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The novel dopamine D_4 receptor agonist (PD 168,077 maleate): Doses with different effects on locomotor activity are without effect in classical conditioning

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Abstract

Conditioning is normally selective to the most likely predictors of motivationally significant events and some dopamine (DA) agonists produce dysfunction in this process. Moreover, the DA D_4 receptor is implicated in normal and abnormal functions that have some dopaminergic basis (e.g., in attention deficit hyperactivity disorder and schizophrenia). We therefore used locomotor activity to identify doses of a novel D_4 receptor agonist (PD 168,077) with contrasting behavioral effects (over the range 0.064–1 mg/kg). Doses that either did (0.064 mg/kg) or did not (0.5 mg/kg) significantly increase activity were then tested using aversive and appetitive procedures, in which conditioning was reflected in decreased and increased response rates, respectively. Associating a signal with food or foot shock is normally reduced in trace conditioning, when stimulus events are separated in time. Similarly, animals normally learn relatively little about background stimuli that do not well predict food delivery or the onset of shock. Both doses of PD 168,077 were without effect on conditioning, whether appetitive or aversive, and irrespective of how informative the predictive stimulus was. Thus, we find no evidence that the D_4 receptor has any likely effect on associative learning or its disorder. Furthermore, D_4-mediated hyperactivity was dissociable from cognitive effects.

Keywords: Conditioning; Dopamine D_4 agonist; Locomotor activity; PD 168,077; Rat

1. Introduction

There is good general evidence for the role of dopamine (DA) in both aversive (Feenstra et al., 2001) and appetitive (Harmer and Phillips, 1998; Dalley et al., 2002) conditioning, as well as in the association of neutral stimuli prior to any reinforcement (Young et al., 1998). However, it is not clear whether such effects are mediated via actions at DA D_1, D_2, D_3, or D_4 receptors, via further receptor subtypes yet to be identified, or through some combination of receptor activations.

We have chosen to investigate the D_4 receptor subtype in associative learning because of its likely role in disorders in which selective learning is impaired. For example, interest in the DA D_4 receptor subtype has been increased by the finding that D_4 receptor binding is elevated in the striatum of (postmortem) schizophrenic brains relative to other DA receptor subtypes (e.g., D_2 and D_3; Seeman et al., 1993). Moreover, clozapine, an atypical antipsychotic used in treating refractory schizophrenia and without the side effects of other neuroleptics, shows a 5- to 10-fold higher affinity for D_4 than for D_2 and D_3 receptors (Van Tol et al., 1991; Wilson et al., 1998). Consistent with an involvement of the D_4 receptor in psychiatric disorder, in the normal brain, there is evidence to suggest that this receptor modulates catecholaminergic activity in rat hippocampus (Pugsley et al., 2002).

It must be said that the evidence for a role of the D_4 site in schizophrenia remains inconclusive (for reviews, see Bristow et al., 1997a; Wong et al., 2000). In preclinical tests, a selective D_4 antagonist failed to antagonize amphetamine-induced hyperactivity, or to reverse an apomorphine-induced deficit in prepulse inhibition (Bristow et al., 1997b). However, with the exception of a conditioned avoidance

Abbreviations: ADHD, attention deficit hyperactivity disorder; CS, conditioned stimulus; DMSO, dimethyl sulfoxide; LI, latent inhibition; UCS, unconditioned stimulus.

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test, the preclinical work did not examine the involvement of the D_4 site in associative learning.

Despite the slightly disappointing evidence with respect to schizophrenia, there is better evidence for the role of the D_4 site in attention deficit hyperactivity disorder (ADHD; Oak et al., 2000). Thus, the resulting interest in D_4 receptor pharmacology, and the involvement of the site in normal and abnormal functions, led us to examine the effects of a novel D_4 (partial) agonist, PD 168,077 (Glase et al., 1997), in tests of associative learning.

Associative learning is normally restricted to the most likely predictors of motivationally significant outcomes like food or foot shock (Dickinson, 1980). Because schizophrenia and ADHD are characterised by attentional impairment, there has also been considerable interest in how the DA system is involved in selective learning processes (for reviews, see Gray et al., 1991; Moser et al., 2000; Weiner and Feldon, 1997). Treatments such as amphetamine impair selective learning, so that animals condition even to a less informative predictor (conditioned stimulus, CS) of the outcome (unconditioned stimulus, UCS) of interest. For example, a weak CS can be set up as irrelevant through prior exposure (in a latent inhibition, LI, procedure). It turns out that impairments in selective learning are seen at doses of amphetamine that produce locomotor hyperactivity but not at doses that produce stereotypy (Staton and Solomon, 1984; Weiner et al., 1987, 1988). It has therefore been suggested that locomotor hyperactivity and effects on selective learning share a common neural substrate in the nucleus accumbens (Weiner, 1990; Weiner and Feldon, 1997). In addition, motor hyperactivity is now widely used to model ADHD (Zhang et al., 2002). Accordingly, in the present study with a novel DA compound, we used locomotor activity to establish functionally contrasting doses of PD 168,077 in the conditioning procedures.

Assessing effects on conditioning can easily be confounded by the role of the DA system in reward processing or in shock sensitivity. In particular, there is evidence from knockout mice that the D_4 receptor is involved in unconditioned fear reactions, although surprisingly in this study, there was no secondary effect on associative learning (Falzone et al., 2002). Unconditioned effects often result in the appearance of an effect on conditioning through an alteration of the impact of the food or foot shock UCS (Ikemoto and Panksepp, 1999; Killcross et al., 1994; Joseph et al., 1996).

In the present study, we compared the effects of PD 168,077 in both appetitive and aversive tasks in order better to distinguish motivational effects from effects on associative learning. In each case, we provided rats with both discrete target CS and a diffuse extended stimulus that provided an experimental background as an alternative CS. In addition, the level of conditioning supported by the target CS was manipulated by the introduction of a trace interval for comparison with the levels of learning seen in contiguously conditioned control groups. This manipulation enabled us to test for any drug-induced increase in conditioning, seen either to the relatively less informative CS in the trace conditioned group, or to the less informative background stimulus that was present throughout the session. Conversely, a comparison of the contiguously conditioned groups would tell us whether PD 168,077 might instead impair conditioning. Drug treatments were administered only during conditioning stages of the experimental procedures. Their effects on the strength of the conditioned associations formed were, in each case, assessed to be drug-free in later extinction tests of responding to the experimental stimuli in the absence of the UCS. In addition, in the appetitive task variant, responding was monitored over the course of acquisition under the drug treatments.

In the same aversive procedure, we have previously found that amphetamine consistently increased conditioning to the background stimulus, and increased conditioning to the trace stimulus at the foot shock intensity used in the current experiment (Norman and Cassaday, 2003). Thus, the purpose of the present study was to test the role of the DA D_4 receptor subtype in mediating amphetamine's effects in aversive conditioning and to test the generality of its role in conditioning with the use of appetitive procedures also.

2. Methods

All the procedures were carried under the UK Animals Scientific Procedures Act (1986) (http://www.homeoffice.gov.uk/animalsinsp/index.htm; project licence no. PPL 40/2019), fully consistent with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (1996).

2.1. Drugs

PD 168,077 (*N*-[4-(2-cyanophenyl)piperazin-1-ylmethyl]-3-methoxybenzamide) maleate was dissolved in dimethyl sulfoxide (DMSO) and injected at 1 ml/kg body weight (intraperitoneally). In Experiment 1, we examined the effects of five doses on locomotor activity (0.064, 0.12, 0.25, 0.5, and 1 mg/kg). Rats were injected according to the same schedule each day, 10 min prior to the test session. In the conditioning experiments, rats were in each case divided into three drug groups—vehicle (DMSO), low-dose PD 168,077 (0.064 mg/kg), and high-dose PD 168,077 (0.5 mg/kg)—administered 15 min before the conditioning stages only (i.e., on 1 day in Experiment 1 and on each of 3 days in Experiment 2).

2.2. Experiment 1: locomotor activity

2.2.1. Animals

Eighteen male Wistar rats (380–454 g; Charles Rivers, UK) were housed in pairs. The rats were maintained on a

12-h light–dark cycle (lights on 8:00 a.m.–8:00 p.m.). Throughout Experiment 1, food and water were available ad libitum.

2.2.2. Apparatus

Twelve individually wired photocell activity cages [40 (L) × 25 (W) × 18 cm (H)] were used (Medical Physics, Queens Medical Centre, Nottingham, UK). Each cage had two photocells, an emitter, and a transmitter interfaced to a computer (Macintosh IIcx). Breaks in the middle photocell beam (9 cm from the bottom of the cage) registered counts that were recorded by the computer to provide a measure of general locomotor activity.

2.2.3. Procedure

On the first day, the rats were simply left for 1 h to habituate to the photocell cages. They were then divided into six groups: vehicle and five different doses of PD 168,077 (0.064, 0.12, 0.25, 0.5, and 1 mg/kg). Activity counts were recorded for a total of 20 min after placing the rat in the cage. This procedure was repeated over three consecutive days.

2.3. Experiment 2: aversive trace conditioning

2.3.1. Animals

Seventy-two male Wistar rats (254–394 g; Charles Rivers) were housed as in Experiment 1. Food was freely available; however, water access was restricted to that provided in the experimental sessions (usually 15 min/day) and, in the home cage, a further 1 h/day (after the experimental sessions) between 11 and 12 p.m. or between 2 and 3 p.m.

2.3.2. Apparatus

Fully automated chambers were set within sound-attenuating casings that contained ventilating fans (Cambridge Cognition, Cambridge, UK). The inner conditioning chambers were plain steel plate boxes [25 × 25 × 22 cm (H)], with a Plexiglas door 19 × 27 cm at the front. The roof was steel plate with a loud speaker inset through which the auditory stimulus was presented. The floor was a grid with steel bars 1 cm apart and 1 cm above the upper lip of a sawdust tray that was a further 7 cm deep. The water spout was mounted in the same wall as the levers, 5 cm above the floor and connected to a lickometer supplied by a pump. A photobeam detected each broken contact with the water spout as a lick and this triggered water delivery from the pump, set to deliver 0.05 ml per lick. The levers were retracted throughout the experiment. Two stimuli were used as the target (noise) and background (flashing light), respectively. There was no other illumination in the chamber apart from when water was available, the water sprout was illuminated. Foot shock was delivered through the grid floor by a constant current shock generator (MISAC Systems, Newbury, UK). Stimulus control was by a RISC PC programmed in Basic, with additional interfacing using an Arachnid extension (Cambridge Cognition).

2.3.3. Procedure

The experiment was run over a 3-week period and the behavioral procedure involved four steps: preconditioning (to establish stable baseline response rates), conditioning (to establish the association between noise CS and foot shock UCS), reshaping (to reestablish the baseline licking response), and, finally, extinction tests (of the strength of the conditioned association to the target and background stimuli).

2.3.3.1. Pre-experimental. Rats were first shaped (over 3 days) to take water from the drinking spout in the experimental chambers. On the following 10 days, they were provided with access to water in their designated conditioning boxes for a total of 15 min after first contacting the water spout. Latency to first lick on the last two pre-experimental days was measured to check that the groups' readiness to drink was well matched prior to conditioning. Throughout the session, there was no presentation of any experimental stimulus.

2.3.3.2. Conditioning. All the rats were conditioned with two 1-s duration (1 mA) foot shocks in a 15-min conditioning session. The foot shock UCS was on both occasions preceded (with or without the interpolated trace interval) by the 5-s duration CS (mixed frequency noise set at 80 dB). Throughout the 15-min conditioning session, an experimental (flashing light) background stimulus was continuously present. The rats were without access to water and there was nothing to record.

2.3.3.3. Reshaping. Rats were placed in the boxes as in the pre-experimental phase and given access to water, as previously. Drink latencies were again recorded to check the rats' readiness to drink prior to the extinction tests and (24 h after conditioning) to provide a measure of general suppression to the contextual cues provided by the experimental chambers.

Reshaping was also essential to reestablish drinking after conditioning (and to allow some extinction of contextual conditioning prior to testing suppression to the experimental stimuli). The recorded drink latency had a cut-off after 15-min exposure, and rats were later given additional exposure to their designated box until they drank freely.

2.3.3.4. Extinction tests. Rats were placed in the boxes with water available as at reshaping. Once drinking had commenced (after the first 50 licks, A period), rats were presented with one of the experimental stimuli (the mixed frequency noise or the flashing light), counterbalanced over 2 days (so that rats receiving the target stimulus on the first day received the background stimulus on the second day). Stimulus presentation continued for the remainder of the session (15 min) and the latency to complete to the next 50 licks (B period) was recorded. The strength of the condi-

tioned response was calculated as the suppression ratio: A/(A + B).

2.4. Experiment 3: appetitive trace conditioning

A subset of 48 male Wistar rats from Experiment 2 (277–419 g) were used, housed as above. All group allocations were counterbalanced for the rats' previous experimental experience. Water was freely available. In the home cage, food was restricted to 5 g/100 g rat body weight and adjusted as necessary to prevent further weight gain in rats over 400 g.

2.4.1. Apparatus

The apparatus for this study is similar to that used in the previous experiment, except that a food magazine replaced the water spout. The food magazine was a metal well with a Plexiglas front flap hinged at the top and was illuminated by a light on the top inside wall of the well when the programme was running. Food pellets were automatically delivered into the magazine well, as programmed, and the nose pokes were registered by the breaking of a photobeam behind the flap as it was pushed open.

Two stimuli were used as the target (noise) and background (flashing light). There was no other illumination in the chamber apart from in the magazine from where food pellets were delivered. Again stimulus control and data collection were performed by a RISC PC programmed in Basic, with additional interfacing using an Arachnid extension (Cambridge Cognition).

2.4.2. Behavioral procedure

2.4.2.1. Pre-experimental. On the first day, each rat was placed in its allocated conditioning box with access to food pellets in the magazine and shaped to nose poke. On the second and third days, rats were placed singly in the boxes and allowed to nose poke for 10 unsignalled rewards, delivered on a variable interval schedule over a 10-min session.

2.4.2.2. Conditioning. For 3 days, rats were conditioned with 10 signalled rewards (UCSs) presented on a variable interval over a 30-min session. The target CS was in each case a 5-s broadband noise, set at 80 dB. During this time, a continuous flashing light stimulus was presented in the background. The contiguously conditioned group received rewards at the offset of each CS presentation, whereas the trace group were exposed to a 60-s delay between CS offset and UCS delivery. Drugs were administered prior to conditioning sessions, as described below.

We recorded the number of nose pokes in the following response bins: (a) 'Pre-noise' in the 5 s before CS presentation; (b) 'Noise' in the 5 s of CS presentation; (c) 'Post-noise' in the 5 s following CS offset; (d) 'Trace' during the 60-s interval between CS and UCS (in the trace conditioned rats); and (e) 'Residual' in the remainder of the session not included in the aforementioned response bins.

2.4.2.3. Extinction. This session was carried over 2 days as a drug-free measure of the levels of conditioning. Rats were presented with only one stimulus, either the mixed frequency noise or the flashing light in the absence of any UCS deliveries. In either case, there were 10 presentations on a variable interval over the 30-min session. Extinction tests were counterbalanced for the order in which conditioning was tested to the noise CS and the flashing light background stimulus. Nose pokes were recorded in the same response bins as for conditioning, except that there was no trace interval in use.

2.5. Data analyses

All statistical tests used analysis of variance (ANOVA) with an α of .05. Locomotor activity was analysed by one-way ANOVA by Drug with repeated measures (Bins) over the 20 min of activity monitoring. The dependent variable was the activity count in each of four 5-min bins, averaged over the 3 days of testing to get the best estimate of the onset of drug effects. There were six levels of the drug to determine a dose–response, and post-hoc comparisons between the doses were performed by *t* test (two-tailed).

The conditioning studies were analysed in 2×3 designs. Trace had two levels (0 and 60-s conditioned groups) and Drug had three levels (vehicle, low dose of PD 168,077, and high dose of PD 168,077). In addition, there were repeated measures (Days) to check the course of conditioning in the appetitive procedure. Levels of learning were measured by suppression ratios and the number of nose pokes, respectively, in the aversive and appetitive procedures. Prior to assessing drug effects on responding to the stimuli of interest, analyses were first carried out to check for pre-existing differences between the rats allocated to the different experimental conditions-to-be, in each case both pre-experimentally, on the pre-conditioning days used to establish the baseline responses of licking or nose poking, and immediately pre-stimulus on experimental days on which the level of conditioning was assessed. In addition to examining effects on conditioning to target, we also took measures of variables sensitive to the level of contextual conditioning to the experimental apparatus. In the aversive procedure, at reshaping, this measure was provided by the latency to first lick after the foot shocks delivered on the preceding day. In the appetitive procedure, we measured nose pokes in the remainder of the session (excluding all responses made during and immediately before or after stimulus presentations).

3. Results

3.1. Experiment 1: locomotor activity

Activity clearly fell off over the course of 20-min testing and differences between the drug treatments in

use were most apparent in Bin 1 (Fig. 1). This pattern of effects was confirmed statistically by a main effect of Bins [$F(3,36) = 86.42$, $P < .001$] and a Bins × Drug interaction [$F(15,36) = 3.76$, $P = .001$]. The overall effect of Drug was insignificant [$F(5,12) = 2.42$]. In Bin 1, drug-treated groups at doses of 0.064–0.25 mg/kg were significantly more active than the saline-treated controls [$t(4) = 4.41$ and 4.33, $P < .05$, respectively]. This effect reduced with increasing dose and, by 0.5 mg/kg, the difference was insignificant [$t(4) = 2.22$]. There were no significant differences between any of the drug groups by Bin 2 of testing [maximum $t(4) = 1.54$].

Since most of the increased locomotor activities were observed within the first 5 min of activity testing, this study showed that PD 168,077 was behaviorally active 15 min post-injection. For the subsequent conditioning studies, we selected the low dose with the largest effect on activity (0.064 mg/kg) and the higher dose at which effects on activity became insignificant (0.5 mg/kg).

3.2. Experiment 2: aversive trace conditioning

3.2.1. Pre-conditioning drink latencies

These were well matched across the different conditions-to-be. On both of the final two sessions of pre-conditioning (Days 9 and 10), there was neither significant effect of Trace or Drug, nor any Trace × Drug interaction [maximum $F(1,66) = 2.80$, $P > .05$, for the Day-10 Trace condition-to-be].

3.2.2. Reshaping latency

There were no effects of Drug, Trace, nor Drug × Trace interaction on the time to start drinking (all Fs < 1). One rat (from the 0.5 mg/kg dose trace condition), which did not drink within the 15 sessions, was then given additional exposure to its designated box until it drank freely.

3.2.3. Extinction tests

3.2.3.1. Noise target. There were no significant effects on the A period, confirming that response rates were comparable prior to presentation of the CS [maximum $F(2,66) = 2.19$ for the Drug × Trace interaction]. The suppression ratios (Fig. 2) showed a significant effect of Trace [$F(1,66) = 68.03$, $P < .001$]. However, there was no effect of Drug, nor any Drug × Trace interaction [maximum $F(2,66) = 2.38$, for the effect of Drug].

3.2.3.2. Light background. There were no significant effects on the A period [maximum $F(1,66) = 3.30$ for the effect of Trace]. The suppression ratios showed neither a significant effect of Trace or Drug, nor of the Drug × Trace interaction [maximum $F(1,66) = 1.25$, for the effect of Trace].

3.3. Experiment 3: appetitive trace conditioning

One rat (from the vehicle trace condition) was excluded as it failed to show the baseline nose poking response. Two further rats were later excluded because of technical error (both were vehicles, one was from the contiguous condition and the other from the trace condition).

3.3.1. Pre-conditioning

There was no effect of Trace or Drug × Trace interaction (Fs < 1) on the average number of nose pokes on the second and third days of magazine training. However, there was a significant effect of Drug (condition-to-be) on this measure

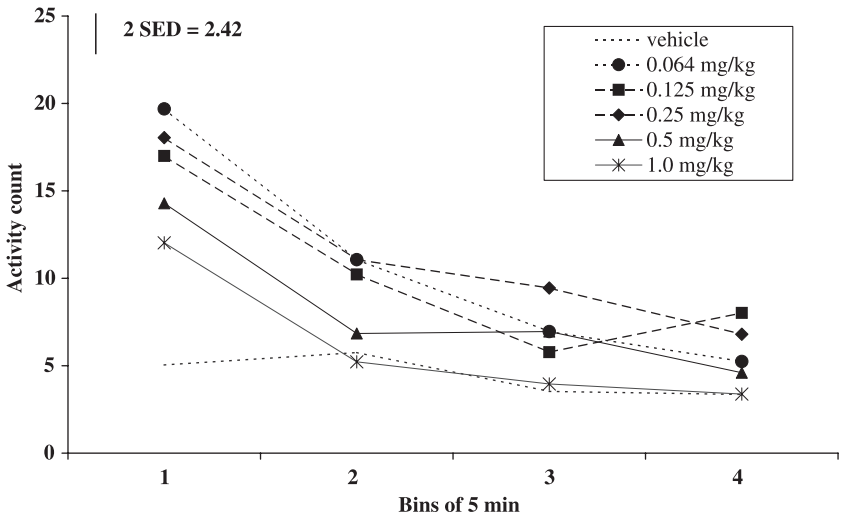

Fig. 1. Average activity counts per 5-min bin over three 20-min sessions. Drug groups given PD 168,077, over the range 0.064–1 mg/kg. Vehicle-injected rats received an equivalent volume of DMSO. Bar at top left shows two standard errors of the difference of the mean (2 S.E.D.), taken from the appropriate stratum of the ANOVA, to allow approximate between-groups comparisons.

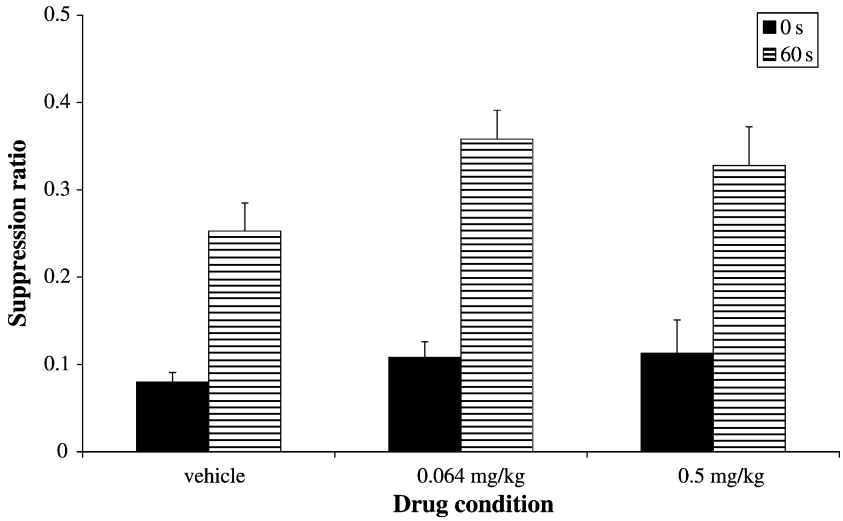

Fig. 2. Conditioned suppression of drinking to the target noise stimulus measured as the suppression ratio for each of the (0 and 60 s) trace conditions. Drug groups were given PD 168,077 at 0.064 or 0.5 mg/kg. Vehicle-injected rats received an equivalent volume of DMSO. For the histograms, individual error bars show standard errors of the mean to allow approximate visual comparisons between groups.

of baseline response rates [$F(2,39) = 4.61$, $P < .05$], suggesting that (in the conditioning phase) we might need to adjust for any drug effects on pre-stimulus response rates in order to assess effects on conditioning.

3.3.2. Conditioning

3.3.2.1. Pre-noise responding. In spite of the difference observed between groups prior to conditioning, there were no differences between the groups on the number of nose pokes made during the pre-stimulus over the course of conditioning. There was no effect of Days, no significant interaction with Trace or Drug, and the Days × Trace × Drug interaction was also insignificant [maximum $F(4,78) = 1.61$, for the three-way interaction]. This lack of effect on responding prior to CS presentation meant that the number of nose pokes in the following 5-s noise presentation could be taken to reflect (drug effects on) the level of conditioning (rather than some nonspecific effect of drug treatments on activity influencing the baseline nose-poking response).

3.3.2.2. Noise responding. Acquisition was confirmed by a significant effect of Days on the number of nose pokes made during the stimulus [$F(2,78) = 27.71$, $P < .001$] (Fig. 3). There was also a significant Days × Trace interaction [$F(2,78) = 20.87$, $P < .001$]. This confirms that whilst the

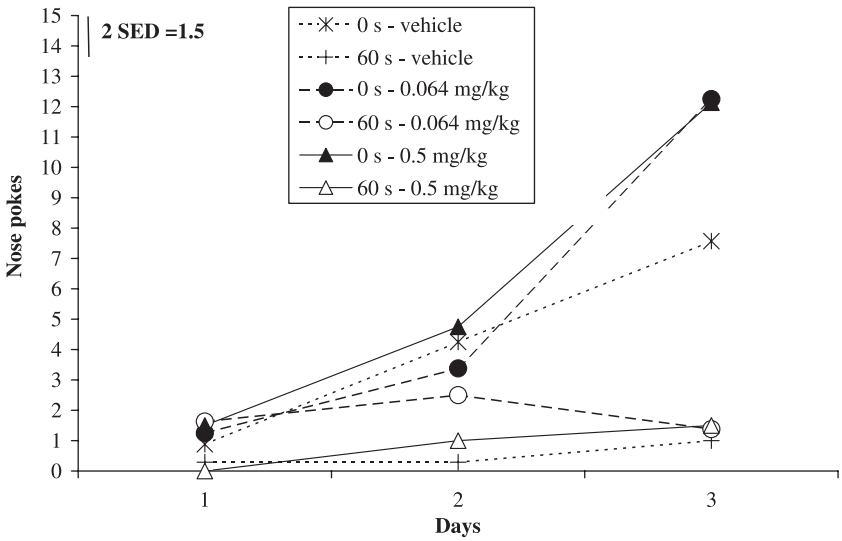

Fig. 3. Nose poking over three sessions' conditioning to the target noise stimulus for each of the (0 and 60 s) trace conditions. Drug groups were given PD 168,077 at 0.064 or 0.5 mg/kg. Vehicle-injected rats received an equivalent volume of DMSO. Bar at top left shows two standard errors of the difference of the mean (2 S.E.D.), taken from the appropriate stratum of the ANOVA, to allow approximate between-groups comparisons.

groups conditioned at 0 s successfully associated the noise stimulus with food, those in the trace groups (conditioned at a 60-s delay) formed little (if any) association between the CS and the UCS. Since there were no significant interactions by Drug [maximum $F(4,78) = 1.53$, for the three-way interaction], PD 168,077 was without effect on conditioning, irrespective of how informative the predictive stimulus was.

3.3.2.3. Post-noise responding. There was again a significant effect of Days [$F(2,78) = 37.29$, $P < .001$] and Days × Trace interaction [$F(2,78) = 3.63$, $P < .05$]. However, there were no significant interactions involving Drug [maximum $F(4,78) = 1.82$, for the Days × Drug interaction], so PD 168,077 was also without effect on responding to food delivery.

3.3.2.4. Residual responding. The nose pokes in the remainder of the session (excluding those responses made in any of the above response windows) were both stable over time and unaffected by the drug. There was no effect of Days and no interactions with Trace or Drug [maximum $F(2,78) = 1.73$, for the Days × Trace interaction].

3.3.3. Extinction to target

3.3.3.1. Pre-noise responding. There were no significant differences between the groups [maximum $F(1,39) = 2.31$, for the effect of Trace].

3.3.3.2. Noise responding. The extinction testing confirmed that whilst conditioning had been weakened by the introduction of the 60-s interval, it was unaffected by treatment with PD 168,077. There was a main effect of Trace [$F(1,39) = 13.79$, $P = .001$], but both the main effects of Drug and the Trace × Drug interaction were insignificant [maximum $F(2,39) = 1.24$, for the interaction].

3.3.3.3. Post-noise responding. Again there was a main effect of Trace [$F(1,39) = 5.73$, $P < .05$] that arose because the 60-s conditioned group nose poked less. As would be expected, both the main effects of Drug and the Trace × Drug interaction were still insignificant [maximum $F(2,39) = 1.05$, for the interaction].

3.3.3.4. Residual responding. The nose pokes in the remainder of the session were not different between the groups [maximum $F(2,39) = 1.31$, for the interaction].

3.3.4. Extinction to background

3.3.4.1. Pre-light responding. There were no significant differences between the groups (all Fs < 1).

3.3.4.2. Light responding. The extinction testing confirmed that differences in conditioning to the noise target had not resulted in differences in conditioning to the experimental background stimulus (all Fs < 1).

3.3.4.3. Post-light responding. As would be expected, there were also no differences in the expectation of food (all Fs < 1).

3.3.4.4. Residual responding. This was also equivalent across the groups [maximum $F(2,39) = 1.85$, for the main effect of Drug].

4. Discussion

The different levels of conditioning produced in the trace conditioned groups and by the weakly predictive alternative background stimulus together provided a strong test of the effects of PD 168,077 on associative learning. Learning that a stimulus (e.g., noise) predicts events such as food or foot shock is normally reduced when these events are separated in time. This effect was clearly seen here, in both aversive and appetitive procedures. Similarly, animals normally learn relatively little about background stimuli, as general context does not reliably predict food delivery or the onset of foot shock. In both appetitive and aversive procedures, conditioning to the experimental background stimulus (flashing lights presented for the duration of the session) was similarly unaffected by treatment with PD 168,077. This experimental stimulus differed from the more conventional context provided by the experimental chambers in that it was unimodal and only present during the conditioning and the test. Thus, since reshaping latencies (in the aversive procedure) and residual responding (in the appetitive procedure) were also unaffected by PD 168,077, there was no evidence for any involvement of the D_4 receptor site in conditioning to conventional contextual stimuli either. Thus, throughout these experiments, PD 168,077 was without effect on conditioning, whether motivated by food or foot shock, and irrespective of how informative the predictive stimulus was.

4.1. Could ceiling or floor effects have masked drug effects on conditioning?

The weaker conditioning to the target CS in the trace conditioned group and the weaker conditioning to the alternative background stimulus would have been sensitive to any increase in conditioning produced by PD 168,077. The stronger conditioning in contiguously conditioned groups would have been sensitive to any impairment in conditioning produced by PD 168,077. Similarly, in the appetitive procedure, there was no indication that ceiling or floor effects could account for the observed effects. Fig. 3 shows that there was plenty of room for 0 s to show impairment and for 60-s conditioned groups to show improvement in associative learning.

4.2. Were the test doses appropriate?

Experiment 1 used locomotor activity to identify doses of PD 168,077 that either did (0.064 mg/kg) or did not (0.5 mg/kg) significantly increase activity relative to vehicle-treated rats. The use of doses with contrasting effects in activity meant that any consistent effects on conditioning could not be attributed to simple motor effects. Moreover, in the aversive procedure, learning was reflected in reduced (i.e., conditioned suppression of lick) responding, whereas in the appetitive procedure, learning was reflected in increased (i.e., nose poke) responding.

4.3. Was the timing of the drug injections appropriate?

Our experimental sessions were all 30 min or less with a 10- to 15-min drug-to-test interval. Since the effects on locomotor activity were seen within 15 min of drug administration, this time course meant that activity effects could not contribute to the behavioural effects seen in the on-the-baseline conditioning tests. However, this does not mean that the drug was ineffective within 15 min. Little is known about the pharmacokinetics of PD 168,077, but for in vivo tests of this compound, it has been acceptable to sum data over a 1-h period (Clifford and Waddington, 2000).

In general, where full drug effect–time studies are done, behavioural dissociations emerge. For example, the duration of action of the benzodiazepine alprazolam depends on the experimental contingencies in use (Simpao et al., 2001). Similarly, cocaine has different time-dependent effects on fixed ratio and locomotor responding (Lau et al., 1999). Thus, the assessment of cognitive effects can be improved if compounds are tested after the disappearance of sedative or stimulatory effects. The rationale here was that to test conditioning when nonspecific effects had subsided (presumably in consequence of habituation to the experimental chambers or acute tolerance to drug with respect to locomotor activity) would help with the clean assessment of on-the-baseline conditioning. In other words, although we used hyperactivity to identify a behaviourally effective dose, for the appetitive procedure, we were not interested in effects mediated by hyperactivity.

4.4. Do nonspecific effects confound interpretation of the results?

Activity as measured by photobeam interruptions lacks behavioral resolution (Clifford and Waddington, 2000). However, in the appetitive conditioning procedure, we were also able to assess effects of PD 168,077 on the same baseline nose-poking response used to assess the strength of the CS–UCS association. This meant that particular nonspecific drug effects relevant to the expression of the conditioned response of interest could be discounted. As well as this 'on-the-baseline' test of associative learning, conditioning was also assessed 'off-the-baseline' in drug-free extinction tests in both aversive and appetitive procedures (in which drug effects on response rates could not be an issue). These extinction tests confirmed that PD 168,077 did not affect the (expression of) associative learning.

As would be expected given that the results on conditioning were negative, there was no evidence that PD 168,077 affected unconditioned responding to the UCS (Killcross et al., 1994; Falzone et al., 2002), although we could only measure this directly in the appetitive procedure. Moreover, in addition to evaluating its effects on contiguously conditioned groups, comparison across differently motivated tasks also meant that we could exclude drug effects on the processing of food and foot shock stimuli.

4.5. Did we use fair tests of selective learning?

Despite the evidence to link the D_4 receptor with schizophrenia, the preclinical evidence that D_4 antagonists might be antipsychotic has (to date) been disappointing (Bristow et al., 1997a). However, this evidence has largely been based on the antagonism of amphetamine-induced effects in tests perhaps better suited to the identification of drugs acting on D_2 receptors. There is rather better evidence that the D_4 site is involved in ADHD (for a review, see Oak et al., 2000). Thus, in the absence of evidence on the behavioral profiles of D_4-selective drugs, it made sense to look at associative learning in tests not specifically developed to identify further D_2 compounds using hyperactivity effects as a basis for the selection of doses. However, consistent with earlier preclinical tests (Bristow et al., 1997b), there was in fact no indication that PD 168,077 impaired selective learning by increased conditioning to less informative stimuli as we might expect in an effective model of attentional impairment. In contrast to the effects seen with amphetamine (Norman and Cassaday 2003), PD 168,077 neither promoted conditioning over the trace interval used to render the target CS less informative, nor increased conditioning to background stimuli with less predictive value.

5. Conclusions

The lower dose of PD 168,077 was chosen on the basis of its effectiveness in producing locomotor hyperactivity. This effect showed that the drug treatment was behaviourally active but the hyperactivity was not sustained for the duration of the conditioning tests. The appetitive procedure was on-the-baseline, so it was important to test conditioning when nonspecific effects had subsided. In fact, there was no evidence that PD 168,077 had any effect on associative learning in either aversive or appetitive procedures. This was despite the provision of alternative predictive stimuli, relatively more and less informative with respect to UCS delivery. In contrast, the conditioned suppression parameters used here were sensitive to the effects of amphetamine at the hyperactivity-inducing doses

used in LI procedures (Norman and Cassaday, 2003). Animals can show acute tolerance to drug with respect to locomotor activity and it is highly unlikely that both doses of PD 168,077 were simply ineffective for the duration of the conditioning tests. Thus, present findings suggest that further effect–time studies will be necessary to specify the relationship between the locomotor and attentional effects of dopaminergic compounds.

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