

Chapter 4.

Role of the nucleus accumbens core and shell in Pavlovian–instrumental transfer

Abstract. When an initially neutral stimulus has been paired in Pavlovian fashion with an appetitive outcome such as food, noncontingent presentation of this conditioned stimulus (CS) can enhance ongoing instrumental responding, a phenomenon termed Pavlovian–instrumental transfer (PIT). In its simplest form, PIT is assessed by presenting a CS for food while the subject is responding in extinction on a single lever for the same food. It has previously been shown that the nucleus accumbens, and particularly the core subdivision, is critical for this form of PIT (Hall *et al.*, 1999). However, behavioural studies have shown that PIT can be subdivided into a general, motivating effect of the Pavlovian CS, and a response-specific PIT effect, seen as a further enhancement when the Pavlovian and instrumental outcomes are the same (see Dickinson & Balleine, 1994). In the present study, rats received lesions to the core or shell of the nucleus accumbens before being tested on a response-specific PIT task. In the Pavlovian phase, one stimulus, CS(pel), was paired with pellet delivery, while a second stimulus, CS(suc), was paired with sucrose solution. The subjects were then trained to respond on two levers, with one lever producing pellets and the other producing sucrose. On test, lever-pressing was recorded in extinction while the stimuli were presented noncontingently. Control subjects ($n = 6$) showed a selective enhancement of lever-pressing on the lever paired with the same outcome as the Pavlovian CS; this is the response-specific PIT effect. Core-lesioned subjects ($n = 4$) showed a general enhancement of responding during the CS, but this was not specific to one response. Shell-lesioned animals ($n = 4$) showed no PIT. It is suggested that in this task, the shell is required for the ‘vigour’ and the core for the ‘direction’ of the potentiation of responding by a noncontingent, appetitive stimulus. This pattern closely resembles that previously observed for the potentiation of responding for conditioned reinforcement by psychostimulant drugs injected into the nucleus accumbens (Parkinson *et al.*, 1999b).

INTRODUCTION

Pavlovian CSs can have effects on operant responding when presented noncontingently, even when responding has never been associated with the CS. This was first demonstrated by Estes (1943; 1948), who found that noncontingent presentation of an appetitive CS, previously paired with food, would elevate the rate of instrumental responding for the same food in a test conducted in extinction — a phenomenon now known as Pavlovian–instrumental transfer (PIT). Estes used the simplest form of PIT, in which the instrumental outcome is the same as the Pavlovian US. As discussed in Chapter 1 (p. 26), PIT has since been subdivided behaviourally (see Dickinson, 1994, pp. 66–68; Dickinson & Balleine, 1994): the CS has a general, motivating effect (Dickinson & Dawson, 1987b; Balleine, 1994), but also potentiates, selectively, an action whose outcome is the Pavlovian US (Colwill & Rescorla, 1988; Colwill & Motzkin, 1994).

The nucleus accumbens (Acb) is an important neural site mediating the ability of Pavlovian CSs to invigorate and direct behaviour, as discussed in Chapter 1 (p. 46). It has previously been shown that PIT, in its simplest form, depends upon the nucleus accumbens core (AcbC), though not the shell (AcbSh)

(Hall *et al.*, 1999). However, it is not known how the AcbC and AcbSh contribute to response-specific PIT, or the potentiation of responses whose outcomes are unrelated to the US.

In the present experiment, the response-specific PIT effect was assessed in rats with lesions of the AcbC or AcbSh, using the experimental design of Colwill & Motzkin (1994, Experiment 2). To assess response-specific PIT, subjects were food-deprived, and two CSs were associated with different appetitive USs (chow pellets or sucrose solution). Next, two instrumental responses (left and right lever-presses) were trained for the two reinforcers used as USs in the Pavlovian phase. Finally, responding was tested in extinction while the CSs were presented noncontingently; response-specific PIT was inferred if one or both stimuli were capable of differentially affecting the two responses.

General PIT was assessed in the same subjects. In an attempt to detect general PIT in subjects who had already experienced several extinction sessions, the observation that PIT underlies the irrelevant incentive effect was used (Dickinson, 1986; Dickinson & Dawson, 1987b). Subjects were given further Pavlovian conditioning sessions, and retrained to respond on the lever producing the pellet outcome. They were shifted from a state of hunger to one of thirst, and their responding for pellets was again assessed in extinction while the CSs were presented. When subjects are thirsty, the CS for liquid sucrose solution predicts an outcome relevant to their current motivational state, and thus should produce strong Pavlovian conditioned motivation (Dickinson, 1986; Dickinson & Dawson, 1987b). General PIT was inferred if the CS for sucrose elevated responding for pellets. (Strictly, this assessment should also be made relative to an unpaired stimulus, though one was not available in the present experiment for technical reasons; in lieu of this, a comparison was made with responding in the interstimulus interval, but also with the CS for pellets, which was expected to suppress responding in thirsty animals; Balleine, 1994.) This test therefore relies on subjects' ability to use a CS to retrieve information about the US and assess its relevance to the current motivational state, as well as their capacity to show general (non-response-specific) PIT.

The experimental design is shown in Table 12.

Table 12. Design of the present experiment (S1, S2 = stimuli; L1, L2 = levers).

Training	Test 1 (Specific PIT)	Retraining	Test 2 (General PIT)
Hungry	Hungry	Hungry	Thirsty
S1 → pellet	S1: L1 v. L2	S1 → pellet	S1: L1
S2 → sucrose	S2: L1 v. L2	S2 → sucrose	S2: L1
L1 → pellet	ISI: L1 v. L2	L1 → pellet	ISI: L1
L2 → sucrose			
	Specific PIT inferred if one or both stimuli differentially affects the level of the two responses.		General PIT inferred if L1(S2) > L1(ISI).

METHODS

Six subjects (JP1, JP3, JP4, JP5, JP7, JP8) received excitotoxic lesions of the AcbC (see Chapter 2, p. 64, for coordinates) and had prior experience of an autoshaping task. Seven subjects (JP11, JP12, JP13, JP14, JP15, JP16, JP17) received lesions of the AcbSh and had prior experience of a simple visual discrimination task using a touchscreen. Six control subjects were used, of which two had received sham AcbC operations (JP6, JP9), two had sham AcbSh operations (JP19, JP20) and two were unoperated (JP2, JP10). No subjects had prior experience of the stimuli, reinforcers or responses used in the present task.

'Response-specific' and 'general' Pavlovian–instrumental transfer tests

Subjects were maintained at 85% of their free-feeding mass. Water was always available in the home cage during training except where stated. The two reinforcers used were 0.05 ml of 20% w/v (= 200 g/l = 0.58 M) sucrose so-

lution (the dipper was normally raised and was lowered briefly to collect liquid), and one 45-mg chow pellet (Rodent Diet Formula A/I, Noyes, Lancaster, NH). Stimulus S1 consisted of the left and right stimulus lights (2.8 W bulbs) above the levers, flashed together at 3 Hz. Stimulus S2 was a clicker relay operated at 10 Hz. A 2.8 W houselight was illuminated at all times.

Subjects were distributed evenly into the counterbalancing conditions listed in Table 13.

Table 13. Counterbalancing conditions for the response-specific PIT test.

Counterbalancing condition	Pavlovian	Instrumental	Sessions
0	S1→sucrose, S2→pellet	Left → sucrose, right → pellet	Begin with sucrose
1	S1→pellet, S2→sucrose	Left → sucrose, right → pellet	Begin with sucrose
2	S1→sucrose, S2→pellet	Left → pellet, right → sucrose	Begin with sucrose
3	S1→pellet, S2→sucrose	Left → pellet, right → sucrose	Begin with sucrose
4	S1→sucrose, S2→pellet	Left → sucrose, right → pellet	Begin with pellet
5	S1→pellet, S2→sucrose	Left → sucrose, right → pellet	Begin with pellet
6	S1→sucrose, S2→pellet	Left → pellet, right → sucrose	Begin with pellet
7	S1→pellet, S2→sucrose	Left → pellet, right → sucrose	Begin with pellet

Phase 1: Pavlovian training. Stimuli S1 and S2 were presented alternately in 2-min components, with no lever present. During each stimulus, the appropriate reinforcer was delivered on a RT 30-s schedule. Each component was separated from the next by a 2-min interstimulus interval (ISI). The session began with an ISI component and ended after 5 of each type of component had been presented. The stimulus–reinforcer assignment and the first reinforcer of the session were counterbalanced as shown in Table 13. Subjects were trained for 10 sessions with one session per day.

Phase 2: Instrumental training. For six 30-min sessions, animals were presented with a single lever that was reinforced on an RI schedule. No other stimuli were present. The lever used alternated across sessions, with half of the subjects receiving the pellet lever first and half the sucrose lever. The parameter of the RI schedule was 2 s for the first pair of sessions, 15 s for the second pair and 30 s for the third. For a further four sessions, both levers were present and reinforced on independent RI 30-s schedules. All sessions began with the insertion of the lever(s) and ended with lever retraction.

Phase 3: Pavlovian reminder. One further Pavlovian session was given, using the same schedule as Phase 1.

Phase 4: Instrumental extinction. As PIT is best observed after a degree of instrumental extinction has occurred (Dickinson *et al.*, 2000; A. Dickinson, personal communication, 7 May 1999), one 8-min session was given in which both levers were available but not reinforced.

Phase 5: Response-specific transfer test. Animals remained food-deprived for two sessions on the specific transfer test, in which both levers were available but not reinforced. Two-minute light and clicker stimuli were presented in alternation, with a 2-min ISI between each, until five of each stimulus had been presented. Assessing performance in the absence of the stimuli gave a measure of baseline lever-pressing. The session began with an ISI and lasted 40 min. As the stimulus presentation order was always ISI→S1→ISI→S2, half the rats received the stimulus associated with sucrose first, half received the stimulus indirectly associated with the left lever first, and half received the stimulus that occurred first in Pavlovian training; these three divisions were orthogonal (Table 14).

Phase 6: Retraining. Pavlovian retraining was given exactly as before for 3 sessions. This was followed by instrumental training in which only the pellet lever was present; three reinforced sessions were given using an RI 30-s schedule, followed by a single 5-min extinction session.

Phase 7: General transfer test. Once the animals had been fed after the final retraining session, they were placed on a 23-h water deprivation schedule with food freely available. On the next and subsequent day, they received a general transfer test in which only the pellet lever was available, though it was not reinforced. Three components were presented (light, clicker, no stimulus) in the same manner as for the specific transfer test, and responding was measured in each component.

As the specific and general PIT tests were conducted using the same subjects, it is important that the first test should not be able to bias the results of the second. This was the case: thus, retraining did not alter any of the Pav-

lovian or instrumental contingencies experienced by the subjects. The specific PIT test consisted of extinction trials to S1 and S2, but these were equal in number and duration. Furthermore, differential extinction on the two levers was not problematic, for the general PIT test was concerned with responding on a single lever. Finally, responding in extinction in the presence of S1 and S2 (in the specific PIT test) was unlikely to affect performance on the general PIT test: since no reinforcers were delivered, S1 and S2 could not become positive instrumental discriminative stimuli.

Table 14. Counterbalancing conditions for response-specific PIT test (continued from Table 13).

Counterbalancing condition	Begin with sucrose or pellet stimulus?	Begin with stimulus associated indirectly with left/right lever?	Begin with stimulus that occurred first or second in Pavlovian training?
0	sucrose	L	1
1	pellet	R	2
2	sucrose	R	1
3	pellet	L	2
4	sucrose	L	2
5	pellet	R	1
6	sucrose	R	2
7	pellet	L	1

RESULTS

Histology

Following histological analysis, the control group included six subjects (nos. JP2, JP6, JP9, JP10, JP19, JP20). Throughout the behavioural analyses, no differences were evident between subjects that had received sham AcbC surgery, sham AcbSh surgery, or no surgery; these subjects were therefore pooled to form a single sham group. In the core group, two rats were found to have lesions of the entire Acb (JP1, JP3) and were excluded from analysis, leaving four with bilateral core lesions only (JP4, JP5, JP7, JP8). In the shell group, one animal was found to have a septal lesion (JP14) and two to have no shell damage (JP16, JP17); these animals were excluded, leaving four with bilateral shell lesions (JP11, JP12, JP13, JP15).

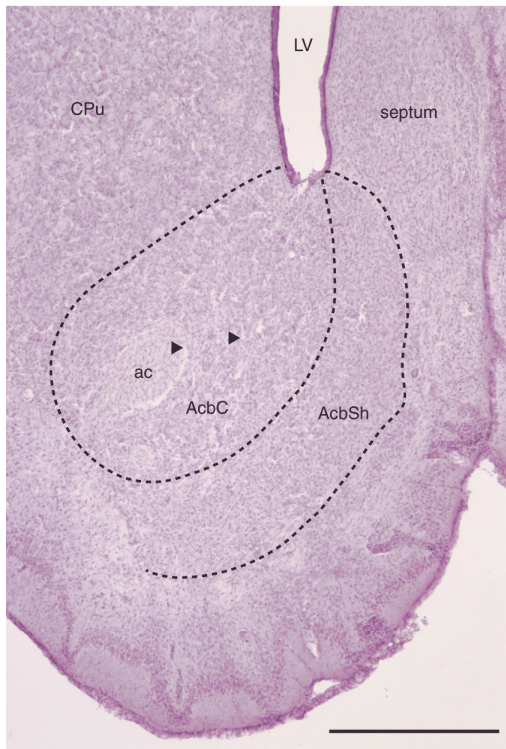
Lesions of the AcbC encompassed most of the core subregion; neuronal loss and associated gliosis extended in an anteroposterior direction from approximately +2.5 mm to +0.5 mm relative to bregma, and did not extend ventrally or caudally into the ventral pallidum or olfactory tubercle. Damage to the ventromedial caudate–putamen was occasionally seen; damage to the AcbSh in these animals was restricted to the lateral edge of the dorsal shell or the superior edge of the lateral shell. Representative photomicrographs of AcbC lesions are shown in Figure 43 and Figure 44; schematics of the lesions are shown in Figure 45.

Lesions of the AcbSh encompassed the medial shell; neuronal loss and associated gliosis extended in an anteroposterior direction from approximately +2.2 mm to +1.0 mm relative to bregma. There was very little damage to the AcbC, the lateral septum, or the medial ventral pallidum. Representative photomicrographs of AcbSh lesions are shown in Figure 46 and Figure 47; schematics of the lesions are shown in Figure 48.

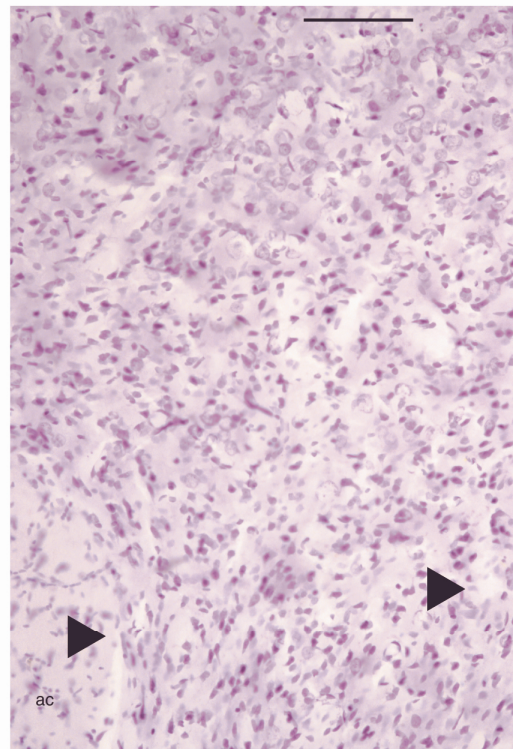
Unfortunately, the post-histological groups were not evenly counterbalanced. The core group were distributed evenly across counterbalancing conditions 1, 3, 5 and 7 (one rat per cell); thus they were counterbalanced for response/outcome assignment and stimulus presentation order, but not for stimulus/outcome assignment: all core-lesioned subjects received light→pellet and clicker→sucrose conditioning. The other groups were better counterbalanced. The shell group were in conditions 1, 4, 5 and 7. The sham group were in conditions 0, 2 (two rats), 4, 5 and 6.

Nucleus accumbens core photomicrographs (cresyl violet staining)

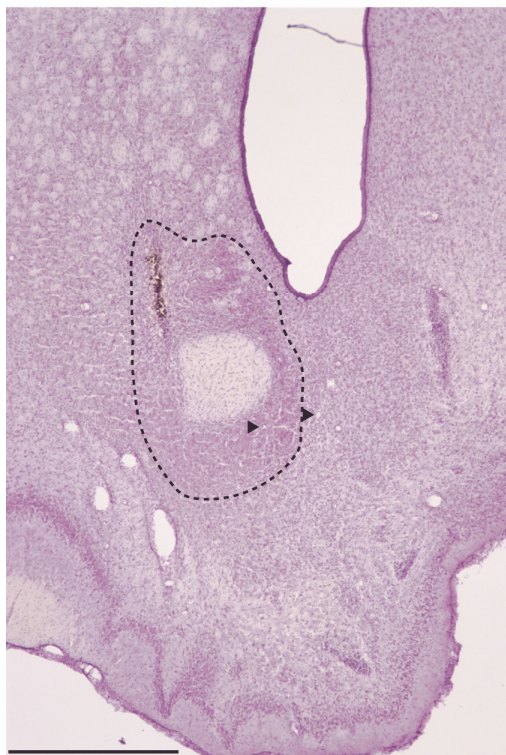
A. Sham-operated rat (low power)



B. Sham-operated rat (high power)



C. Core-lesioned rat (low power)



D. Core-lesioned rat (high power)

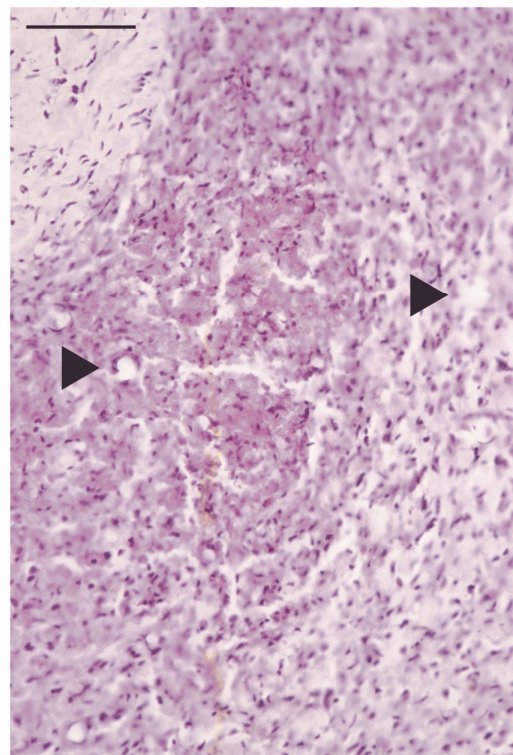
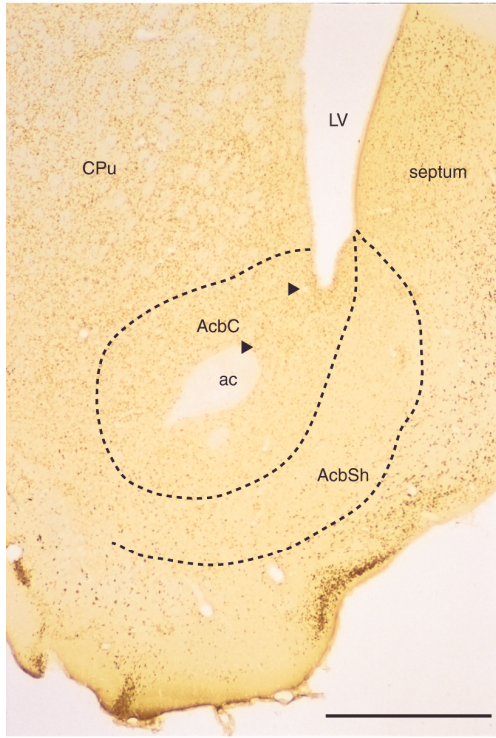


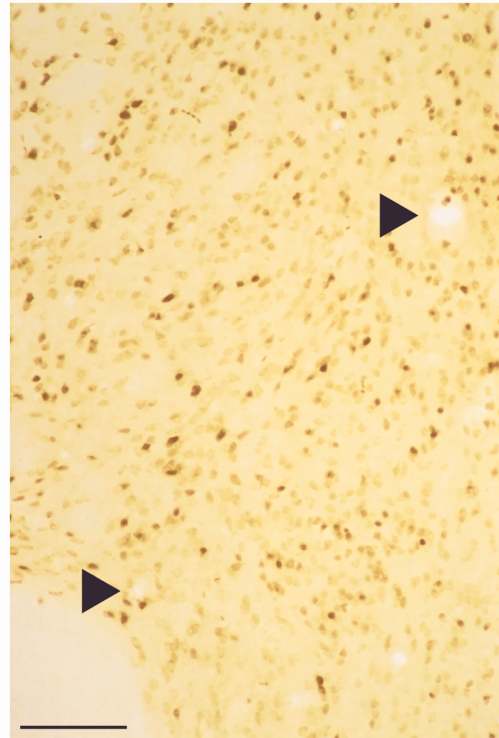
Figure 43. Lesions of the AcbC: photomicrographs of sections at approximately 1.2 mm anterior to bregma, stained with cresyl violet. **A & B:** sham-operated rat (ac, anterior commissure; CPu, caudate–putamen; AcbC, nucleus accumbens core; AcbSh, nucleus accumbens shell; LV, lateral ventricle). **C & D:** core-lesioned rat. Dotted lines show the extent of the lesion. There is tissue collapse within the lesion and the lateral ventricle is slightly expanded. **Left-hand panels** are low-magnification views (scale bars are 1 mm); **right-hand panels** are high-magnification views (scale bars are 0.1 mm). Arrowheads indicate the position of identical structures in corresponding pairs of photomicrographs.

Nucleus accumbens core photomicrographs (*NeuN immunocytochemical staining*)

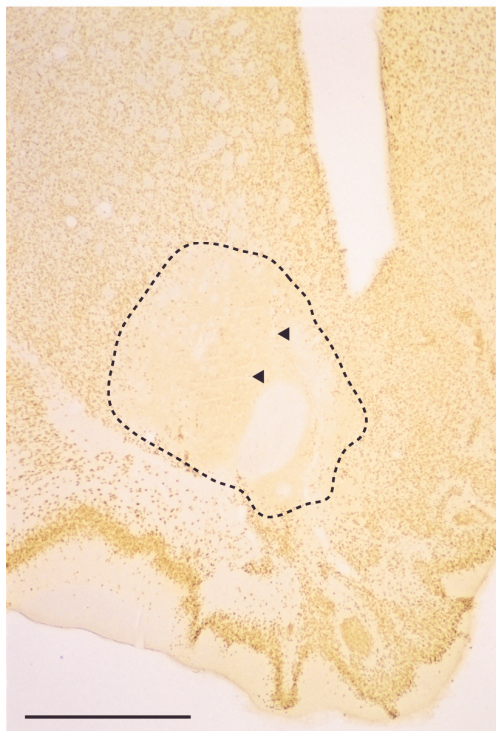
A. Sham-operated rat (low power)



B. Sham-operated rat (high power)



C. Core-lesioned rat (low power)



D. Core-lesioned rat (high power)



Figure 44. Lesions of the AcbC: photomicrographs of sections at approximately 1.2 mm anterior to bregma, stained with NeuN antibody. **A & B:** sham-operated rat (ac, anterior commissure; CPu, caudate–putamen; AcbC, nucleus accumbens core; AcbSh, nucleus accumbens shell; LV, lateral ventricle). **C & D:** core-lesioned rat. Dotted lines show the extent of the lesion. **Left-hand panels** are low-magnification views (scale bars are 1 mm); **right-hand panels** are high-magnification views (scale bars are 0.1 mm). Arrowheads indicate the position of identical structures in corresponding pairs of photomicrographs.

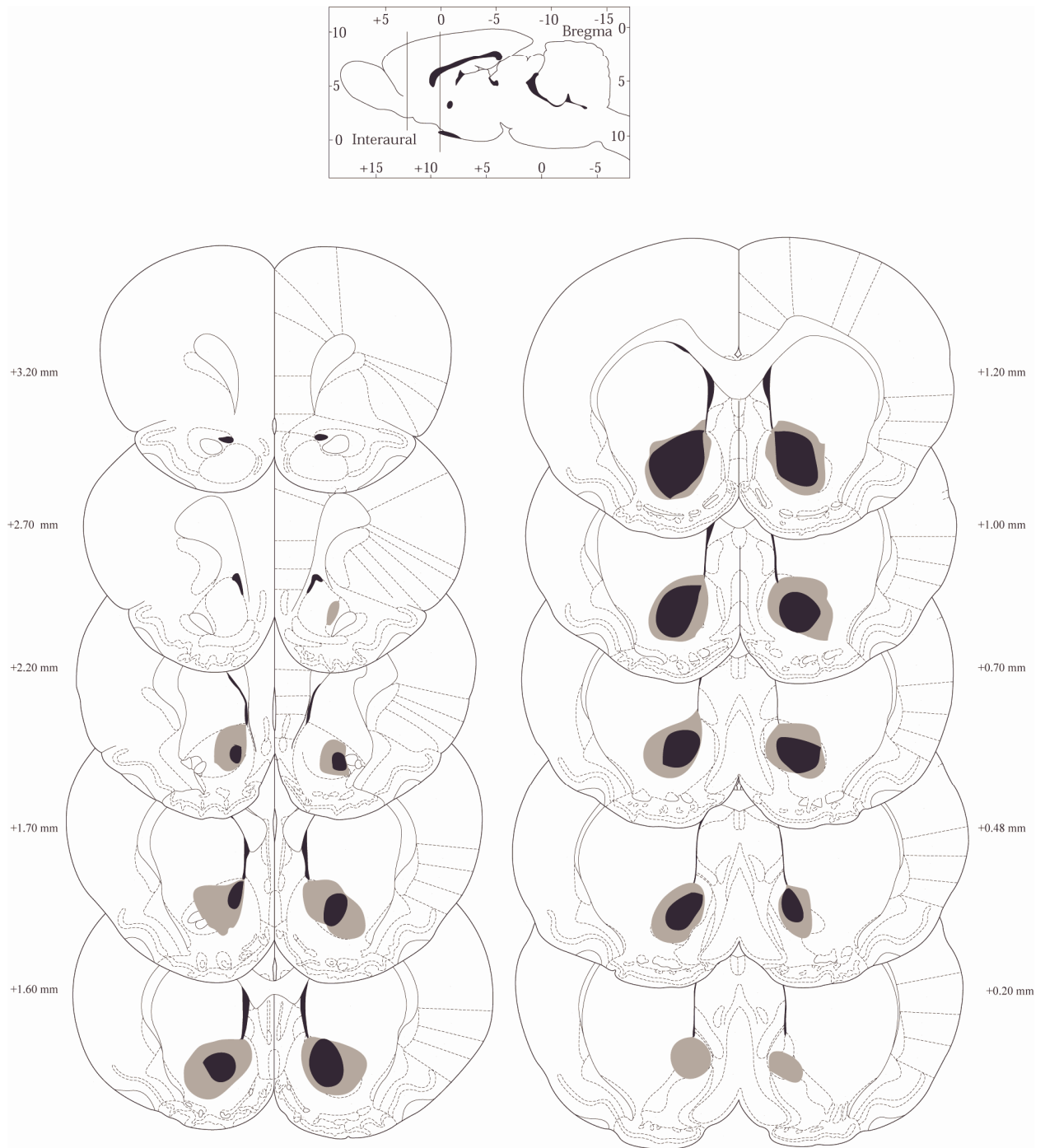
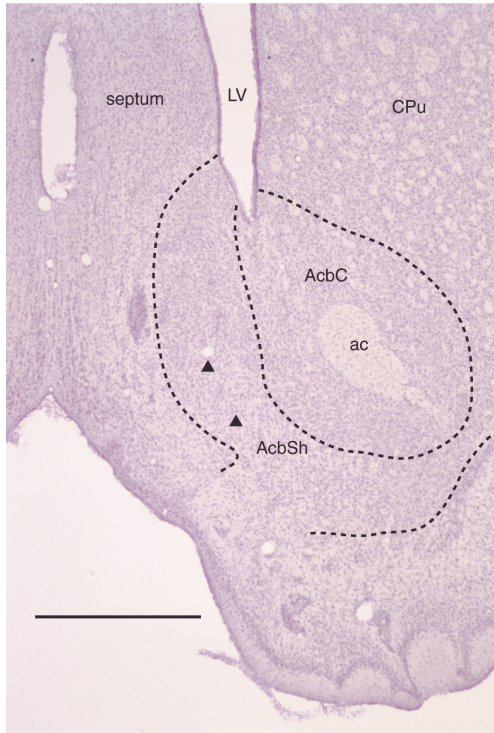
Nucleus accumbens core: schematic of lesions

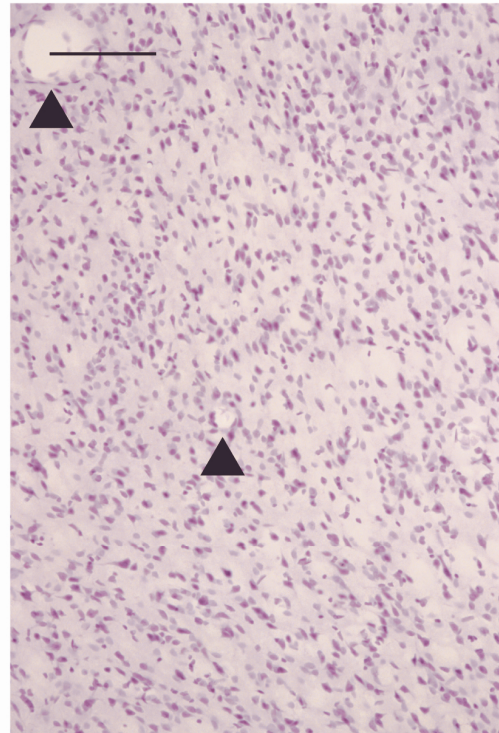
Figure 45. Schematic of lesions of the AcBc (subjects JP4, JP5, JP7, JP8). Grey shading indicates the extent of the largest area of neuronal loss, and black the smallest. Diagrams are taken from Paxinos & Watson (1998).

Nucleus accumbens shell photomicrographs (cresyl violet staining)

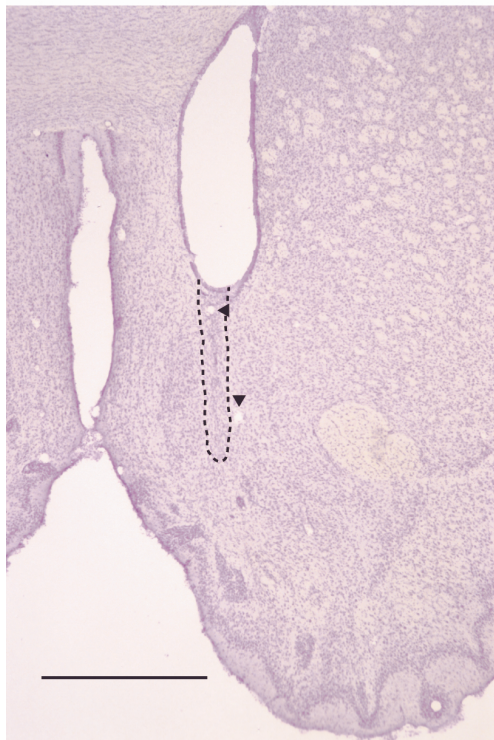
A. Sham-operated rat (low power)



B. Sham-operated rat (high power)



C. Shell-lesioned rat (low power)



D. Shell-lesioned rat (high power)

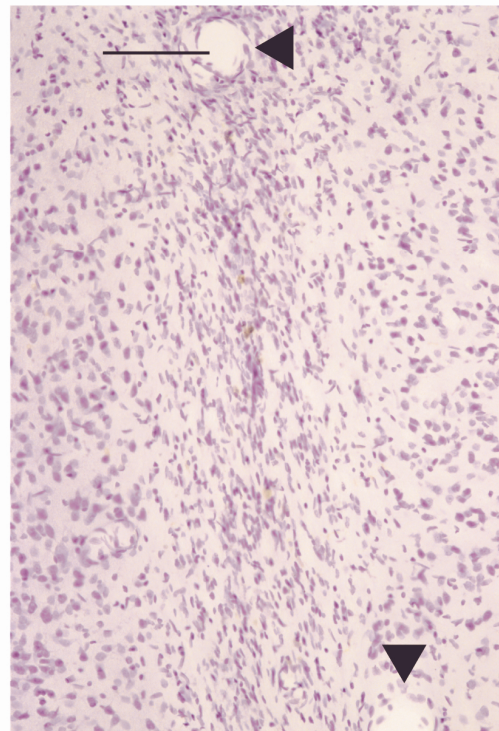
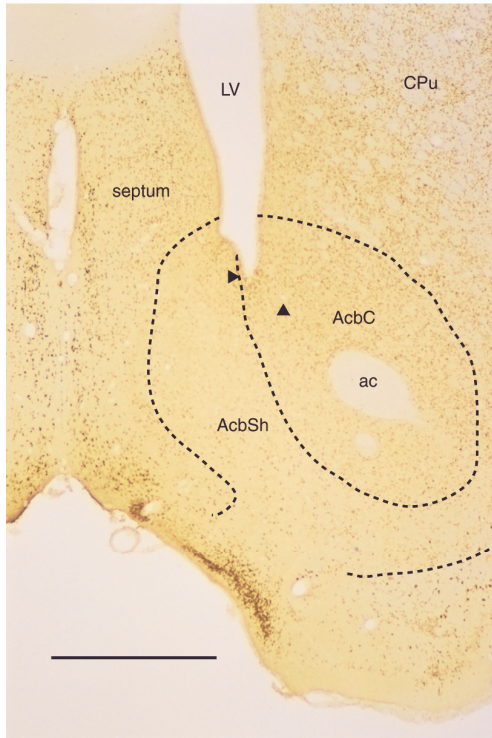


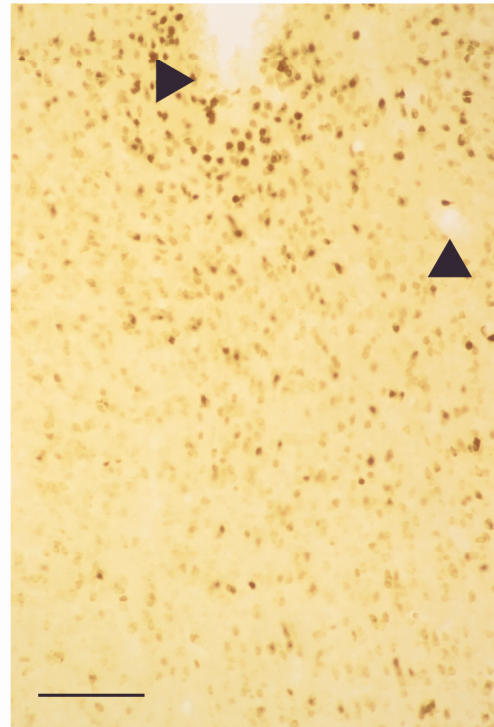
Figure 46. Lesions of the AcbSh: photomicrographs of sections at approximately 1.0 mm anterior to bregma, stained with cresyl violet. **A & B:** sham-operated rat (ac, anterior commissure; CPu, caudate–putamen; AcbC, nucleus accumbens core; AcbSh, nucleus accumbens shell; LV, lateral ventricle). **C & D:** shell-lesioned rat. Dotted lines show the extent of the lesion; the lesioned area has collapsed and there is some ventricular expansion. **Left-hand panels** are low-magnification views (scale bars are 1 mm); **right-hand panels** are high-magnification views (scale bars are 0.1 mm). Arrowheads indicate the position of identical structures in corresponding pairs of photomicrographs.

Nucleus accumbens shell (NeuN immunocytochemical staining)

A. Sham-operated rat (low power)



B. Sham-operated rat (high power)



C. Shell-lesioned rat (low power)



D. Shell-lesioned rat (high power)

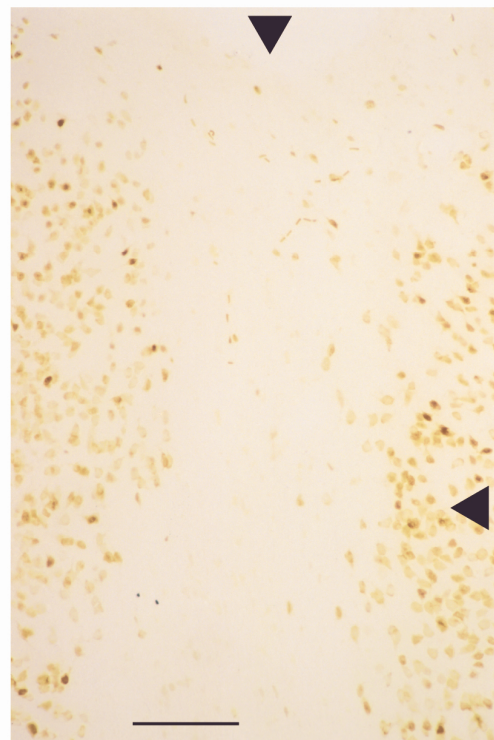


Figure 47. Lesions of the AcbSh: photomicrographs of sections at approximately 1.0 mm anterior to bregma, stained with NeuN antibody. **A & B:** sham-operated rat (ac, anterior commissure; CPu, caudate–putamen; AcbC, nucleus accumbens core; AcbSh, nucleus accumbens shell; LV, lateral ventricle). **C & D:** shell-lesioned rat. Dotted lines show the extent of the lesion. **Left-hand panels** are low-magnification views (scale bars are 1 mm); **right-hand panels** are high-magnification views (scale bars are 0.1 mm). Arrowheads indicate the position of identical structures in corresponding pairs of photomicrographs.

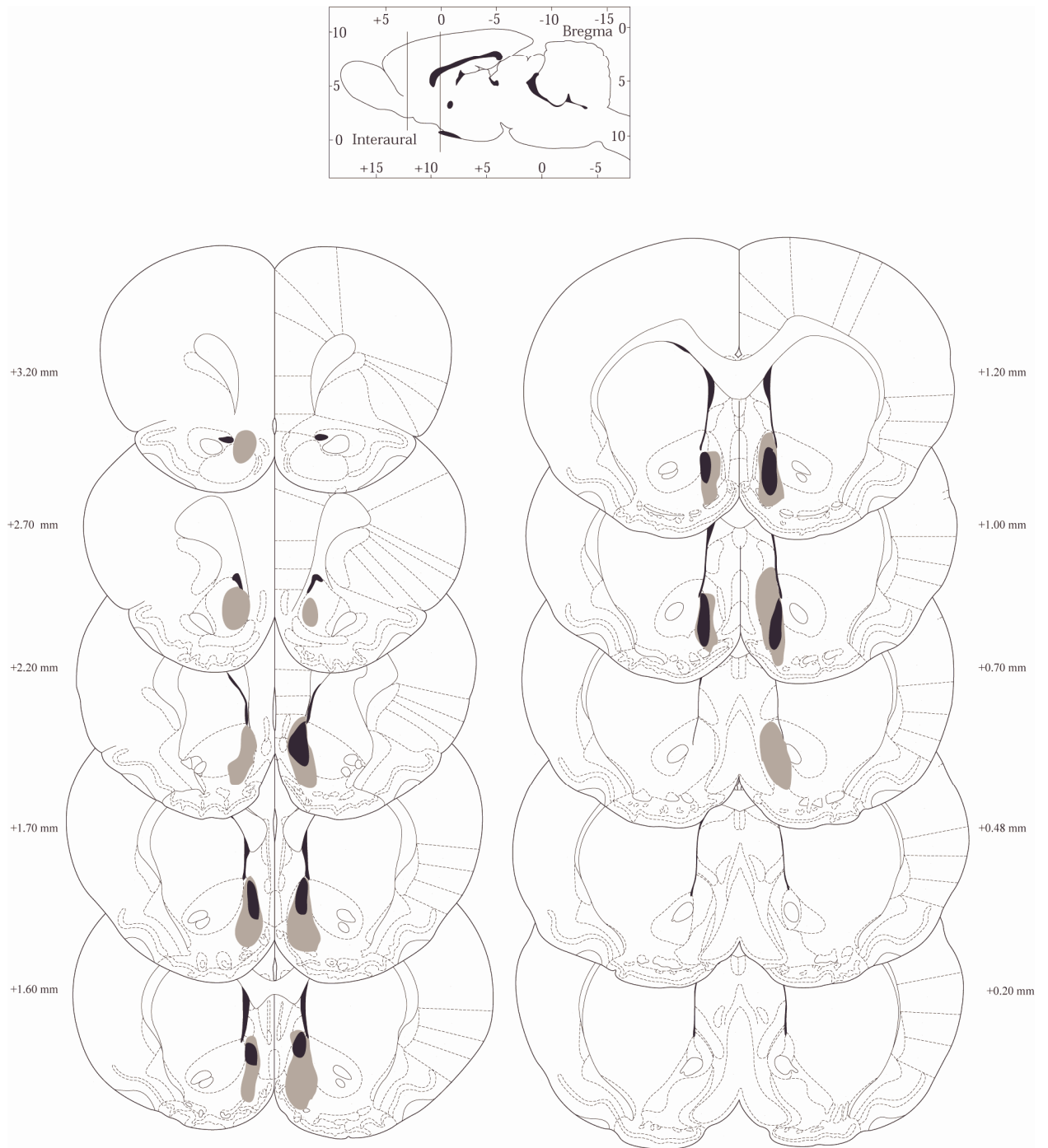
Nucleus accumbens shell: schematic of lesions

Figure 48. Schematic of lesions of the AcbSh (subjects JP11, JP12, JP13, JP15). Grey shading indicates the extent of the largest area of neuronal loss, and black the smallest. Diagrams are taken from Paxinos & Watson (1998).

Pavlovian training

The groups did not differ in their approach behaviour during Pavlovian training. Figure 49 shows approach to the food alcove during the two stimuli, CS(pel) and CS(suc), relative to the ISI. In this task, the measure of approach behaviour is not a pure measure of conditioning (be it Pavlovian or instrumental approach): as food is delivered during the stimuli, approach may reflect unconditioned responding.

Analysis of ratios of responding from initial training sessions using the model $\text{group}_3 \times (\text{stimulus}_2 \times \text{session}_{10} \times S)$ demonstrated a main effect of session ($F_{9,99} = 3.328, p = .001$) and stimulus ($F_{1,11} = 19.506, p = .001$), with nosepoking being greater during the sucrose CS. There was no session \times stimulus interaction ($F_{9,99} = 1.745, \text{NS}$). However, there was no significant effect of group, and no interactions involving group ($F_s < 1.22$).

Nor did the groups differ during the ‘reminder’ session, for which a separate ANOVA was conducted. Again, responding was higher during the sucrose stimulus ($F_{1,11} = 8.553, p = .014$) but there were no group differences (group: $F_{2,11} = 1.571, \text{NS}$; group \times stimulus: $F_{2,11} = 2.14, \text{NS}$).

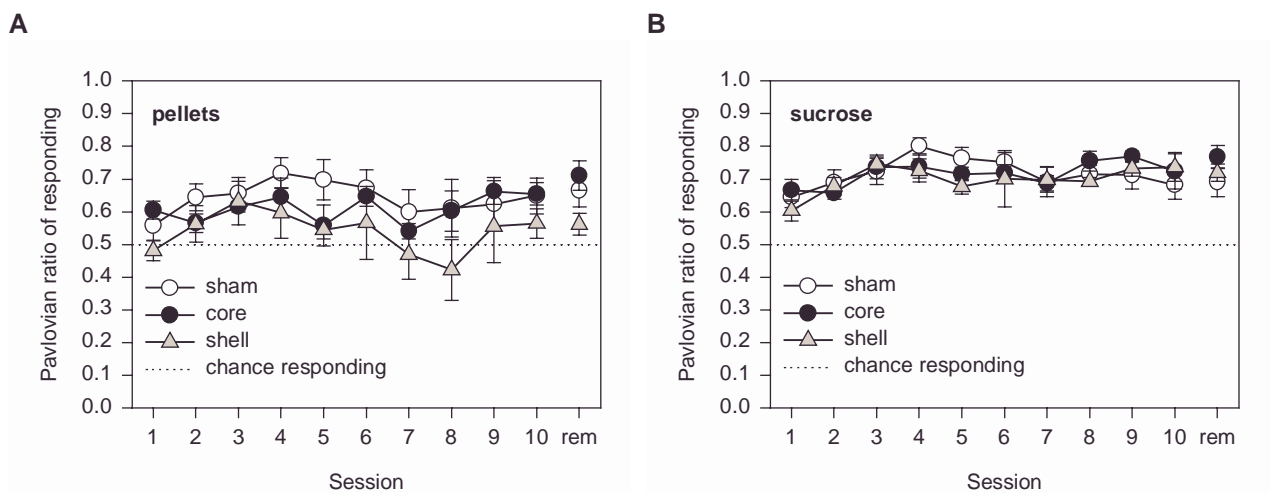


Figure 49. Approach to the food alcove during Pavlovian training, measured by the nosepoke detector. The ratio of responding is the approach time during reinforcer delivery, as a proportion of approach during reinforcer delivery plus approach during the ISI. It is not a pure measure of conditioning, as unconditioned responding to the food may contribute. **A:** Approach during pellet delivery. **B:** Approach during sucrose delivery. The reminder session is indicated by *rem*. The two graphs show approach relative to the same ISI data.

Instrumental training

All groups acquired the lever-press responses at the same rate (Figure 50A). The total number of lever-presses in each session was square-root transformed and data from training sessions 1–10 were analysed using the model $\text{group}_3 \times (\text{session}_{10} \times S)$. This showed a main effect of session ($F_{7,133,70.785} = 42.528, \tilde{\epsilon} = .715, p < .001$) but no group differences ($F_s < 1.23, \text{NS}$).

The core group displayed a slightly stronger preference for the pellet lever than the other two groups. From session 7, when two levers were available, the preference for the sucrose lever was calculated as (sucrose responses) \div (total responses) and subjected to ANOVA. These data are shown in Figure 50B, where it can be seen that all groups responded almost equally on both levers by the end of training (proportions close to 0.5). However, the ANOVA demonstrated a main effect of group ($F_{2,11} = 4.296, p = .042$), with no effect of session and no interaction ($F_s < 1$). Pairwise comparisons with a Sidak correction suggested that this difference was due to the core group having *lower* preference scores than shams (i.e.

preferring the pellet lever more), $p = .052$, with no difference between the shell group and shams ($p = .216$) or between the core and shell groups ($p = .841$).

The rate of responding in extinction did not differ between the groups (Figure 50A), though the core group continued to prefer the pellet lever (Figure 50B). Separate one-way ANOVAs were conducted for the extinction session, which demonstrated no difference in total lever-pressing between the groups ($F_{2,11} = 1.171$, NS). However, the preference for the pellet lever in the core group increased: there was a significant main effect of group ($F_{2,11} = 4.141$, $p = .046$), and Dunnett's test showed that the core group had lower preference scores than shams ($p = .03$) but the shell group did not ($p = .248$).

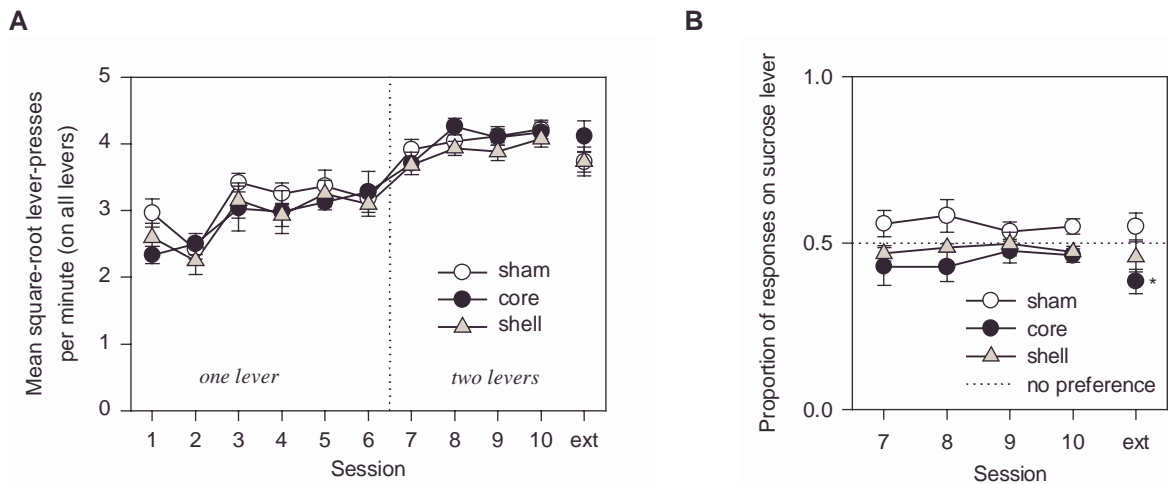


Figure 50. Acquisition of instrumental responding. **A:** Responses per minute, summed over all levers present. The session length was 30 min; the schedule progressed from RI 2 s to RI 30 s as described in the Methods. From session 7 onwards, two levers were concurrently available; *ext* indicates the extinction session. **B:** Proportion of responses made on the sucrose lever (* $p < .05$ relative to shams on the extinction day).

Response-specific PIT

Response rates for the two levers during each stimulus condition are shown in Figure 51, with rates of nose-poking in Figure 52.

Lever-pressing

The sham group displayed a response-specific PIT effect, in that the CS for pellets selectively enhanced responding on the pellet lever. The core group, which preferred the pellet lever slightly, displayed PIT, but this was not specific: the CS for pellets potentiated responding on both levers. The shell group displayed no PIT. The CS for sucrose was less effective than the CS for pellets in producing PIT, across the groups.

Lever-press data were square-root transformed before analysis. As the groups were not evenly counterbalanced for stimulus/outcome assignment, a four-way ANOVA including this term was first performed. This failed to demonstrate any effect of the stimulus/outcome assignment ($F_s < 1.477$, $p > .255$); consequently this term was removed from further analyses.

An ANOVA using the model $\sqrt{(\text{lever-presses})} = \text{group}_3 \times (\text{response}_2 \times \text{stimulus}_3 \times S)$ revealed a response \times stimulus \times group interaction ($F_{4,22} = 3.741$, $p = .018$), in addition to a main effect of response ($F_{1,11} = 5.15$, $p = .044$) and stimulus ($F_{2,22} = 7.646$, $p = .003$). No other terms were significant ($p > .1$). The response \times stimulus \times group interaction was analysed further by considering simple interaction terms; that is, testing for a response \times stimulus interaction in each group.

In the sham group there was a significant response \times stimulus interaction ($F_{2,10} = 8.312, p = .007$), indicating that the pattern of responding across the two levers was differentially affected by the stimuli and implying a response-specific PIT effect. Further analysis showed that the stimulus \times response interaction was due to the CS(pel) selectively potentiating responding on the pellet lever (simple effect of stimulus for the pellet lever, $p < .001$, but not for the sucrose lever, $p = .071$; pairwise comparisons for the pellet lever showed that only the CS(pel) elevated responding, $p = .005$); neither stimulus affected responding on the sucrose lever ($F_{2,10} = 3.489, p = .071$).

In the core group, there was no response \times stimulus interaction ($F < 1$), though these animals demonstrated both a preference for the pellet lever (main effect of response, $F_{1,3} = 26.05, p = .015$) and some response-independent PIT (main effect of stimulus, $F_{2,6} = 24.273, p = .001$; indeed, the pellet CS elevated responding on the sucrose lever, $p = .018$). Pairwise comparisons showed that responding during the CS(pel) was significantly higher than during the ISI ($p = .002$). It was also higher than during CS(suc), though this did not reach significance ($p = .08$); there was no difference in responding between the ISI and CS(suc) ($p > .5$).

The shell group demonstrated no response \times stimulus interaction ($F_{2,6} = 3.159, p = .116$), nor a main effect of either response or stimulus ($F_s < 1$). The lack of an effect of the CSs was not due to differences in the baseline level of responding that obscured PIT; comparison of ISI responding between shell- and sham-lesioned rats using the model group \times (response \times S) revealed no differences ($F_s \leq 1.214, NS$).

These results therefore demonstrate a Pavlovian–instrumental transfer effect in normal rats that is response-specific in that Pavlovian CSs for two reinforcers differentially affected lever-pressing for those reinforcers. Shell-lesioned animals demonstrated no Pavlovian–instrumental transfer, while core-lesioned animals demonstrated transfer, but this transfer lacked response specificity.

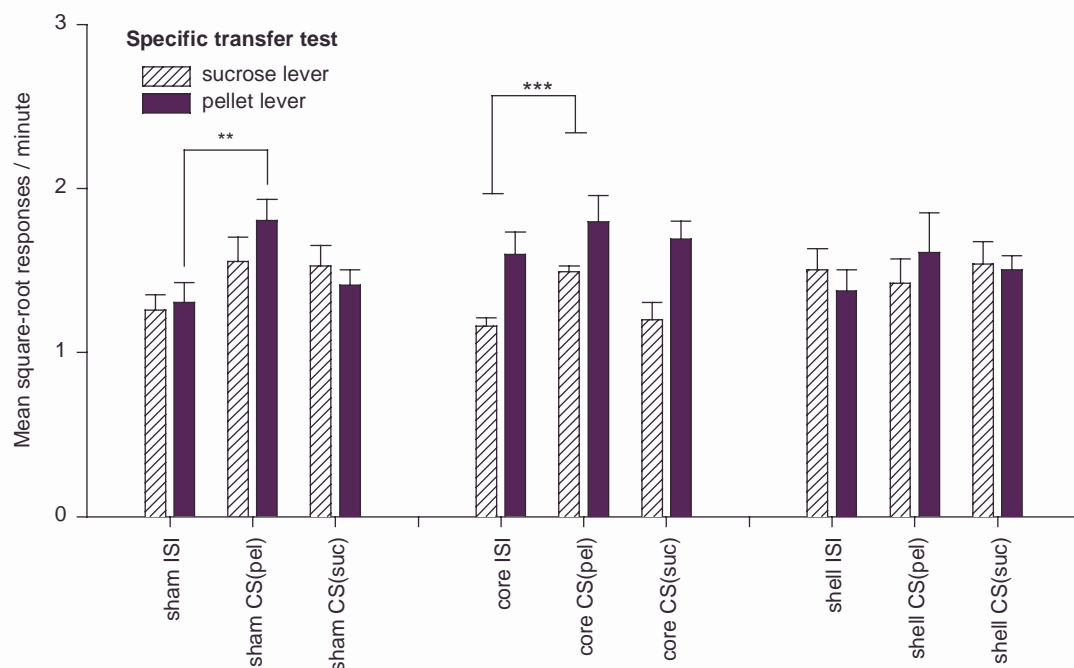


Figure 51. Lever-pressing during the specific PIT test. Sham-operated controls exhibited a specific Pavlovian–instrumental transfer effect, with the Pavlovian CS for pellets selectively potentiating responding on the lever trained with the pellet outcome. Core-lesioned animals exhibited a PIT effect in response to the CS for pellets, but the potentiation was not response-specific. Shell-lesioned animals exhibited no transfer effect. (** $p < .01$; *** $p < .001$.)

Supplemental analysis

For the purpose of comparison with a study of PIT by Hall *et al.* (1999), who used a single test session and a design with a single lever that produced a pellet reinforcer, data from only the first session of response-specific PIT testing were taken, and responding on the pellet lever was analysed in isolation, using the model $\text{group}_3 \times (\text{stimulus}_3 \times S)$. This revealed a main effect of stimulus ($F_{1,777,19,548} = 4.929$, $\tilde{\epsilon} = .889$, $p = .021$); the group \times stimulus interaction escaped significance ($F_{3,554,19,548} = 2.542$, $\tilde{\epsilon} = .889$, $p = .078$) and the main effect of group was not significant ($F < 1$, NS). Overall, the pellet CS elevated responding relative to the ISI ($p = .026$) but the sucrose CS had no effect ($p = .351$). This was also true of the sham group considered alone (pellet CS *v.* ISI, $p = .004$; sucrose CS, $p = .85$). However, in this analysis, no effect of either stimulus was detectable for the core or the shell groups ($F_s < 1$, NS).

Nosepoking

Neither CS affected the rate of nosepoking in core- and shell-lesioned subjects. In the sham group, there was a tendency for the CS(pel) to elevate nosepoking, but this was ambiguous statistically. To analyse nosepoking, the rate of nosepoking was calculated and subjected to a square-root transform to improve homogeneity of variance before an ANOVA was performed using the model $\text{group}_3 \times (\text{stimulus}_3 \times S)$. This revealed a significant stimulus \times group interaction ($F_{4,22} = 4.081$, $p = .013$). Simple effects analysis showed that nosepoking differed among the three stimulus conditions in the sham group ($F_{2,10} = 4.304$, $p = .045$); though no condition was different from any other by *post hoc* pairwise comparisons ($p > .18$), inspection of Figure 52 suggests that the effect was due to elevation of nosepoking by the CS for pellets. In the core and shell groups, there was no effect of the stimulus (core: $F_{2,6} = 3.82$, $p = .085$; shell: $F_{2,6} = 2.334$, NS).

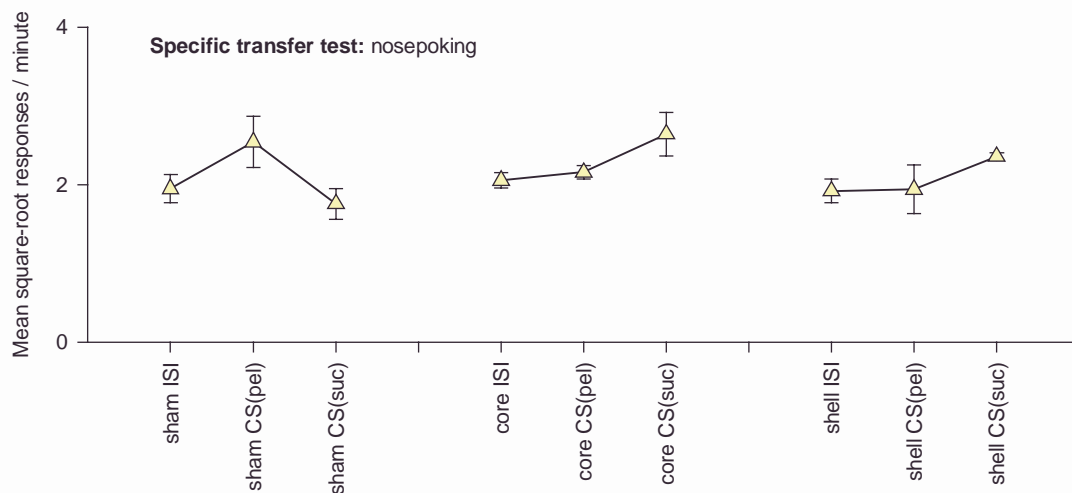


Figure 52. Nosepoking during the specific PIT test. While the stimulus condition affected the rate of nosepoking in the sham group, as detected by a main effect in an ANOVA, no one stimulus condition was significantly different from another by *post hoc* pairwise comparisons. The stimuli did not affect the rate of nosepoking in the core- or shell-lesioned rats.

Retraining

Retraining data are shown in Figure 53.

The groups did not differ in their approach behaviour during Pavlovian retraining. Analysis of the ratios of responding for the Pavlovian sessions was performed using the model $\text{group} \times (\text{session}_3 \times S)$. Once more, this showed greater approach while the CS(suc) was on and sucrose was being presented than dur-

ing the pellet stimulus ($F_{1,11} = 7.394, p = .02$), but no other terms were significant (closest to significance was session \times group, $F_{4,22} = 2.229, p = .099$).

The three groups reacquired the instrumental response at approximately the same rate, but the core-lesioned rats maintained a higher rate of responding in the subsequent extinction session. Analysis of square-root-transformed lever-press data for the reinforced training sessions showed that responding increased over the three sessions ($F_{1,288,14,173}, \tilde{\epsilon} = .644, p = .02$), but no differences between groups were significant (group: $F_{2,11} = 2.356, p = .141$; group \times session: $F_{2,577,14,173} = 1.173, \tilde{\epsilon} = .644, \text{NS}$). However, the core-lesioned group responded significantly more than shams on the extinction day (univariate ANOVA, $F_{2,11} = 4.613, p = .035$; Dunnett's test showed that the core group responded more than shams, $p = .024$, but the shell group did not, $p = .767$).

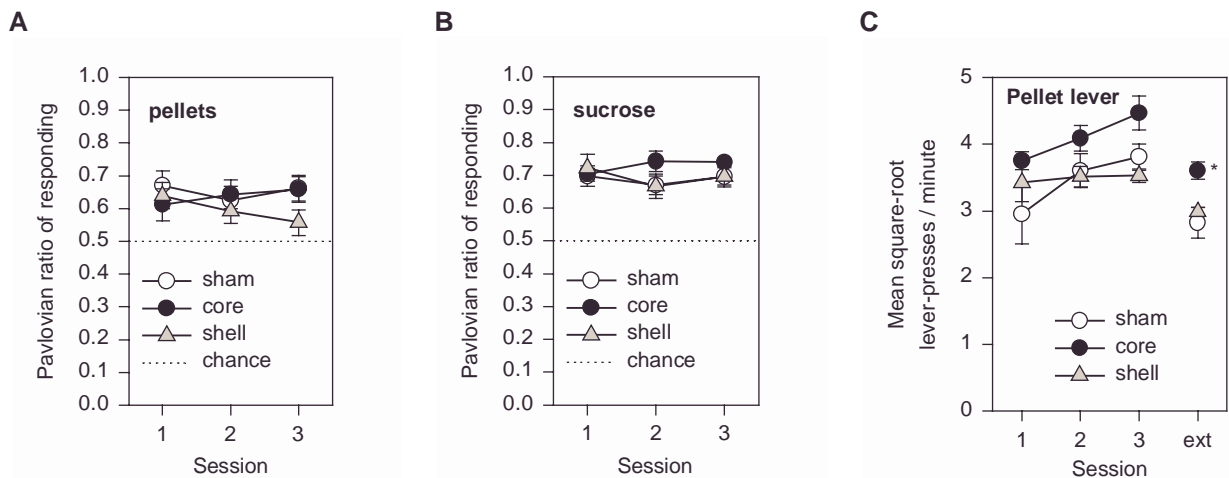


Figure 53. Retaining data before the general PIT test. Panels **A & B** show ratios of responding for the two stimuli in the Pavlovian retraining sessions; as for Figure 49, the ratio of responding is calculated from the time spent approaching the food alone using the formula $CS/(CS+ISI)$. Panel **C** shows responding on the pellet lever (the only lever available) during instrumental retraining and a further extinction session, plotted to the same scale as Figure 50A (* $p < .05$ relative to shams).

General PIT

Lever-pressing was not affected by stimulus presentation in the general transfer test, and the core group responded more than the other groups (Figure 54). Following square-root transformation, an ANOVA was performed using the model $group_3 \times (stimulus_3 \times S)$. This showed that the effect of stimulus presentation did not reach significance ($F_{1,54,16,94} = 3.366, \tilde{\epsilon} = .77, p = .069$) and that there was no stimulus \times group interaction ($F < 1$), though there was a main effect of group ($F_{2,11} = 5.861, p = .019$). The group difference was due to the core group responding more than the other two ($p = .017$), which did not differ from each other ($p > .24$).

However, the Pavlovian stimuli did affect the rate of nosepoking (Figure 55): the CS for sucrose elevated nosepoking relative to the ISI, while the CS for pellets was less effective. Analysis of square-root-transformed nosepoke rates revealed a main effect of stimulus ($F_{2,22} = 8.766, p = .002$), though no main effect of group ($F < 1$) and no stimulus \times group interaction ($F_{4,22} = 2.072, p = .119$). The effect of the stimulus condition could be attributed to greater responding during the sucrose stimulus than the ISI ($p = .007$), with responding during the pellet stimulus at an intermediate level (pellet stimulus *v.* ISI, $p = .058$; pellet *v.* sucrose stimulus, $p = .211$; overall means, in units of square-root responses per minute: ISI 1.393 ± 0.123 , pellet stimulus 1.623 ± 0.174 , sucrose stimulus 1.924 ± 0.11).

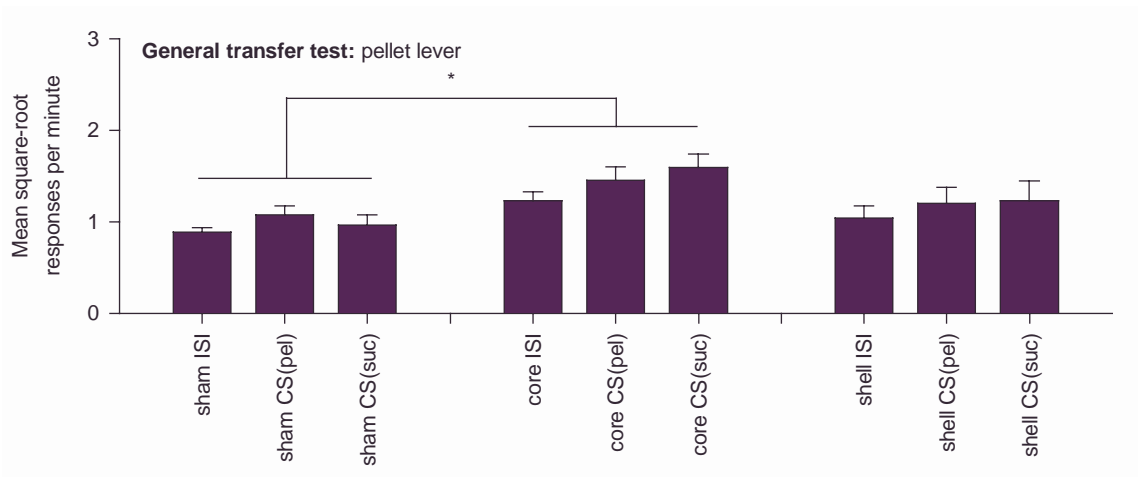


Figure 54. Lever-pressing during the first general transfer test. Responding was not affected by the stimuli, though the core group responded more than the other two groups.

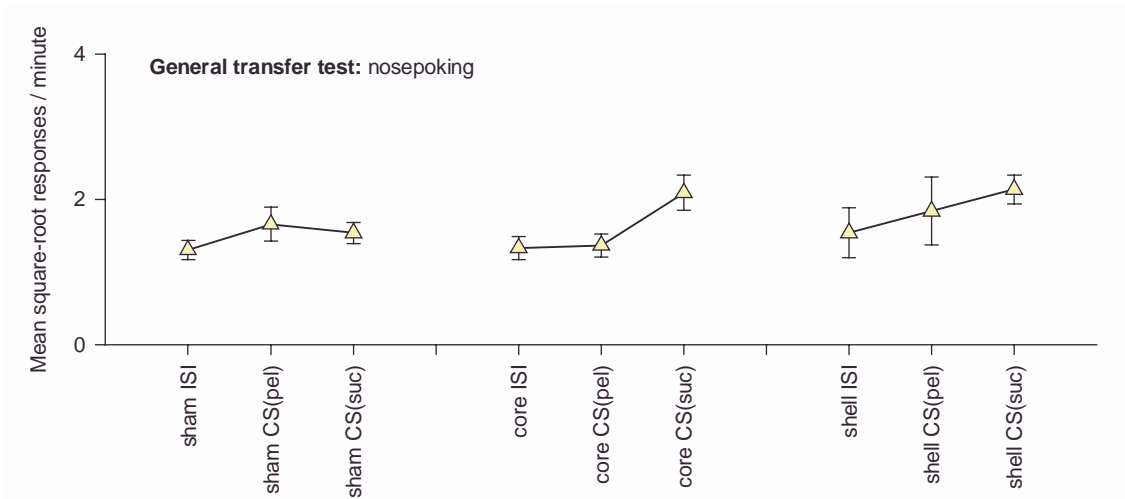


Figure 55. Nose-poking during the general transfer test. The CS(suc) increased the rate of nose-poking over the ISI, but there were no differences between the groups.

On the supposition that transfer to nose-poking rather than lever-pressing occurred because nose-poking was the current prepotent response, further training was given to encourage lever-pressing on test. The rats were returned to the food-deprivation state and given a single Pavlovian training session followed by 8 instrumental sessions with the pellet lever (RI-30s schedule), using the methods described previously. After this, the two-day general transfer test was repeated with subjects water-deprived.

However, this second test was not successful. By this stage the subjects had had extensive experience with extinction sessions (both of the Pavlovian stimuli and the instrumental responses), and responded at very low levels. Responding increased across the instrumental retraining sessions, and inspection of the data suggested that the core group maintained a higher rate of responding, but this was not a significant difference ($p \geq .147$). During the second general transfer test, core-lesioned animals responded more than shams (effect of group: $F_{2,11} = 9.246$, $p = .004$; pairwise comparisons established that the core group differed from the other two groups, which did not differ from each other). However, there was no effect of stimulus presentation on lever-pressing ($F_s \leq 1.571$, NS) or the rate of nose-poking ($F_s < 1.23$, NS).

DISCUSSION

Despite the small number of subjects in the present experiment, response-specific PIT was successfully demonstrated in the sham group: the pellet CS selectively enhanced responding on the lever producing pellets. In core-lesioned subjects, PIT was observed but was not response-specific, while shell-lesioned subjects exhibited no PIT.

‘General’ PIT, the potentiation of responding for one reinforcer by a CS for another, was not successfully obtained in the sham group. As this effect has reliably been observed in other, broadly similar, experimental designs (Dickinson, 1986; Dickinson & Dawson, 1987a; Dickinson & Dawson, 1987b; Balneine, 1994), two features of the present design probably contributed to this failure. Firstly, the results of the response-specific PIT test suggested that the CS for pellets was more effective than the CS for sucrose (the CS for sucrose had no effect on any subjects in this test). Despite the attempt to make the CS for sucrose more salient by rendering the subjects thirsty, this CS was ineffective during the general PIT test except to elevate nose-poking slightly. Had the CS for pellets been presented to hungry subjects responding for sucrose, an effect might have been observed. Indeed, general PIT *was* observed in core-lesioned subjects during the response-specific transfer test, in that the CS for pellets elevated responding on the sucrose lever. Secondly, the general PIT test was conducted after subjects had experienced several extinction sessions (both for the CSs and the responses) as part of the response-specific PIT test. The attempt to conserve subjects was perhaps overly ambitious, and general PIT may be easier to demonstrate in experimentally naïve subjects. This is *not* to imply that the effect is biologically unimportant (as discussed in Chapter 1, p. 27, it underlies the irrelevant incentive effect, probably of great functional significance), but simply that the extinction procedure used to demonstrate the effect guarantees that it will be ephemeral (cf. conditioned reinforcement; Mackintosh, 1974, p. 237).

The psychological basis of response-specific PIT

The present results support some, but not all, previous theories of the psychological basis of response-specific PIT. Several slightly different experimental designs have been used to demonstrate this effect (reviewed by Colwill & Motzkin, 1994). For example, Colwill & Rescorla (1988) trained two groups of subjects, each experiencing a single CS — for one group, the CS was paired with pellets, and for the other group, it was paired with sucrose (see Chapter 1, p. 26). The subjects were then trained to press a lever for pellets and pull a chain for sucrose in separate sessions (of course, the experiment was counterbalanced in this respect). As discussed by Dickinson (1994, p. 67), this meant that subjects learned to press the lever at a time when the contextual cues were associated with pellets and pull the chain when these cues were paired with sucrose solution. Consequently, the presentation of the Pavlovian CS on test may have helped to reinstate the conditions under which one of the actions was trained. (This explanation emphasizes the role of the stimuli that *elicit* instrumental responses, not the consequences of those responses.)

The present design was essentially that of Colwill & Motzkin (1994, Experiment 2), and the results support their conclusions regarding the psychological basis of the effect. As a within-subjects design was used, all animals experienced two Pavlovian CSs paired with two different reinforcers. These CSs were trained in alternation. Furthermore, the two instrumental responses were trained concurrently. This design minimizes differential contextual associations of the Pavlovian CSs, the instrumental responses, and the reinforcers. It is therefore less obvious that the CS reinstated the conditions under which the action was trained. The alternative, more likely explanation of the present data is that the CS potentiates actions based on a comparison of the US with the *outcome* of the instrumental response, as argued by Colwill & Motzkin (1994).

One other feature of the present behavioural results is worth noting. While some previous studies have found that a Pavlovian CS exerts its response-specific effect by *depressing* responses that do *not* share an outcome with the CS (e.g. Colwill & Rescorla, 1988; Colwill & Motzkin, 1994), the present results provide further evidence that a CS can selectively potentiate responses with which it does share an outcome (see also Baxter & Zamble, 1982). It is not at present clear why these difference was found, particularly as the present experiment was very similar in design to that of Colwill & Motzkin (1994), and suggests that CS/S^D differences may not be as critical as previously suggested in determining the direction of the effect (cf. Colwill & Rescorla, 1988). Possible explanations include differences in the rate of baseline responding, the degree of food deprivation, and the degree to which transfer occurs to behaviours other than instrumental responding, but this remains an area for further investigation.

The contribution of the Acb to PIT

It is difficult to draw a clear picture of the role of the Acb in PIT from the experiments conducted to date, as some studies appear contradictory.

The present experiment suggests that the AcbSh is required for PIT *per se*, perhaps providing the ‘vigour’ of PIT, while the AcbC is required to ‘direct’ this potentiation to a particular response when that response shares an outcome with the CS. These results provide further support for the claim that the Acb is critically involved in the impact of Pavlovian CSs upon behaviour (see Chapter 1 and Parkinson *et al.*, 2000a). It seems unlikely that these deficits were due to a failure to discriminate the two instrumental responses, as both core-lesioned (e.g. Chapter 7; Parkinson *et al.*, 1999b) and shell-lesioned (e.g. Parkinson *et al.*, 1999b) rats have been shown able to discriminate two levers for the purposes of responding. The pre-existing preference of the core group for the pellet lever undoubtedly complicates interpretation a little, but cannot easily explain the lack of response specificity in this group; the preference did not lead to a ‘ceiling effect’, for at response rates of ~4/min, core-lesioned subjects were certainly not responding maximally on the pellet lever (they responded at rates of ~16/min during instrumental acquisition, for example).

These results closely resemble the effects of core/shell lesions on the potentiation of responding for conditioned reinforcement by intra-Acb amphetamine (temporarily designated ‘amphetamine potentiation of conditioned reinforcement’, APCR). Parkinson *et al.* (1999b) showed that shell lesions abolished APCR, while core lesions removed the response selectivity of APCR without abolishing APCR itself.

The present results also show some correspondence to those of Corbit & Balleine (2000a), who found that AcbSh lesions abolished transfer in a variant of the response-specific PIT procedure. They found no effect of AcbC lesions on PIT, although only a single lever was present at any one time during their test, which may therefore have been less sensitive to deficits in response specificity (or in the ability to switch between responses as a result of CS presentation). An additional procedural difference was that Corbit & Balleine used a ratio schedule to demonstrate PIT. As discussed in Chapter 1 (p. 28), Lovibond (1983) showed that simple PIT may have a different psychological basis under ratio and interval schedules (possibly relating to the relative contributions of habitual and goal-directed behaviour; see Chapter 1), and it is not yet known how this relates to the involvement of the AcbC.

It is not so easy to reconcile the present data with those of Hall *et al.* (1999), who tested rats with a ‘simple’ PIT task, testing elevation of responding on a single lever by a CS for the same outcome. Hall *et al.* found that shell lesions had no effect on PIT, while core lesions completely abolished the effect. Intuitively, response-specific PIT has much in common with simple PIT: the response-specific test is the simple PIT test with another response available. On the basis of the results of Hall *et al.*, it would be expected

that AcbC lesions would abolish PIT entirely. If both sets of results are accepted, the puzzling conclusion is that response-specific PIT engages a (core-independent) process that does not contribute significantly to simple PIT (because core-lesioned animals showed some PIT in the former situation, but not the latter), but that this extra process is not response specificity itself (as the core-lesioned subjects did not show response specificity). A similar argument may be made from the finding that shell lesions impaired PIT in the present study, but not in that of Hall *et al.* (1999).

However, procedural differences do exist between the two studies. Hall *et al.* (1999) used only a pellet lever and a CS for pellets, and tested over a single 30-min session. In an attempt to see if this difference accounted for the discrepant findings, data from the present study were analysed in an analogous manner (p. 141). This analysis, while detecting PIT in the sham group, failed to detect PIT in the core or shell groups. It is possible, therefore, that the two-day test is more sensitive, and that this accounts for the detection of a PIT effect in the core group, though this cannot explain the differences in findings for the shell group. Perhaps measuring an additional response, as in the present experiment, simply increases the power to detect PIT. Indeed, as Figure 51 (p. 140) shows, the greatest PIT effect observed in the core-lesioned subjects was elevation of responding on the sucrose lever by the pellet CS! In the study of Hall *et al.* (1999), the CS did elevate the rate of one other behaviour, nose-poking in the food alcove — though even using this measure, core-lesioned subjects were impaired relative to shams (J. Hall, personal communication, 8 June 1999).

Additionally, technical failings of the present study must be taken into account. This experiment was based on a small number of subjects (sham 6, core 4, shell 4); though this does not alter any of the conclusions regarding these subjects, it brings a sense of caution to the interpretation of the results as representative of all sham-, core-, or shell-lesioned rats. Also, following histological analysis, the counterbalancing of the groups was incomplete. While an attempt was made to detect bias resulting from this failure of counterbalancing (p. 139), and none was found, failure to find any effects of the counterbalancing conditions may simply have been due to low statistical power and the ‘unbalanced’ counterbalancing may have contributed in some way to the results.

To summarize, while the present results are consistent with work concerning the role of the AcbC and AcbSh in APCR, surprising differences from previous studies of simple PIT emerged. As these differences suggest that PIT operates in a highly counter-intuitive manner, it would be well worth while replicating the present study with larger group sizes to give more effective counterbalancing, perhaps with a larger sucrose reinforcer in order to observe an effect of the CS for sucrose.

The relationship between PIT and conditioned reinforcement

Neither the AcbC nor the AcbSh appear to contribute to the basic phenomenon of conditioned reinforcement; however, they are both critically involved in the artificial phenomenon of APCR (Parkinson *et al.*, 1999b). Wyvell & Berridge (2000) have found that intra-Acb amphetamine potentiates PIT, implying that intra-Acb amphetamine has effects that cannot be explained solely in terms of conditioned reinforcement. It may be fruitful to ask whether the converse is true: can the contribution of the Acb to APCR be explained in terms of PIT, or do both phenomena need to be subsumed within a wider description?

PIT is clearly not analogous to conditioned reinforcement itself. As discussed in Chapter 1 (p. 27), general PIT does not affect choice behaviour (unlike CRf), although once a CS has been earned in a conditioned reinforcement task, it might be capable of boosting responding through PIT. Response-specific PIT might contribute to CRf (though this would require more than first-order associations; see Chapter 1, p. 31), but there is no direct evidence for this suggestion. Furthermore, PIT and CRf have been dissoci-

ated neurally; lesions of the CeA impair simple PIT (Hall *et al.*, 1999), but do not impair CRf (though the effect of intra-accumbens amphetamine upon CRf is abolished; Robledo *et al.*, 1996). Similar results have been reported by Killcross *et al.* (1998), using a task in which prolonged presentation of a putative conditioned reinforcer did indeed produce an elevation of responding (interpretable as PIT); this elevation was sensitive to CeA lesions but the CRf effect itself was not. Conversely, lesions of the BLA, which impair CRf (Cador *et al.*, 1989; Burns *et al.*, 1993), do not affect simple PIT (Killcross *et al.*, 1998; Hall *et al.*, 1999). Finally, lesions of the AcbC or AcbSh do not impair the acquisition of a new response with CRf (Parkinson *et al.*, 1999b), but these regions contribute to PIT (in a way that is still not completely clear: present experiments; Hall *et al.*, 1999; Cardinal *et al.*, 2000a; Corbit *et al.*, submitted).

However, there is a striking match between the neural bases of PIT and APCR. The present results suggest that the AcbSh is required for PIT *per se*, while the AcbC is not required for PIT but is required to ‘direct’ this potentiation to a particular response. Similarly, Parkinson *et al.* (1999b) showed that shell lesions abolished APCR, while core lesions removed only the response selectivity of APCR. The analogy may be continued: APCR depends upon Acb dopamine (Taylor & Robbins, 1986; Cador *et al.*, 1991; Wolterink *et al.*, 1993), while noncontingent presentation of an appetitive CS elevates Acb dopamine (specifically in the AcbC; Bassareo & Di Chiara, 1999; Ito *et al.*, 2000). PIT may also involve Acb dopamine, as it is abolished by systemic dopamine antagonists (Dickinson *et al.*, 2000) and enhanced by intra-Acb amphetamine (Wyvell & Berridge, 2000). Both APCR (Robledo *et al.*, 1996) and PIT (Hall *et al.*, 1999) depend on the CeA, probably because the CeA influences Acb dopamine via the VTA (see Chapter 1, pp. 43/47/49). Furthermore, lesions of the BLA remove the source of information to the Acb regarding conditioned reinforcement that determines which lever APCR acts upon (Cador *et al.*, 1989; Burns *et al.*, 1993); similarly, BLA lesions impair the response selectivity of PIT (Blundell & Killcross, 2000a) but do not abolish the basic PIT effect (Hall *et al.*, 1999; Blundell & Killcross, 2000a). Core lesions can sometimes abolish PIT (Hall *et al.*, 1999), and they also abolish APCR, in that the ability of amphetamine to potentiate responding for a conditioned reinforcer in a selective manner is lost, though amphetamine still potentiates responding in a nonselective manner in AcbC-lesioned animals (Parkinson *et al.*, 1999b). Shell lesions abolish APCR (Parkinson *et al.*, 1999b) and can abolish PIT (present experiments; Corbit & Balleine, 2000a), though not in all tasks (Hall *et al.*, 1999). Thus, though ambiguities remain, it may be reasonable to suppose that APCR reflects artificial activation of the system by which *noncontingent* Pavlovian CSs normally increase the probability of instrumental responses (PIT). This system appears to play a minor role in responding for CRf under normal situations (thus, responding for conditioned reinforcement survives AcbC and AcbSh lesions; Parkinson *et al.*, 1999b), possibly reflecting the fact that typical CRf experiments use brief conditioned reinforcers that cannot significantly potentiate responding via PIT.

Finally, as the Acb is also necessary for autoshaping (the AcbC, but not the AcbSh; Parkinson *et al.*, 2000c), a task in which the response is Pavlovian locomotor approach, the Acb must be capable of influencing several kinds of response. The Acb appears to mediate the motivational influence of noncontingent Pavlovian CSs on instrumental and locomotor behaviour — an influence that has been termed *incentive salience* (Robinson & Berridge, 1993; Berridge & Robinson, 1998), or Pavlovian incentive value (Dickinson *et al.*, 2000). One of the greatest remaining problems of Acb function is to understand the manner in which information passing through the Acb is encoded, and modified by this Pavlovian influence, and how the AcbC and AcbSh interact — apparently in different ways for different tasks — to provide this motivation.