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ORIGINAL INVESTIGATION

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Selective destruction of brain serotonin neurons by 5,7-dihydroxytryptamine increases responding for a conditioned reward

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Abstract *Rationale:* Previously, we have shown that increasing 5-hydroxytryptamine (5-HT) activity attenuates responding for conditioned reward (CR), and the response potentiating effect of *d*-amphetamine on this behaviour. *Objectives:* The present experiments examined the effects of reducing 5-HT function on responding for CR. *Methods:* In experiment 1, thirsty rats were trained to associate a CS+ with water delivery. The neurotoxin 5,7-DHT was then injected into the dorsal and median raphe nuclei. Subsequently, rats were treated with intra-accumbens *d*-amphetamine (1, 3, 10 µg) or saline and given access to two levers. One lever delivered the CS+ (now termed a CR), while the other was inactive. In experiment 2, the lesion was carried out prior to conditioning, and approach behaviour to the water magazine was measured during CS+ periods. Subsequently, rats were allowed to respond for the CS. In experiment 3, non-deprived rats learned to associate a CS+ with 10% sucrose; these animals also experienced a CS– which was not paired with sucrose. During a test phase responses on the two levers delivered either the CS+ or the CS–. *Results:* 5,7-DHT substantially reduced 5-HT levels in striatum and hippocampus. In experiment 1, responding for the CR was enhanced by both *d*-amphetamine and 5-HT depletion in an additive fashion. In experiments 2 and 3, the discriminative control over behaviour exerted by the CS+ was not affected by 5-HT depletion. However, compared to control animals 5-HT-depleted rats showed higher levels of operant responding for the CR. *Conclusions:* Serotonin depletion selectively enhances responding for CR. Although 5-HT depletion did not potentiate the effects of *d*-amphetamine, it is suggested that CRs activate the mesolimbic dopamine system, and that removal of an inhibitory influence of 5-HT on the activity of this system results in increased responding for CR in 5,7-DHT-treated rats.

Key words Serotonin · 5,7-dihydroxytryptamine · Conditioned reward · Amphetamine · Nucleus accumbens · Motivation

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Introduction

Rats will learn to respond for an environmental stimulus such as a light or a tone if that stimulus has been paired previously with a primary rewarding stimulus such as food, water (e.g. Taylor and Robbins 1984, 1986; Cador et al. 1991; Kelley and Delfs 1991) or certain types of drugs (Davis and Smith 1987). Similarly, rats will show approach behaviour to distinct environments that have been paired with rewarding drugs (e.g. Hoffman 1989). Presumably, these environmental cues have gained important motivational significance because they predict the occurrence of the primary reward. Provided that rats approach or respond for these stimuli they can be described as conditioned rewards (CRs). In an operant procedure in which rats have the opportunity to press a lever to deliver a stimulus previously paired with food or water, *d*-amphetamine (Taylor and Robbins 1984, 1986; Kelley and Delfs 1991) or dopamine (Cador et al. 1991) injected into the nucleus accumbens potentiates responding for this CR. This increased responding usually occurs in the absence of any change in responding on an inactive lever, indicating that behaviour is specifically directed towards the CR. The effect of amphetamine requires endogenous dopamine release since it is prevented by prior treatment with the dopamine neurotoxin 6-hydroxydopamine (Taylor and Robbins 1986). Thus, in-

creasing mesolimbic dopamine function enhances behaviour directed towards conditioned rewarding stimuli.

The results of these behavioural studies are also paralleled by electrophysiological and neurochemical evidence. Stimuli that predict the occurrence of reward activate midbrain dopamine neurons in non-human primates (Schultz et al. 1993, 1997; Mirenowicz and Schultz 1996). Although dopamine release in these tasks has not yet been determined, studies in rats have demonstrated increased dopamine release in the nucleus accumbens in response to stimuli that have been paired with food (Phillips et al. 1993), or with self-administered cocaine (Gratton and Wise 1994) or amphetamine (Di Ciano et al. 1998). Overall, these results provide strong evidence for the involvement of mesolimbic dopamine systems in mediating incentive motivation (Beninger 1983; Fibiger and Phillips 1986).

Previous work from our laboratory has demonstrated that manipulation of serotonin (5-hydroxytryptamine; 5-HT) function also alters responding for CR. Systemic injection of the 5-HT releaser *d*-fenfluramine (Fletcher 1995), and intra-accumbens injection of 5-HT (Fletcher 1996) attenuated the ability of *d*-amphetamine to enhance responding for CR. More recently, we have demonstrated that specific activation of 5-HT_{1B} receptors is sufficient to induce this attenuation (Fletcher and Korth 1999). These results are consistent with other behavioural evidence showing that increased 5-HT function in the nucleus accumbens can inhibit dopamine-dependent behaviours such as dopamine-stimulated locomotion (e.g. Jones et al. 1981). While the interaction between 5-HT and dopamine systems is very complex, and probably cannot be described in terms of a simple bi-directional influence of 5-HT on dopamine activity, there is some evidence to indicate that reducing 5-HT activity enhances some dopamine-dependent reward-related behaviours. These include brain stimulation reward (Fletcher et al. 1995), sucrose reinforcement (Wogar et al. 1991), and cocaine self-administration (Loh and Roberts 1990). An acute reduction in 5-HT activity, resulting from injection of the 5-HT_{1A} agonist 8-OH-DPAT into the dorsal or median raphe nucleus, is also sufficient to serve as an unconditioned stimulus that elicits a conditioned place preference (Fletcher et al. 1993). The purpose of the present experiments was therefore to investigate the effects of 5-HT depletion, achieved using the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT), on several aspects of behaviour involved in responding for CR.

Materials and methods

Subjects

Adult male Sprague-Dawley rats (Charles River, Quebec, Canada) weighing 280–320 g at the beginning of each study were used. They were housed individually in either hanging wire mesh cages or clear plastic solid-bottomed cages. The housing room was maintained on a 12-h light/dark cycle (lights on at 08:00 h) and at a temperature of 22±2°C. Animals used in locomotor activity tests had food and water available freely at all times. For rats in the remaining studies, access to food or water was restricted as detailed below in each of the specific experimental procedures. All training and testing was conducted during the light phase. Experimental procedures and manipulations conformed to the guidelines laid down by the Canadian Council on Animal Care, and were approved by the Animal Care Committee at the Centre for Addiction and Mental Health.

Surgery

Approximately 20 min before lesioning, rats were injected IP with 10 mg/kg desipramine HCl (Sigma, St Louis, Mo., USA). They were then anaesthetised with sodium pentobarbital (Somnotol, 45–60 mg/kg IP) and placed in a stereotaxic frame with the incisor bar set 3.3 mm below the interaural line. A 30 g needle was lowered into the intended raphe site and 4 µg 5,7-DHT (5,7-dihydroxytryptamine creatinine sulphate; Sigma) dissolved in 1% ascorbic acid was injected using an infusion pump. Dose refers to the free base. The needle was lowered first to the median raphe and 2 µl 5,7-DHT was infused over a period of 4 min. The needle was left in place for a further 2 min, then raised to the dorsal raphe. Another 2 µl of 5,7-DHT was infused over 4 min, and the needle left in place for an additional 2 min. Sham-lesioned rats underwent an identical treatment except that they received infusions of 1% ascorbic acid only. Co-ordinates were AP +1.2 mm, H 0 mm, V +4.0 (dorsal raphe) and +2.0 mm (median raphe) relative to interaural zero, according to the atlas of Paxinos and Watson (1986).

For rats receiving injections into the nucleus accumbens, bilateral stainless steel guide cannulae (22 g) aimed at the nucleus were implanted immediately after injection of 5,7-DHT or its vehicle using a stereotaxic frame with the incisor bar set at +5 mm. The cannulae were positioned 2.5 mm above the nucleus accumbens according to the co-ordinates (relative to bregma) AP +3.2, L ±1.1, D/V –5.9 mm (Pellegrino et al 1981).

Experiment 1a: effects of post-conditioning 5,7-DHT lesions on responding for CR and the response-potentiating effect of intra-accumbens d*-amphetamine*

Training and testing for all CR experiments were carried out in 4 chambers measuring 28 cm long, 21 cm wide and 21 cm high (Med. Associates Inc., St. Albans, Vt.). Each chamber contained a solenoid operated water dispenser and two retractable response levers, 4,5 cm wide and 7 cm above the floor of the chamber. The centres of each lever were located 6.5 cm either side of a central, recessed dish positioned 3 cm from the floor of the chamber. A red stimulus light was located above each lever, and a sonalert was located behind one stimulus light. Each chamber was illuminated by a house light and housed in a sound-attenuating box equipped with a ventilating fan. The apparatus was controlled, and the data collected, by a 386-SX IBM-type computer.

Two days before behavioural procedures began, rats were water-restricted with water bottles available for 2 h each afternoon. There were three main phases to this experiment. During the first habituation phase subjects were placed in the operant boxes with the house light on, and approximately 2 ml of water in the dish. Both levers were present, and the rats remained in the box until they had completed ten responses on one lever. The following day, conditioning began. During this phase, the levers were retracted, and the animals were trained to associate a compound stimulus with the delivery of 0.05 ml of water. The compound stimulus consisted of a 3-s period of the house light off and both red stimulus lights on. During the last 0.5 s of this 3-s period a tone (2900 Hz at approximately 85 dB) sounded and the water was delivered. This stimulus occurred 30 times, on a random time (RT) 30 s schedule. Daily conditioning sessions were conducted for 9 days and each session lasted on average 15 min. At this point rats underwent surgery for lesions and cannula placement. Ten rats were injected with 1% ascorbic acid, and 11 rats were treated with 5,7-DHT. Following a 1-week recovery period rats were given four more conditioning sessions as described above. The third phase of the experiment then started. During this phase, sessions began with insertion of the levers into the chamber. Pressing the left lever re-

sulted in delivery of the conditioned stimulus described above (now defined as the CR), according to a random ratio (RR) 2 schedule; no water was delivered. Pressing the right lever had no programmed consequences. Rats were subject to four test sessions spaced at 3- to 4-day intervals. Immediately prior to testing, they received bilateral injections of 1, 3 or 10 µg (dissolved in 1 µl saline) *d*-amphetamine or saline into the nucleus accumbens using a needle that extended 2.5 mm beyond the tip of the guide cannula. The drug was delivered over a period of 1 min, with the needle left in place for a further 30 s. The order of *d*-amphetamine injections was counterbalanced as far as possible with approximately equal numbers of animals receiving each dose on a given test day. On drug-free days rats were not placed in the operant chambers.

Experiment 1b: effects of 5,7-DHT lesions on locomotor activity

Locomotor activity was measured in four Plexiglas activity chambers (Med Associates Inc., St Albans, Vt., USA) measuring 40 cm long, 40 cm wide, and 28 cm high. Horizontal movement was detected by two arrays of 16 infrared photobeam detectors 2 cm above the floor of the chamber; a third array positioned 10 cm above the floor detected vertical movement. The software allowed a distinction to be made between repetitive interruptions of the same photobeam, and interruptions of adjacent photobeams. This latter measure was used as an index of ambulatory activity. Beginning 2 weeks after surgery, ten sham-lesioned and 8 5,7-DHT-lesioned rats were habituated to the activity boxes for 1 h a day on each of 3 days. On each of 4 test days rats were placed in the boxes for a 30-min habituation period. At this point, they received bilateral injections of 1, 3 or 10 µg (dissolved in 1 µl saline) *d*-amphetamine or saline into the nucleus accumbens and activity was measured for 1 h. The drug was delivered over a period of 1 min, with the needle left in place for a further 30 s. The order of *d*-amphetamine injections was counterbalanced as far as possible with approximately equal numbers of animals receiving each dose on a given test day. On drug-free days rats were not placed in the activity monitors.

Experiment 2: effects of pre-conditioning 5,7-DHT lesions on approach behaviour and responding for CR

In this experiment, nine rats received injections of 5,7-DHT, and 12 rats were injected with 1% ascorbic acid prior to conditioning. Beginning 1 week after surgery water intake was measured every 24 h for the next 7 days. Water was then made available for 2 h each afternoon; intakes were recorded for 7 days. The rats then began the conditioning phase of the experiment. The same operant chambers as described for experiment 1a were used for this experiment. However, a photobeam was placed at the entrance to the water receptacle to record nosepokes. Conditioning was generally the same as for experiment 1b, except that the conditioned stimulus and water were delivered according to a RT 60-s schedule, with the limitation that a minimum inter-reinforcer interval of 30 s was in effect. The duration of the CS presentation was also extended to 5 s. Sessions lasted for 30 min on average, and occurred once per day for 14 days. Responding during each 5-s CS presentation was recorded, as was responding during the 5 s immediately preceding CS onset. Following the conditioning phase, rats were tested for acquisition of responding for the CR during four consecutive daily 40-min sessions. Each session began with insertion of the two levers into the chamber. Pressing the left lever resulted in delivery of the conditioned stimulus described above (now defined as the conditioned reward), according to a random ratio (RR) 2 schedule; no water was delivered. Pressing the right lever had no programmed consequences.

Experiment 3: effects of pre-conditioning lesions on approach behaviour and responding for CR using a CS+ and CS–

The purpose of this experiment was to examine discriminated approach behaviour using both a CS+ (paired with the primary reinforcer) and a CS– (unpaired with the primary reinforcer) in non-deprived animals. Eight sham-lesioned and 8 5,7-DHT-lesioned rats were first given free access to a 10% w/v solution of sucrose for 3 days, beginning 8 days after lesioning. The sucrose solution was then made available for 2 h per day; water was freely available during the remaining 22 h. Thus, rats were never deprived of food or fluids during this experiment. Intakes of sucrose were recorded on each of the following 6 days. The conditioning phase then began with sucrose available only in the operant chambers. During the conditioning phase rats were placed in the operant chambers, where they were given ten presentations of 0.05 ml 10% sucrose immediately after 10 s CS+ presentations. They also received ten CS– presentations that were not paired with sucrose. These CS presentations were delivered on a RT120 schedule with a minimum inter-stimulus interval of 90 s. For half of the rats the CS+ was a tone (2900 Hz at 85 dB) and the CS– consisted of houselight off and two red stimulus lights illuminated. For the other half of the rats the stimulus designations were reversed. Fifteen conditioning sessions were given, lasting on average 40 min. Nosepokes into the water delivery receptacle were recorded during the CS+ and CS– periods. The latency to collect the sucrose was also recorded. The test phase was similar to that described above for experiments 1a and 2. The session began with both levers inserted into the chamber. Pressing the left lever delivered the CS+ according to an RR2 schedule; no sucrose was delivered. Responding on the right lever delivered the CS– according to a RR2 schedule.

Neurochemical and histological analysis

Rats were decapitated, the brain removed and the hippocampus, striatum, nucleus accumbens and parietal cortex dissected on ice, and then stored at –70°C until analysis. The extent and specificity of the lesions were determined by measuring, using HPLC with electrochemical detection, the levels of 5-HT, dopamine, noradrenaline and their metabolites following extraction in 0.1 N perchloric acid containing 2 µM sodium bisulphite as an antioxidant. For rats in experiment 1a, amine and metabolite levels were measured in all four regions; for rats in the remaining experiments analyses were conducted only on striatal and hippocampal tissue. The analytical system consisted of a Waters 600 Mutlisolvent Pump, a Hichrom 250×4.6 mm column with ODS2 5 µm packing material, an ESA Coulochem 5100 A detector with 5020 Guard Cell and a 5011 Analytical Cell, a TSP AS3000 refrigerated autosampler and a Spectra Physics SP4290 Integrator. The mobile phase comprised 0.822 M acetic acid, 0.094 M sodium acetate, 6% methanol, 0.8 mM octane sulphonate, and 0.124 mM EDTA in purified distilled water filtered through a 0.22 µm nylon filter. Since striatal 5-HT levels were used as one index of the success of the 5,7-DHT lesion it was not possible to verify the placements of the cannulae implanted in the region of the nucleus accumbens. While it is possible that the data analyses included some animals with inaccurate cannula placements, the overall group responses were very similar to those in our previous work in which cannula placements were verified (e.g. Fletcher 1995, 1996).

Statistics

Behavioural data were analysed by two- or three-way analysis of variance. Significant two-way interactions were further analysed by tests of simple main effects. Post-hoc tests were conducted using Tukey's test for pairwise comparisons. Response rates in experiment 1 were subject to square-root transformation to reduce heterogeneity of variance. Neurochemical data were analysed using *t*-tests for independent groups.

Results

Neurochemical verification of the 5,7-DHT lesions

As shown in Table 1 treatment with 5,7-DHT reduced 5-HT and 5-HIAA levels to 3–25% of control levels in striatum and 5–20% in hippocampus in the four separate

Table 1 Regional analysis of 5-HT, 5-HIAA, DA and NA in sham- and 5,7-DHT-lesioned rats

	Striatum				Hippocampus			
	5-HT	5-HIAA	DA	NA	5-HT	5-HIAA	DA	NA
CR (expt 1a)								
Sham	0.6036 (0.04)	0.6477 (0.04)	9.5597 (0.30)	0.0798 (0.01)	0.3347 (0.05)	0.2140 (0.02)	0.0068 (0.001)	0.3316 (0.025)
5,7-DHT	0.1455 (0.05)	0.1676 (0.06)	9.9563 (0.30)	0.0655 (0.01)	0.0166 (0.001)	0.0201 (0.002)	0.0053 (0.001)	0.3046 (0.02)
%	23*	25*	104	82	5*	9*	78	92
Activity (expt 1b)								
Sham	0.4669 (0.1)	0.5391 (0.14)	9.2938 (0.48)	0.0774 (0.01)	0.3272 (0.05)	0.2959 (0.04)	0.0063 (0.001)	0.3987 (0.02)
5,7-DHT	0.0413 (0.01)	0.0496 (0.01)	10.5396 (0.75)	0.0772 (0.01)	0.0427 (0.02)	0.0605 (0.02)	0.0059 (0.001)	0.3984 (0.02)
%	9*	9*	113	100	13*	20*	94	100
CR (expt 2)								
Sham	0.4859 (0.05)	0.5746 (0.05)	9.5582 (0.52)	0.1293 (0.01)	0.2479 (0.05)(0.03)	0.2915 (0.001)	0.0076 (0.03)	0.5166
5,7-DHT	0.0590 (0.02)	0.0949 (0.05)	9.5746 (0.68)	0.1124 (0.01)	0.0152 (0.002)	0.0296 (0.006)	0.0070 (0.004)	0.4533 (0.04)
%	12*	16*	100	87	6*	10*	92	88
CS+ CS– (expt 3)								
Sham	0.6342 (0.14)	0.6970 (0.14)	10.149 (0.61)	0.0860 (0.003)	0.5353 (0.05)	0.5937 (0.06)	0.0130 (0.01)	0.8006 (0.06)
5,7-DHT	0.0192 (0.01)	0.0268 (0.01)	9.474 (0.51)	0.0769 (0.01)	0.0459 (0.02)	0.0621 (0.03)	0.0082 (0.001)	0.6520 (0.07)
%	3*	4*	93	89	9*	10*	63	81

Values represent mean (±SEM) tissue levels expressed as ng/mg tissue. *P<0.01 compared to sham-lesioned controls

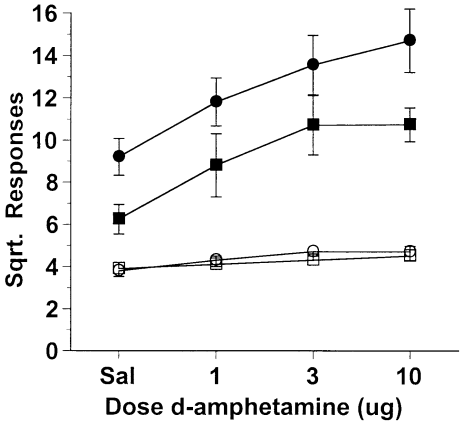

Fig. 1 The effects of *d*-amphetamine and saline (*Sal*) injected into the nucleus accumbens on responding for CR in sham-lesioned and 5,7-DHT-lesioned rats. Values represent the mean (SEM) number of responses following square-root transformation. ■ Sham-CR, □ sham-NCR, ● 5,7-DHT-CR, ○ 5,7-DHT-NCR

groups of rats used in these experiments. Comparable reductions were observed in nucleus accumbens and cortex of rats used in experiment 1a (data not shown). No consistent changes in dopamine or noradrenaline were observed.

Experiment 1a: effects of post-conditioning 5,7-DHT lesions on responding for CR and the response-potentiating effect of intra-accumbens *d*-amphetamine

The results of experiment 1 are shown in Fig. 1. Results of the analysis of variance indicated that overall responding was higher on the CR lever than the NCR lever [F(1,18)=134.08, P<0.001]; that responding was increased by *d*-amphetamine [F(3,54)=8.91, P<0.001]; and that the 5,7-DHT-lesioned rats demonstrated higher responding than sham-lesioned rats [F(1,18)=10.03, P<0.01]. Significant interactions between amphetamine and lever [F(3,54)=8.55, P<0.001] and lesion and lever [F(1,18)=7.15, P<0.02] reflected the fact that each manipulation increased responding on the CR lever (P<0.01) but not on the NCR lever (P>0.1). The interaction between *d*-amphetamine and 5,7-DHT lesion was not significant [F(3,54)=0.15, P>0.9].

Experiment 1b: effects of 5,7-DHT lesions on locomotor activity

The results of this experiment are shown in Fig. 2. Two way analysis of variance conducted on the total 2-h ambulatory counts, shown in Fig. 2, revealed that *d*-amphetamine induced a dose-dependent increase in activity [F(3,48)=17.04, P<0.001]. Neither the main effect of le-

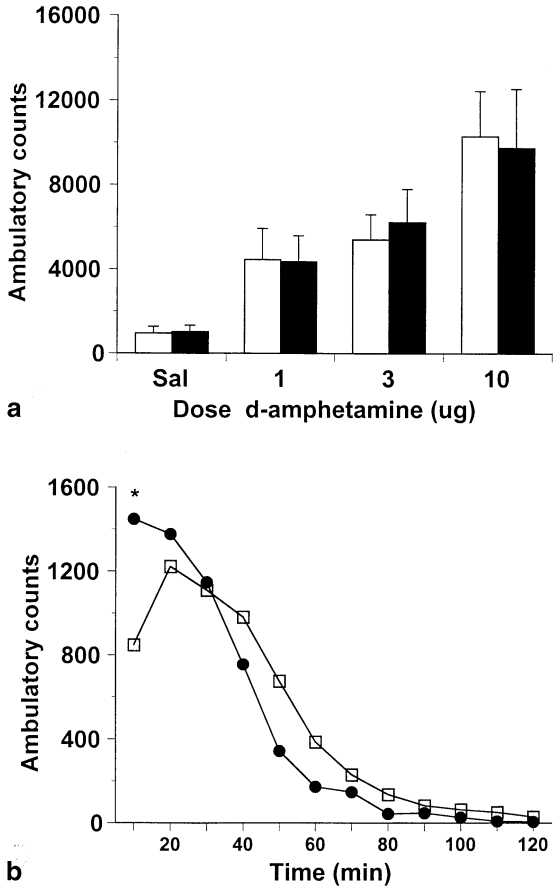

Fig. 2 **a** The effects of saline, 1, 3 and 10 μg *d*-amphetamine injected into the nucleus accumbens on ambulatory counts in sham-lesioned and 5,7-DHT-lesioned rats. *Bars* represent the mean (+SEM) number of counts during the 2-h test. *White bars* sham, *black bars* 5,7-DHT. **b** The mean number of ambulatory counts in each 10-min interval of the 2-h test for sham and 5,7-DHT-lesioned rats, collapsed across dose of *d*-amphetamine. Standard error bars have been omitted for clarity. See text for statistical details. **P*<0.05 compared to sham-lesioned group. □ Sham, ● 5,7-DHT

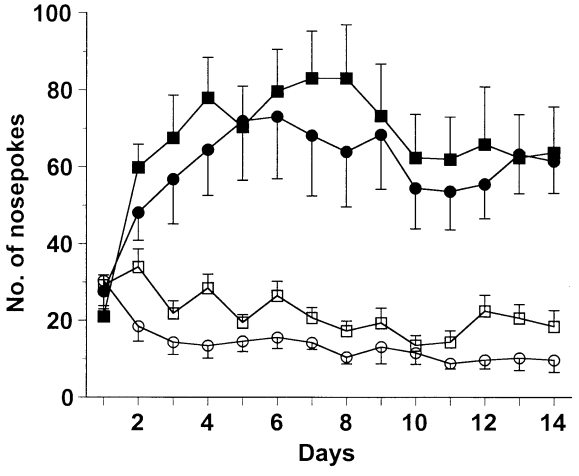

Fig. 3 The number of nosepokes in the water receptacle during 5 s CS periods and the 5 s immediately preceding the CS presentation (*pre-CS*) for sham and 5,7-DHT-lesioned rats. Values are the mean (+SEM) number of responses during each of 14 sessions in which 30 CS presentations were made. ■ Sham CS,● 5,7-DHT CS, □ sham pre-CS, ○ 5,7-DHT pre-CS

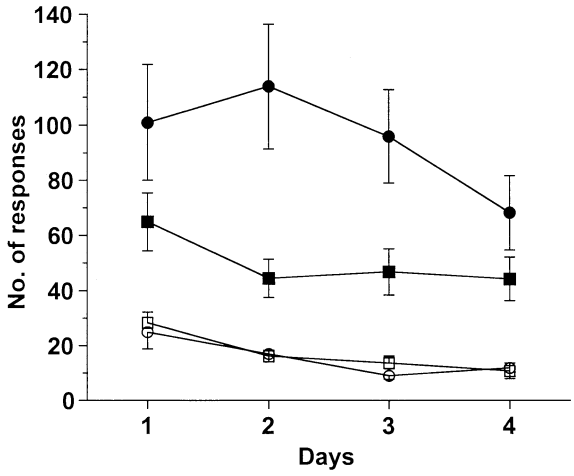

Fig. 4 The number of responses on the lever delivering the conditioned reward (*CR*) and on the inactive lever (*NCR*) for sham and 5,7-DHT-lesioned rats on each of 4 successive test days. ■ Sham-CR, ● 5,7-DHT-CR, □ sham-NCR, ○ 5,7-DHT-NCR

sion nor the lesion×amphetamine interaction were significant [*F*s<0.2 in both cases, NS], indicating that the overall effect of *d*-amphetamine was not modified by 5-HT depletion. Analysis of the time courses of activity did, however, reveal a subtle effect of 5-HT depletion. A three-way analysis of variance on the data for each 10-min bin of the test sessions revealed a significant lesion×time interaction [$F(11,176)=2.08$, $P<0.03$]. As shown in the lower panel of Fig. 2 lesioned rats showed a marginally increased level of activity during the first 10 min and a non-significant reduction in activity over the 40- to 70-min period. Although this interaction did not vary with amphetamine dose [$F(33,528)=0.89$, NS], inspection of the data (not shown) showed that only the effects of the two highest doses of amphetamine were increased in 5-HT-depleted animals during the first 10 min.

Experiment 2: effects of pre-conditioning 5,7-DHT lesions on approach behaviour and responding for CR

Water intakes measured over either 24- or 2-h periods were not affected by 5-HT depletion. Mean (and SEM) daily intakes measured during the 7 days prior to adaptation to water restriction were: sham 37.21 (2.88) ml, 5,7-DHT 39.20 (3.51) ml [$t(15)=0.46$, NS]. Mean (and SEM) 2-h intakes measured during the first 7 days of water restriction were: sham 24.96 (1.11) ml, 5,7-DHT 24.15 (0.38) ml [$t(15)=0.47$, NS]. The effects of the lesion on responding during the CS and pre-CS periods are shown in Fig. 3. Overall, responding was higher during the CS periods compared to the pre-CS periods [$F(1,19)=63.88$, $P<0.001$], and increased over days [$F(13,247)=4.01$, $P<0.001$]. Neither the main effect of lesion [$F(1,19)=1.12$, $P<0.3$] nor any of the interaction terms involving lesion (all *F* ratios <0.7, NS) were significant. During the conditioning phase, the mean laten-

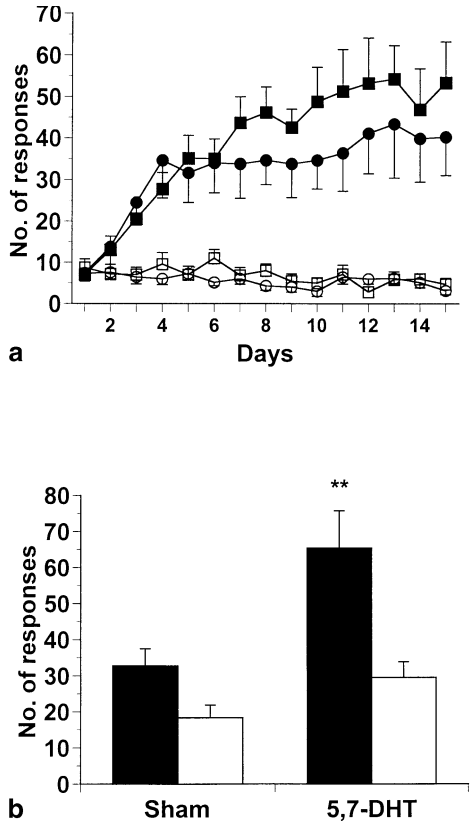

Fig. 5 **a** The number of nosepokes in the water receptacle by sham- and 5,7-DHT-lesioned rats during periods in which the CS+ and the CS– were delivered. Values are the mean (+SEM) number of responses during each of 15 sessions in which ten CS+ and 10 CS– presentations were made. ■ CS+sham, □ CS– sham, ● CS+ 5,7-DHT, ○ CS– 5,7-DHT. **b** The number of responses on the levers delivering the CS+ and CS– for sham and 5,7-DHT-lesioned rats. ***P*<0.01 compared to sham-lesioned group. *Black bars* CS+, *white bars* CS–

cy to collect the water after its delivery did not differ between the two groups. Mean (and SEM) latencies averaged over the last 5 days of testing for the sham and 5,7-DHT-lesioned groups were 2.33 (0.36) and 3.24 (0.78) s, respectively [$t(19)=1.21$, $P>0.2$]. Thus, 5-HT depletion did not alter approach behaviour to the CS. Acquisition of lever pressing for the CS is shown in Fig. 4. Responding was higher on the CR lever than on the NCR lever [$F(1,19)=72.71$, $P<0.001$]. Lesioned animals showed higher overall rates of responding [$F(1,19)=5.1$, $P<0.03$] but as shown by the significant lesion×lever interaction [$F(1,19)=11.67$, $P<0.003$] this increase in responding occurred differentially on the two levers. Tests of simple main effects on this interaction confirmed that the lesion increased responding on the CR lever [$F(1,19)=8.26$, $P<0.01$] but not the NCR lever [$F(1,19)=0.18$, $P>0.6$].

Experiment 3: effects of pre-conditioning lesions on approach behaviour and responding for CR using a CS+ and CS–

Sucrose intakes over the 6 days prior to conditioning did not differ between sham and 5-7-DHT-lesioned rats; mean (and SEM) intakes were 17.89 (2.67) and 15.37 (2.41) ml, respectively [$t(15)=0.74$, $P>0.2$]. The number of nosepokes during CS+ and CS– periods is illustrated in Fig. 5a. Overall, responding was higher during CS+ than the CS– periods [$F(1,14)=43.8$, $P<0.001$], and increased over days [$F(14,196)=14.77$, $P<0.001$]. The significant interaction between the CS type and days [$F(14,196)=19.51$, $P<0.001$] reflects the increased responding during the CS+ periods with time. Neither the main effect of lesion nor any of the interaction terms involving lesion were significant [all F's < 1.6, NS] indicating that the 5,7-DHT lesion did not alter discriminated approach behaviour. Latencies to collect the reinforcer did not differ between the two groups. Mean (and SEM) latencies averaged over the last 5 days of conditioning were: sham 1.84 (0.20), 5,7-DHT 1.61 (0.23) s, [$t(15)=0.88$, >0.2].

Figure 5b shows the number of responses on the lever delivering the CS+ and the lever delivering the CS–. Responding on the CS+ lever was higher than on the CS– lever [$F(1,14)=49.08$, $P<0.001$]. The 5,7-DHT-lesioned rats exhibited an overall greater level of responding than the sham-lesioned rats [$F(1,14)=7.47$, $P<0.02$]. A significant lever×lesion interaction [$F(1,14)=8.98$, $P<0.01$] reflected the fact that the 5,7-DHT-lesioned group showed increased responding on the CS+ lever but not on the CS– lever.

Discussion

Injections of 5,7-DHT into the dorsal and median raphe nuclei induced large reductions in striatal and hippocampal 5-HT and 5-HIAA levels, with no consistent changes in either dopamine or noradrenaline. Since the hippocampus is a major projection area of the median raphe and the striatum is a main projection region of the dorsal raphe (Azmitia 1978), this pattern of 5-HT depletion indicates that the lesion severely damaged ascending 5-HT pathways.

A consistent behavioural effect of 5-HT depletion throughout these experiments was an increase in responding on the lever delivering the CR. This effect occurred irrespective of whether the 5-HT depletion was induced when some conditioning had been established, or prior to the beginning of the conditioning phase. The increased responding on the CR lever also occurred regardless of whether responses on the alternative lever had no programmed consequence (experiments 1a and 2) or delivered a stimulus that had not been paired with the primary reward (experiment 3). Thus, the increased responding for CR in 5-HT-depleted rats does not reflect a non-specific increase in responding, but is selective

for the lever delivering the CR. The finding that spontaneous activity levels were not different in sham and 5,7-DHT-lesioned rats also indicates that the increased lever pressing in lesioned rats was not the result of a generalised increase in motor activity. Deprivation state, and the nature of the primary reinforcer used during conditioning, also did not influence the increased responding for CR in 5-HT-depleted rats because CR responding was increased in deprived animals that received water as the primary reinforcer, as well as in non-deprived rats trained to associate the CS with sucrose. Thus, increased responding for CR following chronic reductions in 5-HT levels appears to be a robust and reliable phenomenon.

The reinforcing efficacy of sucrose is increased in rats treated with 5,7-DHT (Wogar et al. 1991). It is unlikely that this contributes to the observed increase in responding for CR. Firstly, consummatory measures of water and sucrose intakes failed to show an effect of 5-HT depletion. Secondly, latencies to enter the receptacle where the primary reinforcer was delivered were not altered by the lesion. Thirdly, if the lesion enhanced the reinforcing efficacy of the primary reward then this might be expected to increase approach behaviour during periods in which the CS was presented. In fact, lesioned rats showed marginally reduced levels of approach behaviour, but this effect was not statistically significant. This lack of effect of the lesion on discriminated approach behaviour also rules out a further possible explanation for the increased operant responding for CR. It is clear that during conditioning 5,7-DHT-lesioned rats did not differ from sham-lesioned rats in terms of learning the relationship between the CS and the primary reward. Therefore, the increased responding for CR in lesioned rats cannot be accounted for in terms of better learning of the stimulus-stimulus association. The fact that increased responding for CR was observed in animals lesioned after conditioning had been conducted for 9 days also supports this interpretation. Overall, these results suggest that the response-increasing effect of 5,7-DHT-induced depletion of 5-HT is specific to the ability of the CR to elicit and maintain behaviour.

Amphetamine injected into the nucleus accumbens also potentiated responding for CR as previously demonstrated (e.g. Taylor and Robbins 1984; Kelley and Delfs 1991; Fletcher 1995) This effect occurred in both sham and 5,7-DHT-lesioned rats. The lack of a significant interaction between the effects of *d*-amphetamine and 5-HT depletion indicates that the drug-induced increase in responding observed in the lesioned rats appeared to be additive with the effect of the lesion. Consistent with an earlier report (Gately et al. 1986), the locomotor stimulant effects of *d*-amphetamine on total activity levels did not differ between sham and lesioned rats. However, inspection of the time course of locomotor activity suggested that lesioned rats showed an initial potentiation of the effects of *d*-amphetamine during the first 10 min of the test session, but that lesioned rats showed an earlier habituation to the effects of *d*-amphetamine. Although the significance of these changes is unclear, these effects have been noted previously in rats receiving injections of 5,7-DHT into the median raphe (Asin et al. 1983). However, the effect of amphetamine in 5-HT-depleted rats does not resemble the effect that would be predicted if 5-HT depletion was acting to potentiate the effects of amphetamine. We have recently found that 5,7-DHT injections into the dorsal and median raphe nuclei do not alter *d*-amphetamine self-administration (Fletcher et al. 1999). This finding, together with those reported here, provide a convincing body of evidence to support the conclusion that the behavioural effects of *d*-amphetamine are not altered by injecting 5,7-DHT into the raphe nuclei.

Despite the fact that the 5,7-DHT lesions did not potentiate the effects of *d*-amphetamine, it still remains possible that the increased responding for CR in 5-HT-depleted rats involves a potentiation of the effects of endogenous dopamine on this behaviour. Electrophysiological (Schultz et al. 1993, 1997; Mirenowicz and Schultz 1996) and neurochemical (Phillips et al. 1993; Gratton and Wise 1994; Di Ciano et al. 1998) evidence indicates that CRs activate dopamine neurons, leading to the release of dopamine in the nucleus accumbens. This dopamine release has been suggested to control the vigour of responding for CRs (Cador et al. 1989). Two possible sites at which 5-HT depletion could alter the effects of dopamine on CR responding are the nucleus accumbens and the ventral tegmental area (VTA).

The nucleus accumbens receives serotonergic inputs from the raphe nuclei (van Bockstaele and Pickel 1993). Therefore, it is possible that an altered balance of 5-HT-dopamine interactions in 5-HT-depleted rats contributes to the increase in responding for CR. We have found previously that 5-HT acting via 5-HT_{1B} receptors attenuates the response-potentiating effect of *d*-amphetamine at doses that do not induce non-specific deficits in instrumental responding (Fletcher and Korth 1999). This effect contrasts with the observed increase in extracellular levels of dopamine in the nucleus accumbens (Parsons and Justice 1993) and striatum (Galloway et al. 1993) following perfusion of these sites with 5-HT and various 5-HT agonists. We have argued that the behavioural effects of 5-HT agonists may be mediated downstream from dopamine terminals (Fletcher 1996; Fletcher and Korth 1999) to disrupt the flow of reward-related information to the ventral pallidum. The present results show that 5-HT depletion does not alter *d*-amphetamine-stimulated locomotor activity. Similarly, 5,7-DHT treatment does not alter the dynamics of dopamine synthesis within the nucleus accumbens in amphetamine treated animals (Lyness and Moore 1981). Thus, at the present time there is little evidence to indicate that the effects of 5-HT depletion on responding for CR result from altered dopamine function in the nucleus accumbens.

An alternative site for interactions between 5-HT depletion and dopaminergic mechanisms is the VTA, where 5-HT axon terminals make synaptic contacts with dopamine neurons (Herve et al 1987). The 5-HT_{1A} agonist

8-OH-DPAT increases the burst-firing of VTA neurons (Arborelius et al. 1993; Prisco et al. 1994; LeJeune et al. 1997), and this effect has been attributed to an inhibition of serotonergic input to the VTA, via activation of 5-HT_{1A} somatodendritic autoreceptors in the raphe nuclei. Conversely, drugs that elevate synaptic 5-HT levels, such as fluoxetine, inhibit the activity of dopamine neurons (Prisco and Esposito 1995); 5-HT itself potentiates the inhibition of VTA neurons induced by dopamine (Brodie and Bunney 1996). Thus, these results suggest an important inhibitory influence of 5-HT on the activity of mesolimbic dopamine neurons. Within the context of the present experiments it can be hypothesised that the occurrence of the CR activates VTA dopamine neurons leading to release of dopamine in the nucleus accumbens which in turn facilitates further responding for the CR. In rats depleted of 5-HT, the loss of inhibitory 5-HT inputs to dopamine cell bodies could result in increased activation of VTA neurons by the CR, and consequently of dopamine release in the nucleus accumbens, leading to increased CR responding. The additive effects of 5-HT depletion and *d*-amphetamine on CR responding could then be explained in terms of the summation of impulse-dependent dopamine release induced by CR presentation, and impulse-independent release of dopamine by *d*-amphetamine (Westerink et al. 1987; Nomikos et al. 1990). This general hypothesis could be tested using in vivo microdialysis to determine dopamine overflow in the nucleus accumbens of 5-HT-depleted rats engaged in tasks, such as responding for CR, that are known to activate the mesolimbic system. The hypothesis predicts increased dopamine overflow in response to significant motivational stimuli in 5-HT-depleted animals.

An important feature of this mechanism is that it suggests that the influence of 5-HT depletion on dopamine function is observed under conditions in which there is impulse-dependent activation of dopamine neurons. This type of impulse-dependent mechanism potentially could explain the lack of effect of 5-HT depletion on locomotor activity stimulated by *d*-amphetamine, while accounting for the facilitatory effects of reduced 5-HT function on reward-related behaviour, including responding for CR. Evidence suggests that mesolimbic dopamine release is responsible for the increase in locomotor activity elicited by placing rats in a novel, but not a familiar, environment (Hooks and Kalivas 1995; Ladurelle et al. 1995). In the present experiments locomotor activity was measured in a familiar environment, and immediately after a habituation period when spontaneous activity levels were low. Therefore it is reasonable to predict that mesolimbic DA neurons were in a state of relative quiescence at the time that *d*-amphetamine was injected. Consequently, the increased locomotor response to *d*-amphetamine may reflect *d*-amphetamine-stimulated release of dopamine, without an additional component of environmentally induced activation of mesolimbic dopamine activity, as is thought to occur when rats are responding for a CR. Hence, 5-HT depletion itself did not alter activity or potentiate the motor stimulant effects of *d*-amphetamine.

Previous work has shown that a variety of reward-related behaviours including feeding (Fletcher 1991), operant responding for sucrose (Wogar et al. 1991), alcohol intake (Tomkins et al. 1994), lateral hypothalamic self-stimulation (Fletcher et al. 1995), and responding for the dopamine re-uptake inhibitor cocaine (Loh and Roberts 1990) are increased by treatments that reduce 5-HT activity. A common feature of these behaviours is that they all involve direct or indirect activation of mesolimbic dopamine neurons, or increased activity of endogenously released dopamine. Consequently, they evoke impulse-dependent release of dopamine or in the case of cocaine, amplification of the effects of endogenously released dopamine. Conversely, *d*-amphetamine-induced release of dopamine is not dependent on the firing of dopamine neurons, and evidence presented here and elsewhere (Lyness and Moore 1981) suggests that the neurochemical and behavioural effects of *d*-amphetamine are not altered by 5-HT depletion in a manner that is consistent with a potentiation of the effects of *d*-amphetamine. Thus, a key feature in determining whether 5-HT depletion facilitates reward-related behaviour may relate to the degree to which the rewarding stimulus activates endogenous dopamine systems via an impulse-dependent mechanism.

In summary, lesioning ascending 5-HT neurons increases responding for a CR and this behaviour may result from the removal of the inhibition that 5-HT neurons normally exert on the cell bodies of mesolimbic dopamine neurons.

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References

Arborelius L, Chergui K, Murase S, Nomikos GG, Backlund Hook B, Chouvet G, Hacksell U, Svensson TH (1993) The 5-HT_{1A} receptor selective ligands, (*R*)-8-OH-DPAT and (*S*)-UH-301, differentially affect the activity of midbrain dopamine neurons. Naunyn-Schmiedeberg's Arch Pharmacol 347:353–362

Asin KE, Fibiger HC (1983) An analysis of neuronal elements within the median nucleus of the raphe that mediate lesion-induced increases in locomotor activity. Brain Res 268:211–223

Azmitia EC (1978) The serotonin-producing neurons of the midbrain median and dorsal raphe nuclei. In: Iversen LL, Iversen SD, Snyder SH (eds) Handbook of psychopharmacology, vol 9Pl. Plenum Press, New York, pp 233–314

Beninger RJ (1983) The role of dopamine in locomotor activity and learning. Brain Res Rev 6:173–196

Brodie MS, Bunney EB (1996) Serotonin potentiates dopamine inhibition of ventral tegmental area neurons in vitro. J Neurophysiol 76:2077–2082

Cador M, Robbins TW, Everitt BJ (1989) Involvement of the amygdala in stimulus-reward associations: interaction with the ventral striatum. Neuroscience 30:77–86

Cador M, Taylor JR, Robbins TW (1991) Potentiation of the effects of reward-related stimuli by dopaminergic-dependent mechanisms in the nucleus accumbens. Psychopharmacology 104:377–385

Davis WM, Smith SG (1987) Conditioned reinforcement as a measure of the reinforcing properties of drugs. In: Bozarth

MA (ed) Methods of assessing the reinforcing properties of abused drugs. Springer, Berlin Heidelberg New York, pp 199–210

Di Ciano P, Blaha CD, Phillips AG (1998) Conditioned changes in dopamine oxidation currents in the nucleus accumbens of rats by stimuli paired with self-administration or yoked-administration of *d*-amphetamine. Eur J Neurosci 10:1121–1127

Fibiger HC, Phillips AG (1986) Reward, motivation, cognition: psychobiology of mesotelencephalic dopamine systems. In: Mountcastle VB, Bloom FE, Geiger SR (eds) Handbook of physiology: the nervous system, vol 4. American Physiological Society, Bethesda, Maryland, pp 647–675

Fletcher PJ (1991) Dopamine receptor blockade in nucleus accumbens or caudate nucleus differentially affects feeding induced by 8-OH-DPAT injected into dorsal or median raphe. Brain Res 52:181–189

Fletcher PJ (1995) Effects of *d*-fenfluramine and metergoline on responding for conditioned reward and the response potentiating effect of nucleus accumbens *d*-amphetamine. Psychopharmacology 118:155–163

Fletcher PJ (1996) Injection of 5-HT into the nucleus accumbens reduces the effects of *d*-amphetamine on responding for conditioned reward. Psychopharmacology 126:62–69

Fletcher PJ, Korth KM (1999) Activation of 5-HT_{1B} receptors in the nucleus accumbens reduces amphetamine-induced enhancement of responding for conditioned reward. Psychopharmacology 142:165–174

Fletcher PJ, Ming ZH, Higgins GA (1993) Conditioned place preference induced by microinjection of 8-OH-DPAT into the dorsal or median raphe nuclei. Psychopharmacology 113:31–36

Fletcher PJ, Tampakeras M, Yeomans JS (1995) Median raphe injections of 8-OH-DPAT lower frequency thresholds for lateral hypothalamic self-stimulation. Pharmacol Biochem Behav 52: 65–71

Fletcher PJ, Korth KM, Chambers JW (1999) Depletion of brain serotonin following intra-raphe injections of 5,7-dihydroxytryptamine does not alter *d*-amphetamine self-administration across different schedule and access conditions. Psychopharmacology 146:185–193

Galloway MP, Suchowski CS, Keegan, MJ, Hjorth S (1993) Local infusion of the selective 5-HT-1B agonist CP-93,129 facilitates striatal dopamine release in vivo. Synapse 15:90–92

Gately PF, Segal DS, Geyer MA (1986) The behavioral effects of depletions of brain serotonin induced by 5,7-dihydroxytrptamine vary with time after administration. Behav Neural Biol 45: 31–42

Gratton A, Wise RA (1994) Drug- and behavior-associated changes in dopamine-related electrochemical signals during intravenous cocaine self-administration in rats. J Neurosci 14: 130–4146

Herve D, Pickel VM, Joh TH, Beaudet A (1987) Serotonin axon terminals in the ventral tegmental area of the rat: fine structure and synaptic input to dopaminergic neurons. Brain Res 435: 71–83

Hoffman DC (1989) The use of place conditioning in studying the neuropharmacology of drug reinforcement. Brain Res Bull 23: 373–387

Hooks MS, Kalivas PW (1995) The role of mesoaccumbens-pallidal circuitry in novelty-induced behavioral activation. Neuroscience 64:587–597

Jones DL, Mogenson GJ, Wu M (1981) Injections of dopaminergic, cholinergic, serotonergic and GABAergic drugs into the nucleus accumbens: effects on locomotor activity in the rat. Neuropharmacology 20:29–37

Kelley AE, Delfs JM (1991) Dopamine and conditioned reinforcement I. Differential effects of amphetamine microinjections into striatal subregions. Psychopharmacology 103:187–196

Ladurelle N, Roques BP, Duage V (1995) the transfer of rats from a familiar to a novel environment prolongs the increase of extracellular dopamine efflux induced by CCK8 in the posterior nucleus accumbens. J Neurosci 15:3118–3127

LeJeune F, Newman-Tancredi A, Audinot V, Millan MJ (1997) Interactions of (+)- and (–)-8- and 7-hydroxy-2-(di-*n*-propylamino)tetralin at human (h)D_3, hD_2 and h serotonin$_{1A}$ receptors and their modulation of the activity of serotoninergic and dopaminegic neurones in rats. J Pharmacol Exp Ther 280:1241–1249

Loh EA, Roberts DCS (1990) Break-points on a progressive ratio schedule reinforced by intravenous cocaine increase following depletion of forebrain serotonin. Psychopharmacology 101:262–266

Lyness WH, Moore KE (1981) Destruction of 5-hydroxytryptaminergic neurons and the dynamics of dopamine in nucleus accumbens septi and other forebrain regions of the rat. Neuropharmacology 20:327–334

Mirenowicz J, Schultz W (1996) Preferential activation of midbrain dopamine neurons by appetitive rather than aversive stimuli. Nature 379:449–451

Nomikos GG, Damsma G, Wenkstern, D, Fibiger HC (1990) In vivo characterisation of locally applied dopamine uptake inhibitors by striatal microdialysis. Synapse 6:106–112

Parsons LH, Justice JB Jr (1993) Perfusate serotonin increases extracellular dopamine in the nucleus accumbens as measured by in vivo microdialysis. Brain Res 606:195–199

Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates, 2nd edn. Academic Press, New York

Pellegrino LJ, Pellegrino AS, Cushman AJ (1981) A stereotaxic atlas of the rat brain, 2nd edn. Plenum Press, New York, London

Phillips AG, Atkinson LJ, Blackburn JR, Blaha CD (1993) Increased extracellular dopamine in the nucleus accumbens of the rat elicited by a conditioned stimulus for food: an electrochemical study. Can J Physiol Pharmacol 71:387–393

Prisco S, Esposito E (1995) Differential effects of acute and chronic fluoxetine administration on the spontaneous activity of dopamineric neurones in the ventral tegmental area. Br J Pharmacol 116:1923–1931

Prisco S Pagannone S, Esposito E (1994) Serotonin-dopamine interaction in the rat ventral tegmental area; an electrophysiological study in vivo. J Pharmacol Exp Ther 271:83–90

Schultz W, Apicella P, Ljungberg T (1993) Responses of monkey dopamine neurons to reward and conditioned stimuli during successive steps of learning a delayed response task. J Neurosci 13:900–913

Schultz W, Dayan P, Montague PR (1997) A neural substrate of prediction and reward. Science 275:1593–1599

Taylor JR, Robbins TW (1984) Enhanced behavioural control by conditioned reinforcers following microinjections of *d*-amphetamine into the nucleus accumbens. Psychopharmacology 84:405–412

Taylor JR, Robbins TW (1986) 6-Hydroxydopamine lesions of the nucleus accumbens, but not of the caudate nucleus, attenuate responding with reward-related stimuli produced by intra-accumbens *d*-amphetamine. Psychopharmacology 90:390–397

Tomkins DM, Sellers EM, Fletcher PJ (1994) Median and dorsal raphe injections of the 5-HT_{1A} agonist 8-OH-DPAT, and the GABA-A agonist muscimol, increase voluntary ethanol intake in Wistar rats. Neuropharmacology 33:349–358

Van Bockstaele EJ, Pickel VM (1993) Ultrastructure of serotonin-immunoreactive terminals in the core and shell of the rat nucleus accumbens: cellular substrates for interactions with catecholamine afferents. J Comp Neurol 334:603–617

Westerink BHC, Tuntler J, Damsma G, Rollema H, de Vries JB (1987) The use of tetrodotoxin for the characterization of drug-enhanced dopamine release in conscious rats studied by brain dialysis. Naunyn-Schmiedeberg's Arch Pharmacol 336: 502–507

Wogar MA, Bradshaw CM, Szabadi E (1991) Evidence for an involvement of 5-hydroxytryptaminergic neurones in the maintenance of operant behaviour by positive reinforcement. Psychopharmacology 105:119–124