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A neurotensin agonist and antagonist decrease and increase activity, respectively, but do not preclude discrete cue conditioning

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Abstract

There is evidence to suggest that neurotensin (NT) may enhance cognitive function. For example, in aversive trace conditioning, the NT agonist PD149163 selectively increased trace conditioning (Grimond-Billa, *et al*., 2008). The present study, therefore, examined the role of NT in associative learning, tested using an appetitive trace conditioning procedure (0-s or 10-s inter-stimulus-interval [ISI]) with a mixed frequency noise as a conditioned stimulus (CS) and food delivery as the unconditioned stimulus (UCS). The effects of an NT agonist (PD149163, 0.125 and 0.25 mg/kg, Experiment 1) and an NT antagonist (SR142948A, 0.01 and 0.1 mg/kg, Experiment 2) were compared. To take nonspecific effects of these compounds into account, conditioning to the CS was measured as a percentage of total responding, during UCS deliveries and in the inter-trial-interval (ITI). In both experiments, associative learning to the contiguously (0-s) presented CS was demonstrated, although there was a relative reduction in this learning under 0.125 mg/kg PD149163. Counter to prediction, the only effect on trace conditioning was some overall reduction in responding to the CS in the 10-s group conditioned under 0.25 mg/kg PD149163. The NT antagonist was without any effect on appetitive conditioning. However, these NT compounds were not ineffective: decreases and increases in responding in the ITI, ISI and during UCS deliveries seen under PD149163 and SR142948A were dissociable from effects on discrete cue conditioning.

Key words

appetitive conditioning; neurotensin; PD149163; rat; SR142948A; trace conditioning

Introduction

Over the last 30 years since its discovery and characterisation in the brain (Carraway and Leeman, 1973, 1976), a series of pharmacological, anatomical and behavioural studies have pointed to a role for neurotensin (NT) in cognition (Cacéda, *et al*., 2003, 2006). In normal animals, NT and its analogues can act as cognitive enhancers (Azmi, *et al*., 2006; Greidanus, *et al*., 1982; Grimond-Billa, *et al*., 2008). In animal models for schizophrenia, acute treatment with NT or its analogues produces a variety of antipsychotic-like effects, such as reversing phencylidine PCP- and amphetamine-induced behavioural deficits (Cacéda, *et al*., 2003; Nemeroff, 1980). A likely mechanism for such actions is provided by the ability of NT (and its analogues) to reduce the overactivity in the dopamine (DA) system that is otherwise produced by amphetamine (Nemeroff, 1986, Skoog, *et al*., 1986). Additionally, human studies showed that untreated schizophrenics showed decreased NT-like immunoreactivity in the cerebrospinal fluid and that these low NT levels returned to normal after antipsychotic drug treatment (Widerlov, *et al*., 1982).

Therefore, particular interest has been directed towards NT effects on schizophrenic-like symptoms. Attentional disorder, inducing a reduced ability to select between informative and

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uninformative environmental stimuli, is a central feature of schizophrenia (Castner, *et al*., 2004; Sweatt, 2004; Weiner, 1990, 2003) that can be modelled using conditioning procedures in animals to assess selectivity for learning.

Trace conditioning procedures are reliably sensitive to the effects of dopaminergic drugs and lesions (Cassaday, *et al*., 2005a,b; Kantini, *et al*., 2004; Norman and Cassaday 2003) consistent with the possibility that they should identify pro-cognitive effects. In aversive trace conditioning, an NT agonist, PD149163 (at 0.25 but not 1 mg/kg), selectively increased conditioning over the trace interval: there was no such increased conditioning in the 0-s group. At the same time, conditioning to box context was reduced under PD149163 (Grimond-Billa, *et al*., 2008). Appetitive studies are important to establish the generality of observed effects in terms of learning motivated in different ways (Cassaday, *et al*., 2005a, 2008; Horsley and Cassaday, 2007; Kantini, *et al*., 2004; Norman and Cassaday, 2003; Thibaudeau, *et al*., 2007). Therefore, the present study used a standard appetitive procedure (Cassaday, *et al*., 2005a,b, 2008; Nayak and Cassaday, 2003) to test the effects of repeated treatments with an NTR1 selective NT agonist (PD1491623) and a non selective antagonist (SR142948A) over the course of appetitive acquisition. The agonist, PD149163, acts preferentially at NTR1 (Boules, *et al*., 2003; Feifel, *et al*., 1999; Petrie, *et al*., 2004). The antagonist, SR142948A, blocks NTR1 and NTR2 with similar affinity (Vincent, *et al*., 1999). However, there are little data that allow us to exclude a role for any specific NT receptor subtype in the learning processes under test. Two doses of each compound were tested, based on previous dose-related effects (Binder, *et al*., 2001, 2002; Feifel, *et al*., 1999; Grimond-Billa, *et al*., 2008; Shilling, *et al*., 2004).

The specific prediction that treatment with the NT agonist, PD149163, should improve associative learning to the discrete CS across the trace interval (Grimond-Billa, *et al*., 2008) would be consistent with the known effects of haloperidol in the appetitive procedure used here (Cassaday, *et al*., 2005b). SR142948A was without effect in aversive trace conditioning (Grimond-Billa, *et al*., 2008). However, with respect to the DA system, effects in appetitive and aversive trace conditioning procedures are not equivalent (Cassaday, *et al*., 2005a, 2008; Horsley and Cassaday, 2007; Kantini, *et al*., 2004; Norman and Cassaday, 2003). If SR142948A behaves like a DA agonist in the appetitive procedure then, rather than having a demonstrably opposite action to PD149163, it should be expected to increase conditioning to the discrete cue presented without a trace interval (Cassaday, *et al*., 2008).

Methods

Animals

Forty-eight male Wistar rats (Charles Rivers, UK) of mean weights 503 g and 524 g for Experiments 1 and 2, respectively, were used. Rats were counterbalanced for their previous experimental experience, but kept in the same drug condition, as prior testing in aversive conditioning does not interfere with the demonstration of the appetitive (trace) conditioning (Cassaday, *et al*., 2005a, 2008; Nayak and Cassaday, 2003). All training and testing took place during the light phase (between 09.00 and 17.00 h). Animals were housed in pairs (33 × 27 × 13 cm) on a 12:12 h light/dark cycle (lights on 08.00 to 20.00 h).

For the duration of the experiment, food in the home cage was restricted to 5 g/100 g rat body weight to motivate appetitive responding. This basic ration was adjusted (up to a maximum of 20 g per rat per day) to allow further weight gain in rats of below average weight. The average weight loss over the duration of the experiments was less than 5 g. Water was available in the home cage throughout the duration of the study.

All procedures were carried out in accordance with the United Kingdom Animals Scientific Procedures Act 1986, Project Licence number PPL 40/2648.

Drugs

PD149163 (Lys(CH_2NH)-Lys-Pro-Trp-tLeu-Leu-OEt) was generously supplied by the NIMH Chemical Synthesis and Drug Supply Program, and SRI International, Menlo Park, California, USA and SR142948A (2-{[5-(2,6-dimethoxyphenyl)-1-(4-(*N*-(3-dimethylaminopropyl)-*N*-methylcarbamoyl)-2-isopropylphenyl)-1H-pyrazole-3-carbonyl]-amino}-adamantane-2-carboxylic acid hydrochloride by Sanofy-Synthelabo, Toulouse, France, were dissolved in physiological saline and injected at 1 mL/kg body weight (s.c. for PD149163 and i.p. for SR142948A). We investigated the effects of two doses of each compound. In Experiment 1, the 1 mg/kg dose of PD149163 initially selected was too high in that rats were not responding to collect the unconditioned stimulus (UCS) so its effects on conditioning could not be tested. Therefore, both doses of PD149163 were reduced from day 6 onwards and for a subsequent 10 days. Only the data from day 6 of conditioning onwards was used in the analysis. Thus, the drug treatments were as follows: for PD149163, 0.25 mg/kg and 0.125 mg/kg; for SR142948A 0.01 mg/kg and 0.1 mg/kg. Rats were injected according to the same schedule, 15 min (PD149163) or 30 min (SR142948A) before each conditioning session.

Behavioural conditioning apparatus

Experimental testing was conducted within a set of six fully automated ventilated conditioning chambers, housed within sound-attenuating cases containing ventilation fans (Cambridge Cognition, Cambridge, UK). Each of the inner conditioning chambers consisted of a plain steel box (25 × 25 × 22 cm height) with a Plexiglas door (19 × 27 cm) at the front. The floor was a shock grid with steel bars 1 cm apart and 1 cm above the lip of a 7 cm deep sawdust tray.

A recessed food magazine was located on a side-wall of each of the chambers. The magazine was constantly illuminated whenever food was available. Access to the magazine

was via a magazine flap. Nose pokes were recorded by the breaking of the photo beam within the food magazine. The UCS consisted of two 45 mg sucrose pellets dispensed serially into the magazine (Formula F, Noyes Precision Food, New Hampshire, UK).

Two experimental stimuli were available as potential predictors of food delivery. The target stimulus was a noise CS (mixed frequency), presented via a loudspeaker inset on the roof of the chamber, set at 80 dB including background and of 5-s duration. A background stimulus was provided by three wall-mounted stimulus lights and the house light flashing on (0.5 s) and off (0.5 s), continuously throughout the conditioning session. Stimulus control and data collection was done by an Acorn Archimedes RISC computer programmed in Basic with additional interfacing using an Arachnid extension (Cambridge Cognition).

Behavioural conditioning procedure

Allocation to conditioning groups was also counterbalanced by box. Acquisition was conducted for 10 days. On each day, there were 10 pairings of noise CS and food UCS, presented with a 0-s ISI or after a 10-s trace interval.

Pre-conditioning There were two days shaping to accustom rats to eating from the magazine, in which the magazine was preloaded with 10 reward pellets with an additional 5 rewards over 5 min to familiarise rats with the food deliveries. The tray flap door was propped open on day 1 but was closed on day 2 so the rats were then required to nose poke the door open to collect food. Then followed two days of baseline sessions, during which there were 10 unsignalled rewards in 10 min, delivered on a variable interval around 3 min. The total number of nose pokes was recorded. Rats producing only 10 (or fewer) nose pokes on either of these days were given additional shaping at the end of the session. All rats successfully shaped to nose poke and so could proceed to conditioning.

Conditioning Conditioning consisted of 10 signalled rewards presented per session (30 min/session/day) during 10 days under drug treatment. Depending on the experimental group, the food UCS was delivered directly in the 0-s (contiguously conditioned) group or 10-s after CS offset (in the trace-conditioned group). Conditioning trials were presented throughout the 30-min session, on a variable interval, with the constraint that the inter-trial-interval (ITI) was always at least 1.5 times longer than the inter-stimulus-interval (ISI). Throughout the 30 min of acquisition, the background stimulus (flashing lights) was presented continuously. This continuous presentation also encompassed the 10-s ISI, where applicable, that added to the overall duration of the session.

The dependent variables were the number of nose pokes in the following response bins: 5 s before the CS (pre-CS responding); during the 5-s CS presentation (CS responding); during the 10-s trace interval between CS and UCS (ISI); during 5 s after the delivery of the UCS (UCS responding) and in the remainder of the session not included in the aforementioned response bins (ITI responding). The ITI measure excluded responding in the ISI, where applicable. The ITI measure is affected by general level of activity and drug effects thereon, but differences in ITI responding by trace interval reflect differences in the level of contextual conditioning.

Design and analysis

There were six experimental groups run in a $3 \times 2 \times 10$ mixed factorial design. The between subject factors were in each case drug (at three levels of dose) and trace (at levels 0 and 10-s), and to assess effects over the course of acquisition, the repeated measures factor was day. The dependent variable was in each case the number of nose pokes into the food magazine. To separate out drug effects on baseline responding in acquisition, conditioning to the target (noise) CS was analysed as a percentage of responding in the remainder of the session (%CS = [CS/(Pre-CS + UCS + ITI)] × 100).

In the trace group, the responding of the animals during the 10-s trace between CS offset and sucrose delivery (ISI) was also tested for any effects of drug treatments. Drug effects on responding in the ISI were taken into account by using the equivalent average of the ITI ($ITI_{10\text{-s average}}$) as a covariate.

All ANOVAs used an α-level of 0.05. When exploring significant main effects in the absence of interactions, the Student's *t*-tests are collapsed across groups. To explore interactions, we made only the relevant comparisons that were necessary to determine the basis for any differences in conditioning under drug.

Results

Experiment 1: effects of the NT agonist PD149163

Pre-CS responding There was a significant trace effect [$F(1,42) = 6.50$, $P < 0.05$], the main effect of drug approached significance [$F(2,42) = 3.08$, $P = 0.056$] and the drug × trace interaction was significant [$F(2,42) = 3.7$, $P < 0.05$]. As Table 1 illustrates, the interaction arose because the 10-s vehicle group responded more than all other groups. These baseline differences underscore the importance of correcting for non-specific effects on responding to assess drug effects on conditioning. Moreover, there were changes over the course of acquisition in that the main effect of day approached significance [$F(9,378) = 1.89$, $P = 0.053$] and the day × trace interaction was significant [$F(9,378) = 2.18$, $P < 0.05$]. In general terms, this interaction was shown as reduced responding in the pre-CS over days in the 0-s conditioned groups, as might be expected where a strong predictor of the UCS is present, whereas in the 10-s conditioned groups responding changed little. There were no other significant interactions involving Day [$F_{max}(18,378) = 1.44$]. In any event, the %CS

Table 1 Mean nose pokes (SEM) in the contiguously conditioned (0-s) and trace-conditioned (10-s) groups under one of the three drug treatments (saline, 0.125 and 0.25 mg/kg NT agonist PD149163)

ISI	0-s			10-s		
Drug	Saline	0.125 mg/kg	0.25 mg/kg	Saline	0.125 mg/kg	0.25 mg/kg
Pre-CS	1.28 (0.38)	1.31 (0.22)	1.40 (0.29)	3.61 (0.73)	1.69 (0.50)	1.50 (0.40)
UCS	12.78 (2.88)	11.19 (1.16)	10.69 (1.31)	17.18 (2.44)	13.80 (1.88)	8.21 (1.51)
ITI	45.09 (11.53)	46.04 (3.99)	45.19 (5.40)	104.49 (20.06)	62.34 (9.92)	56.98 (8.40)
%CS	28.49 (4.96)	14.84 (2.64)	21.32 (2.45)	5.57 (1.08)	5.27 (1.09)	3.76 (0.80)
ISI				20.20 (4.06)	9.36 (2.21)	4.24 (1.22)

Responding was measured in the following bins: Pre-CS, 5 s before the CS presentation; UCS, 5 s following food delivery; ITI, inter-trial-interval, that is, responding in the remainder of the session excluding all other response bins; %CS, CS/(Pre-CS + UCS + ITI) × 100; ISI, inter-stimulus-interval, that is, responding in the 10-s between CS and UCS presentation.

measure corrected for these fluctuations in responding outside of CS presentations on a day-by-day basis.

UCS responding There was no main effect of trace in the 5 s following UCS delivery ($F < 1$). However, there was a main effect of drug [$F(2,42) = 3.98$, $P < 0.05$] seen as decreased responding under 0.25 mg/kg PD149163: vehicle versus high [$t(30) = 2.56$, $P < 0.05$] and low versus high [$t(30) = 2.01$, $P = 0.053$]; vehicle versus low was non significant, $P > 0.05$ (see Table 1). The drug × trace interaction was not significant [$F(2,42) = 1.65$]. There was a main effect of day [$F(9,378) = 4.33$, $P < 0.001$] because of the general tendency for UCS collection to become more efficient over days. No interaction with day was significant (all Fs < 1).

ITI responding There was a main effect of trace [$F(1,42) = 10.22$, $P < 0.05$] with greater ITI responding in 10-s than in 0-s conditioned groups (see Table 1). There was no main effect of drug or trace × drug interaction [$F_{max} = 2.66$]. However, it is clear from the means (see Table 1) that for the vehicle groups, the 10-s conditioned responded significantly more than the 0-s conditioned groups [$t(14) = 3.08$, $P < 0.01$], whereas for both drug doses this difference was non significant [$t_{max} = 1.51$].

Responding in the ITI period changed by day [$F(9,378) = 6.10$, $P < 0.001$] depending on trace condition [$F(9,378) = 2.191$, $P < 0.05$], with reduced responding over days in 0-s conditioned (as would be expected with an informative CS) but not 10-s conditioned groups. The day × drug interaction was also significant [$F(18,378) = 2.18$, $P < 0.01$] because some overall decrease in responding over days was most apparent in the vehicle-treated groups. The three-way interaction was not significant [$F < 1$].

% CS responding The 0-s conditioned groups produced greater overall responding than 10-s conditioned groups, seen as a main effect of trace [$F(1,42) = 61.88$, $P < 0.001$]. Thus as expected, the introduction of the 10-s interval reduced conditioning. However, this was affected by drug condition as there was a significant main effect of drug [$F(2,42) = 3.71$, $P < 0.05$] and a drug × trace interaction [$F(2,42) = 3.34$, $P < 0.05$]. As Table 1 illustrates, the trace effect remained for each drug condition in that the 0-s conditioned group responded more than the 10-s trace-conditioned group in every drug condition: for vehicle [$t(14) = 3.56$, $P < 0.01$]; for low dose [$t(14) = 2.24$, $P < 0.05$] and for high-dose PD149163 [$t(14) = 3.84$, $P < 0.01$]. The interaction, therefore, arose from the differing pattern of drug effects within the 0-s and the 10-s trace-conditioned groups (see Table 1). For the 0-s conditioned groups, the only significant difference was that low dose responded less than vehicle [$t(14) = 2.68$, $P < 0.05$], whereas for trace-conditioned groups it was the high-dose group that responded significantly less than the vehicle group [$t(14) = 2.78$, $P < 0.05$].

On the analysis of acquisition, there was a significant main effect of day [$F(9,378) = 16.33$, $P < 0.001$] and day × trace [$F(9,378) = 15.42$, $P < 0.001$], day × drug [$F(18,378) = 2.50$, $P < 0.001$] and day × drug × trace [$F(18,378) = 1.96$, $P < 0.05$] interactions. Figure 1 illustrates that the trace effect developed over days for all experimental conditions. The three-way interaction arose because the increase in responding over days in the 0-s conditioned groups was greater under 0.25 mg/kg PD149163 than in the vehicle condition. However, data analysis in effect started at day 6 of conditioning (due to the change of drug dose). Thus, for the 0-s conditioned groups, there was already a difference between drug groups on the first day of comparison. Vehicle had conditioned significantly more than both low and high doses of PD149163 [$t(14) = 2.15$, $P < 0.05$] and [$t(14) = 2.36$, $P < 0.05$], respectively. What is important here is that acquisition, although delayed by drug, was shown in 0-s conditioned groups. Post-hoc tests confirmed that the trace effect was significant for both high and low doses PD149163 from days 8 and 10, respectively, [$t_{min}(14) = 2.28$, $P < 0.05$ and $t_{min}(14) = 2.66$, $P < 0.05$]. However, there was some difference in acquisition measured in 0-s groups by dose of PD149163. Responding under 0.25 mg/kg was significantly lower than responding under vehicle only until day 10, after which performance converged [$t_{max}(14) = 2.52$, $P < 0.05$]; whereas under 0.125 mg/kg responding remained depressed

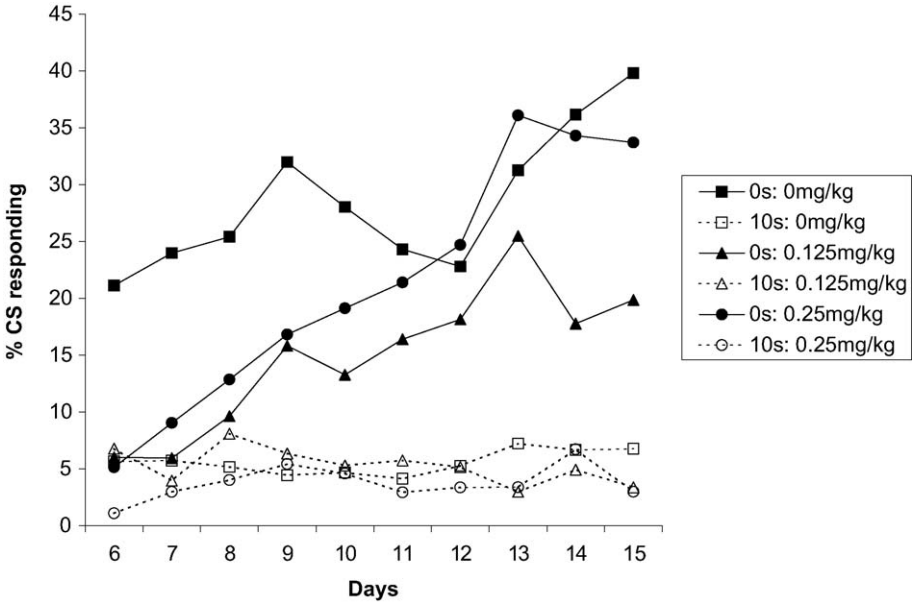

Figure 1 Conditioning to the neurotensin agonist PD149163. Mean number of magazine entries per day during acquisition of conditioning to the discrete CS under 0, 0.125 and 0.25 mg/kg of PD149163. Open symbols and dotted lines represent CS conditioned at 10 s ISI; closed symbols and solid lines represent CS conditioned at 0-s ISI.

relative to vehicle, and significantly so on days 14 and 15 [$t(14) = 2.30$, $P < 0.05$ and $t(14) = 2.92$, $P = 0.011$, respectively].

ISI responding For responding during the ISI in trace-conditioned groups, there was a significant main effect of drug [$F(2,20) = 5.81$, $P = 0.01$] with a dose-related decrease in responding (see Table 1). Vehicle responded significantly more than low [$t(14) = 2.35$, $P < 0.05$] and high-dose groups [$t(14) = 3.77$, $P < 0.01$]. The difference between responding in low and high doses approached significance [$t(14) = 2.03$, $P = 0.063$] with low dose responding more than the high-dose group. There was no effect of, or any interaction with, day [Fs < 1].

Experiment 2: effects of the NT antagonist SR142948A

Pre-CS responding There was a significant effect of trace [$F(1,42) = 5.43$, $P < 0.05$] with greater responding in 10-s than 0-s conditioned groups (see Table 2) and of drug [$F(2,42) = 7.52$, $P < 0.01$] seen as increased responding in the high dose compared with both the vehicle and the low-dose groups [$t(30) = 3.11$, $P < 0.01$ and $t(30) = 2.46$, $P < 0.05$], respectively. This drug effect on responding is taken into account below by analysis of the level of learning in terms of %CS responding.

There was no significant effect of day ($F < 1$), and the day × trace interaction was marginal [$F(9,378) = 1.68$, $P = 0.067$]. No other interaction was significant [F_{max} (18,378) = 1.23].

UCS responding As can be seen from Table 2, the antagonist had an opposite effect on UCS responding to that seen under the agonist. SR142948A increased the number of responses in the 5 s after the UCS presentation, seen as a main effect of drug [$F(2,42) = 5.61$, $P < 0.01$; vehicle versus low $t(30) = 2.53$, $P < 0.05$; vehicle versus high $t(30) = 3.22$, $P < 0.01$; low versus high $P > 0.05$]. There was no effect of trace or trace × drug interaction (Fs < 1).

As the rats became accustomed to the UCS deliveries, there was an effect of day [$F(9,378) = 26.18$, $P < 0.001$] that interacted with trace [$F(9,378) = 2.89$, $P < 0.01$]. The interaction was because the 0-s conditioned groups showed a greater overall increase in responding over days. However, this effect was independent of drug treatment and there were no other significant interactions [$F_{max}(18,378) = 1.41$].

ITI responding There was a main effect of trace [$F(1,42) = 8.22$, $P < 0.01$] seen as higher levels of responding in 10-s than 0-s conditioned groups (Table 2). The main effect of drug was significant [$F(2,42) = 5.50$, $P < 0.01$], with significantly greater levels of responding in the high compared with vehicle and with low-dose groups (Table 2), [$t(30) = 2.77$, $P = 0.01$ and $t(30) = 2.13$, $P < 0.05$], respectively. The trace × drug interaction was not significant [$F < 1$].

Again, there was an overall reduction in responding by day ($F(9,378) = 5.92$, $P < 0.001$), that interacted with the trace condition ($F(9,378) = 7.36$, $P < 0.001$), as the pattern of responding over days differed with 0-s conditioned groups decreasing but 10-s conditioned groups increasing in ITI responding over days.

Table 2 Mean nose pokes (SEM) in the contiguously conditioned (0-s) and trace-conditioned (10-s) groups under one of the three drug treatments (saline, 0.01 and 0.1 mg/kg of NT antagonist SR142948A)

ISI	0-s			10-s		
Drug	Saline	0.01 mg/kg	0.1 mg/kg	Saline	0.01 mg/kg	0.1 mg/kg
Pre-CS	1.83 (0.24)	2.51 (0.43)	3.44 (0.64)	2.75 (0.47)	2.96 (0.32)	5.23 (0.93)
UCS	8.89 (1.77)	12.18 (0.64)	12.98 (1.72)	8.25 (0.41)	11.43 (1.79)	14.29 (2.05)
ITI	69.13 (10.01)	86.28 (8.19)	108.31 (10.96)	103.34 (12.11)	104.11 (11.58)	138.86 (18.71)
%CS	15.93 (1.74)	17.83 (1.43)	15.49 (2.11)	3.92 (0.55)	3.28 (0.81)	5.50 (0.50)
ISI				10.63 (1.34)	15.64 (3.22)	24.21 (5.34)

Responding was measured in the following bins: Pre-CS, 5 s before the CS presentation; UCS, 5 s following food delivery; ITI, inter-trial-interval, that is, responding in the remainder of the session excluding all other response bins); %CS, CS/(Pre-CS + UCS + ITI) × 100; ISI, inter-stimulus-interval, that is, responding in the 10-s between CS and UCS presentation.

The day × drug interaction was significant [$F(18,378) = 1.89$, $P < 0.05$]. This was because there was a greater decrease of responding over days in rats treated with 0.1 mg/kg SR142948A most likely because they initially showed a higher level of responding than rats allocated to the other drug conditions. The day × trace × drug interaction was not significant ($F < 1$).

% CS responding As with the agonist, for the antagonist, the 0-s conditioned groups produced greater responding to the CS than the 10-s conditioned groups [$F(1,42) = 124.52$, $P < 0.001$], see Table 2. The effect of drug and trace × drug interaction was not significant [$F < 1$ and $F(2,42) = 1.46$].

There was a significant effect of day and a day × trace interaction, [$F(9, 378) = 57.15$, $P < 0.001$ and $F(9,378) = 26.29$, $P < 0.001$], respectively. As expected, the interaction resulted from a greater increase in responding over days in the 0-s than in the 10-s conditioned groups, see Figure 2. No other interaction was significant (Fs < 1).

ISI responding Responding during the ISI in 10-s conditioned groups depended on drug [$F(2,21) = 3.49$, $P < 0.05$], with the 0.1 mg/kg treated rats responding significantly more during the ISI than vehicle [$t(14) = 2.47$, $P < 0.05$] (Table 2). Responding during the ISI also increased by day [$F(9,189) = 14.44$,

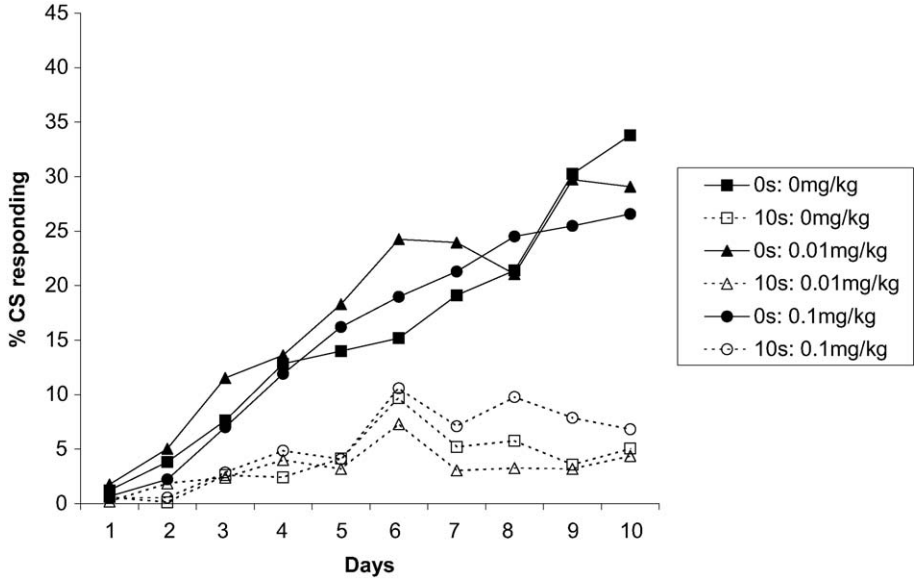

Figure 2 Conditioning to the neurotensin antagonist SR142948A. Mean number of magazine entries per day during acquisition of conditioning to the discrete CS under 0, 0.01 and 0.1 mg/kg SR142948A. Open symbols and dotted lines represent CS conditioned at 10 s ISI; closed symbols and solid lines represent CS conditioned at 0-s ISI.

$P < 0.001$] but this did not interact with drug [$F(18,189) = 1.22$, $P > 0.05$].

Discussion

The NT agonist PD149163 overall reduced conditioning at 0-s ISI and trace conditioning at 10-s ISI, at 0.125 and 0.25 mg/kg, respectively. However, learning was nonetheless shown in the 0-s but not the 10-s drug groups such that the effect of trace was preserved. The NT antagonist SR142948A did not affect discrete cue conditioning. Table 3 summarises the statistical outcomes for each behavioural measure to facilitate comparison across Experiments 1 and 2.

Drug effects on activity and motivation

Treatment with PD149163 significantly depressed unconditioned responding for food (at 0.25 mg/kg) and responding in the ITI (at both 0.125 and 0.25 mg/kg). Effects on activity and motivation cannot be clearly distinguished in this procedure because increased or decreased unconditioned responding for food could well increase or decrease the level of excitatory conditioning to contextual cues and thus increase or decrease overall responding in the ITI. However, as the effect of PD149163 in the ITI was restricted to 10-s trace-conditioned groups it is consistent with reduced contextual conditioning rather than a general effect on activity. The NT antagonist SR142948A had opposite effects on activity or motivation in that it increased unconditioned responding for food (at both 0.01 and 0.1 mg/kg), as well as in the ITI (at 0.1 mg/kg). Importantly, the learning measures in use corrected for such non-specific changes in responding to be expected on the basis of the known motor and motivational effects of NT compounds (Cacéda, *et al*., 2003; Hawkins, 1996; Luttinger, *et al*., 1982; Nemeroff, 1980; Prange and Nemeroff, 1982).

Table 3 Key statistical outcomes (main effects and interactions) for each behavioural measure under PD149163 and SR142948A

PD149163		SR142948A	
Pre-CS	Trace*	Pre-CS	Trace*
	Drug × trace*		Drug**
	Day × trace*		
UCS	Drug*	UCS	Drug**
	Day***		Day***
			Day × trace**
ITI	Trace*	ITI	Trace**
	Day***		Drug**
	Day × Trace*		Day***
	Day × drug**		Day × trace***
			Day × drug*
%CS	Trace***	%CS	Trace***
	Drug*		Day***
	Drug × trace*		Day × trace***
	Day***		
	Day × trace***		
	Day × drug***		
	Day × drug × trace*		
ISI	Drug**	ISI	Drug*
			Day***

Responding was measured in the following bins: Pre-CS, 5 s before the CS presentation; UCS, 5 s following food delivery; ITI, inter-trial-interval, that is, remainder of the session excluding all other response bins; %CS, CS/(Pre-CS + UCS + ITI) × 100; ISI, inter-stimulus-interval, that is, 10-s between CS and UCS presentation.

Statistical significance is shown as $*P \leq 0.05$, $**P \leq 0.01$ and $***P \leq 0.001$. Full details are reported in the text.

Discrete cue conditioning

Rats conditioned to the 0-s CS under both doses of PD149163. Previous findings in the same appetitive procedure with indirect DA agonists showed dose-related increases in conditioning to the 0-s conditioned CS compared with controls (Cassaday, *et al*., 2008). In the present study, the fact that the vehicle-injected control started from a higher baseline means that we cannot exclude the possibility of improved conditioning to the 0-s conditioned CS under 0.25 mg/kg PD149163. In any event, conditioning under 0.125 mg/kg PD149163 was impaired relative to that seen under 0.25 mg/kg (which started from the same baseline). It is possible that the reason for the difference in effects at low- and high-dose PD149163 arises from the introduction of non-specific effects, related to actions at the (lower affinity) NT2 receptor subtype (Petrie, *et al*., 2004) or other actions. However, the percentage responding measure that we have used to determine conditioning to the CS automatically adjusts for non-specific effects on responding. Moreover, it was the lower rather than the higher dose PD149163 that produced some reduction in conditioned responding.

There are other possible reasons for the observed dose-related effects. The dose-response curve exhibited by NT shows an inverted U-shaped relationship (Feifel, *et al*., 1997) with maximal neuroleptic-like actions produced at low doses. In our aversive conditioning procedure, dose-related effects were similarly non-linear in that 0.25 (but not 1) mg/kg PD149163 had a pro-cognitive profile of action (Grimond-Billa, *et al*., 2008).

However, the result obtained under PD149163 was clearly different in the aversive procedure in that 0.25 mg/kg PD149163 selectively increased conditioning to the CS over the trace interval (Grimond-Billa, *et al*., 2008). One possible explanation for this difference between appetitive and aversive findings may be the differential effects of acute and chronic drug regimes (Holtom, *et al*., 2000; Norman, *et al*., 2008). In the present study, there were 15 days of drug administration, whereas in the aversive variant reported by Grimond-Billa, *et al*. (2008) there was a single acute injection of PD149163. Alternatively, the discrepancy between appetitive and aversive procedures may be due to task motivation (Cassaday, *et al*., 2005a, 2008; Horsley and Cassaday, 2007; Kantini, *et al*., 2004; Norman and Cassaday, 2003; Thibaudeau, *et al*., 2007).

Treatment with SR142948A significantly increased general responding but had no effect on the strength of CS-UCS association formed, either when contiguously (0-s) conditioned or when these events were separated in time by the 10-s trace interval.

Drug effects on ISI and ITI responding

Overall differences in ITI responding most probably relate to drug effects on general activity. However, in untreated animals, differences in contextual conditioning can be produced by the introduction of a trace interval (Odling-Smee, 1975; Rawlins and Tanner, 1998; Rescorla and Wagner, 1972). In the present study, this effect was shown in that vehicle-injected rats that were 10-s trace conditioned responded more in the ITI. Treatments that decrease or enhance contextual conditioning (as distinct from having general effects on activity) should show this effect selectively in trace-conditioned groups. In the present study, there was evidence for reduced contextual conditioning under PD149163 in that the increased responding in 10-s trace relative to 0-s conditioned rats was not seen under drug.

ISI responding was also examined to test for additional effects in the local context of the 10-s trace interval over which rats learn to anticipate food delivery (Kaplan and Hearst, 1982). This too was reduced under 0.125 and 0.25 mg/kg PD149163. This finding suggests an additional effect of the drug on conditioning to the context of the trace interval because general differences in activity seen in the ITI were adjusted for statistically. Moreover, in the case of PD149163, effects on activity were anyway distinguishable in that effects on ITI responding were seen only in the 10-s trace-conditioned groups. Therefore, the reduction in ITI and ISI responding under PD149163 clearly points to reduced contextual conditioning.

By contrast under SR142948A, effects on ITI responding were statistically independent of the trace effect, consistent with a general drug effect on activity. This was increased at 0.1 mg/kg. The equivalent effect (increased responding under 0.1 mg/kg SR142948A) in the ISI could reflect increased conditioning to the local context of the trace interval, but this conclusion can only be tentative given the general effect in the ITI. In any event, this effect was distinguishable from the increased responding for the UCS under SR142948A in that the latter effect was seen at both drug doses rather than restricted to the 0.1 mg/kg condition.

Conclusions

In aversive trace conditioning, we found previously that PD149163 showed a profile consistent with cognitive enhancement (Grimond-Billa, *et al*., 2008). This was shown as increased conditioning over the trace interval, at the same time that conditioning to context was reduced. Similarly – using the same appetitive procedure as the present study – low-dose haloperidol increased conditioning over the trace interval, consistent with cognitive enhancement (Cassaday, *et al*., 2005b). There was no drug-induced increase in conditioning over the trace interval in the present study. By contrast, there was in fact some evidence for reduced conditioning in the 10-s trace-conditioned group treated with 0.25 mg/kg PD149163. However, consistent with the earlier findings (Grimond-Billa, *et al*., 2008), contextual conditioning was reduced under PD149163. Follow-up studies should determine whether the difference in trace conditioning under PD149163 apparent between aversive and appetitive procedures is inherent to the task motivation – as lesion studies would seem to suggest (Cassaday, *et al*., 2005a; Thibaudeau, *et al*., 2007) – or related to the chronicity of treatment in systemic drug studies. Because appetitive conditioning necessarily requires a number of days, this issue will best be addressed by chronically pre-treating rats before aversive conditioning.

With respect to SR142948A, the present study confirms the earlier finding in the aversive procedure (Grimond-Billa, *et al*., 2008) that, at the doses tested to date, this compound is without effect on associative learning.

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