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Short communication

Serotonergic modulation of 3,4-methylenedioxymethamphetamine (MDMA)-elicited reduction of response rate but not rewarding threshold in accumbal self-stimulation

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Abstract

In a fixed interval 5-s rate-frequency function paradigm with rats, 3,4-methylenedioxymethamphetamine (MDMA; 0.5, 2 and 4 mg/kg) dose-dependently decreased response rate for nucleus accumbens self-stimulation while both D-amphetamine (0.3 and 1 mg/kg) and cocaine (5 and 15 mg/kg) increased response rates. The highest dose of MDMA caused a cessation of responding in many of the rats tested, but in those rats that continued to respond a significant reduction in frequency threshold for self-stimulation was seen. Cocaine and amphetamine dose-dependently reduced frequency threshold in all rats tested. The non-specific serotonin antagonist, methysergide (5 mg/kg), reversed the inhibitory effects of MDMA on response rates and caused all rats to respond following MDMA (4 mg/kg). Methysergide did not affect MDMA's threshold-lowering properties and when administered alone methysergide had no effect on self-stimulation. These results suggest serotonergic involvement in the performance but not reinforcement-modulating effect of MDMA in the self-stimulation paradigm.

Keywords: 3,4-Methylenedioxymethamphetamine (MDMA); D-Amphetamine; Cocaine; Methysergide; Self-stimulation; Performance; Reinforcement; 5-HT

Over the past 10 years, the use of 3,4-methylenedioxymethamphetamine (MDMA) has grown rapidly to the point where it is now one of the most widely used illicit drugs in the Western world. As the use of this drug has escalated, concerns have been raised about MDMA's addictive potential and possible neurotoxicity [35].

Accumulated evidence has shown a central role for the neurotransmitter serotonin (5-HT) in mediating the behavioural effects and discriminative stimulus properties of MDMA. In drug discrimination studies, MDMA generalises to serotonergically active agents such as fenfluramine and 1-(3-trifluoromethylphenyl)piperazine (TFMPP) [31,32]. In startle reflex tests, the excitatory effects of MDMA are prevented by 5-HT uptake inhibitors and by depletion of central 5-HT with 5,7-dihydroxytryptamine [17]. Similarly, D-MDMA-induced hyperactivity is antagonised by 5-HT reuptake inhibitors and by prior depletion of 5-HT with *p*-chlorophenylalanine [6]. Furthermore, it is reported that a range of MDMA's effects, such as those on drug discrimination [31,32], operant responding [29], conditioned place preference [2], locomotor activity [11] and analgesia [7] are modified by various serotonergic antagonists.

Like most drugs that are euphorigenic in humans, MDMA dose-dependently lowers the rewarding threshold for self-stimulation of the medial forebrain bundle (MFB) in rats [13]. A reinforcing effect of MDMA is also shown in the conditioned place preference paradigm in rats [1,2,33] and in the drug self-administration paradigm in baboons [18]. The amphetamine-like hyperactivity seen in rats following MDMA administration is also consistent with this drug exerting a positively reinforcing effect [10,11].

There is uncertainty regarding the extent to which this positively reinforcing effect of MDMA is serotonergically mediated. The non-selective serotonergic antagonist, methysergide, was found to potentiate MDMA-induced

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hyperactivity in rats [11] while the 5-HT_3 antagonist MDL-72222 prevented the MDMA-induced conditioned place preference [2]. However, other studies suggest that it is MDMA's effect on dopamine (DA), rather than serotonin that is critical for reinforcement. Thus 6-OHDA lesions of the nucleus accumbens (NAS) were found to attenuate MDMA-induced hyperactivity [10]. Further, in the self-stimulation paradigm, the 5-HT_2 receptor antagonist, LY-53857, did not affect the threshold-lowering effect of MDMA on MFB self-stimulation [3].

The present study aimed to further examine the potential role of 5-HT in MDMA's reinforcing effects using a self-stimulation paradigm. Firstly, given the general similarities in the behavioural effects of MDMA, D-amphetamine and cocaine [9,13,21,22,25] we examined the similarities of the effects of these three compounds on self-stimulation (Experiment 1). Secondly, the non-specific serotonin antagonist, methysergide [19], was used to determine the possible role of serotonin in mediating MDMA's effects on self-stimulation (Experiment 2).

In these experiments, a fixed interval (FI) 5-s rate-frequency function paradigm was utilised to allow a sensitive distinction between reward from performance effects [20,30,36]. Further, both threshold and response rate measures were gathered to give a complete profile of each drug on reward and performance [20]. NAS self-stimulation was the target behaviour in these experiments. Previous studies of MDMA's effects on self-stimulation have utilised MFB self-stimulation [3,13]. However, given the probable role of the NAS in mediating MDMA's rewarding effect [10], and the general paucity of psychopharmacological studies of NAS self-stimulation, it was thought that this was an interesting target site for investigation.

Subjects were male Wistar rats (University of Sydney SPF Facility, Little Bay, Australia), pair housed with free access to water and standard lab chow. The colony room was maintained at 22 ± 1°C and illuminated on a 14 h light/10 h dark cycle. All experimentation occurred during the light cycle. Nine drug naive rats were used in Experiment 1. At the end of this experiment, a re-test with MDMA 2 mg/kg showed no significant sensitisation in total responding ($P > 0.81$), maximal rate ($P > 0.71$) or threshold ($P > 0.31$). Thus six of these rats served as the subjects of Experiment 2 and received drug testing approximately 10 days after the final dose of Experiment 1.

Rats, weighing about 260–340 g at the time of surgery, were stereotaxically implanted with stainless monopolar electrodes in the NAS (AP, +1.2–2.2; ML, ±1.4; DV, −7.0) [28] under anaesthesia with a mixture of Ketalar (ketamine, 100 mg/kg; Parke Davis) and Rompun (xylazine hydrochloride, 1 mg/kg; Bayer). About 10 days after the surgery, the rats were individually trained in one of four operant chambers ($37 \times 25 \times 32$ cm), to press a single lever for cathodal rectangular pulses of brain stimulation. The stimulation was produced by a programmable stimulus generator and continuously monitored on an oscilloscope. The experimental process and data collection were under the control of a PDP 11/20 computer system.

In the FI 5-s rate-frequency function paradigm, train duration and pulse width were fixed at 0.5 s and 0.2 ms respectively, and the current intensity was adjusted for individual rats to produce approximately half maximal responding. The test procedure consisted of nine 2-min blocks with a 40-s time-out interval between each block. Stimulation frequency was varied across blocks in nine descending steps of 0.1 $\log_{10}$ unit from 158 to 25 Hz. The first block was set as a warm-up session and data for the first minute of each other block was discarded to allow for adjustment to stable responding following the shift from one block to the next. When the variation in total responding was less than 10% and the variation in frequency threshold was not more than 0.1 $\log_{10}$ unit across 3 consecutive sessions, the response of a rat was considered stable and drug testing commenced.

There were three dependent variables measured in each test session:

(1) *Total responding* which is the sum of responses during the entire 8-min data-collecting period. This measure reflects a drug's effects on both performance and reinforcement in self-stimulation [20,36].

(2) *Maximal rate* which refers to the highest rate (bar pressings/min) reached across the entire session. This is an indicator of changes in performance [36].

(3) *Frequency threshold* which is a specific measure of reinforcement and is defined as the log unit of the pulse frequency at which response rate reached half of the maximal rate seen under vehicle treatment [36].

In certain cases following drug administration, frequency threshold could not be determined because the maximal rate obtained was less than half the maximal rate seen under vehicle treatment. Such cases were omitted from the statistical analysis of threshold changes. With some drug treatments the minimum rate seen was above the half-maximal rate seen under vehicle treatment. In such cases, the lowest stimulation frequency was used as a conservative value for the frequency threshold and was included in the statistical analysis.

MDMA hydrochloride (National Institute on Drug Abuse, USA), D-amphetamine sulfate (May and Baker, UK), cocaine hydrochloride (Australian Pharmaceutical Industries, Australia) and methysergide hydrogen maleate (Sandoz, Switzerland) were dissolved in 0.9% sterile saline. All injections were administered by i.p. route in a volume of 1 ml/kg body weight. Doses were expressed as the weight of salt form. The selection of drug doses was based on previous work in this or other labs [8,11,21,22,29].

In both experiments, a within-subjects design was used in which every rat received each drug treatment. The order of treatments was randomised and there was an interval of at least 72 h between any two treatments. Drug testing or maintenance training continued for one session per day for 6 days per week.

Raw data for total responding and maximal rate as well as $\log_{10}$ units of frequency threshold were used for statistical analyses. The data for total responding comprised scores for all the rats tested, whereas the data for maximal rate and threshold only included scores for those rats whose data were able to be used in threshold determination. In Experiment 1, due to some subjects' premature loss of electrode assemblies or the failure to meet the criterion for threshold calculation, the sample sizes were unequal. For this reason, the data were subjected to matched-sample Dunn test and harmonic means of sample sizes were applied to replace real numbers [12]. In Experiment 2, all data sample sizes were equal and thus analysed by the one-way analysis of variance (ANOVA) with repeated measures followed by the Duncan test.

At the end of experiments, rats were killed and a small direct current lesion was made around the electrode tip. The brain was frozen and sliced, stained with Toluidine blue and examined under a microscope with reference to the atlas of Paxinos and Watson [28]. Only those data from the rats with confirmed placements in the NAS were included in the following results.

Fig. 1 presents the effects of MDMA, D-amphetamine and cocaine on NAS self-stimulation in Experiment 1.

Total responding. MDMA decreased total responding in a dose-dependent manner with a marked effect at 4 mg/kg ($P < 0.01$). At this dose, five of nine rats displayed complete cessation of lever pressing and all nine rats exhibited the ''serotonin syndrome'' characterised by low body posture and head weaving, accompanied by copious salivation. In contrast, increased responding was seen with both the low and high doses of D-amphetamine (both Ps < 0.05) and the lower dose of cocaine ($P < 0.01$). The higher dose of cocaine facilitated responding in most of the rats but decreased total responding in two rats. The difference between cocaine 15 mg/kg and the vehicle control failed to reach a significant level mainly due to this large variation in responding.

Maximal rate. MDMA tended to decrease maximal rate of self-stimulation although non-significantly (all Ps $>$ 0.05). In contrast, the higher dose of D-amphetamine ($P <$ 0.01) and both lower and higher doses of cocaine ($P < 0.01$ and $P < 0.05$, respectively) significantly increased maximal rate.

Frequency threshold. In the four rats with whom threshold determinations could be made following MDMA (4 mg/kg), a significant reduction was seen ($P < 0.05$). The thresholds seen following D-amphetamine ($P < 0.01$) as well as that by both doses of cocaine (both Ps < 0.01) were also significantly reduced.

Fig. 2 shows the interaction of methysergide with MDMA or D-amphetamine on the dependent variables. Compared with the vehicle control, methysergide itself did not cause a statistically reliable change in total responding, maximal rate and threshold (all Ps > 0.05).

Total responding. Statistical analysis by a one-way

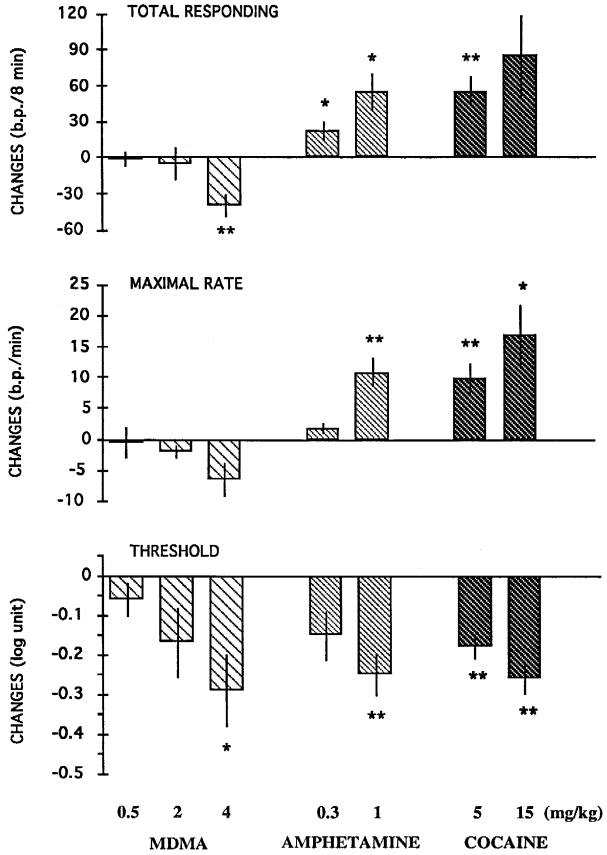

Fig. 1. The effects of MDMA, D-amphetamine and cocaine on total responding (upper), maximal rate (middle) and frequency threshold (lower) of NAS self-stimulation. Each column (vertical bar) represents mean difference (±S.E.M.) in values between drug and vehicle treatments. $n = 7$–9 for the total responding measure, $n = 4$–9 for the maximal rate and threshold measures. * $P < 0.05$, ** $P < 0.01$, compared with the vehicle control. b.p. = bar pressings.

ANOVA with repeated measures confirmed a significant overall effect in this measure ($F_{6,30} = 7.965$, $P < 0.01$). When combined with methysergide, MDMA dose-dependently increased total responding with a significant effect at the highest dose ($P < 0.01$). In contrast to the case of MDMA alone (see the above results), under the combination treatment all six rats in this experiment responded well to brain stimulation and no ''serotonin syndrome'' was observed. Also, combination of both doses of D-amphetamine with methysergide yielded a marked increase of total responding (both Ps < 0.01).

Maximal rate. There was a statistically reliable treatment effect in the measure ($F_{6,30} = 8.996$, $P < 0.01$). In a dose-related fashion, MDMA plus methysergide produced higher maximal rate than vehicle control, with a significant effect at the combined, highest dose of MDMA ($P < 0.01$). So did both combined doses of D-amphetamine plus methysergide (both Ps < 0.01).

Threshold. A marked overall effect in this measure was found in the statistical analysis ($F_{6,30} = 3.724$, $P < 0.05$). In a dose-dependent manner, the combination of methy-

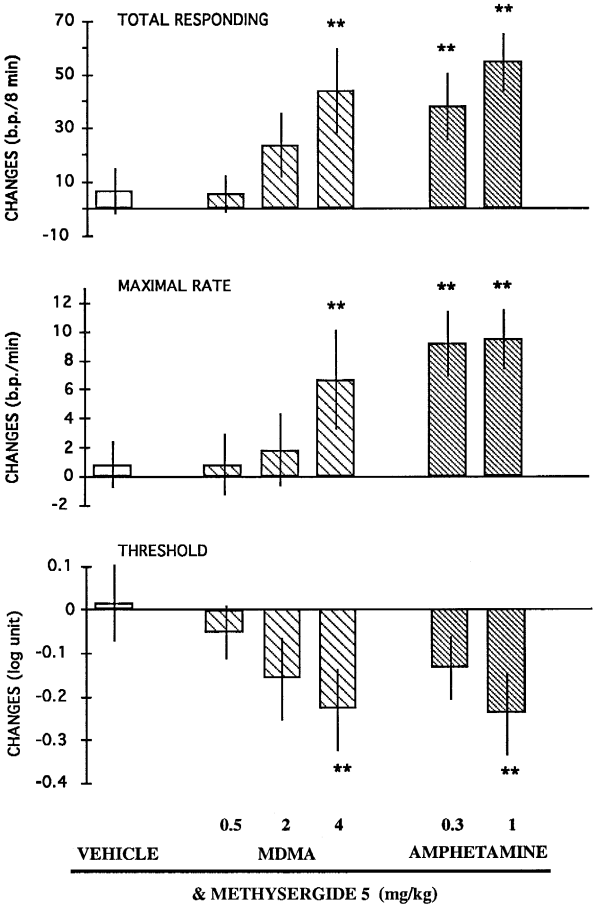

Fig. 2. The influence of methysergide on the effects of MDMA or D-amphetamine on total responding (upper), maximal rate (middle) and frequency threshold (lower) of NAS self-stimulation. Each column (vertical bar) represents mean difference (±S.E.M.) in values between drug and vehicle treatments. $n = 6$ for all three measures. * $P < 0.05$, ** $P < 0.01$, compared with the vehicle control. b.p. = bar pressings.

sergide with MDMA or D-amphetamine reduced thresholds for NAS self-stimulation. The threshold of a combination of methysergide with the highest dose of MDMA ($P < 0.01$) or D-amphetamine ($P < 0.01$) was significantly lower than that of the vehicle control.

Three major findings are evident from the above results. Firstly, MDMA depressed total self-stimulation responding and tended to depress maximal rate, indicating an inhibitory effect of this drug on operant performance. On the other hand, in rats that continued to respond following MDMA administration, MDMA markedly lowered the threshold for brain stimulation, suggesting a facilitatory effect of MDMA on the reinforcement of NAS stimulation. Secondly, both D-amphetamine and cocaine significantly increased response rates and lowered rewarding thresholds, displaying a similarity with MDMA in modulating reinforcement, but a difference in affecting performance. Thirdly, methysergide dramatically reversed the inhibitory effect of MDMA on response rates and augmented the facilitating effect of lower dose of D-amphetamine on the same measures, without affecting the threshold-lowering effect of either drug.

The inhibitory effect of MDMA on performance in NAS self-stimulation is comparable with previous findings obtained with other species and operant paradigms. Thus, MDMA decreased response rates of pigeons for food in both components of a multiple FI 3-min/FR 30 schedule [24], and MDMA disrupted operant responding of rats in a drug discrimination paradigm [26] and of mice lever pressing for a liquid reinforcer [29]. In *Patas* monkeys, MDMA reduced responding for food under a multiple schedule [37]. The minimum effective doses of MDMA in these previous studies range from 2.0 to 4.0 mg/kg which agree with the doses reported here that suppressed total responding for self-stimulation.

The threshold-lowering effect of MDMA in the present study parallels those seen in earlier reports using self-stimulation [3,13], although the present study extends this earlier finding to include the NAS as another electrode site where MDMA reduced self-stimulation thresholds. The reinforcing effect suggested by this is in agreement with the effect seen with MDMA in the drug self-administration [18] and conditioned place preference paradigms [1,2,33].

An interesting finding in the present study is that the performance effect of MDMA is distinct from those of D-amphetamine and cocaine. This is in keeping with earlier findings that MDMA differed from D-amphetamine on affecting pigeon's schedule-controlled performance [4]. It has been reported that, in self-stimulation, amphetamine up to 4 mg/kg still displays facilitating effect on response rate [8] and cocaine does not impair rat's performance capacity even reaching a high dose level of 30 mg/kg [38]. Although very high doses of D-amphetamine or cocaine are likely to cause reduction of response rate, this is different to MDMA where response inhibition is evident at more moderate relative dose levels.

Drug discrimination studies have demonstrated that D-amphetamine is able to substitute for MDMA but the latter fails to substitute for the former, suggesting differences in MDMA and D-amphetamine subjective effects [26]. Correspondingly, the present study reveals that MDMA is dissimilar to D-amphetamine in performance effects but similar to it in reinforcement-modulating effect. The discriminative stimulus properties of D-amphetamine have been linked to dopaminergic systems [34], but those of MDMA are suggestive of serotonergic and dopaminergic mediation [31,32]. An excitatory role for dopamine in both performance and reinforcement in self-stimulation has been supported by a wide range of studies (for refs. see [36]). There also exists ample evidence for an inhibitory role of serotonin in self-stimulation behaviour (e.g. [16,23,27]). In view of MDMA's actions on both dopaminergic and serotonergic systems [14,32], it seems likely that the inhibition of performance seen in the present study is attributed to an activation of serotonergic systems, while the augmentation

of reinforcement may be due to enhanced dopaminergic function.

This speculation is supported by the facts that methysergide reversed the inhibitory effect of MDMA on responding for self-stimulation but did not affect the drug's lowering effect on threshold. This effect of methysergide is parallel to previous reports, in which the serotonergic antagonist partially reversed MDMA's suppression of investigatory responding [5] and significantly reversed the analgesic effects of MDMA on hot-plate test [7]. In this study, the inhibition of performance by MDMA was concomitant with ''serotonin syndrome'', while the reversal of this inhibitory effect by methysergide accompanying disappearance of the syndrome. This implies that MDMA-elicited depression of performance might be due to the stereotyped behaviour induced, which could interfere or compete with operant responding. Methysergide is a serotonergic antagonist with high affinities for the 5-HT_1-like and 5-HT_2 receptors [19]. Montgomery et al. [23] have reported that the 5-HT_{1A} receptors play a role in depressing lateral hypothalamic self-stimulation. We have also observed that the 5-HT_2 receptor antagonist, ketanserin, does not affect the inhibitory performance effect of MDMA in NAS self-stimulation (Lin et al., unpublished data). It is conjectured that the reversal of MDMA's effect on responding by methysergide may therefore involve the 5-HT_1-like receptors.

The failure of methysergide to affect MDMA's threshold-lowering effect confirms an earlier abstract suggesting that the 5-HT_2 receptor is not involved in this aspect of MDMA's effects [3]. In parallel, ketanserin does not affect the reinforcement-modulating effect of MDMA in NAS self-stimulation (Lin et al., unpublished data). More interestingly, the 5-HT reuptake inhibitor, fluoxetine, on its own, significantly reduces total responding and maximal rate without affecting the threshold in a self-stimulation paradigm identical to that used here (Lin et al., unpublished data). Taken together, these data indicate that the performance effect of brain stimulation is highly susceptible to changes in serotonergic function. On the other hand, its reinforcing effect is relatively insensitive to manipulation of this system.

Interaction between serotonergic and dopaminergic systems in mediation of animal's behaviours has been documented [11,27]. Also, previous evidence supports an inhibitory role of 5-HT in self-stimulation [16,23,27]. In general, augmentation of serotonergic activity inhibits the activation of dopaminergic systems, and suppressed serotonergic function is associated with increased dopaminergic activity [11,27]. The reversal of MDMA's inhibitory effects by methysergide could be explained as a result of blocking the serotonergic suppression of dopaminergic function.

It is worth mentioning that, in varying degrees, methysergide augmented the performance effects of the lower but not the higher dose of D-amphetamine without affecting the stimulant's effect on threshold. The augmentation by methysergide may also be interpreted as a result of an interaction between 5-HT and dopamine. That is, the weak dopaminergic effect of the lower dose D-amphetamine may have been exaggerated by the blockade of tonic inhibition from 5-HT. On the other hand, the dopaminergic function activated by the higher dose may be strong enough to overcome the serotonergic inhibition, so methysergide fails to exert any influence on it. The ineffectiveness of the antagonist in D-amphetamine's threshold measure rules out a serotonergic involvement in this respect. Interestingly, these data seem to lend indirect support for the notion described above, that is, 5-HT involvement in the performance but not the reinforcement-modulating effects of MDMA.

Methodologically, the criterion for determining thresholds in rate-frequency function can be variable. In the present study, the half-maximal rate seen under vehicle treatment was chosen as a unique criterion for all conditions. This was subject to the basic principle in pharmacological experimentation, i.e., comparison between drug and vehicle control should be conducted under same conditions except treatments. Alternatively, thresholds could be estimated by using the half-maximal rate of individual curves if considering possible fluctuation of thresholds caused by performance effects of drugs. We have compared the threshold values derived from both methods and found that although there were differences between the two sets of data, these differences did not generally correlate with corresponding changes in maximal rate. Moreover, most of the difference values (in 12/13 drug treatments) were less than or equal to 0.06 log unit and the highest one (at D-amphetamine 0.3 mg/kg alone) was 0.08 log unit. Practically, in the curve-shifting method, such minor fluctuation of threshold (< 0.1 log unit) generated by performance factors is considered of no significance [15]. Another methodological issue in this study is that the lowest stimulation frequency was taken as the threshold under some drug treatments where the minimum rates seen were beyond the half-maximal rate seen under vehicle treatment. These inexact data could be improved by extending the stimulation to lower levels, but we failed to do so due to some technical limitation at the time of conducting this study. Also, the use of a second, inactive lever might help to determine whether the increased performance was specific to the reinforced lever.

In summary, the purpose of the present study was to investigate the potential role of serotonin in MDMA-induced effects on accumbal self-stimulation. The behavioural profile of MDMA seems to differ from those of D-amphetamine and cocaine. Methysergide is able to reverse MDMA's inhibition of response rates but fails to affect its lowering of threshold. The results are suggestive of a role for serotonin in mediation of MDMA's action on performance effect rather than on reinforcing effect of the self-stimulation.

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