and thereby enhances the central effect of the two precursors. The aromatic amino acid decarboxylase in the brain, an enzyme unaffected by PCPA and AMPT, would then convert the immediate precursors to 5-HT and DA in the respective nerve terminals.

Data analysis

The baseline firing rate was defined as the average firing rate of the cell during a 2–5 min epoch before drug injection. The effect of MDMA was determined by comparing the firing rate of the cells after drug administration with that during the baseline period. Subsequently, the results are expressed as a percentage of baseline firing rate. The distribution of responses was analyzed using logistic regression with the appropriate contrasts. Comparisons of dose-response curves were made using two way analysis of variance (ANOVA) and difference between specific means were tested using the Student-Newman-Keuls test. Student's *t*-test was used to test for differences between IC_{50} values between two groups. The criterion for significance is set at $P < 0.05$.

RESULTS

In naive control rats, at 25.6 mg/kg (cumulative dose), (±)-MDMA inhibited the firing of 16/19 (84%) mPFc cells by ≥20% of baseline firing rate; 3/19 (16%) cells were excited by ≥20% baseline firing rate (Figs. 1 and 2). Overall, MDMA inhibited the cells with an IC_{50} of 17.2 mg/kg extrapolated from the cumulative dose-response curve (Fig. 3). If only cells which were inhibited by MDMA were included, the average IC_{50} for MDMA's effect was 8.0 ± 2.3 mg/kg (n = 15, Fig. 4). Relatively long recovery times (1–5 min) were needed before the neurons resumed firing spontaneously (Fig. 1). Granisetron (0.5–4 mg/kg) partially or fully reversed the inhibitory effect of MDMA on 4/7 (57%) mPFc cells but was ineffective on 3/7 (43%) cells. Metergoline (0.5–4 mg/kg) partially or completely reversed this inhibitory response in 9/10 (90%) cells and had no effect on the remaining 1/10 (10%) cell (Fig. 1).

In rats that were pretreated with PCPA to deplete the 5-HT content in the brain[9,25], MDMA was significantly less effective in inhibiting the firing activity of mPFc neurons (Figs. 1 and 2). At 25.6 mg/kg (cumulative dose), only 3/11 (27%) of mPFc cells were inhibited by ≥20% baseline firing rate (Fig. 2). Of these 3, only one was inhibited by ≥50% baseline firing rate. Interestingly,

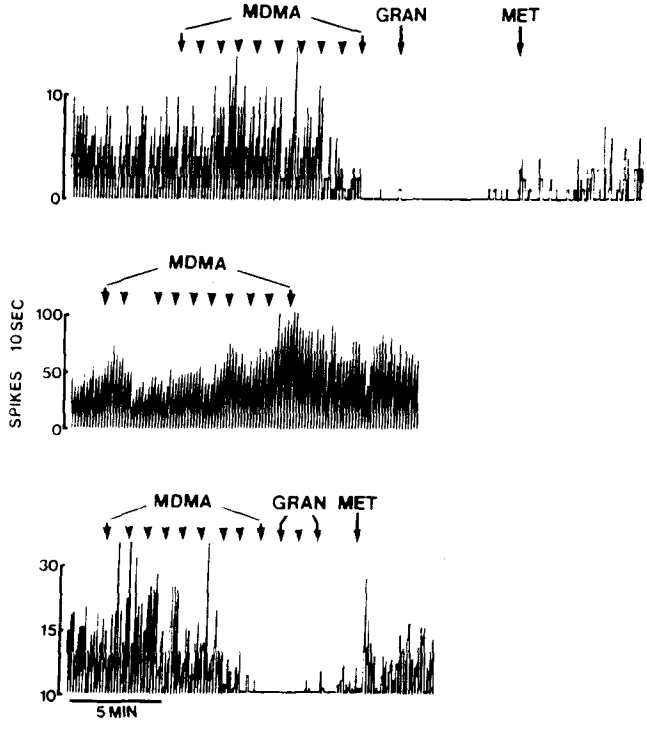

Fig. 1. Representative rate histograms showing the effect of MDMA on the firing of spontaneously active mPFc neurons in control, PCPA- and AMPT-treated animals. Top: this rate histogram shows the inhibitory effect of MDMA on a typical mPFc cell. Granisetron (GRAN, 1 mg/kg) and metergoline (MET, 1 mg/kg) partially reversed its effect. MDMA had similar inhibitory action on the majority of mPFc neurons. (Middle) After PCPA-treatment, MDMA's inhibitory effect was significantly reduced. MDMA (cumulative dose: 25.6 mg/kg) had no effect on this particular neuron. MDMA inhibited only a minority of mPFc cells. Other cells were excited by MDMA. Bottom: in AMPT-treated animals, MDMA was able to inhibit the firing of mPFc neurons, as illustrated by this cell. GRAN (1 mg/kg) and MET (1 mg/kg) reversed MDMA's action.

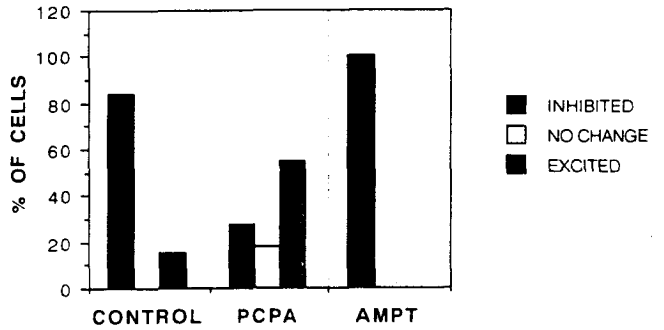

Fig. 2. The effect of MDMA (25.6 mg/kg, cumulative dose) on the firing activity of mPFc neurons in control, PCPA- and AMPT-treated animals. In control rats, MDMA inhibited the firing of 16/19 (84%) mPFc cells by ≥20% baseline firing rate; 3/19 (16%) cells were excited by ≥20% baseline firing rate. In PCPA-pretreated rats, MDMA inhibited 3/11 (27%) mPFc cells, excited 6/11 (55%) cells and had no effect on the remaining 2/11 (18%) cells. This distribution of responses is significantly different from that seen in control. In AMPT-pretreated rats, MDMA inhibited 0/6 (100%) of mPFc cells. This distribution of responses is not different from that seen in control.

for many neurons, at the higher doses, MDMA produced a modest excitatory effect: at 25.6 mg/kg, 6/11 (55%) cells were excited by ≥20% baseline (Fig. 2). The remaining 2/11 (18%) cells were unaffected by MDMA. Overall, after PCPA treatment, mPFc cells were not inhibited by MDMA (Fig. 3). It was impossible to calculate IC_{50} values because the majority of the cells were not inhibited by ≥50% baseline firing rate even after 25.6 mg/kg of MDMA was given. When only the group of cells which were inhibited by MDMA was considered, the dose-response curve for MDMA was

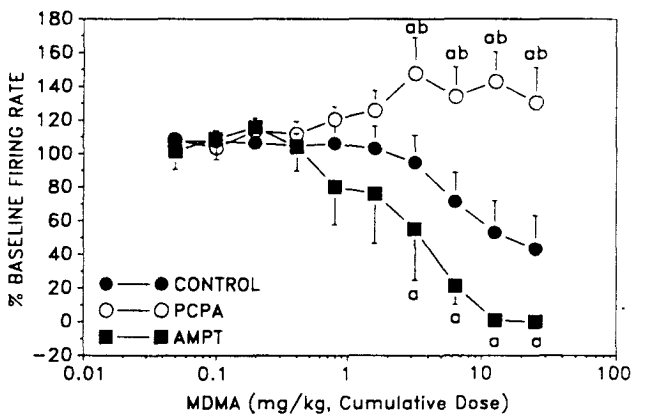

Fig. 3. Cumulative dose-response curves of MDMA's effect on the firing rates of all the mPFc cells studied in control, PCPA- and AMPT-treated animals. MDMA dose-dependently inhibited the firing of mPFc cells in control rats (filled circles). The dose-response curve is significantly shifted to the right after PCPA treatment (open circles) and significantly shifted to the left after AMPT treatment (filled squares). All the cells regardless of whether they were inhibited, excited or unaffected by MDMA were included in plotting the dose-response curves. a, $P < 0.05$, significantly different from controls. b, $P < 0.05$, significantly different from AMPT-treated animals.

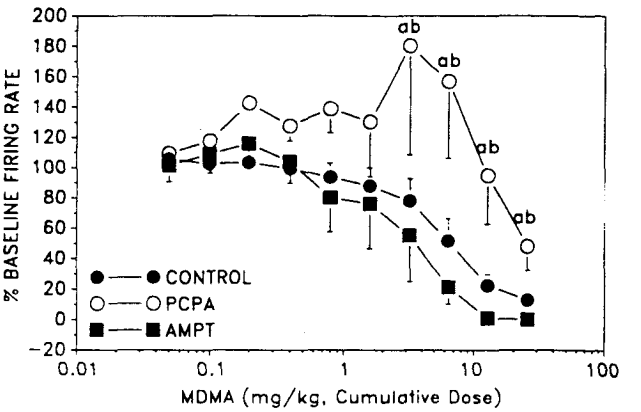

Fig. 4. Cumulative dose-response curves for MDMA's effect on the firing rates of mPFc cells which were inhibited by MDMA in control, PCPA- and AMPT-treated animals. MDMA dose-dependently inhibited the firing of mPFc cells in control rats (filled circles). In PCPA-treated animals (open circles), MDMA was less potent in inhibiting mPFc cells, resulting in a dose-response curve which is significantly different from that seen in controls. In AMPT-treated animals (filled squares), MDMA inhibited mPFc cells in a manner which is not different from that in control. a, $P < 0.05$, significantly different from controls. b, $P < 0.05$, significantly different from AMPT-treated animals.

shifted to the right significantly compared to that of control (Fig. 4). Importantly, in PCPA-treated animals, after the administration of a low dose of benserazide (20 mg/kg) and 5-HTP (a cumulative dose of 50–200 mg/kg, i.v.)[23], MDMA was able to inhibit the firing of the majority (9/12, 75%) of the cortical neurons studied (Figs. 5 and 6). Only 1/12 (8%) cell was excited and the remaining 2/12 (17%) cells were unaffected. This profile of response is not significantly different from that for

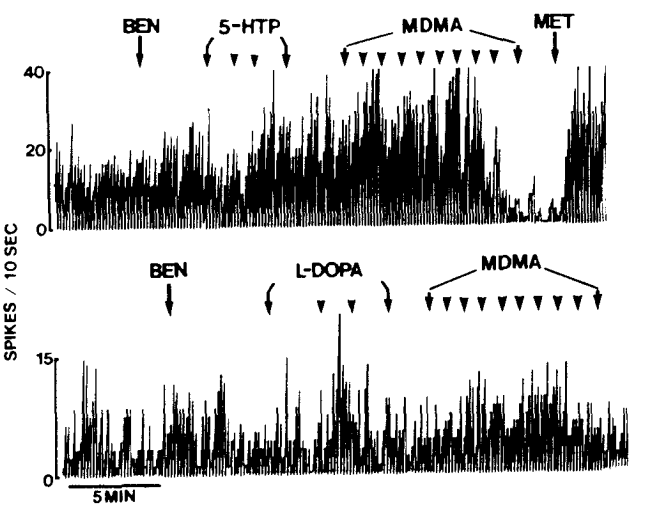

Fig. 5. Representative rate histograms showing the effect of MDMA on mPFc cells of PCPA-treated animals subjected to 5-HTP or L-DOPA pretreatment. Top: 5-HTP pretreatment reinstated MDMA's inhibitory action. Metergoline (MET, 0.5 mg/kg) reversed the effect. Bottom: L-DOPA pretreatment did not reestablish MDMA's inhibitory effect.

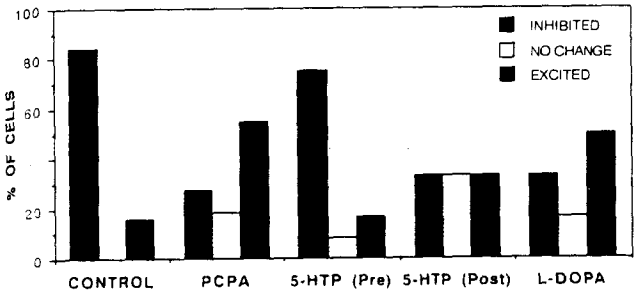

Fig. 6. The effect of MDMA on the firing rates of mPFc neurons in control and PCPA-treated animals. In control rats, at 25.6 mg/kg (cumulative dose), MDMA inhibited 16/19 (84%) and excited 3/19 (16%) mPFc neurons whereas in PCPA-treated rats, MDMA's effect was altered such that only 3/11 (27%) cells were inhibited, 6/11 (55%) cells were excited and the remaining 2/11 (18%) cells were not affected. In PCPA-treated rats, 5-HTP given before MDMA administration reinstated MDMA's profile to that as seen in controls: 9/12 (75%) mPFc cells were inhibited, 2/12 (17%) cells were excited and 1/12 (8%) cell was not affected. This distribution in not significantly different from that in controls. When L-DOPA was substituted for 5-HTP, MDMA's profile remained significantly different from that for controls. Interestingly, when 5-HTP was given after MDMA administration, MDMA's effect was not reestablished with 2/6 (33%) mPFc cells inhibited, 3/6 (50%) cells excited and 1/6 (17%) cell unaffected by MDMA.

controls. The substitution of L-DOPA for 5-HTP was without effect (Figs. 5 and 6). In PCPA-treated rats, 5-HTP and benserazide in the absence of MDMA insignificantly increased the firing rates of mPFc neurons by 3 ± 12% (n = 13). Interestingly, when 5-HTP was given after MDMA administration, MDMA's effect was not reinstated (Fig. 6).

In rats that were pretreated with AMPT to deplete the brain's newly synthesized DA, MDMA dose-dependently inhibited 6 of 6 (100%) mPFc neurons (Figs. 1 and 2). The population response to MDMA was not significantly different from that of control and the average IC_{50} (3.0 ± 1.2 mg/kg, cumulative dose) is not significantly different from that for the majority of the cells in controls which were inhibited by MDMA (Figs. 2 and 3).

DISCUSSION

In the present study, we have demonstrated that (±)-MDMA, when given acutely by a systemic route, predominantly inhibited the firing rates of the great majority of spontaneously active mPFc cells in control rats. This is consistent with previous studies showing that the intravenously administration of MDMA inhibited the firing of substantia nigra DA cells[8,12] and that superfused MDMA inhibited the firing of dorsal raphe neurons in a slice preparation[21].

Our data further suggest that MDMA acts indirectly on mPFc cells by releasing endogenous 5-HT. PCPA, which has been shown to deplete 5-HT in the brain, significantly and drastically attenuated the inhibitory action of MDMA. The administration of 5-HTP prior to MDMA in the PCPA-treated rats reinstated the inhibitory action of MDMA. Presumably, following the conversion of 5-HTP to 5-HT in the nerve terminals, MDMA was able to release the 5-HT and subsequently suppress the firing of mPFc cells. It is known that 5-HTP (and benserazide) alters the blood pressure and heart rate of the animal. However, this effect apparently cannot account for the action of 5-HTP in reinstating the effect of MDMA because L-DOPA (and benserazide) which would induce similar changes in sympathetic function[3] did not restore the action of MDMA. Interestingly, when 5-HTP was given after MDMA, MDMA's inhibitory profile was not reestablished. It is known that in addition to a 5-HT-releasing action, MDMA blocks the reuptake of 5-HT[19]. In fact, this is consistent with our observation that the recovery from MDMA-induced suppressant effect was relatively slow. Possibly, the same mechanism could interfere with the uptake of 5-HTP into the nerve terminals. Consequently, if an insufficient amount of 5-HTP reaches the nerve terminals for the enzymatic conversion to 5-HT, MDMA would not be effective in releasing 5-HT and suppressing the firing activity of mPFc cells.

Preliminary data showed that the inhibitory effect of MDMA was reversed by the 5-HT_3 antagonist granisetron and the $5\text{-HT}_1/5\text{-HT}_2$ antagonist metergoline. This finding is consistent with the idea that postsynaptic 5-HT receptors play a role in mediating MDMA's effect. Furthermore, the involvement of receptors other than the 5-HT_2 subtypes, which is believed to mediate the effect of hallucinogens, may contribute to the pharmacological and psychotropic profiles of MDMA.

On the other hand, DA does not seem to play a critical role in mediating MDMA's inhibitory effect in the mPFc. The depletion of brain DA content by AMPT did not alter MDMA's suppressant action on mPFc cells. In fact, there was a tendency for the inhibitory action of MDMA to be enhanced. However, it should be pointed out that our results do not rule out the possibility that DA may have a more important role in mediating MDMA's action in other brain regions such as nucleus accumbens, which contains a high concentration of DA. The idea that MDMA's action on mPFc cells is primarily mediated via the 5-HT system but not the DA system is further supported by the fact that iontophoretically applied 5-HT invariably suppresses the firing rates of mPFc cells[2,10]. In contrast, the iontophoresis of DA on mPFc cells in layers I–II produced little effect[4].

A minority of mPFc neurons in control rats was modestly excited (up to 30% baseline firing rate at the highest cumulative dose given) by MDMA. Several mechanisms could account for this effect. For example, it

is possible that the MDMA-induced release of 5-HT interacts with 5-HT_2 receptors which could lead to excitation[1,6]. The percentage of cells excited versus inhibited by MDMA thus could reflect relative activation of 5-HT_2 versus 5-HT_1/5-HT_3 receptors in the mPFc. The role of the different subtypes of 5-HT receptors in producing these effects could be ascertained by studying the ability of selective or relatively selective 5-HT receptor antagonists in reversing these effects. Alternatively, the excitatory response could be the result of disinhibition. It is conceivable that some 5-HT-containing neurons impinge on an inhibitory interneuron. MDMA could therefore inhibit this inhibitory interneuron thus disinhibiting the mPFc neuron. It is noteworthy that racemic MDMA was used in this study. The possibility that the individual optical isomers may have distinct pharmacological profiles is currently being explored.

In conclusion, the results obtained from the present study indicate that the systemic administration of (±)-MDMA markedly inhibits the firing rates of mPFc neurons by releasing endogenous 5-HT. The action exerted by MDMA upon mPFc cells could be important in explaining its psychotropic effect in humans.

Acknowledgement. We wish to thank ShuXin Tian for confirming the recording sites histologically and for working on the photographs. We also wish to thank the supply of (±)-MDMA from NIDA. This work was supported by USPHS Grants MH-41440 and MH-00378 (RSDA) to R.Y.W.

REFERENCES

1 Ashby, Jr., C.R., Jiang, L.H., Kasser, R.J. and Wang, R.Y., Electrophysiological characterization of 5-hydroxytryptamine$_2$ receptors in the medial prefrontal cortex: a microiontophoretic study, *J. Pharmacol. Exp. Ther.*, 252 (1990) 171–178.
2 Ashby, Jr., C.R., Edwards, E. and Wang, R.Y., The action of serotonin in the medial prefrontal cortex: mediation by serotonin$_3$-like receptors, *Brain Research*, in press.
3 Baum, T. and Shropshire, A.T., Inhibition of efferent sympathetic nerve activity by 5-hydroxytryptophan and centrally administered 5-hydroxytryptamine, *Neuropharmacology*, 14 (1975) 227–233.
4 Bunney, B.S. and Aghajanian, G.K., Dopamine and norepinephrine innervation in the rat prefrontal cortex: pharmacological differentiation using microiontophoretic techniques, *Life Sci.*, 19 (1976) 1783–1792.
5 Carpenter, M.B. and Sutin, J., Cerebral cortex. In: *Human Neuroanatomy*, 8th edn., Williams and Wilkins, Baltimore, 1983, pp. 704–705.
6 Davies, M.F., Deisz, R.A., Prince, D.A. and Peroutka, S.J., Two distinct effects of 5-hydroxytryptamine on single cortical neurons, *Brain Research*, 423 (1987) 347–352.
7 Johnson, M.P., Hoffman, A.J. and Nichols, D.E., Effects of the enantiomers of MDA, MDMA, and related analogues on [^{3}H]serotonin and [^{3}H]dopamine release from superfused rat brain slices, *Eur. J. Pharmacol.*, 132 (1986) 269–276.
8 Kelland, M.D., Freeman, A.S. and Chiodo, L.A., (±)-3,4-Methylenedioxymethamphetamine-induced changes in the basal activity and pharmacological responsiveness of nigrostriatal dopamine neurons, *Eur. J. Pharmacol.*, 169 (1989) 11–21.
9 Koe, B.K. and Weissman, A., *p*-Chlorophenylalanine: a specific depletor of brain serotonin, *J. Pharmacol. Exp. Ther.*, 154 (1966) 499–516.
10 Lakoski, J.M. and Aghajanian, G.K., Effects of ketanserin on neuronal responses to serotonin in the prefrontal cortex, lateral geniculate and dorsal raphe nucleus, *Neuropharmacology*, 24 (1985) 265–273.
11 Logan, B.L., Laverty, R., Sanderson, W.D. and Yee, Y.B., Differences between rats and mice in MDMA (methylenedioxymethylamphetamine) neurotoxicity, *Eur. J. Pharmacol.*, 152 (1988) 227–234.
12 Matthews, R.T., Champney, T.H. and Frye, G.D., Effects of (±)3,4-methylenedioxymethamphetamine (MDMA) on brain dopaminergic activity in rats, *Pharmacol. Biochem. Behav.*, 33 (1989) 741–747.
13 Nichols, D.E., Differences between the mechanism of action of MDMA, MBDB, and the classic hallucinogens. Identification of a new therapeutic class: entactogens, *J. Psychoactive Drugs*, 18 (1986) 305–313.
14 Paxinos, G. and Watson, C., *The Rat Brain in Stereotaxic Coordinates*, Academic Press, New York, 1988.
15 Schmidt, C.J., Acute administration of methylenedioxyamphetamine: comparison with the neurochemical effects of its N-desmethyl and N-ethyl analogues, *Eur. J. Pharmacol.*, 136 (1987) 81–88.
16 Schmidt, C.J., Neurotoxicity of the psychedelic amphetamine, methylenedioxymethamphetamine, *J. Pharmacol. Exp. Ther.*, 240 (1987) 1–7.
17 Schmidt, C.J., Levin, J.A. and Lovenberg, W., In vitro and in vivo neurochemical effects of methylenedioxymethamphetamine on striatal monoaminergic systems in the rat brain, *Biochem. Pharmacol.*, 36 (1987) 747–755.
18 Shulgin, A.T. and Nichols, D.E., Characterization of three new psychotomimetics. In *The Pharmacology of Hallucinogens*, R.C. Stillman and R.E. Willette (Eds.), Pergamon Press, New York, 1978, pp. 74–83.
19 Steele, T.D., Nichols, D.E. and Yim, G.K.W., Stereochemical effects of 3,4-methylenedioxymethamphetamine (MDMA) and related amphetamine derivatives on inhibition of uptake of [^{3}H]monoamines into synaptosomes from different regions of rat brain, *Biochem. Pharmacol.*, 36 (1987) 2297–2303.
20 Stone, D.M., Merchant, K.M., Hanson, G.R. and Gibb, J.W., Immediate and long-term effects of 3,4-methylenedioxymethamphetamine on serotonin pathways in brain of rat, *Neuropharmacology*, 26 (1987) 1677–1683.
21 Sprouse, J.S., Bradberry, C.W., Roth, R.H. and Aghajanian, G.K., MDMA (3,4-Methylenedioxymethamphetamine) inhibits the firing of dorsal raphe neurons in brain slices via release of serotonin, *Eur. J. Pharmacol.*, 167 (1989) 375–383.
22 Wang, R.Y., Dopaminergic neurons in the rat ventral tegmental area. III. Effects of D- and L-amphetamine, *Brain Res. Rev.*, 3 (1981) 153–165.
23 Wang, R.Y. and Aghajanian, G.K., Inhibition of neurons in the amygdala by dorsal raphe stimulation: mediation through a direct serotonergic pathway, *Brain Research*, 120 (1977) 85–102.
24 Wang, R.Y. and Aghajanian, G.K., Collateral inhibition of serotonergic neurones in the rat dorsal raphe nucleus: pharmacological evidence, *Neuropharmacology*, 17 (1978) 819–825.
25 Wang, R.Y., de Montigny, C., Gold, B.I., Roth R.H. and Aghajanian, G.K., Denervation supersensitivity to serotonin in rat forebrain: single cell studies, *Brain Research*, 178 (1979) 85–102.
26 Yamamoto, B.K. and Spanos, L.J., The acute effects of methylenedioxymethamphetamine on dopamine release in the awake-behaving rat, *Eur. J. Pharmacol.*, 148 (1988) 195–203.