Appetitive overshadowing is disrupted by systemic amphetamine but not by electrolytic lesions to the nucleus accumbens shell

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Abstract

There is evidence that the indirect dopamine (DA) agonist amphetamine (AMP) can disrupt selective learning in an aversive overshadowing task, consistent with a role for the DA system in this form of salience manipulation. In the following experiments we assessed in the male Wistar rat: (1) whether amphetamine disruption of overshadowing extends to an appetitively motivated overshadowing task; and (2) whether selective electrolytic lesions to the n.acc (shell versus core subfields) disrupt appetitively motivated overshadowing. The experiments used sucrose reward pellets as the unconditioned stimulus (UCS). In each case, a conditioned stimulus (CS, light) was either conditioned alone or in compound together with a more intense CS (noise or tone). The presence of overshadowing was demonstrated as reduced conditioning to the light when it had been previously conditioned in compound compared to when it had been conditioned alone. It was predicted that AMP and lesions to the n.acc shell would disrupt overshadowing. AMP was found to abolish overshadowing at 0.5 mg/kg, but not at 1 mg/kg. Contrary to prediction, the shell lesioned animals did not differ from shams.

The results of Experiment 1 add to the evidence that the DA system can moderate salience processing of weaker predictors, also in cases where CS salience is manipulated directly via the physical intensities of the stimuli, as here. However, in terms of the brain structures involved, Experiment 2 suggests that overshadowing is moderated by projections of the DA system without n.acc.

Keywords

appetitive conditioning, overshadowing, amphetamine, nucleus accumbens, dopamine, rat

Introduction

The experiments reported here assume particular importance because of the recent resurgence of interest in schizophrenia as a state of aberrant salience processing (Gray, 1995, 2004; Kapur, 2003). This has revitalised the idea that for people with schizophrenia, weak predictors present in the environment can become abnormally salient and as a consequence, result in disrupted selective learning. The role of the dopamine (DA) system in aberrant learning has been tested in learning tasks that present weak predictors experimentally (e.g. Solomon et al., 1981; Weiner et al., 1981, 1988, 1996; Tai et al., 1995; Jongen-Reblo et al., 2002; Norman and Cassaday, 2003; Kantini et al., 2005; Cassaday et al., 2005a, b). However, these learning tasks manipulate the salience of the learning cue indirectly, e.g. by giving the animals previous experience of the cue in question or removing it in time from the outcome to be predicted. Overshadowing, by contrast, manipulates the effect of relative salience in the most fundamental way in that it tests the effects of different physical intensities of conditioned stimuli (CSs) on learning.

Overshadowing was first described by Pavlov (1927) and is usually identified by decreased conditioned responding to a CS when it has been previously conditioned in compound with a more intense CS compared to when it has been conditioned alone. Overshadowing has not been extensively investigated neuropharmacologically, but it can be abolished by treatment with systemic amphetamine (AMP) (O’Tuathaigh and Moran, 2002, 2004). To
date, such overshadowing studies have been conducted in aver-sively motivated task variants. It is particularly important to test the
generality of these findings because it is known that in other selec-
tive learning tasks the effects of dopaminergic drugs and lesions in
aversive and appetitive task variants are not necessarily equivalent
(Killcross et al., 1994; Norman and Cassaday, 2003; Kantini et al.,
2004; Cassaday et al., 2005b). In Experiment 1, we therefore tested
AMP effects in an appetitive overshadowing procedure, to compare
effects with those previously reported in an aversive procedure

Comparison of the effects of systemic AMP with those of lesions
provides the first step in determining the likely neural substrates of
overshadowing. To date, studies have shown DA depletion in frontal
cortex disrupts overshadowing (Oades et al., 1987). In contrast, les-
ions of the hippocampus in both rats and pigeons leave overshad-
owing intact in both conditioned suppression (Garrud et al., 1984)
and appetitively motivated visual discrimination (Good and Macphail,
1994) procedures. There are as yet no studies concerning effects of
lesions to the DA-rich n.acc. on overshadowing but given the connec-
tivity of the n.acc. to both frontal cortex and hippocampus
(Groenewegen et al., 1987; Zahm and Brog, 1992; Zahm, 2000) and
the evidence that drugs which affect DA disrupt overshadowing, in-
vestigating the n.acc with respect to overshadowing is a logical pro-
geression (Experiment 2).

Within the n.acc, shell and core sub-regions are known to have
dissociable roles in aspects of associative learning (Parkinson
et al., 2002; Phillips et al., 1993; Wilson et al., 1995). Selective
lesions of the shell and core leave Pavlovian appetitive condi-
tioning intact (Corbit et al., 2001; Hall et al., 2001) so changes in
the baseline level of conditioning after such lesions should not
preclude testing their effects in appetitive overshadowing.
Moreover, the shell-core distinction has already proved critical in
examining the role of n.acc. in selective learning. e.g. measured
as latent inhibition (LI). In LI, stimulus salience is reduced by a
series of non-reinforced presentations of a stimulus that normally
retards conditioning to that stimulus when it is subsequently
paired with a reinforcing event (Lubow, 1973). Conditioning to the
pre-exposed discrete stimulus is enhanced by lesions of the shell
and by treatment with AMP (Solomon et al., 1981; Weiner
et al., 1981, 1988, 1996; Tai et al., 1995; Jongen-Relo et al.,
2002; Pothuizen et al., 2005), whereas core lesions leave LI in-
tact (Weiner et al., 1996; Jongen-Relo et al., 2002; Pothuizen
et al., 2005). Other research has shown that lesions incorporating
both shell and core also spare or enhance LI (Konstanti and
Kafetzopoulos, 1993; Weiner et al., 1999; Gal et al., 2005).
Therefore, in LI, an intact n.acc. is a critical component of the
neural circuitry necessary for normal selectivity. Moreover, when
restricted lesions are tested shell and core sub-regions are found
to have clearly dissociable roles (Weiner, 2003).

Thus following on from the well-documented role of the DA
system in LI and recent reports of AMP effects in aversive over-
shadowing (O’Tuathaigh and Moran, 2002, 2004), in the following
experiments, we test the general prediction that AMP and shell
lesions should similarly increase conditioning to a less salient (‘over-
shadowing’) CS, here tested for the first time in an appetitive
overshadowing procedure.

Methods

Animals

On arrival in the laboratory, rats were given free access to food and
water and were handled daily for a minimum of 2 weeks. Throughout
animals were caged in pairs on a 12:12 h light/dark cycle and tested
during the light phase. All testing was conducted between 9:00 h
and 16:00 h. Rats were food deprived, receiving a basic ration of 5 g
per 100 g of body weight (up to a maximum of 20 g per rat per day),
that was adjusted to allow further weight gain in rats of below av-
erage weight. Water was available in the home cage throughout the
duration of the experiment.

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

Experiment 1  Forty-eight experimentally naïve male Wistar rats
(Charles Rivers, UK) weighed between 200 g and 250 g at the start
of the experiment.

Experiment 2  Rats were allowed to reach 200 g in body weight
prior to surgery. The amount of food provided was subsequently ad-
justed in order to maintain weights as close to 200 g as possible for
optimal fit with the anaesthetic delivery apparatus. Weight gain was
monitored daily during this time.

Eighty naïve male Wistar rats (Charles Rivers, UK), of mean
weight 228 g (range 201–265 g) underwent surgery. Sixty rats were
given a n.acc lesion (30 each of core and shell) and 20 rats were sham
operated (10 rats at core coordinates and 10 at shell coordinates).
Rats were weighed daily during the first two post-operative weeks
and weekly thereafter. After surgery, rats were caged alone for one
week, before re-introduction to their cage-mates and paired housing.
Two rats became aggressive after repairing and were thus caged
alone permanently. Five rats died, two during surgery, the other three
were humanely killed post-operatively on veterinary advice; two due
to respiratory infections and one following the onset of tremor.

Thus 75 male Wistar rats (Charles Rivers, UK) underwent test-
ing and weighed between 313 g and 491 g at the start of food de-
privation. The entire sample of rats had previously participated in
both aversive and appetitive trace conditioning procedures (see
Cassaday et al., 2005b) and were approximately 5 months post-
surgery at the start of the present experiment. Rats were allocated
to behavioural conditions counterbalanced for their previous exper-
imental experience and had a break of 5 weeks between the previ-
ous trace experiment and the current overshadowing experiment.

Treatments

Experiment 1: systemic amphetamine  D-amphetamine (Sigma
Aldrich, Dorset, UK) was dissolved in physiological saline and
injected in 1 mL/kg body weight, at doses 0.5 mg/kg and 1 mg/kg
i.p. Control rats received an injection of saline at equivalent vol-
ume. Prior to pre-conditioning, a sensitising injection of AMP or
saline to the control group was given to the rats 15 min prior to
placement in the experimental chambers. During acquisition,
treatments were given 15 min prior to the conditioning sessions. Probe and extinction tests for overshadowing were conducted drug free.

**Experiment 2: surgery** Lesions were made bilaterally using the following coordinates (based on the atlas of Paxinos and Watson, 1997): Core – AP +2; ML ±1.6; DV −6.5 and AP +2.4; ML ±1.6; DV −6.3; Shell – AP +1; ML ±0.8; DV −7 and AP +1.5; ML ±0.8; DV −7. The ML coordinates were taken with reference to the saggital sinus. The DV coordinates were taken from the dura mater; AP coordinates used were with reference to the original position of bregma. At each coordinate, 2 mA DC constant current of 7 s duration were delivered symmetrically to each hemisphere using an electrolytic lesioning device (UGO Basile, Verese, Italy). After each current delivery, the electrode was left in place for an additional 2 min. Sham-operated rats were prepared exactly as above and the electrode was in each case lowered but no current was passed. A fresh electrode was used for each rat. Full details of the surgery have been reported elsewhere (Cassaday et al., 2005b).

**Apparatus**

Six 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 × 25 × 22 cm high) 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. Mounted in one wall were two retracted levers (not used), three stimulus lights and a food magazine. A loudspeaker for the presentation of auditory stimuli was set in the roof. The food 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).

**Stimuli**

In Experiment 1, two experimental stimuli were used (in a between subjects design) as predictors of food delivery; rats received either a 5 s flashing light [three wall mounted stimulus lights and the house light, flash rate (on/off) 0.5 s] as the predictor of food delivery or a flashing light + noise compound (5 s duration) as predictor of food. The noise was a mixed frequency noise presented at 85 dB (A) via a loudspeaker inset on the roof of the chamber. In Experiment 2, the stimuli used were identical, except the auditory element of the compound was an 85 dB (A), 2 kHz pure sine wave tone. Stimulus control and data collection was by an Acorn Archimedes RISC computer programmed in Basic with additional interfacing using an Arachnid extension (Cambridge Cognition).

**Procedures**

**Behavioural** In Experiment 1 rats received 13 days of acquisition; during this phase the light alone or the compound (light plus noise) signalled subsequent sucrose pellet delivery. Rats received 10 CS-UCS pairings in 30 min. In Experiment 2, rats received 18 days of acquisition, receiving eight CS-UCS (here, the compound CS was light plus tone) pairings in 20 min on each day of acquisition. In both experiments, overshadowing was examined in extinction tests of conditioning to the light CS. These tests were in each case followed by tests of conditioning to the overshadowing auditory CS. Stimuli were delivered as discrete 5 s presentations (10 presentations in Experiment 1 and eight presentations in Experiment 2) and no sucrose was delivered during this phase.

**Preconditioning** Rats were already magazine trained. There were two days of baseline, during which there were 10 unsignalled rewards in 10 min, delivered on a variable interval schedule. The total number of magazine entries was recorded.

**Acquisition** Acquisition consisted of 10 (Experiment 1) or eight (Experiment 2) signalled rewards presented over 30 or 20 min respectively. The sucrose pellets UCS was delivered contiguously with CS (light alone or compound) offset. Conditioning trials were conducted over 30 or 20 min (respectively). Conditioning trials were presented throughout the sessions on a variable interval schedule.

The dependent variables were the number of nose pokes in the following response bins: 5 s prior to the CS (pre-CS responding); during the 5 s of the CS (CS responding) and in the remainder of the session (ITI). Since there is general agreement that n.ace DA is involved in the processing of rewards and evidence suggests that DA is released in the n.ace, in response to both appetitive UCSs and CSs (Hernandez and Hoebel, 1988; Young et al., 1992; Robbins and Everitt, 1996; DiChiara, 1998) we also measured responding in the 5 s following delivery of the sucrose pellet UCS (UCS responding) in order to distinguish effects that could be mediated unconditionally.

**Overshadowing tests** There were 10 (Experiment 1) or eight (Experiment 2) stimulus presentations over 30 or 20 min (respectively) but this time in the absence of any food deliveries. The number of magazine entries was recorded 5 s prior to stimulus onset (pre-CS), during the CS (light or noise/tone), and during the ITI. Data from UCS responding is not relevant here, because at test no UCS was delivered.

In order to determine the development of overshadowing, a test to the light alone was administered following 9 (Experiment 1) or 14 days (Experiment 2) of acquisition. In both cases the decision was taken to undertake further conditioning and results for these tests are therefore not reported. Further tests to the light on day 14 (Experiment 1) and day 19 (Experiment 2) showed overshadowing, thus these are reported as the critical overshadowing extinction tests of conditioning to the light. In each case, tests of conditioning to the auditory stimulus were conducted on the days immediately subsequent. In the case of Experiment 1, all extinction tests were conducted drug free.

**Histology**

All the rats used in Experiment 2 were deeply anaesthetized with 1 mL of Sagatal (Sigma Aldrich, Dorset, UK) and were perfused transcardially with 100 mL of 0.9% physiological saline followed by 100 mL of fixative (10% formaldehyde in 0.1 M potassium buffer). The brains were then removed whole and placed in jars containing
100 mL fixative for a minimum of 4 weeks. Then, 24 h prior to histology, the brains were removed from the fixative solution and immersed in 20% sucrose solution at 4–5°C. The brains were then blocked and coronal sections 60 μm in thickness were taken using a freezing microtome (MSE Ltd, cooling unit model 130439). Every third section was retained, mounted on a slide and stained with Cresyl Violet. Lesion size and location were assessed with reference to the atlas of Paxinos and Watson (1997) using a microscope (Olympus, BH2).

Design and statistics

In each experiment there were six experimental groups run in an independent 3 × 2 factorial design, with either drug (at levels saline, 0.5 mg/kg and 1 mg/kg) or lesion (at levels sham, shell and core) and overshadowing [at levels compound (Experiment 1: light + noise; Experiment 2: light + tone) and light alone (LC)] as between subjects factors. To assess effects over the course of acquisition, Days was included as a repeated measures factor. The dependent variable was in each case the number of magazine entries. In Experiment 2, two rats’ data were excluded if the animal failed to enter the magazine a sufficient number of times to collect all the sucrose pellet rewards on more than 50% of conditioning sessions.

For the critical overshadowing tests, to separate out potential differences in baseline responding from drug and lesion effects, CS responding, was determined as a percentage of that seen in the remainder of the session, calculated as CS = [CS/(Pre-CS + CS + ITI)]*100. This calculation automatically excluded four cases in which the denominator was zero: for Experiment 1, there were two exclusions from the light analyses, in Experiment 2, there was one such exclusion from each of the tone and light analyses. Statistical analyses were conducted using Analysis of Variance with alpha set at 0.05. Planned pair-wise comparisons used independent t-tests. We made only the comparisons necessary to explore the interactions in critical test data and to confirm the absence of any effect of the lesions. This means that the inflation of the family-wise error rate was minimal (Howell, 2002).

Results: Experiment 1

Preconditioning baseline responding

There were no significant main effects shown by drug or overshadowing group allocation [maximum F(1, 40) = 2.52], thus groups were well matched prior to acquisition.

Acquisition

Pre-CS There was a significant effect of Days [F(12, 480) = 2.05, p < 0.05] which reflected minor fluctuations in overall mean responding over the 13 days of acquisition. No other main effects or interactions in the Pre-CS were significant [maximum F(12, 480) = 1.07]. Therefore, baseline responding between groups remained equivalent during acquisition.

CS responding There was a significant effect for days which reflected a progressive increase in responding as conditioning progressed [F(12, 480) = 60.3, p < 0.001]. This acquisition was not affected by drug or by overshadowing (i.e. light alone versus light + noise compound conditioning) group, nor did these factors interact significantly with each other, or with days [maximum F(2, 40) = 2.89]. Thus AMP did not affect the speed at which rats learned, nor did it affect the overall level of learning about either of the CSs used. Both the light and compound CSs attracted equivalent levels of associative strength; the addition of a more salient stimulus (noise) to the light did not increase the overall level of conditioned responding compared to the light alone.

UCS responding There were no main effects for drug or overshadowing group and these factors did not significantly interact with each other, or with days [maximum F(12, 40) = 1.94]. There was a significant effect for days [F(12, 480) = 4.52, p < 0.001] because of some initial fluctuations in responding during early acquisition. Thus, rats (regardless of drug or behavioural condition) were equally capable of responding and motivated to enter the food magazine to obtain sucrose pellets.

ITI responding There was no evidence of differences in baseline responding between trials in acquisition [maximum F(12, 480) = 1.75].

Overshadowing tests of conditioning to light

Pre-CS Analysis of levels of baseline responding measured in the Pre-CS period during extinction to the light produced no significant effects for prior drug or overshadowing group and no significant interaction between these factors [maximum F(2, 40) = 0.53].

Percentage CS responding Analysis of magazine entries during the light CS (as a percentage of total responding during the session) showed no significant main effect for prior drug [F(2, 38) = 2.54] or overshadowing (F(1, 38) = 0.40). However, there was a significant interaction between prior drug and overshadowing [F(2, 38) = 3.68, p < 0.05] (see Fig. 1).

Planned comparisons showed that overshadowing was present in both the (previously) saline [t(14) = 2.02, p <0.05, one-tailed] and 1 mg/kg AMP treated rats (see below) but not in the 0.5 mg/kg AMP group [t(13) = 1.27]; in fact, the expected pattern of responding was reversed at the latter dose. The effect of AMP was not confined to the overshadowing group in that although overshadowing was clearly abolished under 0.5 mg/kg AMP, there was no statistical difference between saline and 0.5 mg AMP groups [t(13) = 0.82]. However, the light control group is critical to allow for effects of AMP on conditioning (in the absence of the cue competition that normally reduces conditioning to the light in the compound conditioned overshadowing group). Despite a similar reduction (to that seen at the 0.5 mg/kg AMP dose) in conditioning in the light control group at the 1 mg/kg dose, overshadowing was intact under 1 mg/kg AMP [t(11) = 1.93, p < 0.05, one-tailed].
Noise tests

Pre-CS Analysis of the pre-CS showed no significant main effects or interactions [maximum F(2, 40) = 3.14].

Percentage CS responding Analysis of responding to the noise as a percentage of total responding during the session showed significant main effects for overshadowing group and prior drug [minimum F(2, 38) = 9.96, p < 0.001] and a significant interaction between these factors [F(2, 38) = 3.68, p < 0.05]. Responding to the noise at test reflects unconditioned responding to the novel stimulus [for whom this stimulus is novel] and conditioned responding to the overshadowing CS in the light + noise group. Comparing drug effects between the overshadowing (light + noise) groups (see Fig. 2) showed that AMP reduced conditioned responding to the noise compared to saline controls [minimum t(13) = 2.50, p < 0.05]; there was no significant difference in responding between the two drug groups [t(13) = 0.65]. As expected, there was virtually no unconditioned responding in the control (light alone) group, and there were no differences by drug treatment [maximum t(14) = 1.13].

Results: Experiment 2

Histology

As would be expected with the electrolytic method, the lesions were variable in size. The criteria for retention within each lesion condition were: (1) that the extent and location of the damage should be consistent with that seen on other animals in the same lesion condition; and (2) that the lesion should damage the intended (shell versus core) target and encroach little if at all bilaterally on the alternative subfield.

Seven rats were excluded on the basis of histology: four in the shell-lesioned group and three from the core lesioned group. This left a sample size of 68 rats: 24 core lesion, 24 shell lesion, 10 core sham and 10 shell sham operated animals (prior to statistical exclusion, see Design and Statistics above). The shell and core lesions retained in...
the study were clearly and reliably distinguishable. The largest and smallest extents of the lesions retained are shown in Fig. 3. Although the largest lesions show some encroachment into the alternative subfield, this was only the case in the two largest lesions retained in the experiment, and this damage was, in any case, unilateral. There was inevitably some non-specific damage after the electrolytic lesions; e.g., there was generally some damage to the caudate putamen after core lesions, and damage to medial structures after shell lesions. Full details of the histology are reported elsewhere (Cassaday et al., 2005b).

Responding of shell sham and core sham operated animals in acquisition was compared; no statistically important differences in responding were seen [maximum F(1, 16) = 3.55] so the sham treatments were collapsed for the analyses reported below.

Preconditioning baseline responding

There was a significant interaction between lesion and days [F(2, 62) = 4.47, p < 0.05]. Both shell and core lesioned rats reduced their responding from day 1 to day 2 of baseline, in contrast, sham operated animals increased their responding from day 1 to 2. There were no other significant effects shown by lesion or overshadowing group allocation [maximum F(1, 62) = 3.53].

Acquisition

Pre-CS With the exception of a significant interaction between days and overshadowing group [F(17, 1054) = 1.92, p < 0.05] there were no other significant main effects or interactions [maximum F(2, 62) = 1.67]. Means for the days × overshadowing group interaction that the interaction reflected non-systematic fluctuations in the data over time.

CS responding There was greater learning to the compound CS compared to the light alone CS overall shown by a significant effect for overshadowing; this was also significant in interaction with days [minimum F(17, 1054) = 1.95, p < 0.05]. Other than a main effect of days [F(17, 1054) = 13.62, p < 0.05], there were no other significant effects or interactions [maximum F(34, 1054) = 1.13]. Therefore, lesion did not affect differential rates of learning between the conditioning groups either overall or over days.

UCS responding In acquisition, lesion significantly affected overall UCS responding [F(2, 62) = 3.57, p < 0.05], with shell lesioned animals responding the most (mean ± SEM = 16.96 ± 1.05), followed by shams (mean ± SEM = 14.92 ± 1.15) and then core lesioned animals (mean ± SEM = 12.86 ± 1.12). However, all groups responded on average a sufficient number of times to receive the sucrose UCS. No other effects or interactions were significant [maximum F(2, 62) = 1.33, p = 0.25].

ITI responding Lesion had no effect on responding in the ITI either independently or in interaction with days [maximum F(2, 62) = 1.25]. There was an overall effect for days [F(17, 1054) = 2.86, p < 0.001] and this interacted significantly with overshadowing group [F(17, 1054) = 3.52, p < 0.001]. This reflected differences in responding over days between the groups: the light alone group showed a progressive reduction in nose-poking from 112.31 on day 1, to 78.97 on day 18, in contrast, group light + tone increased nose-poking from day 1 (52.04) to day 18 (83.29). Levels of ITI responding were therefore equivalent by the final day of acquisition. No other effects or interactions were significant [maximum F(1, 62) = 1.10].

Overshadowing tests of conditioning to light

Pre-CS Neither lesion, nor overshadowing (or their interaction) affected baseline responding as measured by the pre-CS: maximum F(2, 62) = 1.84.

Percentage CS responding Analysis of responding to the presentation of the light CS as a percentage of responding across the session confirmed significant overshadowing [F(1, 60) = 9.08, p < 0.005]. There was no statistical evidence to suggest that lesion affected the expected differential levels of learning in the light alone and light + tone groups, nor did lesion exert any independent effects [maximum F(2, 60) = 0.98]. Figure 4 shows that overshadowing was preserved within each lesion condition. Statistically, there were no differences by lesion, neither within the light alone group nor within the light + tone groups [maximum t(24) = 1.9].

Tone tests

Pre-CS Analysis of responding during the pre-CS showed no significant effects or interactions [maximum F(2, 62) = 0.48].

Percentage CS responding Analysis of responding to the tone as a percentage of responding across the session showed no significant effect for overshadowing group [F(1, 60) = 3.58, p = 0.06] and no effect for lesion or significant lesion × overshadowing group interaction [maximum F(2, 60) = 1.34]. Figure 5 shows that lesion had no effect on conditioned responding to tone in the overshadowing

![Figure 4](image-url)
but not 1 mg/kg) of amphetamine resulted in a disruption of overshadowing the effects of systemic amphetamine and shell lesions in acquisition and at extinction showed no systematic differences in

In Experiment 1 baseline responding measured at preconditioning, in acquisition and at extinction showed no systematic differences in responding by drug or by conditioning group allocation. Nor was there any evidence of a drug effect on collection of the sucrose UCS in acquisition. In Experiment 2, there were again no differences between groups at preconditioning. The shell lesioned group responded more to collect the food reward but there was no effect of either lesion on acquisition. Moreover, in order to adjust for effects of AMP or lesion treatments on an individual basis, for the critical overshadowing tests CS responding was analysed as a percentage of overall responding in the session. Thus, we can exclude the possibility that differences in unconditioned responding for food or changes in general activity levels could confound interpretation of the effects of treatments on conditioning.

The rats used in Experiment 2 were not behaviourally naïve however there is no evidence that transfer effects can account for the results seen. Animals were counterbalanced for prior experimental experience. Statistical analysis confirmed that the overshadowing produced in the control groups was comparable between Experiments 1 and 2.

**Effects on conditioning**

In the present study the course of acquisition was unaffected by AMP or lesion treatments. Previous studies have found that treatment with AMP can reduce the expression of prior learning about the experimental stimuli measured drug-free at test (Kantini et al., 2004; Norman and Cassaday, 2004). However, this effect cannot explain the abolition of overshadowing observed here. Statistically, we found no main effect of (prior) drug but there was an interaction between (prior) drug and conditioning group. This means that the consequences of treatment with AMP were different in the different behavioural conditions. If the present result was a straightforward consequence of reduced expression of prior learning under 0.5 mg/kg AMP, statistically there should have been a main effect of drug. On the contrary, there was an interaction in the absence of such a main effect, and the failure to show overshadowing under 0.5 mg/kg AMP was shown as a reversal of the normal pattern of higher conditioning in the light controls compared with the overshadowing group. Mere state-dependent effects (Overton, 1964) are an unlikely account of the present pattern of results because a state-dependent shift in the level of expressed conditioning should affect both overshadowing and light alone groups and in the same direction. It is possible that state-dependent effects of drug could have reduced the expression of prior learning in the present study, but any such shift in the level of expressed conditioning was not so great as to preclude the demonstration of overshadowing in the 1 mg/kg treated group. Therefore, changes in the light alone control group cannot be sufficient explanation of the loss of overshadowing under 0.5 mg/kg AMP because the same change was seen under 1 mg/kg AMP but under this dose – despite a shift in the level of control group conditioning supported – the overshadowing effect was intact.

AMP also reduced responding to the noise that was conditioned in compound with the light in overshadowing groups. However, this reduction in conditioning to the noise cannot explain the abolition of overshadowing seen at 0.5 mg/kg AMP because 1 mg/kg AMP also reduced conditioning to the noise, but at this dose overshadowing

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**Comparison of overshadowing effect between experiments**

Analysis of the key dependent variable (percentage CS responding to the light tests) confirmed that the experiments were comparable. There was no difference in overall level of conditioned responding between the two experiments [F(1, 32) = 0.33], nor was there any difference between experiments in interaction with overshadowing group [F(2, 32) = 0.05].

**Discussion**

This study is the first to investigate the effects of systemic amphetamine administration on an appetitive form of overshadowing. In addition, we tested the effects of lesions to shell and core accumbens, subregions previously demonstrated to show dissociable roles in another form of selective learning, i.e. LI (Weiner et al., 1996; Jongen-Relo et al., 2002). Contrary to expectation, we found that for overshadowing the effects of systemic amphetamine and shell lesions were not equivalent; although administration of a low dose (0.5 mg/kg, but not 1 mg/kg) of amphetamine resulted in a disruption of overshadowing, rats with a shell lesion showed intact overshadowing.

**Do baseline levels of activity or motivation confound interpretation of the results?**

In Experiment 1 baseline responding measured at preconditioning, in acquisition and at extinction showed no systematic differences in groups [maximum t(19) = 1.59], nor was there an effect of lesion on unconditioned responding in the light alone control groups [maximum t(21) = 0.63].
was intact. Therefore, neither general learning impairment, nor reduced expression thereof, nor changes in conditioning to the noise provide any obvious account of the abolition of overshadowing seen at the 0.5 mg/kg dose. Similarly, in the case of n.acc lesions, any conditioned or unconditioned effect of the shell lesion on responding to the overshadowing tone stimulus was insufficient to abolish overshadowing.

**Low but not high dose amphetamine abolished overshadowing**

The lower 0.5 mg/kg dose AMP clearly abolished overshadowing. The fact that the higher 1 mg/kg left this aspect of selective learning intact might reflect a curvilinear relationship between levels of extracellular DA and specific effects on learning. Alternatively the different doses might be affecting different DA-ergic structures or pathways. There is little data available from appetitive selective learning studies (where drug is given chronically) with which to make a comparison. However, differential effects by dose of AMP have been found in a trace conditioning procedure, in which both 0.5 and 1.5 mg/kg reduced the acquisition and expression of appetitive conditioning, but only the 0.5 mg/kg dose promoted the acquisition of anticipatory responding later in the trace interval when food delivery was imminent (Kantini et al., 2004). In appetitive LI, using a discrete CS to test the impairment in later learning that should be produced by stimulus preexposure, only a 1.5 mg/kg (but not 0.5 mg/kg) dose of AMP reduced this normal LI effect, but the reduction was statistically marginal so the appetitive LI procedure in use was not sufficiently sensitive to AMP effects (Killcross et al., 1994). However, in an appetitive LI procedure using a contextual stimulus, 0.5 mg/kg but not 1.5 mg/kg AMP abolished LI because of increased learning in the pre-exposed group (Norman and Cassaday, 2004; though it should be noted that the effect of increased learning in the preexposed group was seen under drug in acquisition but not when the rats were tested drug free in extinction). Thus there is some evidence from other appetitive procedures (with similar treatment regimes) for dissociable effects at different doses of AMP.

However, the AMP dose that abolished overshadowing is not equivalent to that which abolished aversive overshadowing (1 mg/kg AMP; O’Tuathaigh and Moran, 2002). Unavoidable differences in drug treatment regime (acute in aversive and chronic in appetitive procedures) in consequence of different numbers of conditioning days are a likely account of such discrepancies (Norman and Cassaday, 2003; Kantini et al., 2004).

**Overshadowing intact after shell and core accumbens lesions**

Why was the shell lesion without effect on overshadowing? It is possible that the electrolytic lesion technique did not produce lesions with sufficient selectivity for each subfield, thus preventing the demonstration of differential effects on overshadowing. This is an important consideration given the differential pattern of effects of shell and core lesions seen in LI (Konstandi and Kafetzopolous, 1993; Tai et al., 1995; Weiner et al., 1996; Jongen-Relo et al., 2002; Gal et al., 2005; Pothuizen et al., 2005). However, encroachment into the alternate sub-field was minimal, with only the two largest core lesions retained in the study affecting the shell, and this damage was not bilateral. Moreover, we know already that our lesions were effective. The very same shell lesions produced robust effects in trace conditioning experiments (conducted prior to the overshadowing experiment reported here) and these were very clearly different to those of the same core lesions (reported in Cassaday et al., 2005b). There was further evidence for the effectiveness of the lesions within the present study: although without effect on overshadowing, the shell lesion increased unconditioned responding for food.

**Conclusions and implications**

In conclusion, Experiment 1 shows clearly that AMP effects on overshadowing can be observed in an appetitive variant. This adds weight to the argument that the dopaminergic system moderates the salience of weaker predictors, including cases of overshadowing, in which CS salience is manipulated directly via the physical intensities of the stimuli in use, suggesting that in schizophrenia aberrant salience might be attributed to a wider range of weak cues than previously thought. However, in terms of the brain structures involved, Experiment 2 suggests that, overshadowing is moderated by projections of the DA system without n.acc.

The majority of previous experiments assessing effects of drugs and lesions on selective learning in LI experiments have been aversively motivated but equivalent effects can be demonstrated in appetitive procedures (Killcross et al., 1994; Norman and Cassaday, 2004). Here, Experiment 1 replicated AMP effects on aversive overshadowing (O’Tuathaigh and Moran, 2002, 2004) in an appetitive procedure, although at a different dose (as discussed above). Thus AMP treatment which disrupts both appetitive and aversive LI can also disrupt both variants of overshadowing. This suggests that the reduction in CS salience produced through pairing with a more intense CS in overshadowing has functional similarities to the reduction in stimulus salience produced through pre-exposure in LI, independently of how the task was motivated.

However, counter to prediction, results from Experiment 2 show some dissociation of the effects of drugs and lesions. The effects of low dose AMP were not reproduced by shell lesions in the case of overshadowing. By contrast, LI is known to be disrupted by both lesions to the n.acc shell and systemic AMP, leading to the hypothesis that systemic AMP effects are mediated via effects on DA in this structure. The results of the present study would seem argue against this being the case for overshadowing. One possible explanation for this discrepancy is that, although DA is critical in both cases, where effects are mediated depends on how the task is motivated. If this interpretation is correct, shell lesions should be similarly without effect on appetitive LI, which has yet to be tested.

Finally, shell lesions may fail to produce effects on LI at parameters known to be sensitive to amphetamine, but such lesion effects can emerge if these parameters are adjusted (Pothuizen et al., 2006). It is conceivable that this might also be the case for overshadowing, therefore it is not possible to discount entirely a role for n.acc shell in
overshadowing and it would be useful to follow this study up with experiments using different overshadowing parameters. Alternatively, the anatomical substrates of overshadowing may prove to be different from those of LI irrespective of the motivational variant in use (Garrud et al., 1984; Good and Macphail, 1994).

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Q1: Kantini et al., 2005 in text but 2004 in ref. list.