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RESEARCH PAPER

Dopaminergic mechanisms of reinstatement of MDMA-seeking behaviour in rats

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BACKGROUND AND PURPOSE
Animal models of drug-seeking suggest that exposure to cues associated with self-administered drugs and drug primes might precipitate relapse via activation of central dopaminergic substrates.

EXPERIMENTAL APPROACH
The effects of priming injections of dopamine and 5-HT agonists on drug-seeking and effects of dopamine antagonists on methylenedioxymethamphetamine (MDMA)-produced potentiation of drug-seeking following extinguished MDMA self-administration were examined.

KEY RESULTS
Drug-seeking was produced by exposure to a light stimulus that had been paired with self-administered MDMA infusions and this effect was potentiated by experimenter-administered injections of the dopamine D_2-like receptor agonist, quinpirole, the indirect agonist, amphetamine and the uptake inhibitor, GBR 12909. Drug-seeking was not elicited by the dopamine D_1-like receptor agonist, SKF 81297 or the non-selective agonist, apomorphine. The 5-HT receptor agonists DOI or mCPP also failed to elicit drug-seeking. The 5-HT uptake inhibitor, clomipramine, attenuated drug-seeking produced by the MDMA-associated stimulus but failed to alter the potentiated response produced by GBR 12909. The D_1 receptor antagonist, SCH 23390 or the D_2 receptor antagonist, eticlopride attenuated the potentiation of drug-seeking produced by MDMA.

CONCLUSIONS AND IMPLICATIONS
These data provide evidence of dopaminergic mechanisms in drug-seeking following extinction of MDMA self-administration. Because tissue levels of 5-HT were significantly decreased following MDMA self-administration, we suggest that MDMA begins to preferentially activate dopaminergic substrates to potentiate the drug-seeking response.

Abbreviations
DOI, +/–2,5-dimethoxy-4-iodo-phenylisopropylamine; mCPP, 1-(3-chlorophenyl) piperazine hydrochloride; MDMA, methylenedioxymethamphetamine

Introduction

Drug abuse is a chronic relapsing disorder characterized by a compulsive cycle of drug-seeking and drug-taking. In laboratory studies of abstinent drug abusers, craving and drug-seeking have been produced by exposure to cues associated with self-administered drugs as well as by exposure to the drug itself (Jaffe *et al.*, 1989; Grant *et al.*, 1996; de Wit, 1996; Childress *et al.*, 1999). Drug-seeking has been modelled in laboratory animals through the use of the reinstatement procedure (de Wit and Stewart, 1981) and has been demonstrated following exposure to cues that had been associated with self-administered drug infusions, to doses of the self-administered drug or in response to other drug primes (Shaham *et al.*, 2003).

Reinstatement of drug-seeking has generally been attributed to dopaminergic mechanisms. For example, drug-seeking was produced following administration of the

indirect dopamine receptor agonist, GBR 12909 (Schenk, 2002; Schmidt and Pierce, 2006; Platt *et al.*, 2007) and dopamine D_2-like (De Vries *et al.*, 1999; Koeltzow and Vezina, 2005) but not D_1-like (Self *et al.*, 1996; De Vries *et al.*, 1999; Khroyan *et al.*, 2000) receptor agonists (receptor nomenclature follows Alexander *et al.*, 2009). Additionally, drug-seeking was attenuated by pretreatment with dopamine receptor antagonists (Khroyan *et al.*, 2000; Schenk and Gittings, 2003).

When compared with other drugs of abuse, there is a relative paucity of studies that have examined drug-seeking following 3,4-methylenedioxymethamphetamine (MDMA) self-administration. Drug-seeking has been demonstrated in laboratory rats exposed to cues that had been paired with self-administered MDMA (Ball *et al.*, 2007) or in response to an experimenter-administered injection of MDMA (Schenk *et al.*, 2008), in mice (Trigo *et al.*, 2009) and in monkeys (Banks *et al.*, 2008) exposed to priming injections of MDMA following extinction of self-administration. The mechanisms underlying MDMA-seeking have not, however, been investigated.

Following acute administration, MDMA preferentially increased 5-HT neurotransmission (Green *et al.*, 1995) but chronic exposure to high doses in a binge-like manner produced deficits in various indices of 5-HT neurotransmission that were apparent up to 32 weeks after treatment (Sabol *et al.*, 1996). Additionally, the increase in synaptic levels of 5-HT produced following acute exposure to MDMA was compromised following neurotoxic administration (Shankaran and Gudelsky, 1999; Baumann *et al.*, 2008) and the increase in synaptic levels of dopamine produced following acute exposure to MDMA was enhanced following repeated, neurotoxic (Kalivas *et al.*, 1998) or self-administered (Colussi-Mas *et al.*, 2010) exposure. Thus, following repeated exposure, MDMA shifts from being predominantly an indirect 5-HT agonist to being more of an indirect dopamine agonist. These findings raise the possibility that MDMA-seeking, like drug-seeking following self-administration of other drugs of abuse, might involve dopaminergic mechanisms.

The persistent deficits in 5-HT produced following repeated high-dose exposure to MDMA might be particularly relevant for its continued self-administration and for relapse. There are complex interactions between 5-HT and dopamine but there is some evidence that decreased 5-HT can modify drug-seeking (Tran-Nguyen *et al.*, 1999; Schenk, 2000; Fletcher *et al.*, 2002; Burmeister *et al.*, 2004). Effects of pharmacological manipulations, however, might be dependent on the receptor (Burmeister *et al.*, 2004; Acosta *et al.*, 2005) or on the type of stimulus used to reinstate extinguished self-administration (Burmeister *et al.*, 2003; 2004).

Because the increase in synaptic 5-HT produced by acute exposure to MDMA was reduced following repeated neurotoxic exposure (Shankaran and Gudelsky, 1999; Baumann *et al.*, 2008) and MDMA self-administration decreased 5-HT transporter binding densities (Schenk *et al.*, 2007), we have suggested that MDMA self-administration and drug-seeking become more dependent on dopaminergic mechanisms (Schenk *et al.*, 2007; Schenk, 2009; Colussi-Mas *et al.*, 2010). If so, then pharmacological manipulations of dopaminergic systems would be expected to affect drug-seeking. This hypothesis was tested in the present experiment by examining effects of 5-HT and dopamine receptor ligands on reinstatement of drug-seeking following MDMA self-administration.

Methods

Animals

All animal care and experimental procedures were approved by the Animal Ethics committee at Victoria University. Adult male Sprague-Dawley rats weighing 325–350 g were used. The rats were bred in the vivarium at Victoria University of Wellington and were housed in groups of four until they reached 300–325 g. Thereafter, they were housed individually in standard hanging polycarbonate cages in a temperature- (19–21°C) and humidity- (55%) controlled facility. The colony is accredited by the Office of Laboratory Animal Welfare and was maintained on a 12 h light/dark cycle (lights on at 0700 h). All tests were conducted during the light portion of the cycle (beginning at 0900 h). Food and water were available *ad libitum* except during testing.

Surgery

Rats were implanted with a Silastic catheter (Dow Corning, Midland, MI, USA) in the external jugular vein under deep anesthesia produced by the injection of a combination of ketamine (90 $mg \cdot kg^{-1}$) and xylazine (9 $mg \cdot kg^{-1}$). The external jugular vein was isolated and the tubing was inserted and fixed in place. The distal end of the tubing was passed subcutaneously to an exposed portion of the skull and fitted onto a 2 cm length of 22 ga stainless steel tubing which was then attached to the skull using jeweler's screws embedded in acrylic dental cement.

Testing began 5–7 days following surgery. Each day following surgery, the catheters were infused with 0.1 mL of a sterile saline solution containing heparin (3.0 $IU \cdot mL^{-1}$), penicillin G potassium (250 000 $IU \cdot mL^{-1}$), and streptokinase (8000 $IU \cdot mL^{-1}$) to maintain catheter patency and to prevent infection and the formation of clots and fibroids.

Apparatus

Self-administration tests were conducted at the same time each day in operant conditioning chambers (Medical Associates, ENV-001, St. Albans, VT, USA) equipped with two levers. The testing room was temperature- (19–21°C) and humidity- (55%) controlled. Depression of one lever (the 'active' lever) resulted in a 12.0 s intravenous infusion (0.1 mL) of MDMA. Depression of the other lever (the 'inactive' lever) was without programmed consequence. Coincident with drug infusions was the illumination of a stimulus light located above the active lever.

Rats were maintained in their home cages in the animal facility until testing. Immediately prior to each daily test session, the catheters were flushed with 0.1 mL of the heparin-penicillin-streptokinase solution and the exposed stainless steel tubing was attached to a length of microbore tubing that was connected through a swivel apparatus to a 20 mL syringe housed in a mechanical pump (Razel, Model A

with 1 rpm motor, St. Albans, VT, USA). Drug delivery and data acquisition were controlled by a microcomputer using Medical Associates software.

Procedure

Training. Acquisition of MDMA self-administration was conducted during daily 6 h sessions, as previously reported (Schenk *et al.*, 2003, 2007). Every session began with an experimenter-delivered infusion of drug in order to clear the heparin-penicillin-streptokinase solution from the catheter line. Thereafter, each depression of the active lever [fixed ratio (FR1) reinforcement schedule] resulted in an automatic infusion of MDMA (1.0 mg·kg^{-1}per infusion) paired with a stimulus light located directly above the active lever. This dose has been used in acquisition studies in our laboratory and self-administration is produced within 15 days for about 60% of the MDMA trained rats (Schenk *et al.*, 2003, 2007; Colussi-Mas *et al.*, 2010). Responses maintained by this high dose of MDMA are generally low (Schenk *et al.*, 2003; Daniela *et al.*, 2004, 2006) and so following acquisition (at least two consecutive days of at least seven active lever responses and a preference for the active lever), the dose of MDMA was reduced to 0.5 mg·kg^{-1} per infusion, as we have done in our previous studies. Responding on the active lever was reinforced according to an FR1 schedule until there was less than 20% variation in active lever responses on at least three consecutive days. When this criterion was met, responding was considered stable. The response requirements were then increased to FR2 for a minimum of 3 days and then to FR5. Training required at least 30 days and there were at least 5 days of responding maintained by the FR5 schedule before subsequent tests were conducted. In the present study, 36 rats that acquired self-administration continued for tests of drug-seeking. The average number of days for training was 49 (range = 30–71) and average self-administered MDMA during training was 361.5 mg·kg^{-1} (range = 225.5–527.0 mg·kg^{-1}).

Test. Following acquisition and stabilization of MDMA self-administration, reinstatement tests were conducted. These 6 h daily tests were conducted during a recurring series of 5 days comprised of baseline (Phase 1; 2 days), extinction (Phase 2; 2 days) and reinstatement (Phase 3; 1 day) phases, as previously reported (Schenk *et al.*, 2008). Phase 1 consisted of 2 days of responding that was reinforced according to an FR5 schedule by an infusion of MDMA (0.5 mg·kg^{-1} per infusion) with the associated light stimulus. During the 2 days that comprised Phase 2, the drug solution was replaced with vehicle (3 U heparinized saline) and the light stimulus that had been paired with self-administered infusions was omitted. At the start of Phase 3, rats received a drug injection or combination of drugs and drug-seeking was measured. During Phase 3, responding on the lever that previously resulted in an MDMA infusion was reinforced with an infusion of vehicle and the illumination of the stimulus light.

Different groups received the 5-HT uptake inhibitor, clomipramine (10.0 mg·kg^{-1}, i.p.), the dopamine uptake inhibitor, GBR 12909 (1.0 or 10.0 mg·kg^{-1}, i.p.) or a combination of GBR 12909 (10.0 mg·kg^{-1}, i.p.) and clomipramine (10.0 mg·kg^{-1}, i.p.). Other groups received injections of the dopamine D_1-like receptor agonist, SKF 81297 (1.0, 2.0 or 4.0 mg·kg^{-1}, i.p.), the dopamine D_1/D_2-like receptor agonist, apomorphine (2.0 or 4.0 mg·kg^{-1}, i.p.), the dopamine D_2/D_3-like receptor agonist, quinpirole (0.5 or 1.0 mg·kg^{-1}, i.p.), d-amphetamine (1.0 mg·kg^{-1}, i.p.), the 5-HT_2 receptor agonist, +/–2,5-dimethoxy-4-iodo-phenylisopropylamine (DOI; 1.0 mg·kg^{-1}, i.p.) and the 5-HT releasing stimulant, 1-(3-chlorophenyl) piperazine hydrochloride (mCPP; 0.6 or 2.5 mg·kg^{-1}, i.p.).

Separate groups of rats were pretreated with the dopamine D_1-like receptor antagonist, SCH 23390 (0.02 or 0.04 mg·kg^{-1}, s.c.) or the dopamine D_2-like receptor antagonist, eticlopride (0.0125, 0.025 or 0.05 mg·kg^{-1}, i.p.), 15 or 30 min, respectively, prior to an injection of MDMA (10.0 mg·kg^{-1}, i.p.) at the start of Phase 3.

Doses of the agonists were determined as behaviourally relevant in tests in our laboratory. These doses of SKF 81297, amphetamine and apomorphine produced dose-dependent hyperactivity and the dose of clomipramine blocked hyperactivity produced by MDMA. When tested in various paradigms in our laboratory, doses of 0.6–2.5 mg·kg^{-1} mCPP dose-dependently decreased baseline locomotor activity (Jones *et al.*, 2010) and the dose of 1.25 mg·kg^{-1} increased latency to emerge from a hide box (Jones *et al.*, 2010). The dose of 1.0 mg·kg^{-1} DOI produced muscle twitches (Schreiber *et al.*, 1995) and a decrease in open arm exploration on the elevated plus maze (Bull *et al.*, 2004). In our laboratory, 1.0 mg·kg^{-1} DOI produced significantly more 'wet dog shakes' than vehicle-injected rats (6.5 ± 1.1 compared with 1.0 ± 0.4; unpubl. findings). The dose of GBR 12909 has previously been shown to produce cocaine- and heroin-seeking in a similar paradigm (De Vries *et al.*, 1999; Schenk and Partridge, 1999) and the dose of MDMA has previously been shown to produce MDMA-seeking (Schenk *et al.*, 2008).

For these tests rats were randomly assigned to drug and dose condition. For tests of the effects of the agonists, 2–3 rats were initially assigned to each drug condition (n = 22). Once assigned to a particular drug group, the rats were first tested with all doses of the drug in random order. Once the complete data set was obtained for the effects of this first drug, 13 of the 22 rats were tested with all doses of another drug so that final sample sizes were 4–5 per condition. The rats in each of the initial drug groups were allocated to different drug groups for subsequent tests so that no drug group contained more than one rat with specific prior drug experience. The tests of effects of drug combinations (clomipramine/GBR 12909; SCH 23390/MDMA; eticlopride/MDMA) were conducted on rats that had not been tested previously (n = 5 per group).

Within each drug test condition, rats received all doses of the drug administered in random order. Between tests of different doses, there were at least 2 days of responding maintained by MDMA (0.5 mg·kg^{-1} per infusion, FR5 schedule of reinforcement) followed by extinction and reinstatement tests. Tests of effects of additional doses were conducted only when responding was within 20% of responding produced during initial self-administration trials.

In order to ensure that prior testing with one drug did not affect the results of tests for another drug, at least half of the subjects in each group received that test compound first. For example, in the group of four rats that received GBR 12909, two received GBR 12909 as their first drug, one received DOI and one received mCPP prior to GBR 12909. In the mCPP and

DOI groups, two of the rats were first tested with GBR 12909 or clomipramine and two of the rats had not received any other test. Visual inspection of the data from individual rats in each drug condition suggested that the effects were not different for rats that had received prior testing with another compound. Indeed, effective drug doses produced a high number of responses in all rats regardless of order of administration within a drug test or when tests of multiple drugs were conducted. Because the order of doses was randomized and because some rats in each drug condition received the test drug as a first exposure, it was possible to observe whether dose or drug order affected drug-seeking. In all cases, drugs either failed to produce drug-seeking or produced drug-seeking regardless of whether they were administered as a first test or after tests with another compound.

Locomotor activity tests. In order to ensure that doses of the dopamine receptor agonists that failed to produce drug-seeking were behaviourally relevant, the locomotor activating effects of SKF 81297 and apomorphine were determined. Eight open field chambers (Medical Associates) equipped with two banks of 16 photocells on each wall were used to measure horizontal locomotion. The open field boxes were interfaced with a microcomputer located in an adjacent laboratory. Testing was conducted in the dark between 1000 and 1600 h. White noise was continually present to mask extraneous disturbances. On the test day, separate groups of rats ($n = 8$ per group) were habituated to the apparatus for 30 min after which they received an injection of apomorphine (0, 0.125, 1.0, 2.0 or 4.0 mg·kg^{-1}, i.p.) or SKF 81297 (0, 0.5, 2.0 or 4.0 mg·kg^{-1}, i.p.). Total activity, a compilation of horizontal and vertical activity, was measured for a 60 min period.

Neurochemical measurement of 5-HT and dopamine. Tissue from rats that were not tested for reinstatement ($n = 4$) or from control rats ($n = 8$) that were sham-operated and placed in the operant chambers daily were obtained 5 days following the last session. The number of days of self-administration training ranged from 53 to 67 and the total MDMA self-administered ranged from 312 to 418.5 mg·kg^{-1} (average = 368.1 ± 26.8 mg·kg^{-1}). Thus, these rats had exposure to self-administered MDMA comparable to that for the rats used in the behavioural tests.

The animals were killed by CO_2 asphyxiation. The brains were immediately removed and dissected on ice using a brain block to obtain the frontal cortex, striatum and hippocampus. Tissue samples were stored at –80°C until assay. Samples were homogenized in 10 volumes of 0.1 N perchloric acid and centrifuged at 12 000× *g* for 30 min at 4°C. The supernatant was analysed using a HPLC system (1100 series, Agilent, Santa Clara, CA, USA) with electrochemical detection with a C18 reversed phase column (Agilent Eclipse XDB-C18, 4.6 × 150 mm, 5 µm particle size) and a coulometric detector (Coulochem III, ESA; analysis cell: –175 and +400 mV; guard cell: +450 mV, Chelmsford, MA, USA). The mobile phase consisted of 75 mM NaH_2PO_4, 0.25 mM EDTA, 1.7 mM octane-1-sulphonic acid, 100 µL·L^{-1} triethylamine and 10% (v·v^{-1}) acetonitrile, pH 3, and was delivered at a constant flow rate of 1.0 mL·min^{-1}. Chromatograms were acquired with ChemStation software (Chelmsford, MA, USA) and peak heights of samples were compared with peak heights of standards with known concentrations of 5-HT and dopamine. Concentrations are expressed as ng·mg^{-1} of tissue.

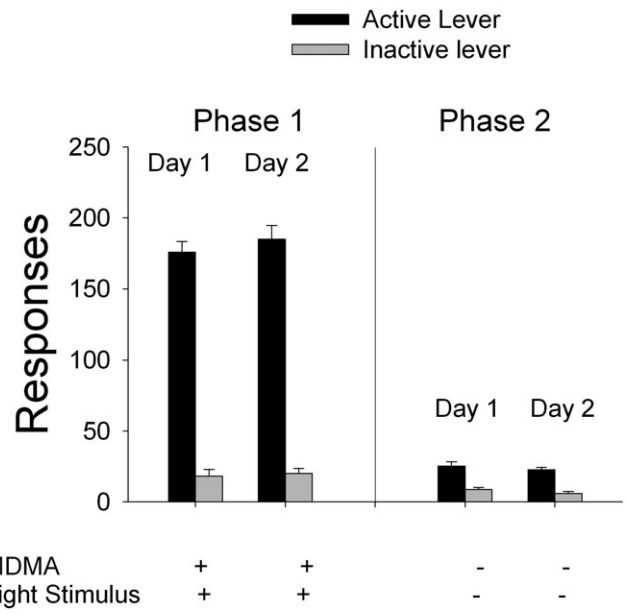

Figure 1

Active and inactive lever responses during the 2 days that comprised Phase 1, when lever responding was reinforced with an infusion of methylenedioxymethamphetamine (MDMA) (0.5 mg·kg^{-1} per infusion) and the illumination of a stimulus light and the 2 days that comprised Phase 2 when the MDMA solution was replaced with vehicle and the stimulus light was omitted. Data are from all 36 rats in the sample. Bars represent the mean number of responses (+SEM).

Data analysis

Responses on the lever that had previously resulted in an infusion of MDMA and on the inactive lever were recorded. The number of responses produced during each 6 h test session was analysed first using repeated measures ANOVAs. When a significant effect of Dose or interaction between Dose and Lever was observed, we used Tukey *post hoc* contrast tests. Neurochemical measurements from self-administering and control rats were compared using *t*-tests.

Materials

Racemic MDMA-HCl was obtained from ESR, Porirua, New Zealand and all other drugs were obtained from Tocris, New Zealand. Intravenous infusions of MDMA dissolved in 3 U heparinized saline were in a volume of 100 µL and intraperitoneal or subcutaneous injections were given in a 1.0 mL saline per kg volume. All drug doses were calculated based on salt weights.

Results

Figure 1 shows the average number of active and inactive responses during the 2 days that comprised each of Phases 1

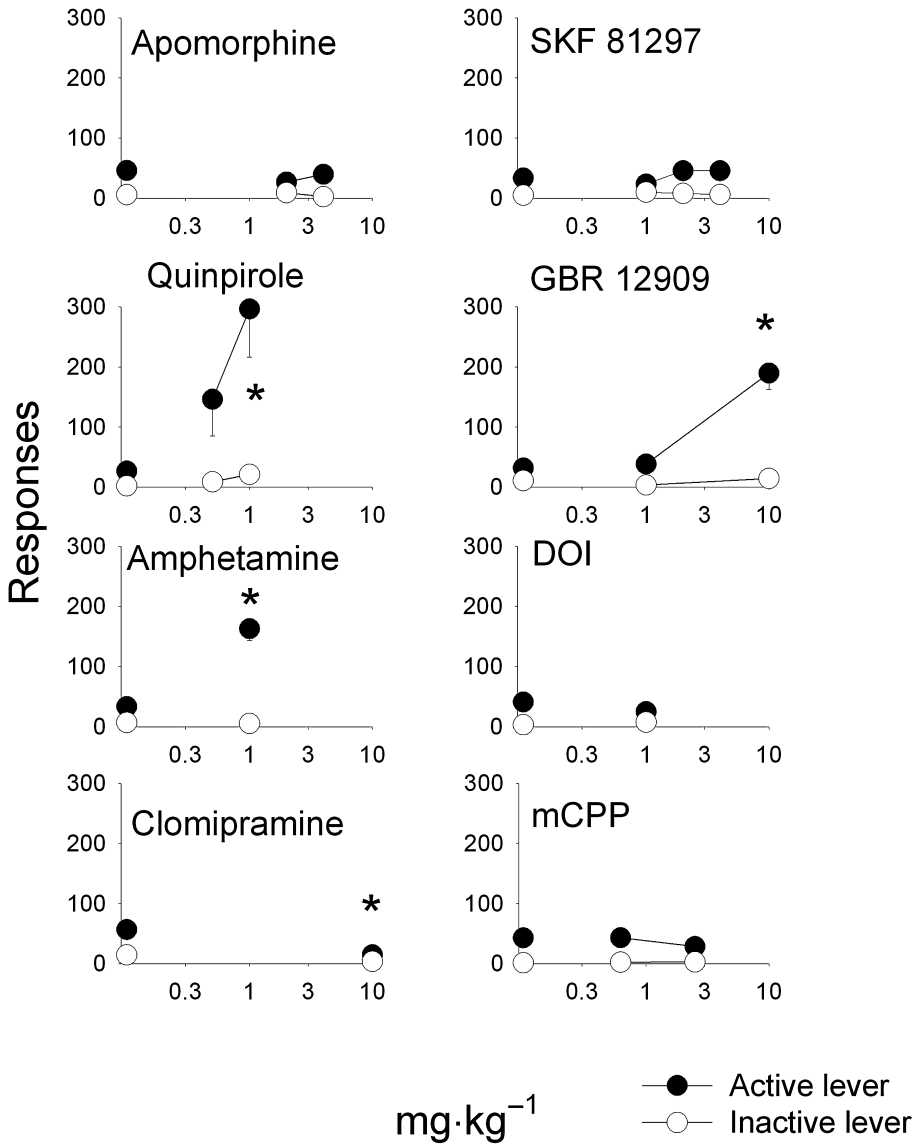

Figure 2

Effects of priming injections of various direct and indirect dopamine and 5-HT agonists on drug-seeking. Symbols represent the mean number of responses produced during Phase 3 (+SEM). Sample sizes are five per group for apomorphine, SKF 81297 and quinpirole and four or all other drugs. The left-most data point indicates responses following injection of the vehicle. Error bars that are not visible are smaller than the size of the symbol. *$P < 0.05$. DOI, +/–2,5-dimethoxy-4-iodo-phenylisopropylamine; mCPP, 1-(3-chlorophenyl) piperazine hydrochloride.

and 2 from all rats in this study. During Phase 1, MDMA infusions with the illumination of a stimulus light located above the active lever maintained a high number of active lever responses and a marked preference for the drug-associated lever was observed on both days that comprised this phase. Average MDMA self-administration was 18.5 mg·kg^{-1}·day^{-1} during the second day of FR5 responding. When the drug and the stimulus light were omitted during the 2 days that comprised Phase 2, active lever responding decreased and less than 20 responses were produced.

Figure 2 shows the effects of the various dopamine (top) and 5-HT (bottom) receptor agonists on drug-seeking during Phase 3. ANOVA revealed a significant increase in responding produced by GBR 12909 [$F(2,6) = 6.140$, $P < 0.05$], amphetamine [$F(1,5) = 36.46$, $P < 0.05$] and quinpirole [$F(1,4) = 11.636$, $P < 0.05$]. *Post hoc* contrast tests revealed significant increases in responding following 10.0 mg·kg^{-1} GBR 12909, 1.0 mg·kg^{-1} amphetamine and 1.0 mg·kg^{-1} quinpirole ($P < 0.05$). Small but non-significant increases in responding were produced by the D_1-like receptor agonist, SKF 81297 [$F(3,12) = 2.492$, not significant (NS)] and the non-selective dopamine receptor agonist, apomorphine [$F(2,8) = 1.441$, NS].Both of the direct 5-HT receptor agonists decreased responding slightly [DOI; ($F(1,3) = 0.78$, NS); mCPP ($F(2,6) = 0.638$, NS)] but clomipramine produced a significant decrease in responding [$F(1,3) = 27.35$, $P < 0.05$].

Figure 3 shows effect of various doses of apomorphine and SKF 81297 on locomotor activity. ANOVA revealed a main

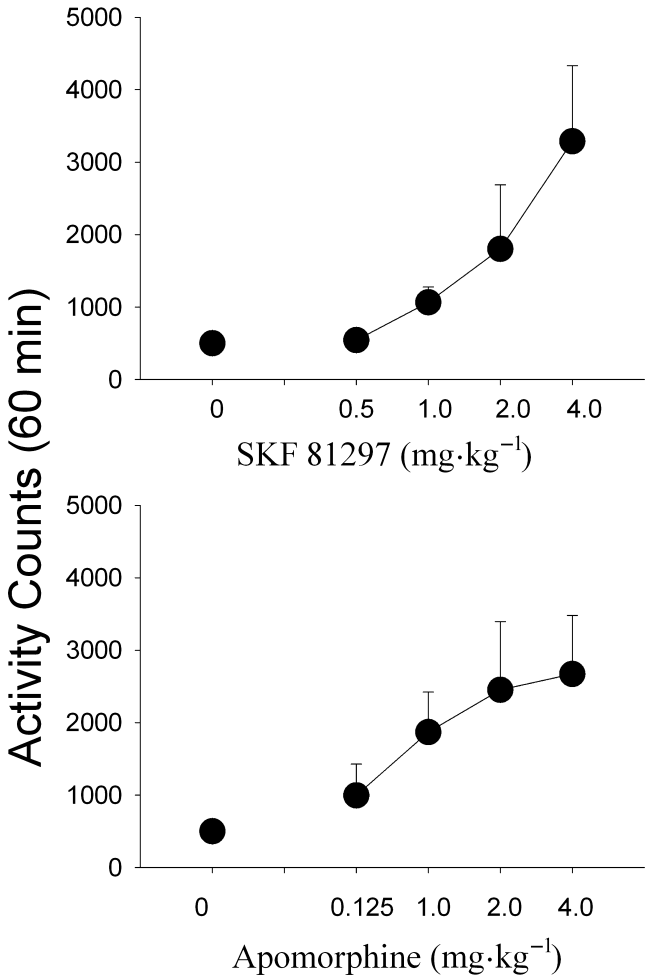

Figure 3

Locomotor activating effects of SKF 81297 (top) and apomorphine (bottom). Symbols represent the mean total number of activity counts (+SEM) during the 60 min period following injection. Sample sizes were eight per group.

effect of SKF 81297 dose [F(4,40) = 4.783, $P < 0.05$] and *post hoc* contrast tests revealed significant increases in activity following the dose of 4.0 mg·kg^{-1}. There was substantial variability in apomorphine-produced hyperactivity. Thus, some rats were particularly responsive to the hyperactive effects whereas for others the drug produced hypoactivity (range of activity scores following administration of: 2.0 mg·kg^{-1} = 751–8064; 4.0 mg·kg^{-1} = 19–5468), probably a reflection of competing motor responses elicited by higher doses of the drug (Harkin *et al.*, 2000). As a result of the high variability in apomorphine-produced hyperactivity, the effect of dose only approached significance [F(4,40) = 2.361, $P = 0.07$]. The homogeneity of variance test failed for these data and therefore a Kruskal–Wallis test was conducted. This test revealed a main effect of apomorphine dose [χ^2 (4) = 8.821, $P = 0.032$].

For many of the drug tests, responding increased following the injection of vehicle solution at the start of Phase 3 relative to responding produced during Phase 2. During both of these phases, lever responding resulted only in the infusion of vehicle but during Phase 3 but not Phase 2 responding also resulted in the illumination of the light stimulus that had been paired with self-administered infusions. Thus, an increase in responding would reflect effects of presentation of the MDMA-associated stimulus on drug-seeking. In order to examine this possibility, the data from all rats in the study were combined and the number of responses during Phase 2 when no light stimulus was presented [active = 19.7 (+1.4); inactive = 6.8 (+1.2)] and during Phase 3 when the light stimulus was presented [active = 41.8 (+5.2); inactive = 7.6 (+1.6)] were compared. Responding was higher when the light stimulus was presented and ANOVA revealed a significant interaction between Test Phase and Lever [F(1,14) = 6.369, $P < 0.01$]. *Post hoc* tests confirmed that responding on the active lever was significantly greater when the light stimulus was presented ($P < 0.05$).

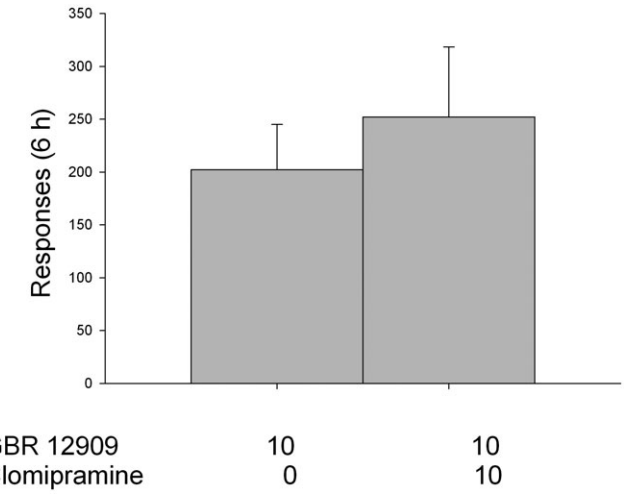

Figure 4

Number of responses (+SEM) produced following separate injections of clomipramine and GBR 12909 at the start of Phase 3 ($n = 5$).

Figure 4 shows the effect of the 5-HT uptake inhibitor, clomipramine combined with the dopamine uptake inhibitor, GBR 12909 on drug-seeking. Clomipramine failed to alter GBR 12909-produced drug-seeking.

Figure 5 shows the effects of the dopamine D_1-like receptor antagonist, SCH 23390, on drug-seeking produced by MDMA (10.0 mg·kg^{-1}). MDMA alone produced a high rate of responding, as previously reported (Schenk *et al.*, 2008), and this was attenuated by pretreatment with SCH 23390. ANOVA revealed a significant interaction between Lever and SCH 23390 Dose [F(2,3) = 10.906, $P < 0.05$] and *post hoc* tests confirmed a significant decrease in active lever responding with increasing doses of SCH 23390 ($P < 0.05$).

Figure 6 shows the effects of the dopamine D_2-like receptor antagonist, eticlopride, on drug-seeking produced by MDMA (10.0 mg·kg^{-1}). The high rate of responding produced by MDMA alone was attenuated by pretreatment with eticlopride. ANOVA revealed a significant interaction between Lever and eticlopride Dose [F(3.12) = 4.338, $P < 0.05$] and *post hoc* tests confirmed a significant decrease in active lever responding with increasing doses of eticlopride ($P < 0.05$).

Tables 1 and 2 show tissue levels of 5-HT and dopamine, respectively, from rats that had self-administered MDMA and sham-operated control rats that had been placed in the self-administration chambers but not had the opportunity to self-administer MDMA. In the three sites measured, levels of 5-HT were significantly reduced (34% frontal cortex; 19.5% striatum; 23.8% hippocampus; $P < 0.05$). Dopamine levels were not significantly altered by MDMA self-administration.

Discussion and conclusions

This study examined the effects of some dopaminergic and 5-hydroxytryptaminergic ligands on drug-seeking produced by exposure to either (i) a light stimulus that had been associated with self-administered MDMA or (ii) the light stimulus coupled with a drug injection. Both paradigms revealed an important role of dopaminergic mechanisms in MDMA-seeking.

A light stimulus that had been paired with self-administered infusions of MDMA reinstated extinguished responding and this effect was potentiated by experimenter-administered injections of the dopamine uptake inhibitor, GBR 12909, the indirect agonist, amphetamine, or the dopamine D_2-like receptor agonist, quinpirole. The dopamine D_1-like receptor agonist, SKF 81297, the non-selective agonist, apomorphine, or the direct $5HT_{2A/C}$ receptor agonists, DOI and mCPP, all failed to alter drug-seeking. In contrast, the 5-HT uptake inhibitor, clomipramine, decreased drug-seeking, as has previously been observed following administration of other 5-HT uptake inhibitors (Schmidt and Pierce, 2006) or the releasing stimulant, fenfluramine (Burmeister *et al.*, 2003; 2004). These data might suggest an inhibitory role of 5-HT but this interpretation must be made cautiously because clomipramine decreased responding on both levers which might reflect a non-specific disruption in the ability to perform the lever press operant.

These data are consistent with findings from other studies that have addressed the role of dopamine in drug-seeking

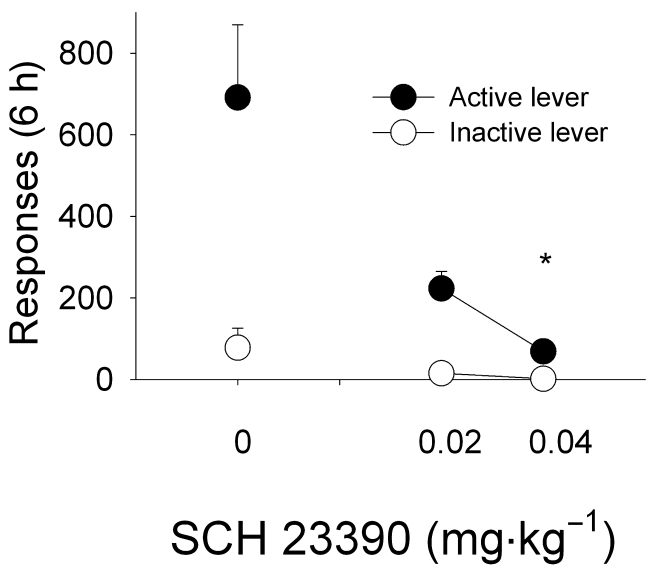

Figure 5
Effects of pretreatment with the dopamine D_1-like receptor antagonist, SCH 23390, on drug-seeking produced by methylenedioxymethamphetamine (10.0 mg·kg^{-1}). Symbols represent the mean (+SEM) number of responses ($n = 5$).

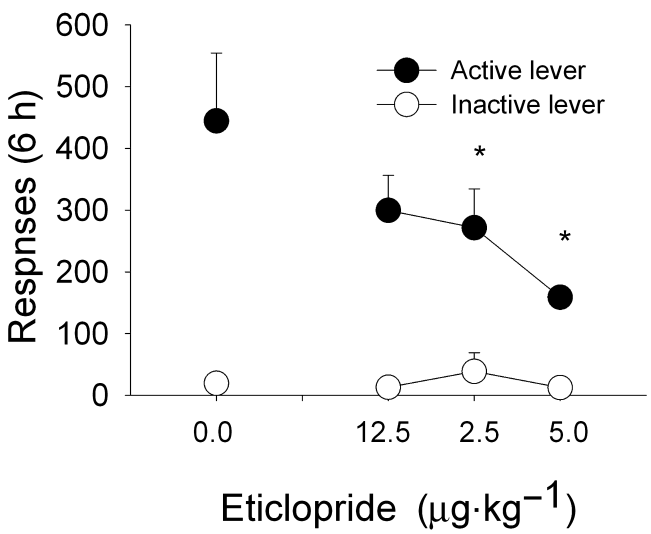

Figure 6
Effects of pretreatment with the dopamine D_2-like receptor antagonist, eticlopride, on drug-seeking produced by methylenedioxymethamphetamine (10.0 mg·kg^{-1}). Symbols represent the mean (+SEM) number of responses ($n = 5$).

Table 1
5-HT levels measured 5 days following the last methylenedioxymethamphetamine (MDMA) self-administration session

Brain region	Control ($n = 8$)	MDMA ($n = 4$)	Statistical results
Frontal cortex	0.68 ± 0.04	0.45 ± 0.09[a]	$t(10) = 2.759$, $P < 0.05$
Striatum	0.46 ± 0.03	0.34 ± 0.03[a]	$t(10) = 2.966$, $P < 0.05$
Hippocampus	0.42 ± 0.02	0.32 ± 0.04[a]	$t(10) = 2.484$, $P < 0.05$

Data are expressed as ng·mg^{-1} of tissue.
[a]$P < 0.05$ versus Control.

Table 2

Dopamine levels measured five days following the last methylenedioxymethamphetamine (MDMA) self-administration session

Brain region	Control ($n = 8$)	MDMA ($n = 4$)	Statistical results
Frontal cortex	0.07 ± 0.01	0.08 ± 0.02	$t(10) = 0.569$, NS
Striatum	14.89 ± 0.67	16.89 ± 1.30	$t(10) = 1.536$, NS
Hippocampus	0.06 ± 0.00	0.07 ± 0.01	$t(10) = 0.976$, NS

Data are expressed as ng·mg^{-1} of tissue.
NS, not significant.

following self-administration of other drugs. It might be surprising therefore that the D_1/D_2 receptor agonist, apomorphine, failed to enhance drug-seeking at doses that increased locomotor activity and were therefore pharmacologically active. Various other studies have also reported that apomorphine either failed to induce drug-seeking (De Vries *et al.*, 1999) or produced marginal increases in responding during the reinstatement test (de Wit and Stewart, 1981, 1983). The differential effects of apomorphine and the indirect receptor agonists that increase synaptic dopamine, like amphetamine and GBR 12909, are likely to reflect the extrasynaptic dopamine D_1-like receptor mediated-effects produced by apomorphine. It is difficult to reconcile this idea, however, with the failure of SKF 81297 to decrease cue-produced drug-seeking. Clearly, more research is required to adequately assess the impact of dopamine D_1 receptor activation on drug-seeking but it is possible that the antagonistic effects of D_1 receptor stimulation only become apparent under conditions that produce higher levels of drug-seeking (D_2 receptor stimulation). If so, then the D_2 receptor agonist properties of apomorphine would be offset by the extrasynaptic D_1 receptor agonist effects. This idea is consistent with the findings that cocaine-produced drug-seeking was also attenuated by a D_1-like receptor agonist that failed to alter drug-seeking when presented alone (Self *et al.*, 1996).

The failure of either of DOI or mCPP to enhance drug-seeking suggests a limited role of the $5HT_{2A/C}$ receptor subtypes in cue-produced drug-seeking. Because clomipramine decreased cue-produced MDMA-seeking, however, we cannot exclude the possibility that other 5-HT mechanisms play an inhibitory role in this response. As more selective ligands become available, it might be possible to attribute specific effects to specific receptor subtypes.

Because MDMA increases synaptic levels of both 5-HT and dopamine and because MDMA alone produced a larger number of responses during the reinstatement test than doses of GBR 12909 tested, the possibility that drug-seeking would be enhanced by a combination of a 5-HT and dopamine reuptake inhibitor was examined. Clomipramine, however, failed to increase the effects of GBR 12909.

None of the drugs produced as much responding as the dose of MDMA tested. Higher doses of these other drugs, however, might have produced more responding because the primary effect of increasing drug dose in a reinstatement paradigm is to increase the time spent responding during the session. Thus, a dose of 1.0 mg·kg^{-1} amphetamine (Schenk and Partridge, 2001) and 10.0 mg·kg^{-1} GBR 12909 (Schenk, 2002) produced responding that was restricted to the first 2 h of the drug-seeking test. Quinpirole-produced drug-seeking (0.5 mg·kg^{-1}) was restricted to the first 30 min of a 1 h test of amphetamine-seeking (Koeltzow and Vezina, 2005), although cocaine-seeking persisted for up to 4 h following administration of this dose (De Vries *et al.*, 2002). Lower doses of MDMA produced a lower number of responses during the 6 h drug-seeking test (Schenk *et al.*, 2008) but the effect of 10.0 mg·kg^{-1} MDMA was persistent and drug-seeking was observed up to 4–5 h following injection (Colussi-Mas *et al.*, 2010). Unfortunately, time-course data were not available in the present study but these other studies suggest that higher doses would have produced greater responses.

The present results are consistent with the results of most studies that have implicated dopaminergic mechanisms in drug-produced potentiation of conditioned reinforcement. Amphetamine (Beninger and Ranaldi, 1992), GBR 12909 (Kelley and Lang, 1989) and quinpirole (Beninger and Ranaldi, 1992) increased responding maintained by conditioned reinforcers but apomorphine (Robbins *et al.*, 1983; Mazurski and Beninger, 1986; Beninger and Ranaldi, 1992) or D_1 receptor agonists (Beninger and Ranaldi, 1992; Ranaldi *et al.*, 1995) did not.

Methylenedioxymethamphetamine, however, decreased responding maintained by a conditioned reinforcer (Fletcher *et al.*, 2002) but increased drug-seeking in the present study. A decrease in responding is consistent with effects of 5-HT agonists on responding maintained by a conditioned reinforcer (see Nic Dhonnchadha and Cunningham, 2008). Dopamine receptor agonists, however, potentiate responding maintained by a conditioned reinforcer. Thus, the MDMA effects in the present study are consistent with a dopaminergic rather than a 5-hydroxytryptaminergic mechanism. A role of dopamine in MDMA-produced drug-seeking was demonstrated when effects of dopamine receptor antagonists were measured. Pretreatment with either the D_1-like receptor antagonist, SCH 23390, or the D_2-like receptor antagonist, eticlopride, decreased responding produced by MDMA.

Because of the critical role of 5-HT in many of the behavioral effects of acute exposure to MDMA, the demonstration of a major role of dopaminergic substrates in drug-seeking might not have been expected. Following repeated exposure to MDMA, however, there were a number of markers of compromised 5-HT neurotransmission (Green *et al.*, 1995). Self-administration also led to decreased 5-HT transporter binding

densities (Schenk *et al.*, 2007) and the present results show that tissue levels of 5-HT were significantly decreased following MDMA self-administration.

Tissue levels were obtained 5 days following self-administration whereas the behavioral data were collected following only two drug-free days. Because 5-HT deficits in mature (40 or 70 days of age) rats treated with MDMA were persistent and comparable deficits (about 30%) in tissue levels of 5-HT were observed between 24 and 168 h following administration (Broening *et al.*, 1994), it is likely that the deficit produced following self-administration would also be comparable despite the small difference in the number of drug-free days. Because the MDMA-produced increase in synaptic dopamine was enhanced following MDMA self-administration (Colussi-Mas *et al.*, 2010), the data support the idea that there is a shift in the 5-HT/dopamine effects of MDMA as a result of self-administration.

A number of studies have shown an inverse relationship between 5-HT and self-administration (Smith *et al.*, 1986; Carroll *et al.*, 1990; Loh and Roberts, 1990). On the basis of these findings, it has been suggested that 5-HT counteracts the reinforcing effects of drugs by diminishing the dopamine response. According to this rationale, drugs like MDMA that increase synaptic 5-HT to a much greater degree than dopamine (Baumann *et al.*, 2008) would be expected to have limited reinforcing effects. Indeed, the initial reinforcing efficacy of MDMA is low, probably as a result of the substantial effects on 5-HT. With repeated exposure, however, deficits in 5-HT and the MDMA-produced increase in synaptic 5-HT were produced. This would be expected to increase the ratio of dopamine: 5-HT produced by subsequent MDMA infusions which would be expected to increase the reinforcing efficacy of MDMA (Wee *et al.*, 2005; Rothman and Baumann, 2006).

Accordingly, repeated exposure to MDMA self-administration and resulting deficits in MDMA-produced 5-HT might be expected to enhance the propensity to self-administer the drug, leading to further deficits in 5-HT neurotransmission, as we have previously suggested (Schenk, 2009). As a result, drug-taking and drug-seeking might become controlled by dopaminergic mechanisms. The current study supports this hypothesis by demonstrating an important role of dopaminergic mechanisms in MDMA-seeking.

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Conflict of interest

None.

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