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MDMA: further evidence that its action in the medial prefrontal cortex is mediated by the serotonergic system

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Systemically administered (±)-MDMA (3,4-methylenedioxymethamphetamine, 'Ecstasy') suppressed the firing rates of the majority of neurons in the medial prefrontal cortex (mPFc). The responses of mPFc cells to (±)-MDMA is mimicked by (+)-MDMA but not (–)-MDMA. Furthermore, pretreatment with fluoxetine (a specific 5-HT uptake blocker) but not GBR 12909 (a specific dopamine uptake blocker) prevented the suppressant action of MDMA. These data support the notion that the 5-HT system mediates (±)-MDMA's action.

MDMA (3,4-methylenedioxymethamphetamine, 'Ecstasy') is a recreational substance of abuse. It has been shown to interact with the brain serotonin (5-hydroxytryptamine, 5-HT) and dopamine (DA) systems, although the DA system is less pronouncedly affected[11]. MDMA potently depletes regional brain 5-HT content both acutely and chronically[20]. The long-term depletion of 5-HT is correlated with the development of 5-HT neurotoxicity[17] which could be prevented by treatment with 5-HT_2 antagonists[19]. The short-term 5-HT content changes may be related to MDMA's behavioral and psychological effects. Interestingly, MDMA-induced acute behavior such as locomotion could be blocked by pindolol (5-$HT_{1A,B}$ and β receptor antagonist) but not by 5-HT_2 receptor antagonists (Geyer et al., personal communication).

We have recently demonstrated that (±)-MDMA's suppressant action on the firing activity of medial prefrontal cortical (mPFc) cells was either abolished or markedly attenuated in animals whose brain 5-HT content was depleted by *p*-chlorophenylalanine (PCPA) but was not affected in animals whose brain dopamine (DA) content was depleted by α-methyl-*p*-tyrosine (AMPT)[15]. Moreover, the precursor of 5-HT, 5-hydroxytryptophan, but not the precursor of DA, L-DOPA, reinstated the suppressant action of MDMA in PCPA-treated rats[15]. Additionally, our preliminary results show that the suppressant action of MDMA was reversed by metergoline (5-$HT_{1,2}$ receptor antagonist) and by granisetron (5-HT_3 receptor antagonist), although the latter's effect was less consistent. To further support the view that the 5-HT system is critical in mediating the action of MDMA, we report here that pretreating the animals with the 5-HT uptake blocker fluoxetine but not the DA uptake blocker GBR 12909 prevented the effect of (±)-MDMA. In addition, (+)-MDMA but not (–)-MDMA mimicked the action of (±)-MDMA.

Male Sprague–Dawley rats (200–250 g, Taconic Farms, NY) 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 and 11.0–11.5 mm anterior to Lambda) according to the atlas of Paxinos and Watson[16] to allow the lowering of a single barrel glass recording electrode (in vitro impedance of 2–4 MΩ, filled with 2 M NaCl saturated with 1% Fast green dye). The effects of the individual isomers as well as racemic MDMA on the firing of spontaneously active mPFc neurons were studied using standard extracellular single unit recording techniques as described in detail elsewhere[26]. The mPFc is chosen because it is a brain region which is involved in cognition and the reward phenomenon and because we have characterized the physiological and pharmacological properties of 5-HT_2 and 5-HT_3 receptors in this region[2,3]. 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).

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To obviate residual drug effects, only one cell was studied in each rat. The response of a mPFc cell was termed 'inhibited' or 'excited' when a drug decreased or increased the basal firing rate by ≥20%. At the end of each experiment, −30 μA of current was passed for 20 min through the electrode to eject Fast green dye and thus mark the location of the electrode tip. The rats were then perfused transcardially and the recording sites were verified histologically.

As shown previously, in control rats, (±)-MDMA predominantly inhibited the firing of spontaneously active mPFc neurons (Fig. 1). Specifically, (±)-MDMA inhibited the firing of the majority (16/19, 84%) and excited the firing of a minority (3/19, 16%) of the neurons (Fig. 2A)[15]. Similarly, (+)-MDMA inhibited the firing of 9/10 (90%) and excited 1/10 (10%) mPFc cells (Fig. 2A). In contrast, (–)-MDMA inhibited the firing of 6/15 (40%) cells, excited 8/15 (53%) cells and had no effect on the remaining one (7%) cell (Fig. 2A). The profile of mPFc cells' responses to (±)-MDMA is statistically different (Logistic regression, $P < 0.05$) from that for (–)-MDMA but is the same as that for (+)-MDMA (Logistic regression, $P > 0.05$). In parallel, when the responses of all the neurons were considered, the cumulative dose–response curve of (–)-MDMA is shifted to the right with respect to that for (±)-MDMA (ANOVA and Student's Newman–Keul's test, $P < 0.05$) and the curves for (±)-MDMA and (+)-MDMA are not different (ANOVA and Student's Newman–Keul's test, $P > 0.05$) (Fig. 2B). The IC_{50} values extrapolated from the curves were 17.1 mg/kg for (±)-MDMA and 6.5 mg/kg for (+)-MDMA. The IC_{50} value for (–)-MDMA cannot be determined because even at the highest cumulative dose, the cells on the average were not appreciably inhibited. When only the cells which were inhibited by MDMA were considered, the IC_{50} values for (±)-, (+)- and (–)-MDMA were 8.0 ± 2.3 (n = 15), 4.2 ± 1.1 (n = 9) and 15.6 ± 4.9 (n = 6) mg/kg, respectively.

The effects of (±)-MDMA were reversed by the 5-HT receptor antagonists metergoline, granisetron and S-zacopride (0.5–2 mg/kg, i.v.) although metergoline's action was the most consistent. When MDMA-induced

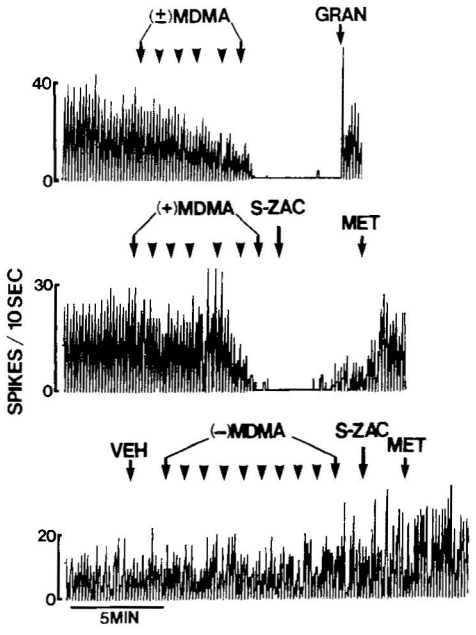

Fig. 1. Representative rate histograms showing the effect of (±)-, (+)- and (–)-MDMA on the firing of spontaneously active mPFc neurons in normal animals. Top: this rate histogram shows the inhibitory effect of (±)-MDMA on a typical mPFc cell. Granisetron (GRAN, 1 mg/kg) reversed its effect. MDMA had a similar inhibitory action on the majority of mPFc neurons. Middle: (+)-MDMA similarly inhibited the firing of this particular mPFc neuron. S-Zacopride (S-ZAC, 0.1–0.4 mg/kg) partially and metergoline (MET, 0.1 mg/kg) completely reversed its effect. The majority of mPFc neurons was similarly inhibited by (+)-MDMA. Bottom: (–)-MDMA had no effect on the majority of mPFc neurons as illustrated by this cell. S-ZAC (0.5–1 mg/kg) and MET (0.5–1 mg/kg) as well as vehicle had no effect on MDMA's action.

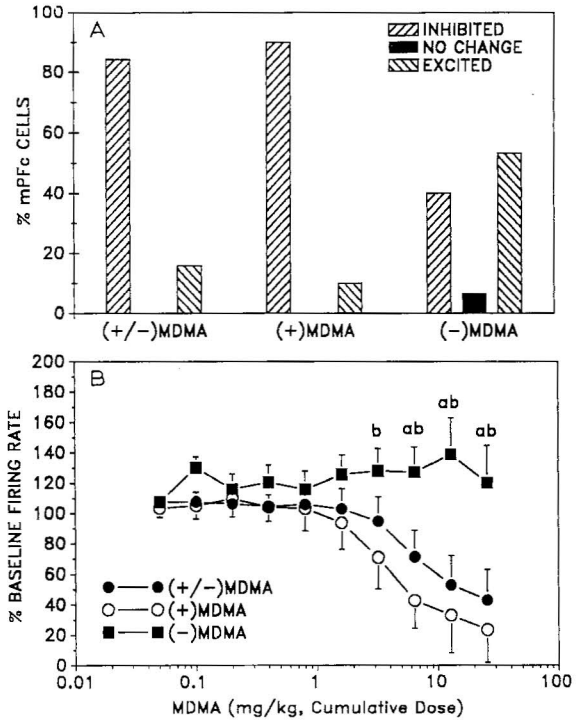

Fig. 2. The effect of (±)-, (+)- and (–)-MDMA (25.6 mg/kg, cumulative dose) on the firing activity of mPFc neurons in normal animals. A: (±)-MDMA inhibited the firing of 16/19 (84%) and excited 3/19 (16%) cells. (+)-MDMA inhibited 9/10 (90%) and excited 1/10 (10%) mPFC cells. (–)-MDMA inhibited 6/15 (40%), excited 8/15 (53%) and had no effect on 1/15 (7%) mPFc cells. The distribution of responses for (±)-MDMA is the same as that for (+)-MDMA but is significantly different from that for (–)-MDMA. B: the cumulative dose–response curves, including all cells studied, for (±)-, (+)- and (–)-MDMA. The curve for (–)-MDMA is significantly shifted to the right compared to those for (±)-MDMA and (+)-MDMA. a, $P < 0.05$, significantly different from (±)-MDMA, b, $P < 0.05$, significantly different from (+)-MDMA. Vertical bars = S.E.M.

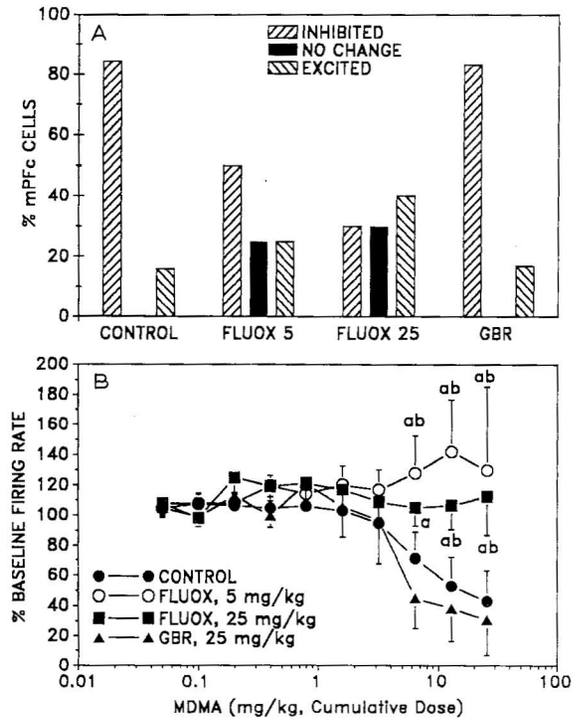

Fig. 3. The effect of fluoxetine (FLUOX) and GBR 12909 (GBR) treatment on mPFc cells' response to (±)-MDMA. A: in animals pretreated with FLUOX (5 or 25 mg/kg, i.p.) (±)-MDMA either excited or had no effect on more mPFC cells compared to controls. In contrast, GBR pretreatment did not have comparable effects. B: cumulative dose–response curves of MDMA's effect on the firing rates of all the mPFc cells studied revealed that FLUOX pretreatment significantly shifted (±)-MDMA's dose–response curves to the right compared to those in controls and GBR pretreated animals. a, $P < 0.05$, significantly different from controls, b, $P < 0.05$, significantly different from GBR-pretreated animals. Vertical bars = S.E.M.

inhibitory effect was considered, metergoline, granisetron and S-zacopride completely reversed its action in 11/25 (44%), 3/9 (33%) and 0/5 (0%) cells, respectively; partially reversed its action in 10/25 (40%), 2/9 (22%) and 3/5 (60%) cells; and did not reverse its action in 4/25 (16%), 4/9 (44%) and 2/5 (40%) cells. The MDMA-induced excitatory effect was completely reversed in 3/6 (50%), partially reversed in 1/6 (17%) and was not reversed in 2/6 (33%) mPFc cells by metergoline.

In animals treated with 5 mg/kg fluoxetine intraperitoneally 15–30 min before the electrophysiological experiment, 4/8 (50%) of mPFc cells were inhibited, 2/8 (25%) cells were excited and the remaining 2/8 (25%) cells were not affected by the drug (Fig. 3A). This distribution narrowly missed being significantly different from that produced by (±)-MDMA in controls (Logistic regression, $P > 0.05$). In animals treated with 25 mg/kg fluoxetine intraperitoneally 15–30 min before recording had begun, 3/10 (30%) cells were inhibited, 3/10 (30%) cells were excited and the remaining 4/10 (40%) cells were unaffected (Fig. 2A). This profile of response is significantly different from that seen in controls (Logistic regression, $P < 0.05$). By contrast, in animals treated with 25 mg/kg GBR 12909 (Research Biochemicals Inc.) intraperitoneally, 5/6 (83%) cells were inhibited and 1/6 (17%) cell was excited, a distribution which is not different from that seen in controls (Logistic regression, $P > 0.05$) (Fig. 2A). The cumulative dose–response curves describing (±)-MDMA's effect on mPFc cells after the aforementioned treatments are shown in Fig. 3B. IC_{50} values extrapolated from these curves were 17.1 mg/kg for controls and 11.6 mg/kg for animals treated with GBR 12909. IC_{50} values could not be determined for animals treated with fluoxetine because even at the highest dose (25.6 mg/kg) studied the cells on the average were inhibited by less than 50% of baseline. Overall, the dose–response curves for controls and the GBR 12909-treated animals were not different (ANOVA and Student's Newman–Keuls's test, $P > 0.05$), whereas those for controls were significantly different from animals which had received either 5 or 25 mg/kg fluoxetine (ANOVA and Student's Newman–Keul's test, $P < 0.05$) 10–30 min before recording began.

The present results show that (±)-MDMA suppresses the firing of the majority of mPFc neurons. The dose which suppresses the firing by 50% is slightly higher than the typical dose used by human users (1–5 mg/kg, p.o.) but is in agreement with doses given to monkeys (3 mg/kg, i.v., LD_{50} = 22 mg/kg) and rats (1–5 mg/kg, i.v., up to 300 mg/kg, p.o., LD_{50} = 32 mg/kg, i.p.)[21].

Our data also show that MDMA's suppressant effect is prevented or markedly attenuated by fluoxetine but not GBR 12909. Our finding is in concordance with the idea that the effect of (±)-MDMA is mediated via the 5-HT but not DA system. For instance, we and others have shown that MDMA-induced electrophysiological effects[12,15,23] and locomotion[6] are prevented by pretreating the animals with PCPA or fluoxetine but not AMPT. Taken together, the data indicate that the effect of MDMA is dependent on its 5-HT-releasing and uptake-blocking properties, actions which have been documented by many biochemical studies[20,24,25].

Our finding that (+)-MDMA is more effective than (–)-MDMA in inhibiting the firing of mPFc cells is consistent with the report that of the two isomers, the (+)-enantiomer has a more potent action in releasing 5-HT[20]. Similarly, Spouse et al.[23] found that (+)-MDMA inhibits the firing of dorsal raphe neurons by releasing 5-HT and that these cells are 2 to 3 times more sensitive to the action of (+)-MDMA than the (–)-isomer. Studies using various behavioral paradigms have also detected differences in the action of the two enantiomers. For example, (+)-MDMA is more potent than (–)-MDMA in drug discrimination tests[7,18], in producing hyperthermia[1]

and in eliciting stereotyped behaviors[10], although this last behavior has not been uniformly observed by others using the racemate[9,14,27].

MDMA has been reported to have a micromolar affinity for postsynaptic 5-HT receptors, especially the 5-HT_2 receptor subtype[4]. However, the (–)-isomer appears to have a higher affinity than the (+)-isomer at this binding site[13]. Interestingly, 5-HT_2 receptors have been implicated in the action of hallucinogens and the (–)-enantiomer of the hallucinogens is the more active one. Our finding that (+)-MDMA is the more active isomer may be related to the fact that MDMA has little or no hallucinogenic properties and indicates that MDMA's mechanism of action may be mediated by 5-HT sites other than (or in addition to) the postsynaptic 5-HT_2 receptors.

In this study, MDMA's suppressant action was reversed by the 5-HT_3 receptor antagonists S-zacopride, granisetron and the 5-$HT_{1,2}$ receptor antagonist metergoline. However, the antagonism produced by S-zacopride and granisetron was less consistent than metergoline. The data suggest that both 5-HT_1 and 5-HT_3 receptors may play a role in mediating MDMA's suppressant effect in the mPFc. Consistent with our finding, Geyer et al. (personal communication) have presented preliminary results showing that 5-HT_1 receptors are important in mediating the MDMA-induced locomotor activity as pindolol effectively blocks MDMA's action. On the other hand, it is tempting to speculate that 5-HT_2 receptors contribute to the occasional excitatory effect produced by MDMA as stimulation of this receptor subtype has been reported to excite or facilitate the excitatory action of glutamate on neurons in the frontal cortex[3,5]. Obviously, more research is needed to further elucidate the involvement of the specific 5-HT_1 receptor subtypes in the action of MDMA.

In summary, although several lines of evidence suggest that MDMA, in addition to the 5-HT system, activates the DA system[8,20,22,27,28], our data indicate that 5-HT, but not DA, plays an important role in MDMA's action in the mPFc. Nevertheless, it is likely that MDMA may have a more substantial effect on the DA-system in other brain areas rich in DA content and innervation.

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