Available online at www.sciencedirect.com
SCIENCE DIRECT
Behavioural Brain Research 160 (2005) 222–235
BEHAVIOURAL BRAIN RESEARCH
www.elsevier.com/locate/bbr

Research report

Electrolytic lesions to nucleus accumbens core and shell have dissociable effects on conditioning to discrete and contextual cues in aversive and appetitive procedures respectively

H.J. Cassaday*, R.R. Horsley, C. Norman

School of Psychology, University of Nottingham, University Park, Nottingham NG7 2RD, UK

Received 20 August 2004; received in revised form 30 November 2004; accepted 6 December 2004
Available online 1 February 2005

Abstract

The nucleus accumbens (n. acc.) has been implicated in conditioning to both discrete and contextual cues but its precise role is as yet controversial because conflicting patterns of effect have been reported. These inconsistencies may relate to the extent to which the lesions used encroach on different subfields of n. acc. and the use of different task variants. The present study compared the effects of selective lesions of shell and core subfields of nucleus accumbens (n. acc.) across aversive and appetitive trace conditioning variants.

In both experiments, an auditory stimulus was contiguous with footshock or food, or presented at a trace interval. A continuous flashing light in each case provided an experimental background stimulus. Conditioning to the cues provided by the experimental chambers was also assessed. Rats with electrolytic lesions to the n. acc. shell and core showed different patterns of effect in aversive (Experiment 1) and appetitive (Experiment 2) variants of this procedure. In Experiment 1, the core lesion reduced the difference between trace and contiguously conditioned groups, in responding to the discrete noise stimulus. However, neither lesion had any detectable effect on contextual conditioning. In Experiment 2, the shell lesion clearly increased contextual conditioning, selectively in the trace conditioned group, but neither lesion had any detectable effect on discrete cue conditioning.

Thus, whilst the shell and core lesions produced dissociable effects on discrete cue and contextual conditioning, the conclusions to be drawn depend on the procedural variant in use.

Keywords: Trace conditioning; Contextual conditioning; Nucleus accumbens; Dopamine

1. Introduction

Previously, the hippocampal formation has been found to have dissociable roles in discrete cue and contextual conditioning [26,30,41]. Recently, a dopaminergic structure, the nucleus accumbens (n. acc.), that receives projections from hippocampus, has become a focus of research into the neural substrates of contextual and discrete cue conditioning because this structure could contribute to effects previously attributed to hippocampus [23,29]. In parallel, the dopamine (DA) hypothesis and related amphetamine model of schizophrenia [5] have prompted considerable interest in using the involvement of the dopaminergic system in selective learning to provide animal models of cognitive dysfunction. To this end, there has hitherto been a particular focus on factors affecting discrete cue conditioning.

Normally animals demonstrate that they have learned to expect motivationally significant events such as foot shock or food (unconditioned stimuli, UCSs) by anticipatory conditioned responses to the discrete cues (conditioned stimuli, CSs) that best predict the occurrence of the UCS. Disorder in these processes provides a likely model for aspects of schizophrenic attentional disorder. For example, in latent inhibition (LI), a series of nonreinforced presentations of a stimulus normally retards conditioning to that stimu-

* Corresponding author. Tel.: +44 115 951 5324; fax: +44 115 951 5124.
E-mail address: helen.cassaday@nottingham.ac.uk (H.J. Cassaday).

0166-4328/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2004.12.012

lus when it is subsequently paired with a reinforcing event [17]. This effect is absent in acutely ill schizophrenia patients [1], after treatment with amphetamine in both humans [6,15] and rats [31,36,37], and after lesions to the DA-rich n. acc. [10,32,38,39]. The fact that such lesions can produce effects resembling those of amphetamine suggests that n. acc. lesions result in DA hyperactivity, possibly due to the destruction of terminals of the hippocampal projection to this region (that are thought to be inhibitory).

However, n. acc. is anatomically heterogeneous, and already divided into medioventral shell and dorsolateral core subregions [42]. Moreover, there is evidence that this anatomical differentiation is, as would be expected on the basis of the differential projections of shell and core [43], reflected in functional dissociations, for both low level behaviours like activity [19,40], as well as in the cognitive processes necessary to LI [35,38,39]. Such dissociations may relate to the differential projections of shell and core sub-regions to motor and limbic regions [43]. With respect to contextual conditioning, motor cortex might be engaged by the exploratory activity necessary to show learning about environmental stimuli. Accordingly, there has also been some investigation of the role of the core and shell sub-regions in discrete cue versus contextual conditioning. Electrolytic lesions centred on the shell have been found to reduce contextual but not discrete cue conditioning in an aversive procedure [29]. By contrast, selective excitotoxic core lesions, have been reported to increase contextual but decrease discrete cue conditioning, again in aversive procedure in which conditioning was unaffected by shell lesions [23].

Thus, there are two problems: (1) these investigations have been based almost exclusively on aversive task variants and (2) they have yielded contradictory results. Despite good evidence for the role of n. acc. in unconditioned responses to reward, relatively little is known about how the dopaminergic system is involved in the associative aspect of appetitive learning and the production of anticipatory conditioned responses. There is some evidence for a role in Pavlovian approach behaviour [24]. However, little (if anything) is known about the role of n. acc. in appetitive contextual conditioning.

In consequence, there are outstanding questions about the role of n. acc. in discrete cue and contextual conditioning, particularly where there is an attentional component to the learning, and the generality of findings across aversive and appetitive procedures. We therefore compared shell and core lesions (made electrolytically as a first step) in trace conditioning, implemented in both appetitive and aversive variants. The neural substrates so far identified as having a role in contextual conditioning should extend beyond aversive conditioning, i.e. they should also mediate appetitive contextual conditioning, but this we need to test.

The trace CS is a less informative predictor of the UCS than the CS immediately followed by the UCS [11]. We used both an aversive off-the-baseline conditioning procedure, like that typically used in LI experiments, and an appetitive on-the-baseline procedure. This allowed comparison across aversive and appetitive tasks and a more detailed examination of effects over the course of (appetitive) acquisition. For both trace and contiguously conditioned groups, an experimental background stimulus was presented continuously throughout the conditioning session and provided an alternative conditioning stimulus. The introduction of a trace interval was predicted to increase the level of contextual conditioning supported.

In addition to testing conditioning to the discrete CS and to the experimental background stimulus, we also measured contextual conditioning to the experimental chambers ('box context') as lick suppression at the start of the first post-conditioning drinking session in the aversive procedure and as the level of background responding in the appetitive procedure. Thus, as well as providing an alternative means (to preexposure) of presenting animals with a weakly predictive CS, the trace conditioning procedure used here provides measures of contextual conditioning, both to the experimental background stimulus and the cues provided by the conditioning chambers. Moreover, the trace interval between CS and UCS should influence the level of contextual conditioning supported. The normal selective learning effect is to show relatively increased contextual conditioning when the discrete stimulus is rendered less informative because of the use of a trace interval [22,27,28]. Treatments that improve selective learning should therefore promote the tendency to form associations with contextual stimuli in trace conditioned groups, particularly within the local context of the trace interval [13]. Treatments that impair selective learning should reduce this apportioning of associative strength.

2. Materials and methods

2.1. Subjects

On arrival in the laboratory, rats were caged in pairs on a 12:12 h light/dark cycle and given free access to food and water. They were handled daily for 2 weeks. The rats' weights on arrival were in the range 150–175 g and they were on free food until they reached 200 g in body weight. The amount of food provided was subsequently adjusted in order to maintain weights as close to 200 g as possible so that the rats were all operated at about the same size: 200 g allowed best fit into the mouth piece of the anaesthetic delivery apparatus. Rats were weighed daily during the first two post-operative weeks, and weekly thereafter.

Eighty naïve male Wistar rats (Charles Rivers, UK), of mean weight 228 g (range 201–265 g) underwent surgery. Thirty rats were randomly allocated to each of the core and shell lesion groups and a total of twenty rats were allocated to the sham condition (10 rats were sham operated at the core coordinates and 10 rats were sham operated at the shell coordinates). After surgery, rats were caged alone for 1 week, before re-introduction to their cage-mates and paired housing. Two rats became aggressive after re-pairing and were thus caged alone permanently. Five rats died, two during surgery, the other three were humanely killed post-operatively (following veterinary advice) due to respiratory infections (two) or the onset of tremor (one).

After a minimum 2-week recovery period, 75 rats were tested in the Experiment 1 aversive procedure. Their weights were all within the range 270–516 g at the start of water deprivation. The same 75 rats were later tested in the Experiment 2 appetitive procedure counterbalanced for their previous experimental experience. We already know that prior testing in aversive conditioning does not interfere with the demonstration of the appetitive (trace) conditioning [20]. All experimental tests were conducted during the light phase.

Rats were water deprived in Experiment 1, receiving 1 h of access to water per day following testing. Food was freely available in the home cage throughout the duration of Experiment 1. There was a 2-week gap between Experiments 1 and 2, during which time water was available ad lib and rats were fed a maintenance ration of at least 15–20 g per rat, adjusted as necessary to allow for healthy weight gain and then to stabilize weights in those over 400 g. Their weights were all within the range 307–545 g at the start of Experiment 2, the appetitive study, for which they were food deprived. A basic ration of 5 g per 100 g of body weight (up to a maximum of 20 g per rat per day), was adjusted to allow further weight gain in rats of below average weight. Water was available in the home cage throughout the duration of the appetitive study.

All procedures were carried out in accordance with the United Kingdom Animals Scientific Procedures Act 1986, Project Licence number PPL 40/2019.

2.2. Surgery

Immediately prior to surgery, each rat was treated with a non-steroidal anti-inflammatory drug (Rimadyl 0.03mls s.c., for 24 h-post-operative pain relief). Anaesthesia was induced via a mouthpiece using isoflurane (5%), nitrous oxide (750 cm^3/min) and oxygen (500 cm^3/min). Subsequently, anaesthesia was maintained with Isoflurane at 2–3% (as required) via a mouthpiece designed to fit the stereotaxic apparatus (David Kopf Instruments).

Lesions were made bilaterally using the following coordinates, based on the atlas of Paxinos and Watson [25] and previous work [38]: 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. A small bone flap was removed so that the ML coordinate could then be 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) attached to an electrode made from an insect pin (German 0.3 mm diameter, size 00; Hillside Books, Canterbury, UK). The pins (with the heads removed) were coated in two thin layers of insulating varnish (RS Components, Northants, UK) with 0.5 mm of the tip exposed. After each current delivery, the electrode was left in place for an additional 30 s. Sham-operated rats were prepared exactly as above and the electrode was in each case lowered but no current was passed.

2.3. Histology

Rats were deeply anaesthetized with 1 ml of Sagatal (Sigma, 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 formalin solution and 'sunk' 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 sledge 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 [25] using a light microscope (Olympus, BH2).

2.4. Experiment 1

2.4.1. Apparatus

Four identical fully automated conditioning chambers, housed within sound-attenuating cases containing ventilation fans (Cambridge Cognition, Cambridge, UK), were used in Experiment 1. Each of the inner conditioning chambers consisted of a plain steel box (25 cm × 25 cm × 22 cm high) with a Plexiglas door (19 cm × 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 (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) as the target stimulus (i.e. the CS paired with the UCS) and a 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 1 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 was by a Acorn Archimedes RISC computer programmed in Basic with additional interfacing using an Arachnid extension (Cambridge Cognition).

2.4.2. Procedure

Rats were handled for approximately 10 min per day for 2 weeks prior to any procedure and water deprivation was introduced the day before shaping began. The one stage conditioning procedure was preceded by shaping and followed by reshaping before the test phase.

2.4.2.1. Pre-conditioning. Rats were shaped (over 2 days) until all drank from the waterspout and individually assigned to a conditioning box for the duration of the experiment, counterbalanced for both lesion and behavioural condition.

There then followed 10 days of pre-conditioning, in which rats drank in the experimental chamber for 15 min each day (timed from first lick). The drinking spout was illuminated throughout, but there were no other stimuli presented in this phase. Latency to first lick was measured as an indicator of habituation to the experimental context. Total number of licks was also recorded each day to assess preexisting differences in drinking (prior to any conditioning).

2.4.2.2. Conditioning. Conditioning was conducted in 1 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 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, with a further 5 min left in the apparatus following the second shock presentation.

Depending on experimental group, the footshock followed either 0s (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 (ISI) that simply added to the overall duration of the session in the trace groups. In the absence of drinking, there was nothing to record.

2.4.2.3. Reshaping. On the day following conditioning, animals were reshaped following the same procedure as in pre-conditioning sessions. This was in order to re-establish drinking after conditioning. Reshaping also provided measures of conditioning to the box context (latency to first lick).

2.4.2.4. Test. 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) in a suppression ratio (measured as A/(A + B), to assess conditioning whilst taking baseline variation into account). These were extinction tests and 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.

2.4.3. Design and analysis

The experiment was run in 3×2 factorial design for later analysis of variance (ANOVA). Thus, 75 rats were assigned to six experimental conditions, counterbalanced for box. The between subjects factors were lesion (at levels sham, shell and core) and trace (at levels 0 and 30 s). The dependent variable to assess conditioning at test was the suppression ratio. Pre-conditioning drink levels were also assessed in a 3×2 mixed design ANOVA with the repeated measures factor of days (at 10 levels) to check for any preexisting differences in total amount drunk.

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).

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 pairwise comparisons that were necessary, in order to determine the basis for any reduction in the trace conditioning effect (e.g., to assess whether this could be mediated by general disinhibition). This means that the inflation of familywise Type 1 error rate was minimal [8].

2.5. Experiment 2

2.5.1. Apparatus

Experimental testing was conducted within a set of six fully automated ventilated conditioning chambers, identical to those described for Experiment 1, except that they were set up for the appetitive task variant. The food magazine (recessed in a side-wall of each of the chambers) 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).

As in Experiment 1, two experimental stimuli were available as potential predictors of food delivery. The target stimulus was different (mixed frequency noise CS), presented via a loudspeaker inset on the roof of the chamber, set at 80 dB including background and of 5 s duration. The background stimulus was the same (three wall mounted stimulus lights and the house light flashing on and off for 5 s).

2.5.2. Procedures

Allocation to conditioning groups was also counterbalanced by box. In Experiment 2, acquisition was conducted over 14 days. On each day there were eight pairings of noise CS and food presented at 10 or 0 s trace. The strength of associative learning was also tested over 2 days' extinction. However, in Experiment 2, the test order was not counterbalanced: the day 1 extinction test was to the background light to get the best possible test of contextual conditioning to this stimulus; the day 2 extinction test was to the noise CS.

2.5.2.1. Pre-conditioning. There were 2 days shaping to accustom rats to eating from the magazine. These gave access to a preload of 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 2 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) nosepokes on either of these days were given additional shaping at the end of the session (total four rats on day 1, with a repeat reshape for one of these rats on day 2).

2.5.2.2. Conditioning. Conditioning consisted of eight signalled rewards presented over 20 min. Depending on the experimental group, the reward (UCS) was delivered directly (in the contiguous group) or 10 s after CS offset (in the trace group). Conditioning trials were presented throughout the 20 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 ISI length. Throughout the 20 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 prior to the CS (pre-CS responding); during the 5 s of the CS (CS responding); during the 10 s trace interval between CS and UCS, where applicable (ISI responding); 5 s after the delivery of the UCS in acquisition (UCS responding); in

the remainder of the session not included in the aforementioned response bins (residual responding). The residual measure excluded responding in the ISI, where applicable.

2.5.2.3. Extinction tests. On day 1, the rats were presented with the light background, on day 2 they were presented with the tone CS. In each case, there were again eight stimulus presentations over 20 min but this time in the absence of any food deliveries. The number of nose pokes was recorded 5 s prior to stimulus onset (pre-CS for the noise; pre-stimulus for the light), during the CS or background (CS or stimulus), and during the remainder of the session (residual responding).

2.5.3. Design and analysis

In most respects the design and analysis was as reported for Experiment 1. There were six experimental groups run in a 3×2 factorial design, counterbalanced for box and previous experimental experience. The between subject factors were lesion (at levels sham, shell and core) and trace (at levels 0 and 10 s), and to assess effects over the course of acquisition, the repeated measures factor was days. The dependent variable was in each case the number of nose pokes into the food magazine. To separate out lesion effects on baseline responding in acquisition, both conditioning to the target stimulus and responding during the UCS deliveries were analyzed using difference measures. Difference measures were similarly used in extinction, where required. In each case, they were calculated as CS (or stimulus) responding minus responding during the equivalent pre-CS (or pre-stimulus) period and UCS responding (i.e. in the equivalent period of food delivery) minus responding during the pre-CS.

In the trace group, the responding of the animals during the 10 s trace between CS offset and sucrose delivery was also tested for any effects of lesion. This response period was broken down into two second bins of time and analysed using a $3 \times 5 \times 14$ repeated measures ANOVA, with bins (5) and days (14) as repeated measures factors. Lesion effects on responding in the ISI needed to be adjusted for those seen in the equivalent average of the inter-trial-interval ($ITI_{10\,s\,average}$). To distinguish effects on anticipatory responding within the trace interval from more general effects on contextual conditioning, the analysis was therefore repeated with the $ITI_{10\,s\,average}$ as a covariate.

3. Results

3.1. Histology

As would be expected with the electrolytic method, the lesions were variable in size. Photographs of representative lesions are shown in Fig. 1. 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 show little if any bilateral encroachment on the alternative (core versus shell) subfield [38,39]. The largest core lesion showed some overlap with the area damaged by the shell lesions, but this was unilateral shell damage in the two cases retained in the study.

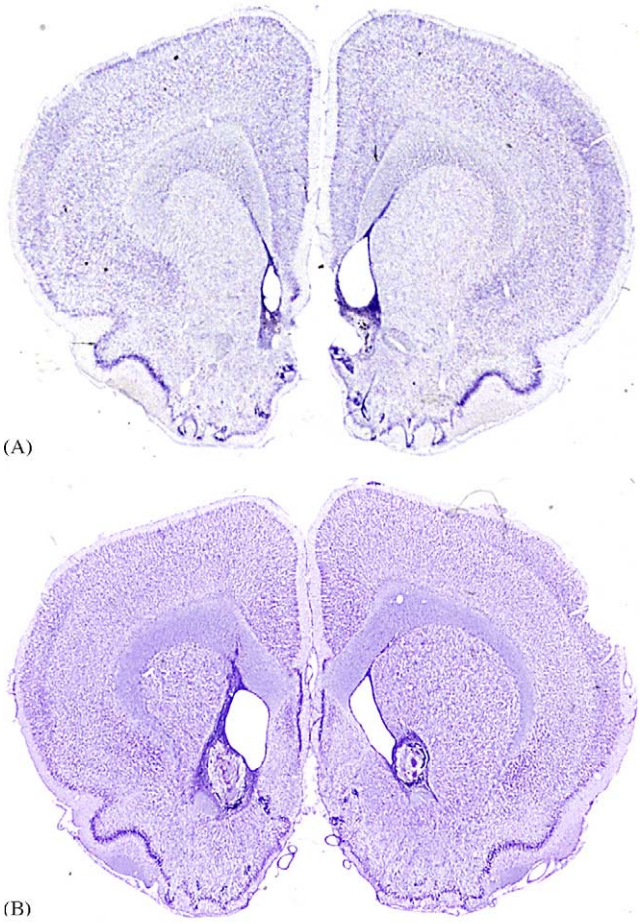

Fig. 1. A: photograph of a representative shell lesion: magnification 7× taken on a Kodak 620 (image adjusted only for contrast); B: photograph of a representative core lesion: magnification 7× taken on a Kodak 620 (image adjusted only for contrast).

Seven rats were excluded: three in the shell-lesioned group, as their lesions failed to meet the first criterion of consistency, because the lesions were located more posteriorly and were much more restricted in extent; in addition, three from the core lesioned one from the shell lesioned group were excluded as their lesions were associated with more extensive non-specific damage, including bilateral damage to the alternative field of n. acc. The largest and smallest extents of the lesions retained are shown in Fig. 2.

In addition to any (mostly very limited) damage to shell in core lesions and vice versa, there was nonetheless inevitably still nonspecific damage after the electrolytic lesions, for example, there was generally some damage to the caudate putamen after core lesions, and damage to medial structures after shell lesions. The largest retained core lesion extended to the anterior commissure, caudate putamen (including the lateral stripe) and the dorsal endopiriform cortex. The largest retained shell lesion extended to the semilunar nucleus, the nucleus of the vertical limb of the diagonal band, and the medial and lateral septal nuclei. Both shell and core lesions

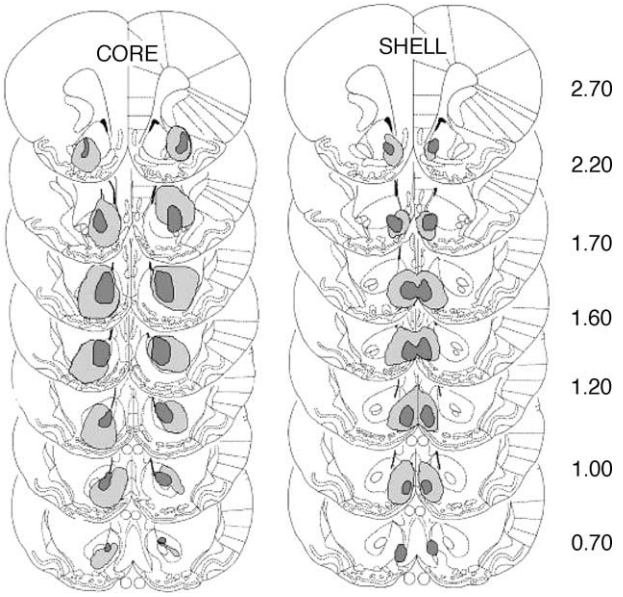

Fig. 2. Drawings of smallest and largest core and shell accumbens lesions superimposed on coronal sections from the atlas of Paxinos and Watson [25] (Figs. 9–15, 2.7–0.7 mm anterior to bregma, Paxinos and Watson, 1997, reproduced with permission). Dark grey fill: small lesion; light grey fill: large lesion.

were associated with damage to the anterior olfactory nucleus (posterior part), the islands of calleja, the medial forebrain bundle and the ventral pallidum. As would be expected, sham-operated animals showed only very minimal evidence of mechanical damage, at worst just very fine electrode tracts and these visible only on microscopic examination. Moreover, there was no evidence on any test measure for a statistical difference between the sham lesions made at the core versus shell lesion coordinates, so all the analyses reported below collapse the sham groups.

3.2. Experiment 1: aversive procedure

All analyses were carried out after the seven histological exclusions (total four from the shell group and three from the core group, see above). After counterbalancing for box, these exclusions left us with the following group sizes: 0 s sham lesion, $n = 9$; 0 s shell lesion, $n = 15$; 0 s core lesion, $n = 11$; 10 s sham lesion, $n = 11$; 10 s shell lesion, $n = 9$; 10 s core lesion, $n = 13$. In addition, due to a technical failure, the data on latency to begin drinking were missing, for day 1 of pre-conditioning only.

3.2.1. Pre-conditioning

Over the 10 days prior to conditioning, there was no overall effect of lesion, trace or the interaction on the total amount drunk in these sessions, all $F < 1$. There was an interaction between days and lesion, $F(18,558) = 2.80$, $p < 0.001$, but this did not arise in consequence of any systematic differences in the pattern of fluctuation. As expected, there was an effect of days, both on the total amount drunk and on the latency to begin drinking, minimum $F(8,496) = 3.54$, $p = 0.001$, reflecting habituation to the experimental chambers. In addition, there was an overall effect of lesion on the latency to begin drinking in the experimental chambers, $F(2,62) = 4.48$, $p < 0.05$. This arose because, prior to any conditioning, the core-lesioned group had the longest drink latencies, significantly longer than those seen in the shell-lesioned group, $t(46) = 2.04$, $p < 0.05$, none of the other comparisons was significant, maximum $t(42) = 1.81$, for core versus sham-lesioned. There were no main effects or interactions to suggest that the groups were not well-matched with respect to trace-condition-to-be, maximum $F(2,62) = 2.71$.

3.2.2. Reshape

After conditioning, on the reshaping day prior to the stimulus tests, there was no evidence of any differences between the groups on the latency to make the first lick, maximum $F(2,62) = 1.14$. This also means that there was no evidence that the level of suppression to the box context was influenced by lesion or trace condition.

3.2.3. Test: background stimulus

There was no evidence that the level of suppression to the experimental background stimulus was influenced by lesion or trace condition, maximum $F(2,62) = 2.58$.

3.2.4. Test: CS

There was an effect of trace, $F(1,62) = 22.61$, $p < 0.001$, an effect of lesion, $F(2,62) = 5.25$, $p < 0.01$, and a lesion × trace interaction $F(2,62) = 3.88$, $p < 0.05$. As expected, there was a clear difference between contiguous and trace conditioned groups in the sham group, $t(18) = 2.81$, $p < 0.05$, and also in the shell group, $t(22) = 4.52$, $p < 0.001$. However, in the core group the difference between contiguous and trace conditioned groups was not significant, $t(22) = 1.15$. The normal difference between the groups was not reduced because of increased conditioning over the trace interval, but rather because the contiguous group was relatively unsuppressed, significantly less so than the corresponding sham group, $t(18) = 2.13$, $p < 0.05$ (see Fig. 3). However, disinhibition in the core group does not provide a convincing explanation of the overall pattern of effects because within the trace condition the only significant differences were between the shell and core and the shell and sham groups, minimum $t(20) = 2.20$, $p < 0.05$, in each case the shell group being the least suppressed.

3.3. Experiment 2: appetitive procedure

After counterbalancing for box, the histological exclusions left us with group sizes of 10 per cell in all groups except the shell and core 10 s groups, in each of which there were 14 rats.

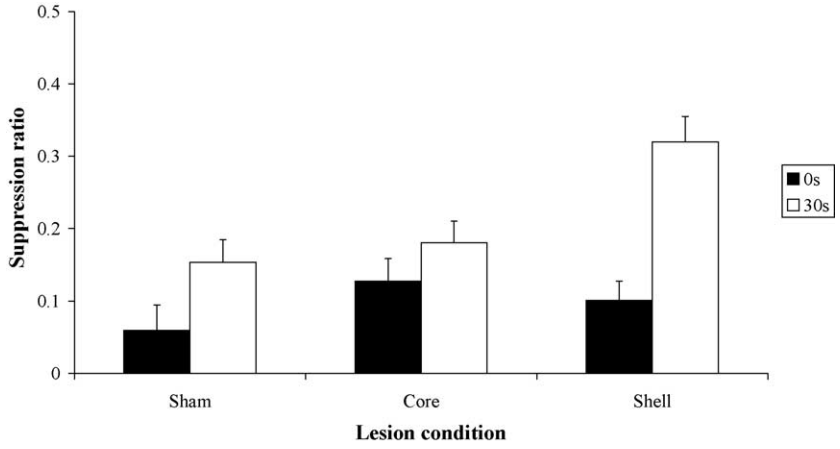

Fig. 3. Experiment 1: discrete cue conditioning measured as mean suppression ratio for sham-, core- and shell-lesioned rats. Bars show the results of single extinction tests, for each lesion condition, with CS previously paired with foot shock either at 0 s (black bars) or at a 30 s trace (white bars). Error bars represent the standard errors of the group mean. Group sizes: 0 s sham lesion, $n = 9$; 0 s shell lesion, $n = 15$; 0 s core lesion, $n = 11$; 10 s sham lesion, $n = 11$; 10 s shell lesion, $n = 9$; 10 s core lesion, $n = 13$.

3.3.1. Pre-conditioning

There were no significant effects of lesion, trace or their interaction, maximum $F(2,62) = 2.37$, indicating that response rates were matched between the groups prior to conditioning. There was only the expected main effect of days, $F(1,62) = 10.61$, $p < 0.005$ because magazine entries increased from day one (mean = 112.09) to day 2 (mean = 132.24).

3.3.2. Conditioning tests

3.3.2.1. Pre-CS responding. Here there were group differences by lesion and by trace, minimum $F(1,69) = 7.33$, $p < 0.01$. Furthermore, there was a significant lesion × trace interaction, $F(2,69) = 3.42$, $p < 0.05$. This arose because shell-lesioned rats in the 10 s trace group showed significantly elevated responding compared to the shell lesion 0 s trace group, $t(22) = 3.12$, $p = 0.005$, and also in comparison with sham 10 s trace rats, $t(22) = 4.11$, $p < 0.001$.

There was no overall effect of days, $F(13,806) = 1.27$, although days did interact significantly with trace, $F(13,806) = 2.49$, $p < 0.005$. This interaction may have arisen because of a small progressive decrease in nose-poking over days in the 0 s trace group (day 1 mean = 3.03, day 14 mean = 1.97), perhaps in consequence of increased anticipatory responding during the CS presentations. In contrast, the 10 s trace group showed generally higher responding but little systematic change in pre-CS nose-poking over days (day 1 mean = 3.99, day 14 mean = 4.77), presumably because, for this group, the noise was not a better predictor than the background contextual stimuli. No other main effects or interactions were significant, maximum $F(26,806) = 1.29$.

3.3.2.2. CS responding. Because there were significant effects on pre-CS response rates, analyses of responding during presentation of the noise used a difference measure (as previously described). There was the predicted effect of trace, both overall, $F(1,62) = 141.53$, $p < 0.001$, and in interaction with days, $F(13,806) = 24.42$, $p < 0.001$. However, there were no effects involving lesion, maximum $F(2,62) = 1.59$. Therefore, there was less conditioning in 10 s compared with 0 s conditioned rats which responded more overall during the course of acquisition (see Fig. 4). This difference between trace and contiguously conditioned groups was seen irrespective of lesion condition. In other words, neither shell nor core lesion affected the development of this trace conditioning effect.

3.3.2.3. ISI responding. On the unadjusted scores, there was a main effect of days, $F(13,1820) = 22.67$, $p < 0.001$, because, as would be expected, responding within the ISI increased over time (from day 1 mean = 0.12, to day 14 mean = 0.64). There was also a significant interaction between days and bins, $F(52,1820) = 1.38$, $p < 0.05$. However, examination of means revealed no consistent pattern to the changes in the distribution of responding over days. A main effect of lesion, $F(2,35) = 6.39$, $p < 0.005$, arose because of the following differences in the means of the lesion groups: shell = 23.50; core = 12.29; sham = 13.86. No other effects or interactions were significant, maximum $F(4,1820) = 1.34$.

However, repeating the above analyses with the ITI average as covariate, there were no significant effects or interactions, maximum $F(4,1768) = 2.13$. This means that once the effects of the lesion in the ITI were taken into account, neither the level of responding within the ISI (nor the distribution of responding within the ISI) were influenced by the stage of conditioning or whether the rats had a lesion.

3.3.2.4. UCS responding. There was no overall effect of trace, $F(1,62) = 0.00$. Although there seemed to be some effect of trace in interaction with days, $F(13,806) = 1.88$, $p < 0.05$, inspection of the means showed little difference in responding by trace over the course of acquisition, only

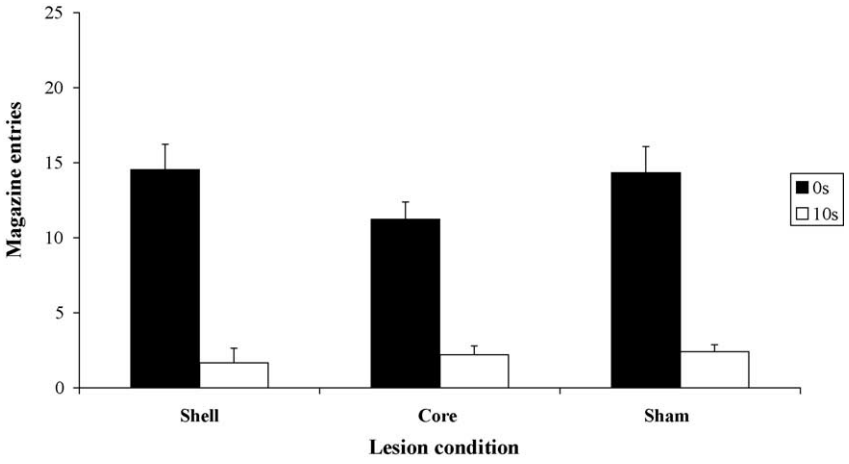

Fig. 4. Experiment 2: discrete cue conditioning measured as mean magazine entries for sham-, core- and shell-lesioned rats. Bars show the results of acquisition tests (collapsed over days) measured as difference scores (CS minus pre-CS). For each lesion condition, CS previously paired with food deliveries either at 0 s (black bars) or at a 10 s trace (white bars). Error bars represent the standard errors of the group mean. Group sizes: 0 s sham lesion, $n = 10$; 0 s shell lesion, $n = 10$; 0 s core lesion, $n = 10$; 10 s sham lesion, $n = 10$; 10 s shell lesion, $n = 14$; 10 s core lesion, $n = 14$.

transient differences in responding on days 1 and 11. More importantly, there was neither an overall effect of lesion, nor a lesion by trace interaction, maximum $F(3,62) = 1.84$. Although there was a main effect of days, $F(13,806) = 9.56$, $p < 0.001$, no other interaction with days was significant, maximum $F(26,806) = 1.01$.

The above analyses suggest that neither behavioural nor lesion condition affected animals' motivation to respond for the sucrose rewards.

3.3.2.5. Residual responding. Analysis of the ITI (residual responding) showed a similar pattern of results to the pre-CS period. There were significant main effects of trace and lesion, minimum $F(1,62) = 8.53$, $p = 0.005$, and a significant interaction between these factors, $F(2,62) = 4.35$, $p < 0.05$. Independent *t*-tests showed that the 10 s conditioned shell group responded significantly more than both the 10 s conditioned sham group and the 0 s conditioned shell group, minimum $t(22) = 3.17$, $p < 0.005$. Responding did fluctuate over time and this was shown statistically as a significant main effect for days, $F(13,806) = 3.49$, $p < 0.001$, which also interacted with trace, $F(13,806) = 3.32$, $p < 0.001$. This interaction arose because of a progressive decrease in 0 s conditioned animals' responding over days, which was not seen in 10 s conditioned rats. This latter group maintained responding over days, consistent with a generally higher level of contextual conditioning. However, no other interactions with lesion were significant, maximum $F(26,806) = 1.42$.

Therefore, as shown in the pre-CS analysis, the shell-lesioned animals group showed elevated responding in the ITI in comparison to sham-operated animals. Moreover, this effect was restricted to the trace (10 s) group. This suggests that the shell lesion increased contextual conditioning as measured by responding in the remainder of the session, selectively in the group with the weak target CS (see Fig. 5A).

3.3.3. Extinction tests

3.3.3.1. Background light stimulus

Pre-stimulus responding. Analysis of the pre-stimulus at test to the background showed that there was again an effect of trace, $F(1,62) = 4.89$, $p < 0.05$. The 10 s group nose poked overall less (mean = 1.83) than the contiguously conditioned group (mean = 3.48). There was no effect for lesion or trace × lesion interaction, maximum $F(2,62) = 2.04$.

Stimulus responding. For responding to background, there were no significant effects or interactions, all $F < 1$. However, responding was at floor, with all groups showing even less responding during the flashing light presentations than during the corresponding pre-stimulus interval. Thus, there was no evidence for any effects on contextual conditioning, or even much contextual conditioning, as measured by responding to the experimental background stimulus.

Residual responding (during light tests). Analysis of responding in the remainder of the session during the light tests showed significant effects of both trace and lesion, minimum $F(2,62) = 7.21$, $p < 0.005$. The lesion × trace interaction did not in this case reach significance, $F(2,62) = 1.84$. However, as shown in Fig. 5B, there was again elevated responding in the 10 s shell group compared to all other groups. Planned comparisons confirmed that the 10 s shell group responded significantly more than both the 0 s shell group, $t(22) = 2.25$, $p < 0.05$, and the 10 s sham group, $t(22) = 2.91$, $p < 0.01$. This confirms that the flashing light was a relatively ineffective stimulus, as already suggested by its failure to increase responding on presentation during extinction tests. Even in the absence of the flashing light stimulus, that should have produced some generalization decrement, the trace-conditioned shell group continued to show enhanced conditioning to the box cues.

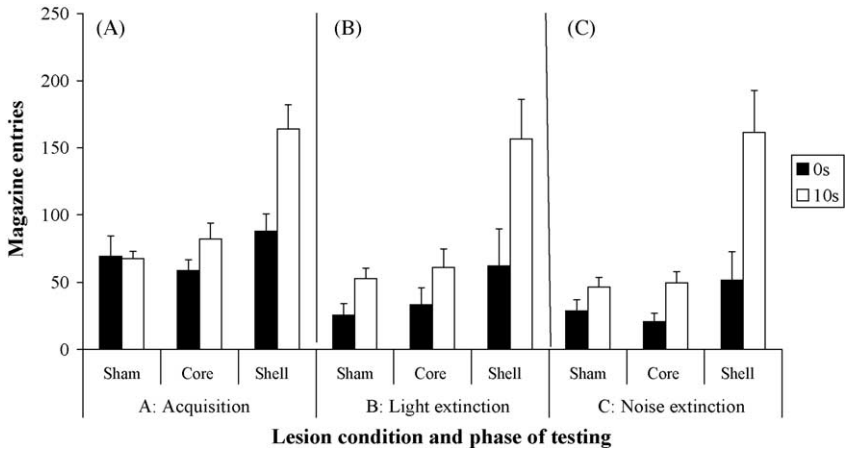

Fig. 5. Experiment 2: contextual conditioning measured as responding in the inter-trial-interval. Bars show the mean magazine entries for sham-, core- and shell-lesioned rats, separately for each phase of the experiment: (A) acquisition (overall average collapsed over days); (B) during the light extinction tests; (C) during the noise extinction tests. For each lesion condition, CS (previously) paired with food deliveries either at 0 s (black bars) or at a 10 s trace (white bars). Error bars represent the standard errors of the group mean. Group sizes: 0 s sham lesion, $n = 10$; 0 s shell lesion, n = 10; 0 s core lesion, $n = 10$; 10 s sham lesion, $n = 10$; 10 s shell lesion, $n = 14$; 10 s core lesion, $n = 14$.

3.3.3.2. Noise CS

Pre-CS responding. Analysis of the pre-CS responding showed a significant main effect for lesion, $F(2,62) = 8.15$, $p < 0.005$, and a significant lesion × trace interaction, $F(2,62) = 4.29$, $p < 0.05$. *T*-tests confirmed that there was elevated responding in the 10 s shell group in comparison to all other groups, minimum $t(22) = 2.19$, $p < 0.05$. By contrast, the 0 s shell group showed no such elevation in responding compared to core lesioned or sham operated rats, maximum $t(18) = 0.79$. The main effect for trace was not significant, $F(1,62) = 3.31$.

CS responding. As would be expected, during noise tests rats continued to show a significant effect of trace, $F(1,62) = 85.49$, $p < 0.001$, because the 0 s conditioned groups (for whom the noise was a better predictor) exhibited higher overall responding to the noise (mean = 11.17), than the 10 s groups (mean = 1.52). Confirming the result seen in acquisition, there was no effect of lesion and there was no lesion × trace interaction, maximum $F(2,62) = 1.89$. Thus, as in acquisition, the difference between 10 and 0 s conditioned groups was not disrupted by the presence of either a core or a shell lesion. Moreover, again confirming the result seen in acquisition, there was no indication of elevated responding to the target stimulus in the 10 s shell group.

Residual responding (during noise tests). Analysis of responding in the remainder of the session provided further confirmation that there was increased nose poke responding in the 10 s shell group. There was a significant lesion × trace interaction, $F(2,62) = 3.64$, $p < 0.05$. There were also overall main effects for lesion, $F(2,62) = 9.54$, $p < 0.001$ and trace, $F(1,62) = 11.55$, $p = 0.001$. The main effect for lesion reflected elevated overall responding in the shell-lesioned groups, but (as would be expected given that there was also a significant interaction) this effect was carried largely by increased responding in the 10 s group (see Fig. 5C). The 10 s shell group responded significantly more than both the 0 s shell group, $t(22) = 2.68$, $p < 0.05$, and the 10 s sham group, $t(22) = 3.07$, $p < 0.01$.

4. Discussion

These experiments tested whether shell or core lesions to n. acc. would increase or impair conditioning to a discrete cue presented with or without a trace interval as well as to contextual cues, in both aversive and appetitive procedures. In Experiment 1 (aversive), the core lesion reduced the difference between trace and contiguously conditioned groups, in responding to the discrete noise stimulus. However, neither lesion had any detectable effect on contextual conditioning, whether this was measured as hesitancy to drink after conditioning in the experimental chamber or conditioned suppression upon later presentation of the flashing light. In Experiment 2, the shell lesion clearly increased contextual conditioning to the cues provided by the experimental chambers, selectively in the trace conditioned group, but neither lesion had any detectable effect on discrete cue conditioning. Again the flashing light background stimulus did not acquire much associative strength (this measure is further discussed below). Thus, whilst the shell and core lesions produced dissociable effects on discrete cue and contextual conditioning, the conclusions to be drawn depend on the procedural variant in use.

4.1. Baseline measures of responding

Before the interpretation of effects on conditioning can be considered, possible effects on baseline responding must be taken into account. In Experiment 1, the aversive procedure, analysis confirmed that the groups were matched on baseline

drinking measures before the start of conditioning. Although the core lesioned group had longer drink latencies this difference did not persist to the reshaping day. Thus there were no systematic group differences in drinking and we took the additional precaution of using suppression ratios on the test days, to adjust for individual variation in lick rate immediately prior to presentation of the experimental stimuli.

In Experiment 2, the appetitive procedure, we were similarly able to distinguish non-specific effects, e.g., on collection of food reinforcement (UCS responding). There was no sign that motor or motivational effects affected the rats' nose poking to collect the sucrose UCS. Whilst there were lesion effects on nose poking in the remainder of the session, these differences were confined to the trace-conditioned groups suggesting that they reflected an associative effect, i.e. contextual conditioning to apparatus cues, rather than a non-specific effect of the lesion, mediated through (e.g.) hyperactivity.

4.2. What do the findings in the aversive procedure tell us?

Contrary to expectation based on the known effect of amphetamine in this procedure [21] and what might be anticipated from the effects of shell lesions in LI [10,32,38,39], the shell lesion did nothing to increase associative learning over the trace interval. In fact the shell trace group learned less than both the sham and the core trace groups and showed, if anything, a bigger difference relative to their contiguously conditioned counterparts. The core lesion, however, reduced the difference between trace and contiguously conditioned groups, and this effect was mediated by decreased conditioning in the contiguously conditioned core group relative to their sham-lesioned counterparts. There was no difference between sham and core trace groups, but general disinhibition is a poor account of the overall pattern of results because most disinhibited of all were the shell trace group and the shell-lesioned animals showed the biggest trace conditioning effect. Thus, these findings suggest that the core lesion reduced whilst the shell lesion tended to increase the differential levels of conditioning produced through manipulation of salience using a trace interval (whilst there was little difference in contiguous groups, in the trace groups, those in the shell condition were significantly less suppressed).

Similarly, excitotoxic n. acc. shell lesions were reported to be without effect in an aversive trace conditioning procedure, whilst lesions to the core impaired conditioning to the CS so that there was no difference between short and long trace conditioned groups [23].

4.3. What do the findings in the appetitive procedure tell us?

The appetitive procedure allowed us to assess the acquisition of associative strength 'on-the-baseline'. However, contrary to the results of Experiment 1, in Experiment 2, neither lesion affected the differential rates of learning about the discrete CS (noise), in either the trace or contiguously conditioned groups.

In the ITI (measured as 'residual responding'), there was increased conditioning to the context provided by the experimental box in the shell-lesioned rats conditioned with a trace interval. However, in the ISI there was no additional effect of lesion on the overall level of responding (or the distribution of responses within the trace).

The subsequent extinction tests confirmed that, as in acquisition, the lesions did not affect the relative apportioning of associative strength to the discrete (noise) CS in trace and contiguous groups. Contrary to expectation, based on the increased excitation to box context seen in the shell-lesioned group, there was no detectable effect of lesion on conditioning to the background stimulus (light). However, responding was at floor, possibly because the unimodal light lacked the necessary characteristics to be an effective context [27]. Moreover, as was the case in acquisition (and on both days) the extinction tests showed there was increased nose poke responding to box context in the shell-lesioned group conditioned over a trace interval.

This selective effect on contextual conditioning (in trace but not contiguous groups) finds no explanation in terms of non-specific effects of the shell lesion (e.g., motor or motivational).

4.4. Contextual conditioning

The experimental background stimulus provided by the flashing lights presented for the duration of the conditioning sessions, provides a method to assess conditioning to a contextual stimulus, over and above the more normal measures of contextual conditioning provided by the level of responding to the conditioning boxes [27]. However, this kind of experimental stimulus necessarily differs from the more naturalistic context provided by the multimodal box cues. The respective measures are also different. In the appetitive procedure, responding to all of the box cues, including the experimental background, was measured by differences in the level of nose poking in the ITI. Then over and above responding between stimulus presentations, we tested conditioning to the experimental light stimulus in extinction tests. In the aversive procedure, the best measure of conditioning to the box cues is provided by latency to drink on the reshaping day. The flashing light has to be absent on the reshaping day or it would be extinguished before the extinction test. Thus, the level of contextual conditioning was quite probably underestimated because of generalisation decrement, and no lesion effect was found on suppression to box context on the reshaping day. However, generalisation decrement did not prevent us from seeing increased responding to box context in the absence of the flashing light in the shell trace-conditioned group in the appetitive procedure.

It is true that this kind of experimental stimulus can suggest different conclusions from more conventional measures

of responding to the more naturalistic multimodal apparatus cues [27,41]. In earlier appetitive experiments, we have found some evidence that nose poke responding can shift from experimental background stimulus to the box cues with prolonged testing, perhaps because of differential latent inhibition to these cues, because the experimental background is a flashing light and this kind of cue is particularly effective in promoting latent inhibition [3]. In earlier aversive experiments, we found that conditioning to an experimental background stimulus was increased both by serotonergic depletion [2] and treatment with systemic amphetamine [21]. In the present study, however, the light supported little conditioning and this component of the contextual conditioning was not increased by either of the n. acc. lesions.

By contrast, we did see a very clear effect on contextual conditioning to the multimodal cues provided by the experimental chambers. Relatively little is known about appetitive contextual conditioning and its neural bases and this effect (that reproduced in both acquisition and extinction tests) is an original finding. There is a theoretical possibility that the apparent increase in contextual conditioning after the shell lesion in fact reflects increased responding within the local context provided by the ISI that generalizes to the ITI. Inevitably, this possibility arises in any trace procedure where increased ITI responding could in principle result from generalization from the ISI. Animals can discriminate these intervals on the basis of their temporal difference [13]. To accommodate the required number of conditioning trials (eight over 20 min) per day, the maximum duration of the ITI was necessarily constrained, but it was always at least 1.5 times the ISI in duration. Moreover, the variability of the ITI should have limited generalization from the fixed (short duration) ISI.

Anyway, there is no evidence for this interpretation of our data and some evidence against it: responses in the ISI were considerably higher than in the ITI for the equivalent average duration and taking the differences in the ITI into account (using analysis of covariance), there was no evidence that the lesion affected responding in the ISI. This is consistent with other studies using the same appetitive procedure in which drug effects in the ITI were independent of those in the ISI [3] and vice versa [12].

By contrast, in the Experiment 1 aversive procedure, there was no indication that either lesion increased aversive contextual conditioning as measured by latency to recommence drinking after conditioning (on the reshape day) or suppression to the experimental background stimulus. Aversive conditioning to context can be increased by lesions of the hippocampus [41] and n. acc. [29]. With the aversive trace conditioning procedure that was used in the present study, we have previously found increased conditioning to background stimuli, after both serotonergic depletion [2] and systemic amphetamine [21], so (as discussed above) the parameters should have been sensitive to any treatments that promote contextual conditioning. More restricted n. acc. core lesions have previously been found to increase aversive contextual conditioning [23]. These lesions were made excitoxically rather than electrolytically but the core lesions impaired discrete cue conditioning (as here), and (as here) alternative discrete stimuli were presented the within the context.

In any event, with procedures as similar as possible, and the same lesions, the increased contextual conditioning in the appetitive trace conditioned group did not reproduce in the aversive procedure. The difference between the trace intervals in use is discussed below.

4.5. Why the difference in findings between the aversive and appetitive procedures?

Within a single motivational system, conditioning with discrete and contextual stimuli could rely on the same or different neural processes. The comparison of appetitive and aversive task variants was intended to test the generality of the observed behavioural effects.

The effect of the core lesion seen in the aversive procedure on the difference between trace and contiguous groups did not reproduce in the appetitive procedure. In fact, there was no sign that either lesion influenced the acquisition of discrete cue conditioning over the trace interval. However, the lesions were not simply ineffective in the appetitive procedure in that we found a marked effect of the shell lesion on contextual conditioning in the trace conditioned group. This difference in contextual conditioning between the aversive and appetitive procedure could be attributable to the difference in the number of conditioning trials that were necessary in the two procedures. In the appetitive trace group the higher number of conditioning trials might have promoted the difference in conditioning between trace and contiguous groups that was so clearly enhanced by the shell lesion.

We also used different trace intervals in the appetitive and aversive procedures. The optimum ISI for conditioning is known to depend on the response system being measured [18] and we needed to use a longer trace interval in the aversive procedure reliably to weaken conditioning to the trace CS. With respect to the comparison made with the appetitive study, there are good theoretical grounds to suppose that the use of a longer trace interval should promote more rather than less contextual conditioning [22,28]. Thus, the difference in the trace interval provides no obvious explanation of the difference between aversive and appetitive variants that we observed here because the shell lesion effect on contextual conditioning was seen in conjunction with the use of a shorter trace interval in the appetitive procedure and absent in aversive procedure that used a longer trace interval (that should promote contextual conditioning). In neither procedure did the shell lesion affect conditioning to the trace CS so although confined to the trace group, the effect on contextual conditioning was independent of any systematic shift in discrete cue conditioning.

In earlier studies we have shown that these trace intervals are suitable for detecting drug and lesion effects on

both conditioning to the trace CS and contextual stimuli, in both aversive [2,21] and appetitive variants [3]. Thus, the use of different trace intervals in aversive and appetitive procedures is an unlikely account of the lack of lesion effect on aversive contextual conditioning reported here.

An additional consideration arises in that effects on associative learning could interact with the different response requirements in appetitive and aversive procedures, in which learning is shown by increased and decreased response rates, respectively. But this would have to be an interaction in the sense that general shifts in responding do not explain the pattern of results that we observed. For example, in the appetitive procedure, the shell-lesioned animals alone showed elevated responding but only to contextual stimuli and this effect was confined to the trace group. Similarly, some difference in the apparent level of conditioning supported in appetitive and aversive procedures, is likely to be produced by differences in the specificity of response elicited [14]. Differences in the specificity of response elicited (consummatory versus preparatory) could in principle contribute to the observed differences in discrete cue versus contextual conditioning. However, any such response differences cannot account for the selective effect in the appetitive trace conditioned group found here.

The lesions were variable (see below) but the use of the same lesioned groups in the aversive and appetitive experiments means that the behavioural differences observed cannot be due to systematic differences in lesion placements between the studies.

Comparing across previous studies done with essentially the same aversive and appetitive procedures, amphetamine too has had inconsistent effects. For example, whilst treatment with systemic amphetamine increased conditioning to background in the aversive variant [21], it had no comparable effect (on responding in the ITI) in an appetitive variant [12]. This difference across task variants is essentially opposite to that seen after the n. acc. shell lesion that increased contextual conditioning in the appetitive but not the aversive procedure. Thus, the differing effects of n. acc. lesions and systemic amphetamine are inconsistent with the argument advanced earlier that such lesions result in DA hyperactivity so that effects of systemic amphetamine and dopaminergic lesions should therefore correspond (as they do for LI). Moreover, we find (using the same procedure) the same increase in contextual conditioning, again confined to trace groups, after treatment with low dose haloperidol [3]. Either such lesions do not necessarily result in DA hyperactivity, or the relationship between functional activity and behaviour is different for different kinds of selective learning task.

4.6. Implications for LI

The results of the present study suggest that the reduction in stimulus salience produced by trace conditioning is functionally different from other methods of reducing stimulus salience, e.g., through LI. This is because to the extent that there was an effect (in the aversive procedure), this was opposite to the pattern of effects of n. acc. shell and core lesions seen in LI, where shell and core lesions reduce and enhance the effect, respectively [10,32,34,38,39]. Of course this difference in task sensitivity might arise because changes in stimulus salience do not provide the best account of LI, e.g., an alternative account is based on the behavioural flexibility that moderates the switch to current contingencies [33–35]. In any event, the present findings suggest that, irrespective of the best explanation of LI, trace conditioning involves different psychological processes from LI, at least when these are tested in aversive procedures.

Moreover, we saw different effects on associative learning depending on whether an aversive or an appetitive procedure was in use. This inconsistency suggests the need for further tests of LI in appetitive as well as aversive procedures. What evidence there is shows that effects (in this case of amygdala lesions) on LI can similarly depend on whether the effect is tested in an aversive or an appetitive procedure [4].

4.7. Neural substrates of effects on conditioning

The lack of effect of shell lesions on discrete cue conditioning is consistent with previous data [23,29]. Parkinson et al. also report impaired discrete cue conditioning after core lesions [23] as was seen here in the aversive procedure. In LI experiments, core (but not shell) lesions have similarly been reported to reduce basic conditioning in the (contiguous ‘non-preexposed’) control groups, in an aversive procedure, as was used here, although this finding seems to depend on the laterality of the lesion placement [38].

The increase in contextual conditioning after the shell lesion is inconsistent with the findings of an earlier study [29]. The lesions were electrolytic as here but encroached on core, and although the trace conditioning procedure was aversive it was different from our aversive procedure. The increase in contextual conditioning after the shell lesion is broadly consistent with the hippocampal input to the shell, given the known role of hippocampus in contextual conditioning [26,30,41]. However, these studies have found evidence for both heightened [41] and impaired contextual conditioning [26,30] after hippocampal lesions. The fact that we saw increased contextual conditioning only in the appetitive procedure, and then only in trace conditioned group, suggests that the motivational basis of the task, the informativeness of the available discrete cues, and perhaps other procedural differences, may be critical.

Electrolytic lesions are not that selective in that they can damage structures outside the intended target and take out fibres of passage, e.g. [16]. This is particularly true of the shell sub-region that has a high proportion of fibres passing through [7]. However, electrolytic lesions are nonetheless useful (as a first step) when they result in behavioural dissociations (as

here). Moreover, it has recently been demonstrated that even excitotoxic lesions, hitherto presumed selective, can extend well beyond the area indicated by conventional Nissl staining [9,10]. Thus, the present findings need to be followed up both with more restricted lesions and the appropriate histological assessment. In particular, little is known about the neural bases of appetitive contextual conditioning, and here we find a very striking effect of a shell lesion that depended on the availability of competing discrete cues.

Acknowledgments

These studies were supported by a Wellcome Trust project grant (reference 055330) and a School of Psychology (University of Nottingham) studentship. We thank Andy Smith and Carl Espin for technical support.

References

[1] Baruch I, Hemsley DR, Gray JA. Differential performance of acute and chronic schizophrenics in a latent inhibition task. J Nerv Ment Dis 1988;176:598–606.
[2] Cassaday HJ, Shilliam CS, Marsden CA. Serotonergic depletion increases conditioned suppression to background stimuli in the rat. J Psychopharmacol 2001;15:83–92.
[3] Cassaday HJ, Nelson AJD, Norman C. Haloperidol can increase responding to both discrete and contextual cues in trace conditioned rats. Behav Brain Res 2005;158:31–42.
[4] Coutureau E, Blundell PJ, Killcross S. Basolateral amygdala lesions disrupt latent inhibition in rats. Brain Res Bull 2001;56:49–53.
[5] Ellison GD, Eison MS. Continuous amphetamine intoxication: an animal model of acute psychotic episode. Psychol Med 1983;13:751–61.
[6] Gray NS, Pickering AD, Hemsley DR, Dawling S, Gray JA. Abolition of latent inhibition by a single 5 mg dose of D-amphetamine in man. Psychopharmacology (Berlin) 1992;107:425–30.
[7] Groenewegen HJ, Wright CI, Beijer AV, Voorn P. Convergence and segregation of ventral striatal inputs and outputs. Annu NY Acad Sci 1999;877:49–63.
[8] Howell DC. Statistical methods for psychology. 5th ed. Belmont (CA): Wadsworth/Duxbury Press; 2002. p. 375–7.
[9] Jongen-Relo AL, Feldon J. Specific neuronal protein: a new tool for histological evaluation of excitotoxic lesions. Physiol Behav 2002;76:449–56.
[10] Jongen-Relo AL, Kaufmann S, Feldon JA. differential involvement of the shell and core subterritories of the nucleus accumbens of rats in attentional processes. Neuroscience 2002;111:95–109.
[11] Kamin LJ. Temporal and intensity characteristics of the conditioned stimulus. In: Prokasy WF, editor. Classical conditioning: a symposium. New York: Appleton-Century-Crofts; 1965, p. 118–47.
[12] Kantini E, Norman C, Cassaday HJ. Amphetamine decreases the expression and acquisition of appetitive conditioning but increases the acquisition of anticipatory responding over a trace interval. J Psychopharmacol 2004;18:1516–26.
[13] Kaplan PS, Hearst E. Trace conditioning, contiguity, and context. In: Commons ML, Herrnstein RJ, Wagner AR, editors. Quantitative analysis of behavior: acquisition, vol. III. Cambridge, MA: Ballinger; 1982. p. 347–70.
[14] Konorski J. Integrative activity of the brain: an interdisciplinary approach. University of Chicago Press; 1967.
[15] Kumari V, Cotter PA, Mulligan OF, Checkley SA, Gray NS, Hemsley DR, et al. Effects of D-amphetamine and haloperidol on latent inhibition in healthy male volunteers. J Psychopharmacol 1999;13:398–405.
[16] Leszczuck MH, Flaherty CF. Lesions of nucleus accumbens reduce instrumental but not consummatory negative contrast in rats. Behav. Brain Res 2000;116:61–79.
[17] Lubow RE. Latent inhibition. Psychol Bull 1973;79:398–407.
[18] Mackintosh NJ. The psychology of animal learning. London: Academic Press Inc. Ltd.; 1974. p. 62–6.
[19] Maldonado-Irizarry CS, Kelley AE. Excitotoxic lesions of core and shell subregions of the nucleus accumbens differentially disrupt body weight regulation and motor activity in rat. Brain Res Bull 1995;38:551–9.
[20] Nayak S, Cassaday HJ. The novel dopamine D_4 receptor agonist (PD 168077 maleate): doses with different effects on locomotor activity are without effect in classical conditioning. Prog Neuropsychopharmacol Biol Psychiatr 2003;27:441–9.
[21] Norman C, Cassaday HJ. Amphetamine increases aversive conditioning to diffuse contextual stimuli and to a discrete stimulus when conditioned at high footshock intensity. J Psychopharmacol 2003;17:67–76.
[22] Odling-Smee FJ. Background stimuli and the interstimulus interval during Pavlovian conditioning. Q J Exp Psychol B 1975;27:387–92.
[23] Parkinson JA, Robbins TW, Everitt BJ. Selective excitotoxic lesions of the nucleus accumbens core and shell differentially affect aversive Pavlovian conditioning to discrete and contextual cues. Psychobiology 1999;27:256–66.
[24] Parkinson JA, Dalley JW, Cardinal RN, Bamford A, Fehnert B, Lachenal G, et al. Nucleus accumbens dopamine depletion impairs both acquisition and performance of appetitive Pavlovian approach behavior: implications for mesoaccumbens dopamine function. Behav Brain Res 2002;137:149–63.
[25] Paxinos G, Watson C. The Rat brain in stereotaxic coordinates. 3rd ed. San Diego, CA: Academic Press; 1997.
[26] Phillips RG, LeDoux JE. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behav Neurosci 1992;106:274–85.
[27] Rawlins JNP, Tanner J. The effects of hippocampal aspiration lesions on conditioning to the CS and to a background stimulus in trace conditioned suppression. Behav Brain Res 1998;91:61–72.
[28] Rescorla RA, Wagner AR. A theory of Pavlovian conditioning: Variations in the effectiveness of reinforcement and nonreinforcement. In: Black AH, Prokasy WF, editors. Classical conditioning II: current theory and research. New York: Appleton-Century-Crofts; 1972. p. 64–99.
[29] Riedel G, Harrington NR, Hall G, MacPhail EM. Nucleus accumbens lesions impair context, but not cue, conditioning in rats. NeuroReport 1997;8:2477–82.
[30] Selden NRW, Everitt BJ, Jarrard LE, Robbins TW. Complementary roles for the amygdala and hippocampus in aversive conditioning to explicit and contextual cues. Neuroscience 1991;42:335–50.
[31] Solomon PR, Crider A, Winkelman JW, Turi A, Kamer RM, Kaplan LJ. Disrupted latent inhibition in the rat with chronic amphetamine or haloperidol-induced supersensitivity: relationship to schizophrenic attention disorder. Biol Psychiatr 1981;17:743–56.
[32] Tai C-T, Cassaday HJ, Feldon J, Rawlins JNP. Both electrolytic and excitotoxic lesions of nucleus accumbens disrupt latent inhibition of learning in rats. Neurobiol Learn Mem 1995;64:36–48.
[33] Weiner I. Neural substrates of latent inhibition: the switching model. Psychol Bull 1990;108:442–61.
[34] Weiner I. The “two headed” latent inhibition model of schizophrenia: modelling positive and negative symptoms and their treatment. Psychopharmacology (Berlin) 2003;169:257–97.
[35] Weiner I, Feldon J. The switching model of latent inhibition: an update of neural substrates. Behav Brain Res 1997;88:11–25.

[36] Weiner I, Lubow RE, Feldon J. Chronic amphetamine and latent inhibition. Behav Brain Res 1981;2:285–6.
[37] Weiner I, Lubow RE, Feldon J. Disruption of latent inhibition by acute administration of low doses of amphetamine. Pharmacol Biochem Behav 1988;30:871–8.
[38] Weiner I, Gal G, Rawlins JNP, Feldon J. Differential involvement of the shell and core subterritories of the nucleus in latent inhibition and amphetamine-induced activity. Behav Brain Res 1996;81:123–33.
[39] Weiner I, Gal G, Feldon J. Disrupted and undisruptable latent inhibition following shell and core lesions. Annu NY Acad Sci 1999;877:723–7.
[40] West CHK, Boss-Williams KA, Weiss JM. Motor activation by amphetamine infusion into nucleus accumbens core and shell subregions of rats differentially sensitive to dopaminergic drugs. Behav Brain Res 1999;98:155–65.
[41] Winocur G, Rawlins JNP, Gray JA. The hippocampus and conditioning to contextual cues. Behav Neurosci 1987;101:617–25.
[42] Zahm DS, Brog JS. On the significance of subterritories in the ‘accumbens’ part of the rat ventral striatum. Neuroscience 1992;50:751–67.
[43] Zahm DS, Heimer L. Two transpallidal pathways originating in the rat nucleus accumbens. J Comp Neurol 1990;302:437–46.