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Selectively increased trace conditioning under the neurotensin agonist PD 149163 in an aversive procedure in which SR 142948A was without intrinsic effect

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Abstract

There is evidence to suggest that neurotensin (NT) may enhance cognitive function. The present study therefore examined the role of NT in associative learning between a conditioned stimulus (CS) and an unconditioned stimulus (UCS). This was tested in a trace procedure using conditioned suppression of drinking with a noise CS and foot shock UCS. We compared the effects of an NT agonist (PD 149163, 0.25 and 1 mg/kg) with those of an NT antagonist (SR 142948A, 0.01 and 0.1 mg/kg). Conditioning after drug treatment was followed by drug-free tests of associative learning. At 0.25 but not 1 mg/kg, PD 149163 selectively increased conditioning over the trace interval: there was no such increased conditioning in the 0 s group. This increased conditioning over the trace is an effect that is reliably produced by dopamine (DA) agonists in the same procedure. However, dissimilar to the effects of DA agonists, conditioning to box context, was reduced under PD 149163. Doses of SR 142948A, selected on the basis of their effects in similar aversively motivated tests of latent inhibition, were without intrinsic effect in the present procedure. The dose-related dissociation between trace and contextual conditioning effects under PD 149163 is considered as cognitive enhancement.

Keywords

aversive conditioning, trace conditioning, neurotensin, PD 149163, SR 1 42948A, rat

Introduction

Following the dopamine (DA) hypothesis and related amphetamine model of schizophrenia (Ellison and Eison, 1983), there has been intensive investigation of how a variety of drugs that modulate the brain DA system affect basic cognitive processes in animal models. In this context, particular interest has been directed toward the tridecapeptide neurotensin (NT) since its discovery and characterization in the brain (Carraway and Leeman, 1973, 1976). Over the last 30 years, a series of studies have demonstrated that NT modulates the DA system (Nemeroff, 1986; Kinkead *et al.*, 1999; Binder *et al.*, 2001a, 2001b). Moreover, NT and its analogues have been found to produce a number of anti-psychotic-like effects, such as reversing PCP- and amphetamine-induced behavioural deficits, in animal models of schizophrenia (Nemeroff, 1980; Cacéda *et al.*, 2003). Consistent with a role for NT in schizophrenia, human studies have shown that untreated schizophrenics have decreased NT-like immunoreactivity in the cerebrospinal fluid (CSF) and that NT levels were returned to normal after anti-psychotic drug treatment (Widerlov *et al.*, 1982). A likely mechanism for such actions is provided by the ability of NT (and its analogues) to reduce the overactivity in the DA system that is otherwise produced by amphetamine (Nemeroff, 1986; Skoog *et al.*, 1986).

With respect to the cognitive processes that we examine here, evidence for disturbed attentional processes in schizophrenics has led to animal models based on dysfunctional associative learning

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(Solomon *et al.*, 1981; Weiner *et al.*, 1981, 1988; Crider *et al.*, 1982; Ohad *et al.*, 1987; Weiner, 1990; Gray *et al.*, 1992; Jones *et al.*, 1997; Kumari *et al.*, 1999). Various studies have shown that in untreated animals, NT and its analogues, may also act more generally as cognitive enhancers (Van Wimersma Greidanus *et al.*, 1982; Shibata and Furukawa, 1988; Azmi *et al.*, 2006). In the present study, we therefore examined the role of NT in aversive conditioning procedures suitable to measure cognitive enhancement as well as to test for effects on attentional aspects of learning that may relate with anti-psychotic potential.

Specifically, we used a trace conditioned stimulus (CS, noise) as a less informative predictor of the unconditioned stimulus (UCS, foot shock) than the CS immediately followed by the UCS (Kamin, 1965) in an aversive conditioned suppression of drinking procedure that we have previously used to test the effects of DA agonists and lesions (Norman and Cassaday, 2003; Cassaday *et al.*, 2005a; Horsley and Cassaday, in press). In this procedure, we also present an experimental background stimulus (flashing light) to provide a later measure of contextual conditioning; and a more conventional measure of contextual conditioning is provided by the initial latency to resume drinking in the experimental chambers after exposure to foot shock (Rescorla and Wagner, 1972; Odling-Smee, 1975; Rawlins and Tanner, 1998).

In the present study, we tested two doses of an NT agonist, PD 1491623: 0.25 and 1 mg/kg, based on previous dose-related effects shown in pre-pulse inhibition (PPI) where PD 1491623 reversed the disruption in PPI otherwise seen under amphetamine (Feifel *et al.*, 1999; Shilling *et al.*, 2004). There is other evidence to show that even the lower dose selected is likely to be behaviourally effective: 0.25 mg/kg PD 149163 is sufficient to increase fos expression in rat cortex (Petrie *et al.*, 2004). We similarly tested two doses of an NT antagonist, SR 142948A: 0.01 and 0.1 mg/kg, based on previous dose-related effects shown in latent inhibition (LI) where SR 142948A showed effects like a DA agonist in that it impaired LI and this effect was reversible by treatment with a DA D2 antagonist (Binder *et al.*, 2001c, 2002). Thus, the doses to be tested were established empirically.

The agonist, PD 149163, has high affinity for NTR1 and negligible affinity for NTR2 (Feifel *et al.*, 1999; Boules *et al.*, 2003; Petrie *et al.*, 2004). The antagonist, SR 142948A, blocks NTR 1 and NTR2 with similar affinity (Vincent *et al.*, 1999). However, there are no data that allow us to exclude a role for any specific NT receptor subtype in the learning processes under test.

We consistently find increased conditioning to less informative stimuli or decreased selectivity in attentional learning using the DA agonists amphetamine and methylphenidate in the same aversive conditioning procedure (Norman and Cassaday, 2003; Horsley and Cassaday, in press). The consistency in these findings in the very same task provides a basis to predict the likely effects of SR 142948A and PD 149163 in the present study. A caveat arises in that regional differences in NT interactions with the DA system (Hanson *et al.*, 1997; Holtom *et al.*, 2000; Binder *et al.*, 2001a) and interactions with other neurotransmitter systems may confound prediction (Sakamoto *et al.*, 1986; Szigethy and Beaudet, 1987; Nakachi *et al.*, 1995; Morin *et al.*, 1996). That said, following from the evidence that in animal models NT agonists can have anti-dopaminergic (Nemeroff, 1980; Feifel *et al.*, 1999; Cacéda *et al.*, 2003; Shilling *et al.*, 2003) and conversely NT antagonists pro-dopaminergic effects (Kinkead *et al.*, 1999; Binder *et al.*, 2001a, 2001b, 2001c, 2002), on these grounds, we would expect that treatment with the antagonist SR 142948A, but not the agonist NT PD 149163, should here increase conditioning to the trace CS and the contextual stimuli.

The prediction for the agonist PD 149163 is that it should improve selectivity for associative learning. This prediction is based on the evidence that NT agonists can reduce cognitive disturbances used to model schizophrenia (Feifel *et al.*, 1999; Caceda *et al.*, 2003; Shilling *et al.*, 2003, 2004). In the trace procedure used here, such an improvement would potentially take the form of relatively increased conditioning to the most informative stimulus available (i.e., the discrete cue whether trace or 0 s conditioned) relative to the background contextual cues.

Materials and methods

Animals

Seventy-two naïve male Wistar rats (Charles Rivers, UK) were used in each experiment, of weight ranges 218–263 and 238–269 g, in Experiments 1 (using NT agonist) and 2 (using NT antagonist), respectively. All animals were caged in pairs on a 12:12 h light/dark cycle (lights on 08.00 to 20.00 h) and handled over two weeks before the start of the experiments. Rats were water deprived, receiving 1 h of access to water per day following every day of the experimental procedure. Food was freely available in the home cage. All procedures were carried out in accordance with the UK Animals Scientific Procedures Act 1986, Project Licence no. PPL 40/2648.

Drugs

PD 149163 (H-Lys–psi(CH_2N)-Lys–Pro–Trp–Tle–Leu-O-Et) was generously supplied by the NIMH Chemical Synthesis and Drug Supply Program, and SRI International (Menlo Park, CA, 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 (Sanofy-Synthelabo, Toulouse, France). Both compounds were dissolved in physiological saline and injected at 1 mL/kg body weight (s.c. for PD 149163 and i.p. for SR 142948A). We investigated the effects of two doses of each compound: for PD 149163, 0.25 and 1 mg/kg; for SR 142948A, 0.01 and 0.1 mg/kg. Rats were injected according to the same schedule, 15 min (PD 149163) or 30 min (SR 142948A) prior to each conditioning session (i.e., they received a single acute injection of each drug). Subsequent to conditioning under drug, tests of associative learning were conducted drug-free.

Behavioural conditioning apparatus

Four identical fully automated conditioning chambers, housed within sound-attenuating cases containing ventilation fans

(Cambridge Cognition, Cambridge, UK), were used. Each of the inner conditioning chambers consisted of a plain steel box ($25 \times 25 \times 22\,cm^3$ height) with a Plexiglas door ($19 \times 27\,cm^2$) 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. Mounted in one wall were two retracted levers (that were not in use), three stimulus lights and a waterspout.

The spout was 5 cm above the floor and connected to a lickometer supplied by a pump. Licks were registered by the breaking of the photo beam within the spout, which also triggered water delivery of 0.05 mL per lick. The waterspout was illuminated when water was available. A loudspeaker for the presentation of auditory stimuli was set in the roof.

Two stimuli were used in this procedure; a mixed frequency noise set at 70 dB (including background) of 5 s duration as the target stimulus (i.e., the CS paired with the UCS) and a continuously presented flashing light as the alternate or background stimulus, provided by the three wall mounted stimulus lights and the house light flashing both on and off for 0.5 s. Footshock of 1 s duration and 0.5 mA intensity was delivered through the grid floor by a constant current shock generator (pulsed voltage: output square wave 10 ms on, 80 ms off, 370 V peak under no load conditions, MISAC Systems, Newbury, UK). Stimulus control and data collection were by an Acorn Archimedes RISC computer programmed in Basic with additional interfacing using an Arachnid extension (Cambridge Cognition).

Behavioural conditioning procedure

This followed a standard procedure that has been reported elsewhere (Norman and Cassaday, 2003; Cassaday *et al.*, 2005a; Horsley and Cassaday, in press): before the behavioural training began, rats were weighed and handled daily for two weeks. At the end of this time, water deprivation was introduced, and, starting the following day, the rats were trained to lick for water in the behavioural conditioning apparatus ('shaping', two days). Shaping led into the following pre-conditioning stage of lick training to establish a stable licking response (10 days). Then, after pre-conditioning was completed, the single day of conditioning was followed by a single 'reshaping' day, to reinstate the licking response before the test phase, and to provide a measure of suppression to the box cues. Finally, tests of the strength of conditioning to the different experimental stimuli were conducted over the final two days of the study.

Pre-conditioning to establish baseline lick response Rats' responses were shaped (over two days) until all drank from the waterspout and each rat was then individually assigned to a conditioning box for the duration of the experiment.

There then followed 10 days of pre-conditioning. Every day, each rat drank in its experimental chamber for 15 min. The drinking spout was illuminated throughout, but there were no other stimuli presented in this phase. The latency to make the first lick was measured as an indicator of habituation to the experimental context.

Conditioning with foot shock Conditioning was conducted in one day following the last pre-conditioning day. No water was available within the chamber and the waterspout was not illuminated. There was a continuous background stimulus (flashing lights) onto which pairings of the 5 s target (noise CS) and footshock (UCS) were superimposed. There were two such conditioning trials. The first pairing of CS and UCS was presented after 5 min of background stimulus had elapsed, and the second pairing was at 5 min after the first test, with a further 5 min left in the apparatus following the second shock presentation.

Depending on experimental groups, the footshock followed at either 0 s (for the contiguous groups) or 30 s (for the trace groups) after target CS offset. The flashing light stimulus was presented throughout the 15 min session, including the 30 s inter-stimulus-interval that simply added to the overall duration of the session in the trace groups.

Reshaping after foot shock On the day following the conditioning, animals were reshaped following the same procedure as in pre-conditioning sessions: each rat drank in its experimental chamber for 15 min. This was done in order to re-establish drinking after conditioning. Reshaping also provided a measure of conditioning to the box context (latency to first lick).

Conditioned suppression tests There were two test days, one for each type of stimulus, with the order of testing counterbalanced such that half the rats were first tested for conditioning to CS (noise) and half the rats were tested for conditioning to the background stimulus (light). The time taken to complete 50 licks prior to any stimulus presentation (the *A* period) provided a measure of any individual variation in baseline lick responding, to be compared with the time taken to complete 50 licks during stimulus presentation (the B-period). The suppression ratio was calculated as $A/(A + B)$, and used to assess conditioning while taking baseline variation into account. These were extinction tests with no further footshock presentation. Both stimuli were presented continuously throughout the session, so in each case the B-period was a maximum of 900 s, for rats that did not complete 50 licks within the 15 min session.

Experimental design and analysis

The experiment was run in a 3×2 independent measures factorial design for later analysis of variance (ANOVA). Thus, 72 rats were assigned to six experimental conditions, counterbalanced for box. The between subjects factors were Drug (at levels saline, low and high dose) and Trace (at levels 0 and 30 s). The dependent variable to assess conditioning at test was the su ppression ratio.

At pre-conditioning and reshaping, latency to first lick provided a measure of habituation and subsequent conditioning to context, respectively, and was analysed in the same 3×2 design. The reshape latencies provided a measure of conditioning to the apparatus contextual cues (in the absence of any experimental stimulus). Pre-conditioning drink levels were also assessed in a $3 \times 2 \times 10$ mixed design ANOVA with the repeated measures factor of days (at 10 levels) to check for any pre-existing differences in total amount drunk.

All ANOVAs used an alpha level of 0.05. Significant main effects and interactions were explored by two-tailed *t*-tests.

When exploring significant main effects in the absence of interactions, the *t*-tests are collapsed across groups. To explore interactions, we made only the relevant pair wise comparisons that were necessary, in order to determine the basis for any reduction in the trace conditioning effect.

Results

Experiment 1: effects of the NT agonist PD 149163

Pre-conditioning: baseline lick responding Latency to first lick over the 10 days of baseline training showed an effect of days [$F(9,594) = 35.193$, $P < 0.0001$]. Thus as might be expected with habituation, rats drank more readily during the session over successive days of the experiment: mean (±SEM) for day 1 = 25.583 (+3.162); mean (±SEM) for day 10 = 1.903 (+0.286). Analysis by allocation to later conditioning and drug groups revealed that these prospective groups were well matched in terms of the rats' readiness to drink before conditioning. There were no overall effects of drug condition-to-be ($F < 1$) or trace condition-to-be [$F(1,66) = 3.139$] or any significant interactions with days (all $F < 1$).

Reshaping: effects on lick latencies Figure 1 shows that there was a significant effect of drug on the latency to first lick [$F(2,66) = 8.025$, $P < 0.001$]. The vehicle group took significantly longer to start drinking than both the low [$t(46) = 2.64$, $P = 0.011$], and high dose rates treated with PD 14963 [$t(46) = 3.28$, $P < 0.01$]. There was no significant effect of trace, or trace × drug interaction [maximum $F(2,66) = 1.014$)].

Conditioned suppression tests: background stimulus The suppression ratios on presentation of the light stimulus were mostly close to 0.5 for all the groups, so there was no sign of any contextual conditioning to this stimulus (Figure 2). Statistically there were no significant effects of drug, trace or their interaction [maximum $F(1, 66) = 1.334$].

Conditioned suppression tests: target stimulus As expected the trace-conditioned groups showed overall less suppression, reflecting weaker conditioning to the CS (see Figure 3). This was seen in the analysis of the suppression ratio as a main effect of trace [$F(2,66) = 32.127$, $P < 0.0001$]. There was also a main effect of drug [$F(1,66) = 4.94$, $P = 0.01$]. Figure 3 shows that this arose because of overall greater suppression in rats treated with 0.25 mg/kg PD 14963.

The drug × trace interaction did not reach significance [$F(2,66) = 2.489$, $P = 0.09$], but it is clear that the main effect of trace could only have arisen because of the significant differences in the level of conditioning in trace and contiguous groups shown within vehicle [$t(22) = 3.984$, $P < 0.001$] and high dose [$t(22) = 3.815$, $P < 0.001$] groups. By contrast, at the 0.25 mg/kg dose PD 149163, the difference between trace and contiguously conditioned groups was not significant [$t(22) = 1.854$]. Consistent with the main effect of drug described above, this loss of the normal trace conditioning effect within the low dose group arose because of increased conditioning over the trace interval under treatment with 0.25 mg/kg PD 149163. Statistically, while the contiguously conditioned groups did not differ from each other after any drug treatment [maximum $t(22) = 1.125$], the level of suppression shown in the trace conditioning groups depended on their drug treatment. The trace group conditioned under 0.25 mg/kg PD

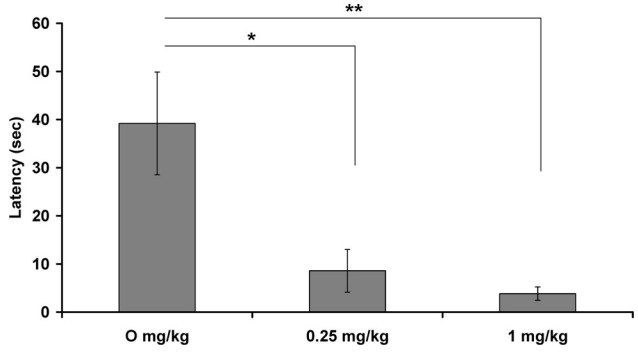

Figure 1 Effects of the NT agonist (PD 149163 at 0.25 and 1 mg/kg) on suppression to the experimental chambers. The level of contextual conditioning is expressed as mean latency to make the first licks (s); shown collapsed across the trace condition that made no difference to the level of contextual conditioning shown; $n = 24$ per group. Error bars show two standard errors of the mean for approximate between groups comparisons

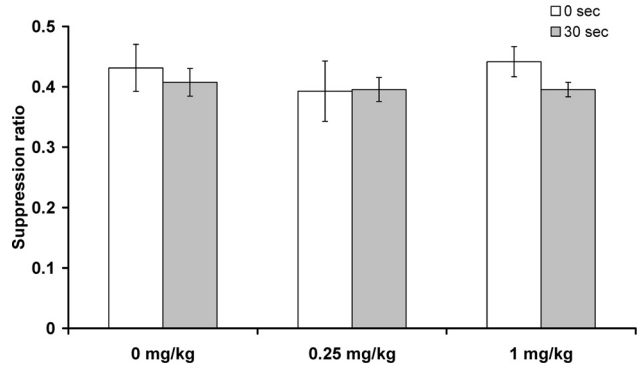

Figure 2 Effects of the NT agonist (PD 149163 at 0.25 and 1 mg/kg) on conditioned suppression to the flashing light stimulus. The level of conditioning is expressed as mean suppression ratio (calculated as $A/(A + B)$; where A was the time taken to complete 50 licks prior to any stimulus presentation and B was the time taken to complete 50 licks during stimulus presentation). Bars show the results by drug and trace condition, in that the CS was previously paired with footshock either at 0 s (white bars) or at a 30 s trace (grey bars); $n = 12$ per group. Error bars show two standard errors of the mean for approximate between groups comparisons

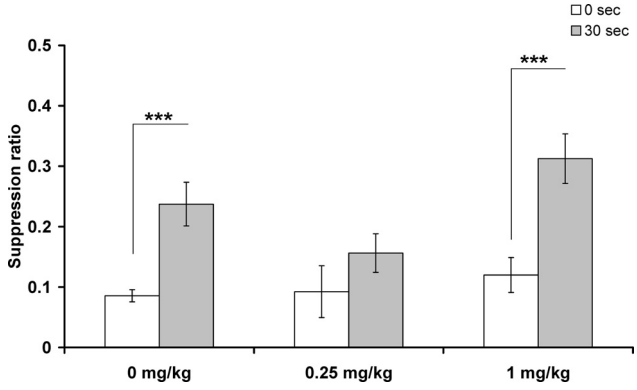

Figure 3 Effects of the NT agonist (PD 149163 at 0.25 and 1 mg/kg) on conditioned suppression to the target noise CS. The level of conditioning is expressed as mean suppression ratio (calculated as $A/(A + B)$; where A was the time taken to complete 50 licks prior to any stimulus presentation and B was the time taken to complete 50 licks during stimulus presentation). Bars show the results by drug and trace condition, in that the CS was previously paired with footshock either at 0 s (white bars) or at a 30 s trace (grey bars); $n = 12$ per group. Error bars show two standard errors of the mean for approximate between groups comparisons.

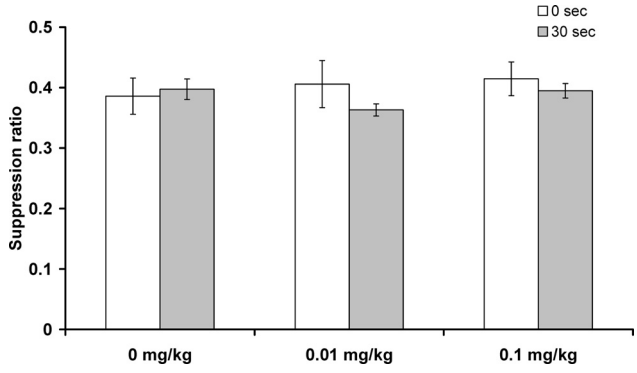

Figure 4 Effects of the NT antagonist (SR 142948A at 0.01 and 0.1 mg/kg) on conditioned suppression to the flashing light stimulus. The level of conditioning is expressed as mean suppression ratio (calculated as $A/(A + B)$; where A was the time taken to complete 50 licks prior to any stimulus presentation and B was the time taken to complete 50 licks during stimulus presentation). Bars show the results by drug and trace condition, in that the CS was previously paired with foot shock either at 0 s (white bars) or at a 30 s trace (grey bars); $n = 12$ per group. Error bars show two standard errors of the mean for approximate between groups comparisons

149163 showed evidence of a stronger association between the CS and the UCS than did the trace groups conditioned under 1 mg/kg PD 149163 [$t(22) = 2.97$, $P < 0.01$]. There was no difference between the others groups [maximum ($t(22) = 1.663$].

Experiment 2: Effects of the NT antagonist SR 142948A

Pre-conditioning: baseline lick responding As in Experiment 1, rats showed habituation to the experimental apparatus as there was a significant main effect of days on both the latency to first lick [$F(9,594) = 10.7$, $P < 0.0001$]. Over successive days of baseline training, rats start to drink more quickly: mean ($\pm$SEM) for day1 = 15.75 ($\pm$0.95); and for day 10 = 4.08 ($\pm$0.80). Again analysis by allocation to later conditioning and drug groups confirmed that these were well matched. There were no overall effects of drug condition-to-be [Fs < 1] or trace condition-to-be [Fs < 1], or any significant interactions with days [Fs < 1].

Reshaping: effects on lick latencies By contrast with the agonist results, the NT antagonist was without any effect on contextual conditioning as measured at reshape. There were no significant effects or interactions on the latency to drink [maximum $F(2,63) = 1.299$].

Conditioned suppression tests: background stimulus Figure 4 shows that again there was little conditioning to the background CS and no increase in the level of conditioning shown under either dose SR 142948A. There were no statistically significant effects of drug, trace or the interaction [all Fs < 1].

Conditioned suppression tests: target stimulus Figure 5 shows that the 0 s were overall more suppressed than the 30 s conditioned groups, shown as a main effect of trace [$F(1,63) = 106.95$, $P < 0.0001$]. However, SR 142948A was without effect on conditioning to the target CS. There was no significant effect of drug, or trace $\times$ drug interaction [both Fs < 1].

Discussion

Learning that a stimulus (here a noise) predicts an outcome is normally reduced when these events are separated in time. This effect was clearly seen in both Experiments 1 and 2. There was also evidence for the role of NT in the associative learning processes tested here, but this was asymmetric in that while the NT agonist PD 149163 selectively increased conditioning over a trace interval at 0.25 but not 1 mg/kg, there was no sign of any effect on associative learning of treatment with the NT antagonist SR 142948A, in either direction. The 0 s conditioned group provides a test for any general reduction in associative learning that might have been produced under SR 142948A but none was seen. The increase in conditioning to the trace CS under 0.25 but not 1 mg/kg PD 149163 was selective in that there was no effect on conditioning in the 0 s conditioned groups under either dose of PD 149163. Arguably further increased conditioning might be difficult to demonstrate given the

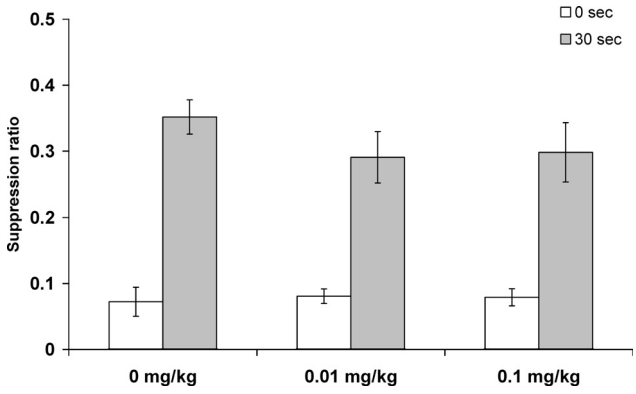

Figure 5 Effects of the NT antagonist (SR 142948A at 0.01 and 0.1 mg/kg) on conditioned suppression to the target noise CS. The level of conditioning is expressed as mean suppression ratio (calculated as $A/(A + B)$; where A was the time taken to complete 50 licks prior to any stimulus presentation and B was the time taken to complete 50 licks during stimulus presentation). Bars show the results by drug and trace condition, in that the CS was previously paired with footshock either at 0 s (white bars) or at a 30 s trace (grey bars); $n = 12$ per group. Error bars show two standard errors of the mean for approximate between groups comparisons

level of suppression shown in the 0 s conditioned groups. However, both doses of PD 149163 significantly reduced conditioning to the contextual cues provided by the experimental chambers. Thus the attentional aspect of learning has clearly been affected by treatment PD 149163 in the present study. Moreover, the effect was clearly dose related in that at equivalent levels of 0 s conditioning, there was no effect on conditioning to the trace CS under 1 mg/kg PD 149163. In line with prediction, based on the evidence that NT agonists can reduce cognitive disturbances used to model schizophrenia (Feifel *et al.*, 1999; Caceda *et al.*, 2003; Shilling *et al.*, 2003, 2004), we consider that the pattern of effects seen under 0.25 mg/kg PD 149163) is pro-cognitive (see below). First, however, it is important to consider the components of PD 149163 action.

Non-specific effects of NT compounds

Treatment with NT agonists has been reported to decrease spontaneous locomotor activity and produce muscle relaxation and even catalepsy (Cacéda *et al.*, 2003; Nemeroff, 1980; Prange and Nemeroff, 1982). In the present study, however, the suppression to the box cues and to the experimental stimuli were tested drug free so any motor effects of drug are not at issue and, in both experiments, the drug and conditioning groups were well matched in terms of the baseline readiness to drink before any drug treatment was given. Moreover, in terms of any possible residual effect of prior drug treatment, as well as the differences in suppression to box cues observed in Experiment 1, the use of the suppression ratios in order to measure learning to the experimental stimuli adjusts for individual differences in baseline response rate. Nonetheless, the rats are conditioned under drug and previous studies report an analgesic effect of NT (Clineschmidt *et al.*, 1979; Osbahr *et al.*, 1981). This raises the possibility that a difference in motivation or hedonic tone could contribute to apparent differences in aversive conditioning under PD 149163. This possibility is rendered unlikely, however, given that the increase in conditioning observed in the trace conditioned group under 0.25 mg/kg PD 149163 is the opposite of what would be expected if sensitivity to foot shock had been reduced. Moreover, suppression to box context was reduced under both doses PD 149163. Any difference in motivation or hedonic tone produced by PD 149163 should have had a general effect on the levels of suppression shown, irrespective of the nature of the conditioning cue.

NT and discrete cue conditioning, with and without a trace interval

When CS offset was contiguous with UCS onset, neither PD 149163 nor SR 142948A had any effect on conditioned suppression tests to the target stimulus, i.e., discrete cue conditioning. Where the level of conditioning was normally reduced by the introduction of a trace interval, the agonist PD 149163 (at 0.25 mg/kg) selectively increased suppression, suggesting that the capacity to make associations with relatively weak stimuli had been increased. This effect was seen on conditioned suppression tests to the target but not the background stimulus and thus restricted to discrete cue conditioning. Moreover, as increased conditioning to the trace CS was shown at the same time as reduced contextual conditioning under PD 149163 as measured by suppression to the box cues (see below), this suggests that treatment with this NT agonist did not generally enhance learning about the wider range of available cues.

The fact that this effect was seen at 0.25 mg/kg but not at a higher dose may be because of opposing (pro- and anti- dopaminergic effects) of NT on the DA system (Gully *et al.*, 1993, 1997; Feifel *et al.*, 1997, 1998; Robledo *et al.*, 1993; Tanganelli *et al.*, 1994). Importantly, although the effects of PD 149163 in discrete cue conditioning were inconsistent with the predicted anti-dopaminergic mechanism of action (Cacéda *et al.*, 2003; Shilling *et al.*, 2003; Feifel *et al.*, 1999), they did suggest a pro-cognitive effect.

NT and contextual conditioning

There were in effect two measures of contextual conditioning: suppression to the box context measured on the reshaping day; and the test of suppression to the flashing light background stimulus. In neither experiment was there any sign for a role of NT in contextual conditioning in so far as this is measured by our unimodal experimental background, though such an effect was clearly demonstrated in earlier studies with DA agonists (Norman and Cassaday, 2003; Horsley and Cassaday, in press). Suppression to the multi-modal box context measured at reshaping, however, was decreased by both doses of PD 149163, suggesting that treatment with the NT agonist decreased the association between the relatively uninformative contextual cues and the shock UCS.

In previous studies using the experimental parameters of the present study, amphetamine and methylphenidate both increased

suppression to both the flashing light stimulus and the cues provided by the experimental chambers (Norman and Cassaday, 2003; Horsley and Cassaday, in press), suggesting that these cues can be functionally interchangeable. In the present study they were not in that PD 149163 affected suppression to box context but not to the experimental flashing light stimulus. However, given that PD 149163 reduced contextual conditioning to the box cues (shown as shorter latency to drink at reshape), the lack of any effect seen in the tests of conditioning to the flashing light may reflect no more than a floor effect in learning (shown as a ceiling on the suppression ratio measure on which higher scores show less learning) because the level of conditioning supported by this stimulus was very low.

Alternatively, this difference may reflect the fact that the measures of contextual conditioning (suppression to box cues measured at reshaping and to the experimental flashing light stimulus at test) are functionally different. Certainly the measures were different. Box context suppression was measured at reshaping and suppression to light was measured in subsequent extinction tests in exactly the same way as suppression to the noise CS. This means that (unlike suppression to the background light stimulus) box suppression is measured with a single latency score (time to start drinking) and there is no equivalent to the A and B periods because no stimuli were presented.

In any event, the effect of PD 149163, seen as reduced suppression to box cues though not to the flashing light, was opposite to that seen under DA agonists (Norman and Cassaday, 2003; Horsley and Cassaday, in press). Contextual and discrete cue conditioning rely on different neural substrates (Winocur *et al.*, 1987; Cassaday and Rawlins, 1997; Parkinson *et al.*, 1999; Pezze *et al.*, 2001). Therefore, the most likely explanation of this apparent discrepancy comes from the evidence that the pro- versus anti-dopaminergic effects of NT are regionally selective (Hanson *et al.*, 1997; Holtom *et al.*, 2000; Binder *et al.*, 2001a). For example, while NT administered to the ventral striatum acts like an anti-psychotic (Nemeroff, 1980; Kalivas *et al.*, 1984), NT infused into the ventral midbrain acts like psychostimulant drugs (Berod and Rostene, 2002; Dobner *et al.*, 2003).

Similarly, differences may arise because of interactions with neurotransmitter systems other than DA. For example, a likely role for NT in attentional aspects of learning is also suggested by its interactions with the cholinergic system (Sakamoto *et al.*, 1986; Szigethy and Beaudet, 1987; Nakachi *et al.*, 1995; Muir *et al.*, 1992; Morin *et al.*, 1996; Matthews, 1999; Azmi *et al.*, 2006) and in particular the cholinergic innervation of the hippocampus (Bennett *et al.*, 1973; Stewart and Fox, 1990).

As was the case for discrete cue conditioning, the NT antagonist SR 142948A was without detectable effect on either measure of contextual conditioning.

Dissociable effects under PD 149163 suggest pro-cognitive properties

We have not typically seen this dissociation between discrete cue and contextual conditioning. Normally (under DA agonists) both conditioning over the trace and to the contextual cues is increased (Norman and Cassaday, 2003; Horsley and Cassaday, in press). We know of no precedent for drug or lesion effects of the pattern observed under PD 149163. In untreated animals there can be an inverse relationship between conditioning to CS and contextual cues, normally taking the form of increased conditioning to contextual cues in trace conditioned rats (Odling-Smee, 1975; Rawlins and Tanner, 1998; Rescorla and Wagner, 1972). Theoretically, there are very well established accounts of this kind of cue competition, both acquisition- (Rescorla-Wagner, 1972) and retrieval-based (Miller and Matzel, 1988). In general terms then, the level of observed conditioning to CS can be moderated by the level of contextual conditioning and one explanation of the effects of PD 149163 could be in terms of the normal mechanisms underlying cue competition effects. For example, the pattern of observed effects could be consistent with a pro-cognitive effect on something like a comparator function, but here the drug effect was mediated at acquisition rather than retrieval, as would need to be the case on the established theory (Miller and Matzel, 1988). However, while 0.25 but not 1 mg/kg PD 149163 increased conditioning to the trace CS, both doses reduced suppression to the box context. Therefore, increased conditioning to the trace CS did not necessarily follow from reduced contextual conditioning under PD 149163.

Whatever the best explanation, the circumstances under which we find improved and reduced conditioning under PD 149163 may be argued to reflect cognitive enhancement in that the salience of an otherwise weak discrete cue was increased, but in a focused way in that conditioning to contextual cues was at the same time reduced. Thus there was no general increase in conditioning under PD 149163. This pro-cognitive effect of 0.25 mg/kg PD 149163 could indicate anti-psychotic potential. Moreover, although LI was not directly examined here, NT antagonists abolish LI (Binder *et al.*, 2001c, 2002). LI to contextual cues has been demonstrated (Norman and Cassaday, 2004) and enhanced LI to contextual cues under PD 149163 could be a mechanism for the reduced contextual conditioning that we observed.

Previous studies show that NT given intracerebroventricularly causes hypothermia and decreased basal locomotor activity, similar to those produced by clinically prescribed anti-psychotic drugs (Nemeroff, 1980). Even more compelling, NT injection either systemically (Shilling *et al.*, 2004) or (consistent with dopaminergic mediation of effects) directly into the nucleus accumbens (Feifel *et al.*, 1997) blocked amphetamine-induced PPI deficits. This is a standard test for assessing potential anti-psychotic efficacy. We have not been able to show any clear effect of haloperidol in the aversive trace conditioning procedure described here (unpublished data). By contrast, we did find clear effects of haloperidol in an appetitive variant of the procedure (Cassaday *et al.*, 2005), of enhanced associative learning. However, effects in this appetitive procedure are different from those seen in the aversive procedure after treatment with DA agonists (Kantini *et al.*, 2004). Moreover, the effects of PD 149163 were here tested in normal untreated rats rather than in a model of DA hyperactivity (Ellison and Eison, 1983).

Many other lines of evidence, both anatomical (Sakamoto *et al.*, 1986; Szigethy and Beaudet, 1987; Nakachi *et al.*, 1995; Morin *et al.*, 1996) and behavioural (Van Wimersma Greidanus *et al.*,

1982; Shibata and Furukawa, 1988) that NT is involved in cognitive processes. Consistent with a more general role in producing cognitive enhancement, PD 149163 has been found to reverse scopolamine-induced deficits in working memory (Azmi *et al.*, 2006).

The NT antagonist SR 142948A was without effect on aversive conditioning

It is possible that only responses activated with an NT agonist are reversible by treatment with an NT antagonist. However, previous studies have shown that SR 142948A can have intrinsic effects when given alone: resulting in, for example, LI disruption (Binder *et al.*, 2001c, 2002).

SR 142948A acts at both NTR1 and NTR2 receptor subtypes (Gully *et al.*, 1997, Betancur *et al.*, 1998), whereas the agonist, PD 149163 has negligible affinity for NTR2 (Boules *et al.*, 2003; Feifel *et al.*, 1999; Petrie *et al.*, 2004). Thus the difference in efficacy of the agonist and the antagonist used in the present procedure could arise because of difference in the selectivity of the compounds for NTR1 over NTR2, or at other receptor subtypes. For example, behavioural differences could arise because of differential affinity for NTR3 or NTR4, but as yet little is known about the functional significance of these sites (Mazella, 2001; Motoi *et al.*, 1999; Vincent *et al.*, 1999; Jacobsen *et al.*, 2001). A more likely explanation of the apparent discrepancy is that conditioning on behavioural measures of interest was already low so that further reductions in conditioning could not be demonstrated.

Conclusions and implications

Increased conditioning over the trace interval (seen here after acute treatment with 0.25 mg/kg PD 149163) is an effect that is reliably produced by indirect DA agonists in the same procedure (Norman and Cassaday, 2003; Horsley and Cassaday, in press). This finding (counter to prediction) might relate with the treatment protocol in use and thus the extent to which DA release is stimulated (Nemeroff, 1980; Kalivas *et al.*, 1984; Hanson *et al.*, 1997; Feifel *et al.*, 1998, 1999; Holtom *et al.*, 2000; Binder *et al.*, 2001a; Berod and Rostene, 2002; Dobner *et al.*, 2003). However, there are alternative non-dopaminergic mechanisms by which PD 149163 might enhance cognition (Sakamoto *et al.*, 1986; Szigethy and Beaudet, 1987; Nakachi *et al.*, 1995; Morin *et al.*, 1996; Azmi *et al.*, 2006).

Even if PD 149163 were active through its effect on DA, procognitive versus psychosis-inducing effects may well depend on its actions in mesocortical versus mesolimbic pathways (Broderick and Piercey, 1998; Austin *et al.*, 2000). Therefore, although systemic drug administration is an appropriate first approach, regional differences in the effects of NT mean that the present findings should be followed up with brain microinjection studies (Kalivas *et al.*, 1984; Berod and Rostene, 2002; Dobner *et al.*, 2003). As they become available, follow-up tests with more selective NT agonists can be used to confirm whether, as would seem likely, the pattern of effects seen here under PD 149163 is mediated exclusively via actions at NTR1.

Finally, follow up studies should test the generality of NT effects in associative learning. First, previous studies have found differences between aversive and appetitive conditioning procedures (Norman and Cassaday, 2003; Kantini *et al.*, 2004; Cassaday *et al.*, 2005a, 2005b). Therefore, it would also be useful to assess the effects of the same NT compounds in the same appetitive trace conditioning procedure that we have used to examine the effects of DA drugs and lesions. Secondly, most of the studies of the effect of NT agonists on cognition and anti-psychotic effects have been done acutely (Azmi *et al.*, 2006; Feifel *et al.*, 1997; Shilling *et al.*, 2004). It would therefore be useful in the light of possible differences between acute and chronic NT administration (Hertel *et al.*, 2002) to test the generality of effects found here under longer term, as well as regionally localized, treatment protocols.

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Author Queries

Q1 Please update Reference Horsley and Cassaday.

Q2 ‘Methods’ has been changed to ‘Materials and methods’ as per style in the head level. Please confirm whether the change made is ok.