Chapter 7. Contributions of limbic and prefrontal circuitry to choice of delayed reinforcement

Abstract. Impulsive choice, the inability to choose a large delayed reward in preference to an immediate but small reward, is an important but poorly-understood phenomenon. As impulsive choice may result from an insensitivity to delayed reinforcement, and limbic corticostriatal circuits have been implicated in reinforcement processes, the present experiments investigated the contribution of components of the prefrontal cortex and ventral striatum to rats' ability to choose a delayed reward. Rats were trained on a two-lever discrete-trial delayed reinforcement task in which they chose one food pellet delivered immediately or four pellets delivered after a delay; this delay increased from 0 to 60 s during each session. Subjects developed a characteristic within-session shift in preference, choosing the larger reinforcer at short delays, but the smaller reinforcer when the delay was long. Once trained, the rats were assigned to matched groups and received excitotoxic lesions of the perigenual anterior cingulate cortex (ACC), medial prefrontal cortex (mPFC), or nucleus accumbens core (AcbC); they were then retested. Lesions of the ACC had no effect on subjects' capacity to choose the delayed reward, or their ability to respond to removal of the delays by choosing the large reward consistently, though ACC-lesioned subjects were slower to collect the larger reward than sham-operated controls. Lesions of the mPFC induced a 'flattening' of the within-session shift in preference, but subjects still responded normally to removal of the delays, suggesting a loss of temporal stimulus control. Lesions of the AcbC dramatically and persistently impaired subjects' ability to choose the large reinforcer when it was delayed, even though subjects discriminated the two reinforcers. It is suggested that dysfunction of the AcbC may be a key element in the pathology of impulsivity. In a different version of the task, intra-accumbens amphetamine was found to have slight but inconsistent effects to reduce preference for the delayed reinforcer, though this effect did not depend on whether the delayed reward was signalled or unsignalled.

INTRODUCTION

Impulsive choice is exemplified by the tendency of an individual to choose a reward that is small, poor, or ultimately disastrous, but is available immediately, in preference to a larger reward that is only obtainable after a period of time (Ainslie, 1975). Impulsive choice may reflect reduced efficacy of delayed reinforcement. It has been considered a normal human characteristic (Aristotle, 350 BC / 1925), but impulsive choice contributes to deleterious states such as drug addiction (Poulos *et al*., 1995; Heyman, 1996; Bickel *et al*., 1999; Evenden, 1999a; Mitchell, 1999) and has been suggested to underlie a number of other clinical disorders, including attention-deficit / hyperactivity disorder (ADHD; Sagvolden *et al.*, 1998; Sagvolden & Sergeant, 1998).

Little is known of the neuroanatomical basis of impulsive choice. However, three lines of evidence suggest the nucleus accumbens (Acb) and its cortical afferents, including the anterior cingulate and me-

dial prefrontal cortices (ACC, mPFC), as candidate structures that may be involved in regulating choice between alternative reinforcers.

First, these structures have been firmly implicated in reinforcement processes. The Acb, once suggested to mediate the reinforcing efficacy of natural and artificial rewards (see Koob, 1992) (and also Wise, 1981; 1982; 1985; 1994)**,** is now thought not to be necessary for this, but instead to be a key site for the motivational impact of impending rewards (reviewed by Robbins & Everitt, 1996; Salamone *et al.*, 1997; Everitt *et al.*, 1999; Parkinson *et al.*, 2000a). Many of its afferents have also been shown to be involved in reward-related learning, including the ACC (Chapter 3; Bussey *et al.*, 1997a; Bussey *et al.*, 1997b; Parkinson *et al.*, 2000c) and mPFC (e.g. Balleine & Dickinson, 1998a; Richardson & Gratton, 1998; Bechara *et al.*, 1999; Tzschentke, 2000).

Second, these regions are important recipients of dopaminergic and serotonergic afferents (Fallon & Loughlin, 1995; Halliday *et al.*, 1995), and pharmacological manipulations of dopamine and serotonin systems have been shown to affect impulsive choice in rats (Sagvolden *et al.*, 1992; Wogar *et al.*, 1993b; Richards & Seiden, 1995; Charrier & Thiébot, 1996; Evenden & Ryan, 1996; Richards *et al.*, 1997a; Evenden, 1998; Bizot *et al.*, 1999; Evenden, 1999b; Evenden & Ryan, 1999; Ho *et al.*, 1999; Richards *et al.*, 1999; Cardinal *et al.*, 2000b; Wade *et al.*, 2000).

Third, abnormalities of these regions have been detected in humans with ADHD, and in animal models of ADHD. Abnormal functioning of prefrontal cortical regions, including medial prefrontal and anterior cingulate cortex, has been observed in ADHD patients (Ernst *et al.*, 1998; Bush *et al.*, 1999; Rubia *et al.*, 1999). In the spontaneously hypertensive rat (SHR), widely used as an animal model of ADHD (Wultz *et al.*, 1990; Sagvolden *et al.*, 1992; Sagvolden *et al.*, 1993; Sagvolden, 2000), differences in dopamine receptor density and gene expression have been observed within the core and shell regions of the Acb (Papa *et al.*, 1996; Carey *et al.*, 1998; Papa *et al.*, 1998; Sadile, 2000). Abnormalities of dopamine release have been detected in the Acb (de Villiers *et al.*, 1995; Russell *et al.*, 1998; Russell, 2000) and prefrontal cortex (Russell *et al.*, 1995), in addition to possible dysfunction in the dorsal striatum and amygdala (Russell *et al.*, 1995; Papa *et al.*, 2000).

Evenden and Ryan (1996) developed a model of impulsive choice in which food-restricted rats choose between a small, immediate reward and a large, delayed reward in discrete trials, the delay to the large reinforcer being increased in steps as the session progressed. The present study investigated the effects of excitotoxic lesions of the ACC, mPFC, and AcbC on performance of a modified version of this task. Potentially, the lesions might affect learning of the task; in order to avoid this confounding factor, subjects were trained before the lesions were made. As it was demonstrated in Chapter 6 that explicit signals present during a delay to reinforcement may affect the response to a behavioural or pharmacological manipulation, the simplest situation was used, with no signals present during the delay to reinforcement. After subjects had been tested post-operatively, all delays were removed from the task to establish whether lesioned subjects remained sensitive to the delays.

Finally, an experiment was conducted to investigate the role of the Acb in the effects of amphetamine on impulsive choice. Amphetamine was injected directly into the Acb before animals chose between a small, immediate reward and a large, delayed reward in discrete trials. As the effects of amphetamine depend in part upon signals present during the delay to reward (Chapter 6; Cardinal *et al.*, 2000b), intra-Acb amphetamine was administered to two groups of subjects, trained with or without a cue stimulus present during this delay. As discussed in Chapter 6 (p. 192), it was anticipated that intra-Acb amphetamine would enhance the conditioned reinforcing properties of such a stimulus, promoting choice of the delayed reward in the cued group.

EXPERIMENT 1. EFFECTS OF LESIONS OF THE ANTERIOR CINGULATE CORTEX

Methods

Twenty-four naïve rats were maintained at 90% of their free-feeding mass and trained on the same delay-of-reinforcement task used in Chapter 6 (*q.v.*). They were first trained to press levers for sucrose pellets. (In Chapter 6, subjects were allowed to respond freely on an FR1 schedule on the left lever until they had acquired at least 50 reinforcers in 30 minutes, and then trained on the right lever, with no limit on the number of reinforcers available in each 30-min session. However, it was observed that subjects tended to acquire responding more rapidly, and thus accrue more reinforcers, on the lever trained second; thus, for all studies in the present chapter, subjects were trained until they had accrued an *overall total* of 50 reinforcers on each lever in turn; when this limit had been reached, the lever was retracted and the session finished.) Next, they were trained to nosepoke to initiate discrete-trial presentations of the levers, before being trained on the main delay-of-reinforcement task for 19 sessions. No cues were present during the delays to reinforcement. After this, they were assigned to matched groups by ranking all subjects according to the regression slope measure (see Chapter 6, p. 174), calculated using data from the last 3 pre-operative sessions. The ranked list was divided into pairs, and from each pair one subject was assigned to the sham group and the other to the ACCX group, at random. It was subsequently ensured that both groups had achieved criterion sensitivity to delay (see Chapter 6), and that there were no significant pre-operative differences in the absolute level of preference.

Subjects then received lesions of the anterior cingulate cortex (ACCX, $n = 12$) or sham lesions (sham, $n = 12$). At the time of surgery, their body mass was 329–379 g. Following recovery, they were retested on the basic task for 7 sessions to obtain a baseline of performance. After this, 4 sessions were given in which all delays were omitted in alternate sessions (DNDN design; D = delays present, N = no delays). Half of the subjects began this test with the delays present, and half with no delays (counterbalanced across groups).

Results

Histology

One subject in the ACCX group died post-operatively (subject H11). Histological analysis revealed that the lesion was incomplete in two subjects (subjects H2, H22), who were excluded, leaving 9 in the ACCX group (H1, H3, H8, H10, H14, H15, H18, H19, H21) and 12 in the sham group (H4, H5, H6, H7, H9, H12, H13, H16, H17, H20, H23, H24). Neuronal loss and associated gliosis in the lesion group extended from ~3.0 mm anterior to bregma to ~0.3 mm posterior to bregma, damaging perigenual Cg1 and Cg2, and in some cases Cg1 more anteriorly. There was no damage to PrL, IL, PCC, or the corpus callosum. Within the ACCX group, there was some heterogeneity; 5 of these animals had lesions encompassing the entire ventral perigenual region, including the ventral portion of Cg2 at 1.6–1.7 mm anterior to bregma (H1, H10, H14, H15, H19; see Figure 82), while 4 did not (H3, H8, H18, H21; see Figure 83). Representative photomicrographs of ACC lesions were shown in Chapter 3 (p. 80).

Anterior cingulate cortex: schematic of lesions

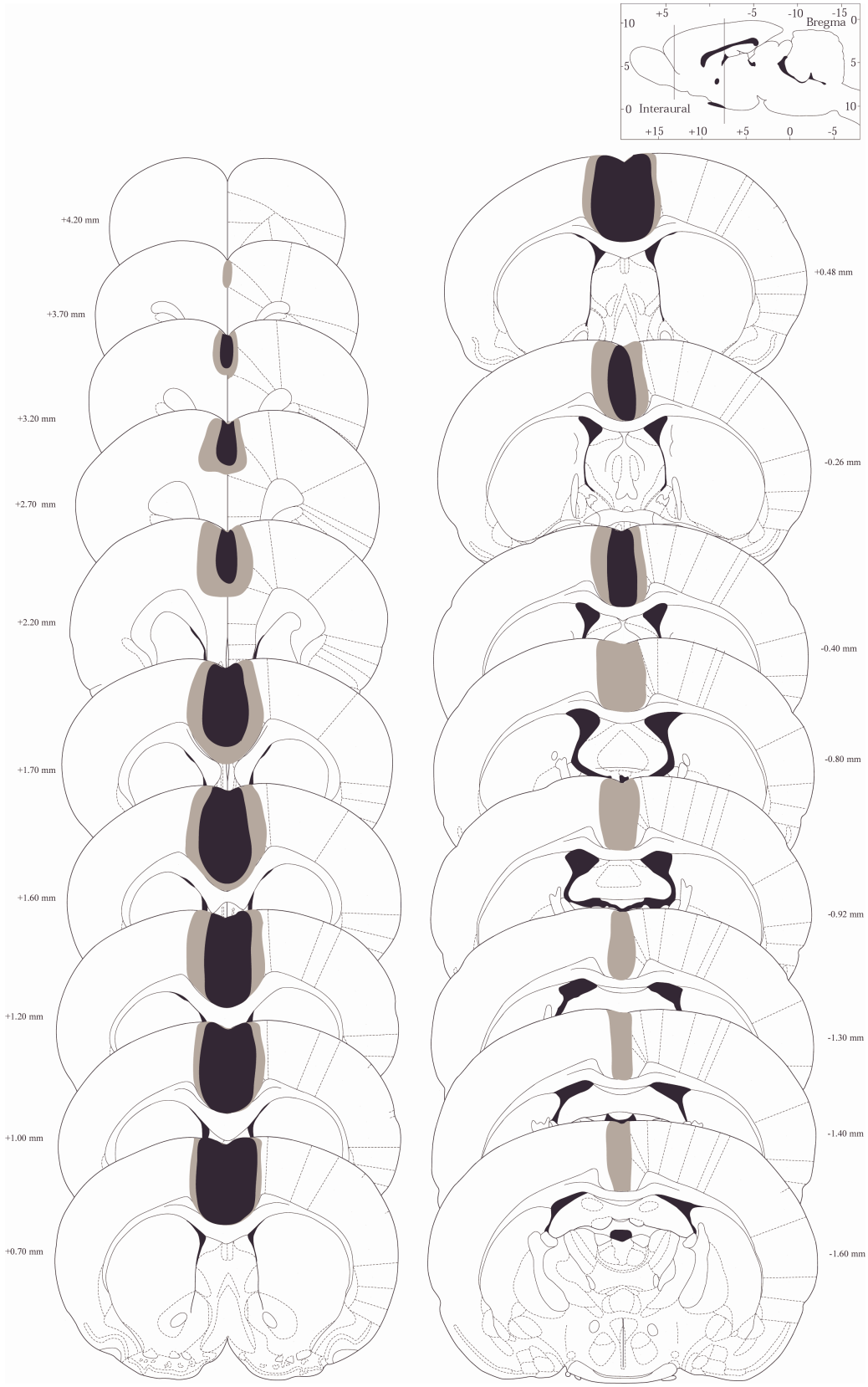

Figure 82. Lesions of the ACC, *including* the ventral perigenual region. Subjects were classified as having whole or partial ACC lesions of the basis of whether the ventral portion of Cg2 in the 'cup' of the genu was lesioned (seen here in sections +1.6 and +1.7 mm from bregma). Grey shading indicates the extent of the largest area of neuronal loss, and black the smallest. Diagrams are taken from Paxinos & Watson (1998). (Subjects: H1, H10, H14, H15, H19.)

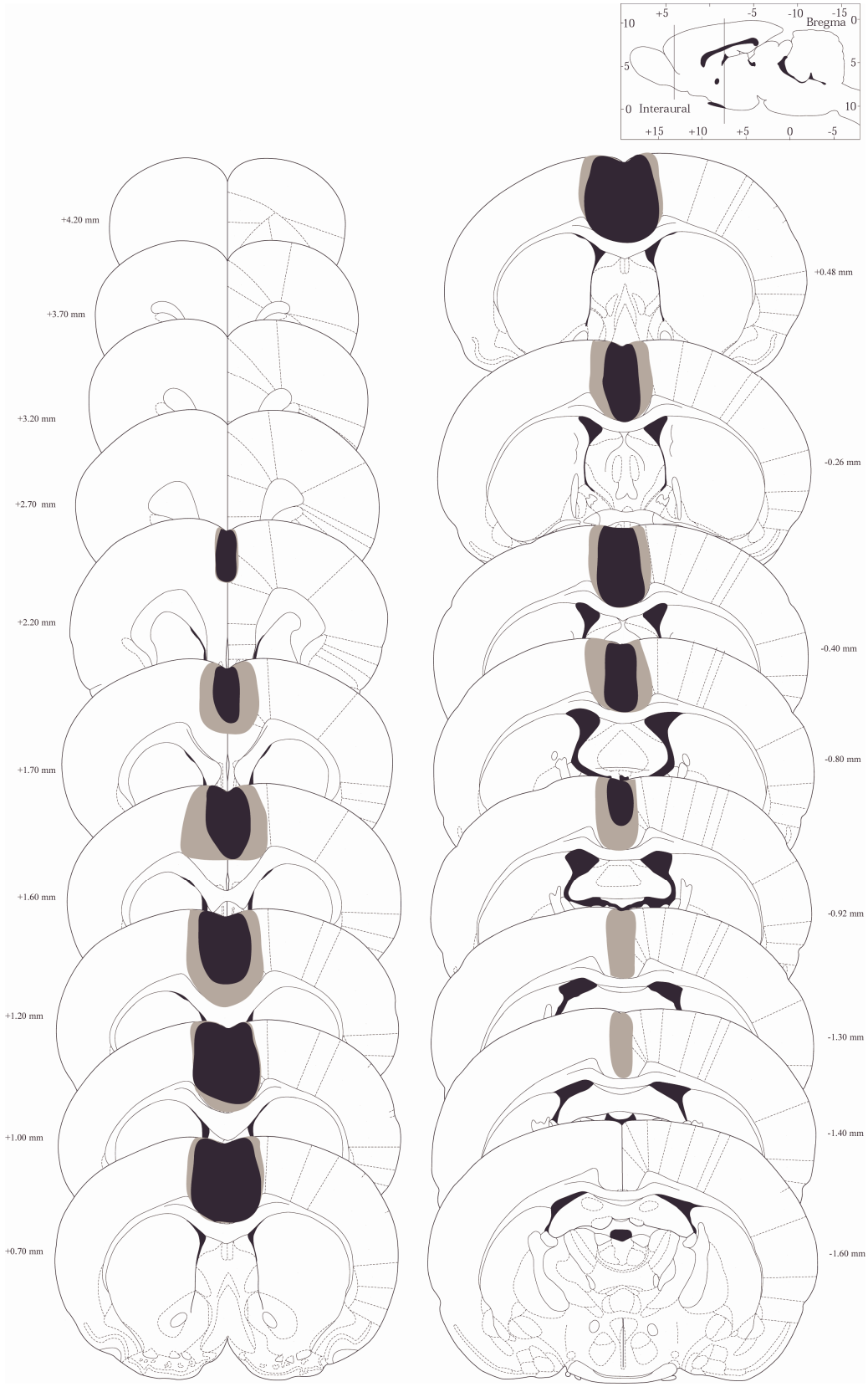

Figure 83. Lesions of the ACC, *excluding* the ventral perigenual region (compare Figure 82). (Subjects: H3, H8, H18, H21.)

Pre-operative acquisition

The groups remained matched after histological selection: there were no differences in the pre-operative pattern of choice (Figure 84A). Choice ratios from the last 3 pre-operative sessions were analysed using the model group × (delay × S). While there was a highly significant effect of delay ($F_{1.858,35.299}$ = 44.349, $\tilde{\varepsilon}$ = .464, p < .001), there was no effect of group and no group × delay interaction (Fs < 1, NS).

Baseline post-operative performance

Choice. There were no differences between sham and ACCX groups in the pattern of choice observed for the 7 baseline sessions (Figure 84B–D). Analysis of choice ratios demonstrated that the effect of delay remained highly significant ($F_{2.404,45.684}$ = 53.46, $\tilde{\varepsilon}$ = .601, p < .001), but there was no effect of group and no group × delay interaction (Fs < 1, NS). The rapidity of the within-session shift in preference, as assessed by the slope measure, did not differ either, and did not alter across the post-operative sessions; analysis using the model group_2 × (session_7 × S) revealed no effect of any term (Fs < 1.101, NS). A separate comparison between shams and the subgroup of ACC-lesioned animals with complete ventral perigenual damage did not alter these conclusions (all terms involving group, Fs < 1, NS).

Omissions. Responding was reliable, with all animals regularly sampling both levers, and the two groups did not differ in the number of omissions made. An analysis of the percentage of trials on which an omission occurred, across all delays, revealed no effect of group ($F_{1,19}$ = 2.686, NS).

Initiation latency. While initiation latencies increased with delay (from 1.20 ± 0.06 s at zero delay to 1.55 ± 0.12 s at the maximum delay), there were no differences between the two groups (delay: $F_{2.639}$ = 11.572, $\tilde{\varepsilon}$ = .66, p < .001; group and delay × group, Fs < 1, NS).

Choice latency. Subjects responded faster on the lever that produced the larger reward, particularly at short delays (mean latencies at zero delay: large reward 0.96 ± 0.05 s, small reward 1.34 ± 0.014 s; at 60 s delay: large reward 0.97 ± 0.05 s, small reward 1.01 ± 0.04 s). However, there were no group differences. An analysis using the model group × (response × delay × S) revealed a response × delay interaction ($F_{2.577,43.804}$ = 5.676, $\tilde{\varepsilon}$ = .644, p = .003), as well as main effects of response ($F_{1,17}$ = 5.195, p = .036) and delay ($F_{2.021,34.357}$ = 4.997, $\tilde{\varepsilon}$ = .505, p = .012). However, no terms involving group were significant (Fs < 1, NS).

Collection latency. Lesioned subjects were slower to collect the larger reward (Figure 84D). An analysis by group × (response × delay × S) revealed a group × response interaction ($F_{1,17}$ = 15.1, p = .001) as well as a main effect of delay ($F_{2.794,47.501}$ = 3.042, $\tilde{\varepsilon}$ = .699, p = .041), reflecting slightly longer collection latencies at long delays. No other terms were significant (closest to significance: response, $F_{1,17}$ = 2.466, p = .135).

Nosepoking during the delay. While there was a small tendency for subjects to spend a greater proportion of time nosepoking at longer delays, no group differences were found. An analysis of the percentage of the delay spent nosepoking, using the model group_2 × (delay_4 × S), revealed an effect of delay ($F_{2.13,40.463}$ = 3.36, $\tilde{\varepsilon}$ = .71, p = .042) but no effect of group ($F_{1,19}$ = 1.663, NS) and no group × delay interaction ($F_{2.13,40.463}$ = 1.938, $\tilde{\varepsilon}$ = .71, NS).

Effect of omitting all delays

Both groups remained sensitive to the removal of delays, shifting their preference towards the large reinforcer under these conditions (Figure 84E). Analysis of choice ratios using the model group_2 × ({Delays versus No Delays}$_2$ × trial block_5 × S) revealed a highly significant interaction between the Delay / No Delay factor and the trial block ($F_{3.302,62.731}$ = 39.346, $\tilde{\varepsilon}$ = .825, p < .001), in addition to main effects of the

Delay / No Delay factor ($F_{1,19}$ = 30.235, p < .001) and the trial block ($F_{4,76}$ = 33.679, p < .001), However, there were no group differences (terms involving group: Fs < 1.392, NS). As in the previous study (Chapter 6), a significant shift of preference persisted in the absence of delays (simple effect of trial block in the No Delay condition: $F_{4,76}$ = 2.542, p = .046), although it was slight.

Summary

Lesions of the ACC did not affect subjects' ability to choose a delayed reward; their pattern of choice was indistinguishable from that of sham-operated controls, and their behaviour remained sensitive to removal of the delays. The only behavioural difference apparent was that ACC-lesioned subjects collected the large reward somewhat slower than controls.

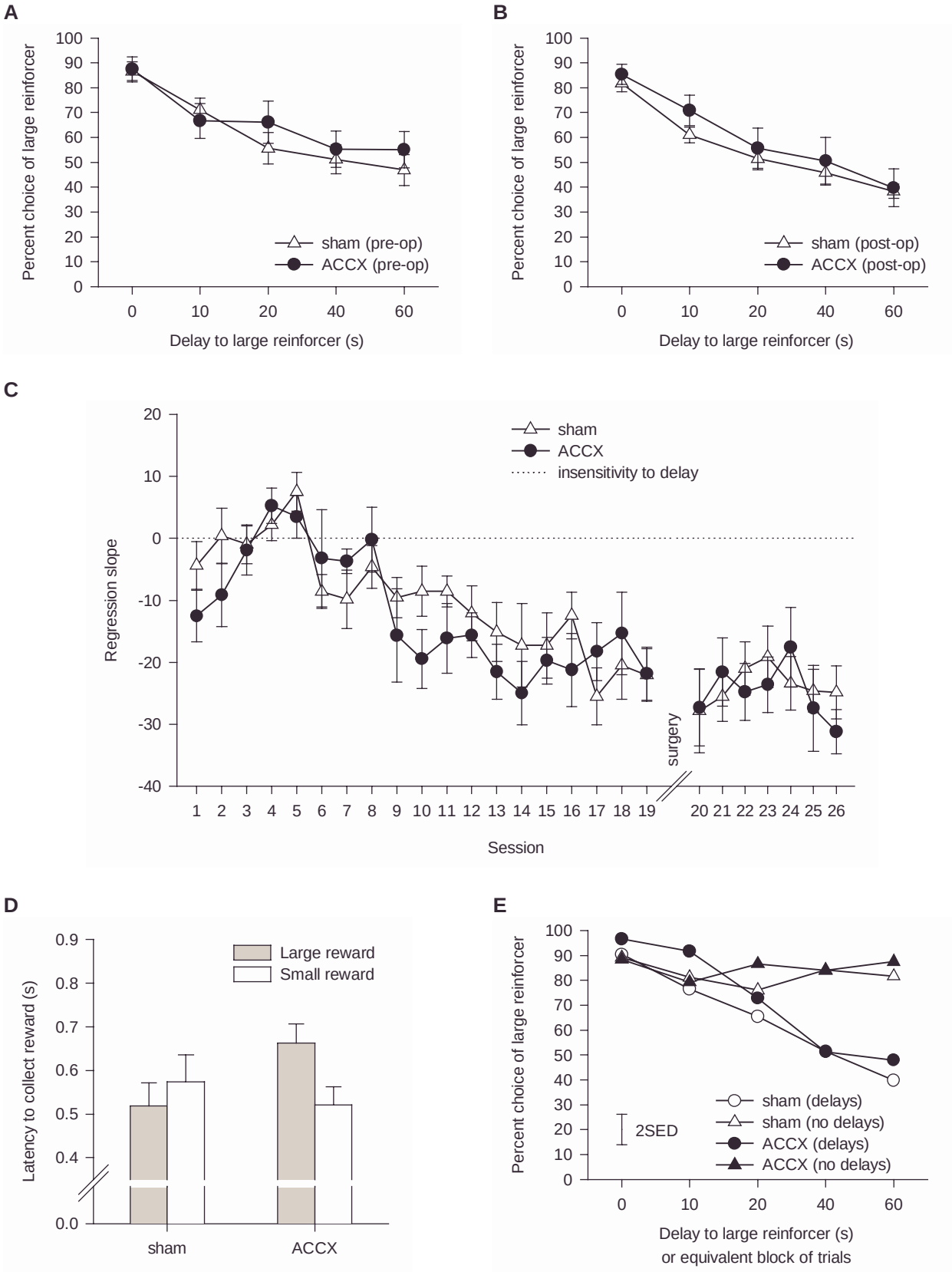

Figure 84. Effects of lesions of the ACC on performance of the delayed-reinforcement choice task. **A:** Pre-operative performance — data from the last 3 sessions preceding surgery. **B:** Post-operative performance — data from the first 7 sessions following surgery. **C:** Slope measures before and after surgery. This slope measure is the linear regression of %choice of the large reinforcer against log(delay + 1 s), calculated for each session. More negative slopes indicate a larger within-session shift from the large to the small reinforcer as the delay lengthens. **D:** Latencies to collect reward post-operatively, averaged across all delays. ACC-lesioned rats were slower to collect the large reward. **E:** Effect of omitting all delays in alternating sessions (*2SED*, twice the standard error of the difference for the three-way interaction).

EXPERIMENT 2. EFFECTS OF LESIONS OF MEDIAL PREFRONTAL CORTEX

Methods

Twenty-four naïve subjects were trained and assigned to two groups as in Experiment 1 (p. 197). They then received lesions of the medial prefrontal cortex (mPFC group, $n = 14$) or sham lesions ($n = 10$). At the time of surgery, they weighed 276–373 g. Following recovery, they were retested on the basic task for 7 sessions. After this, 4 sessions were given in which all delays were omitted in alternate sessions (ABAB design), as before. Finally, a 2-h locomotor test was given.

Results

Histology

There were no postoperative deaths. One rat was excluded from the mPFC group because its lesion was unilateral (M3), and two because the lesion extended beyond the genu posteriorly (M4, M7), leaving 11 in the mPFC group (M6, M9, M10, M12, M13, M14, M16, M18, M20, M22, M24) and 10 in the sham group (M1, M2, M5, M8, M11, M15, M17, M19, M21, M23). Within the mPFC group, neuronal loss and associated gliosis extended from approximately 5.0 to 1.7 mm anterior to bregma. Within this region, there was extensive damage to prelimbic cortex, with damage also occurring in infralimbic cortex, dorsal Cg1, and medial orbital cortex. There was no damage posterior to the genu. Representative photomicrographs are shown in Figure 85, and schematics (indicating the largest and smallest extent of the lesions) are shown in Figure 86.

Medial prefrontal cortex: photomicrographs

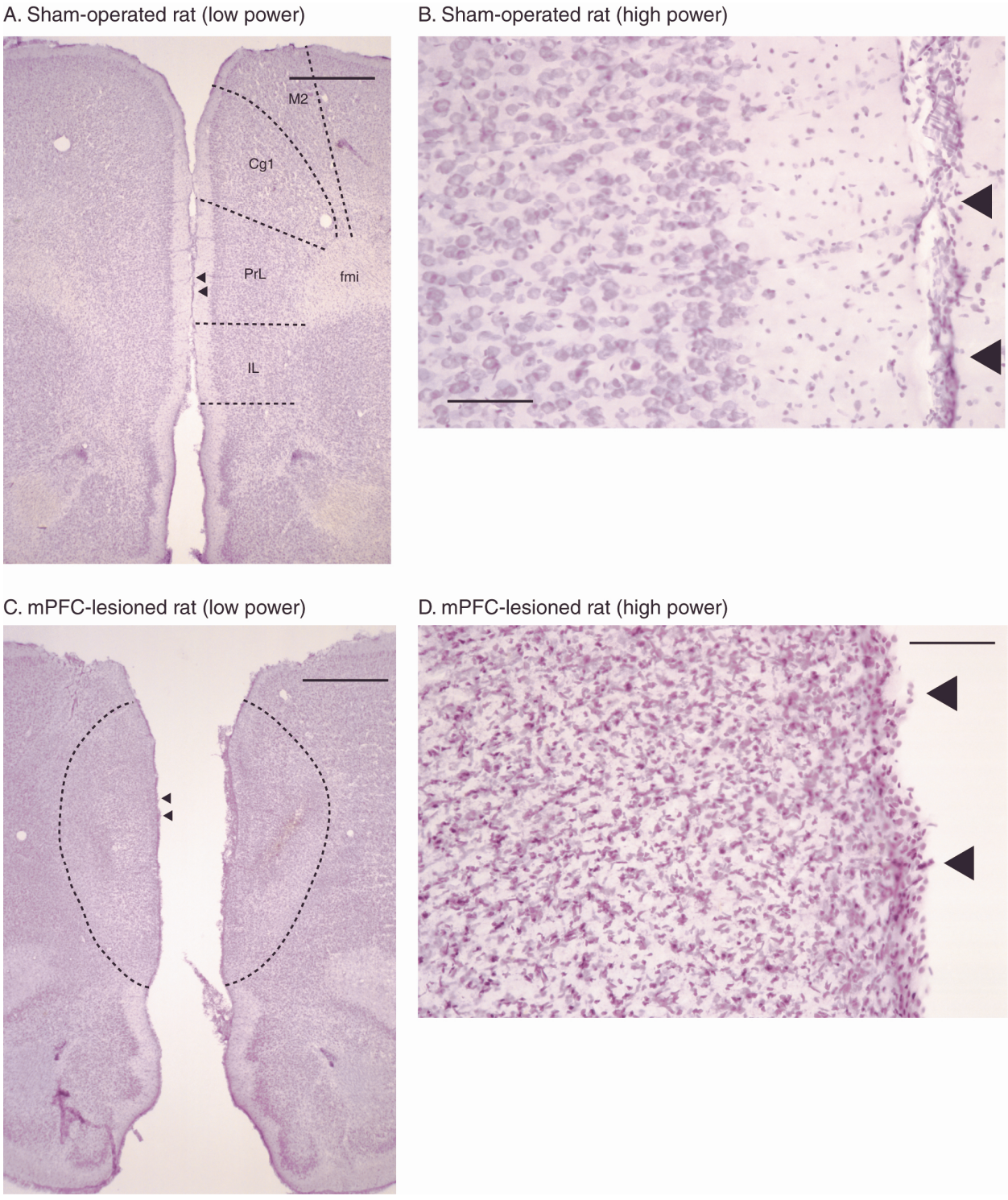

Figure 85. Lesions of the mPFC: photomicrographs of sections at approximately 2.6 mm anterior to bregma, stained with cresyl violet. **A & B:** sham-operated rat (M2, secondary motor cortex; Cg1, cingulate area 1; PrL, prelimbic cortex; IL, infralimbic cortex; fmi, forceps minor of the corpus callosum). **C & D:** mPFC-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.

Medial prefrontal cortex: schematic of lesions

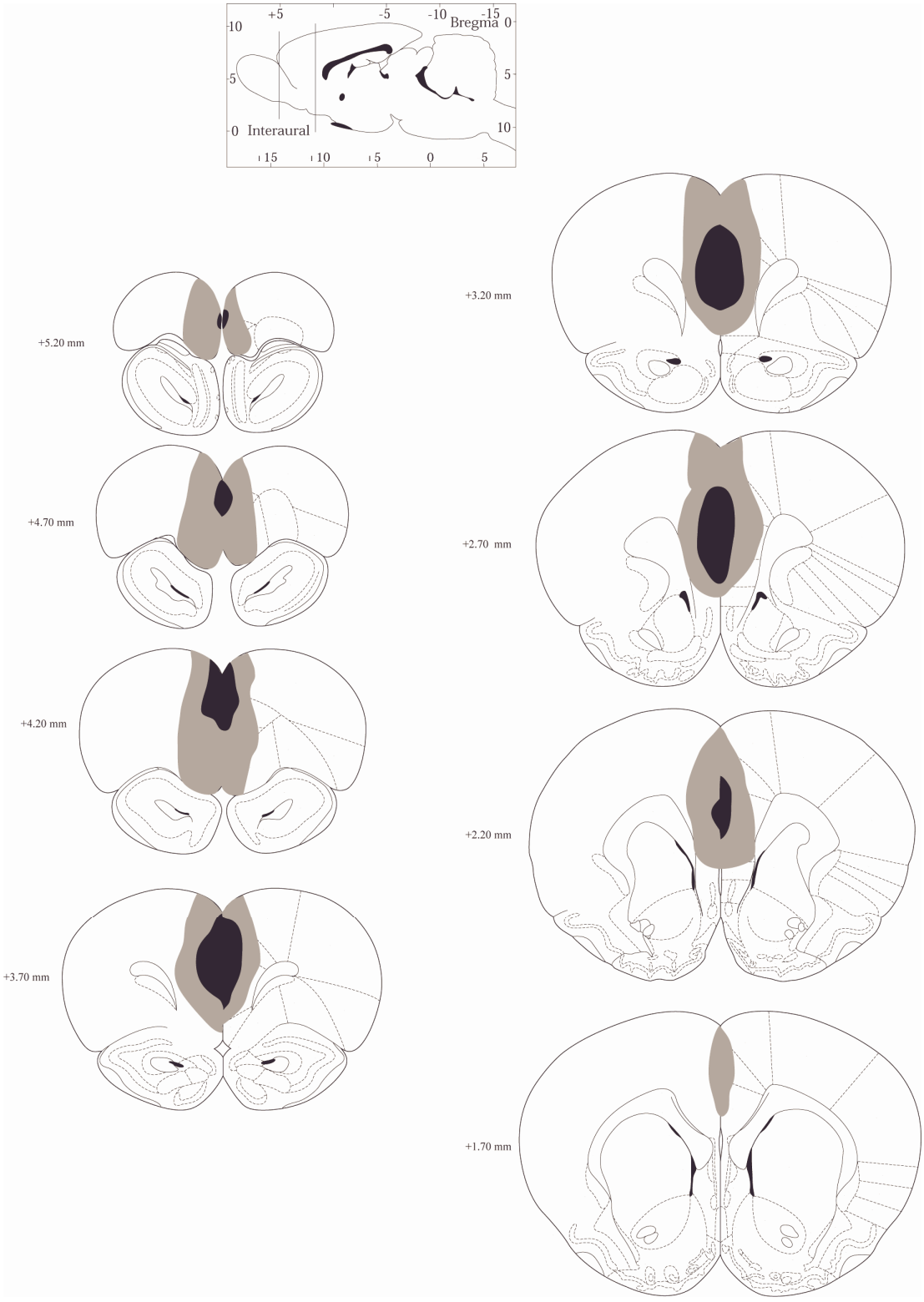

Figure 86. Lesions of the mPFC (subjects M6, M9, M10, M12, M13, M14, M16, M18, M20, M22, M24). Grey shading indicates the extent of the largest area of neuronal loss, and black the smallest. Diagrams are taken from Paxinos & Watson (1998).

Body mass

The two groups did not differ in body mass, either at the start or the end of behavioural testing (*F*s < 1, NS).

Pre-operative acquisition

The groups remained matched after histological selection: there were no differences in the pre-operative pattern of choice (Figure 88A, p. 208). Choice ratios calculated from the last 3 pre-operative sessions were analysed using the model group × (delay × S). While there was a highly significant effect of delay ($F_{2.528,48.033} = 18.429$, $\tilde{\varepsilon} = .632$, $p < .001$), there was no effect of group and no group × delay interaction (*F*s < 1, NS).

Baseline post-operative performance

Choice. Although the mPFC-lesioned group exhibited a within-session shift in preference, this shift was less pronounced than in the sham group (Figure 88B, p. 208). Analysis of choice ratios using the model group × (delay × S) revealed a group × delay interaction ($F_{2.891,54.926} = 3.188$, $\tilde{\varepsilon} = .723$, $p = .032$), in addition to a main effect of delay ($F_{2.891,54.926} = 26.831$, $\tilde{\varepsilon} = .723$, $p < .001$). There was no main effect of group ($F < 1$, NS). Separate analyses of each group demonstrated that both groups shifted their preference from the large to the small reinforcer as the delay increased (effect of delay in the sham group: $F_{2.819,25.367} = 17.499$, $\tilde{\varepsilon} = .705$, $p < .001$; in the mPFC group: $F_{4,40} = 8.87$, $p < .001$). At no *individual* delay was preference different between the two groups (simple effects of group at each delay: *F*s < 1.218, NS).

This interpretation was confirmed by analysis of the regression slope measure, which was substantially higher post-operatively in the mPFC group, indicating a flattened within-session shift in preference (Figure 88C; more negative values of this measure indicate a more pronounced shift from large to small reinforcer across the session). Analysis of slope measures from pre- and post-operative sessions, using the model $\text{group}_2 \times (\text{session}_{26} \times \text{S})$, revealed a highly significant group × session interaction ($F_{16.491,313.333} = 2.286$, $\tilde{\varepsilon} = .66$, $p = .003$), in addition to a main effect of session ($F_{16.491,313.333} = 6.265$, $\tilde{\varepsilon} = .66$, $p < .001$); there was no main effect of group in this analysis ($F < 1$, NS). This interaction was not due to pre-operative differences between the groups: analysis of pre-operative sessions 1–19 revealed a main effect of session ($F_{9.914,188.366} = 7.375$, $\tilde{\varepsilon} = .551$, $p < .001$), but no effect of group ($F_{1,19} = 1.288$, NS) and no group × session interaction ($F_{9.914,188.366} = 1.399$, $\tilde{\varepsilon} = .551$, NS). Post-operatively, however, slope measures were substantially higher (less negative) in the mPFC group, with analysis of post-operative sessions 20–26 revealing a main effect of group ($F_{1,19} = 4.848$, $p = .04$). This pattern did not change during post-operative testing: there was no effect of session ($F_{6,114} = 1.127$, NS) and no group × session interaction ($F < 1$, NS).

Omissions. Responding was reliable, with all animals regularly sampling both levers, and the two groups did not differ in the number of omissions made. An analysis of the percentage of trials on which an omission occurred, across all delays, revealed no effect of group ($F < 1$, NS).

Initiation latency. Subjects were slower to initiate trials as the session progressed and the delays lengthened, but there were no differences between mPFC and sham groups in this respect. Analysis of initiation latencies using the model group × (delay × S) revealed a main effect of delay ($F_{2.456,46.657} = 4.637$, $\tilde{\varepsilon} = 4.637$, $p = .01$) but no significant terms involving group (group: $F_{1,19} = 2.255$, NS; group × delay: $F < 1$, NS).

Choice latency. Lesioned rats were slower to respond on the levers (Figure 88D), and initiation latencies were generally longer for all subjects at the start of the session. Analysis using the model group ×

(delay × response × S) demonstrated main effects of group ($F_{1,16}$ = 7.741, p = .013) and delay ($F_{2.246,35.934}$ = 5.137, $\tilde{\varepsilon}$ = .561, p = .009), but no other significant terms (response × delay: $F_{2.373,37.975}$ = 2.119, $\tilde{\varepsilon}$ = .593, NS; other terms: F < 1, NS).

Collection latency. The lesion did not affect collection latencies. Subjects collected the immediate reward slightly faster than the delayed reward, and were slower to collect rewards as the session progressed; these two tendencies were statistically independent. Analysis using the model group × (delay × response × S) showed main effects of response ($F_{1,16}$ = 6.305, p = .023) and delay ($F_{3.663,58.601}$ = 2.647, $\tilde{\varepsilon}$ = .916, p = .047), bur no other significant terms (Fs < 1.823, NS).

Nosepoking during the delay. The lesion did not affect nosepoking behaviour, and nosepoking occurred at a constant rate at all delays. Analysis using the model group × (delay × S) revealed no effect of any term (group: $F_{1,17}$ = 2.14, NS; other terms: F < 1.454, NS).

Effect of omitting all delays

Both groups remained sensitive to the removal of delays, shifting their preference towards the large reinforcer under these conditions (Figure 88E). Analysis of choice ratios using the model group_2 × ({Delays versus No Delays}$_2$ × trial block_5 × S) revealed a highly significant interaction between the Delay / No Delay factor and the trial block ($F_{2.269,43.116}$ = 29.442, $\tilde{\varepsilon}$ = .567, p < .001) in addition to main effects of the Delay / No Delay factor ($F_{1,19}$ = 22.949, p < .001) and of trial block ($F_{3.253,61.813}$ = 17.117, $\tilde{\varepsilon}$ = .813, p < .001), but there were no significant terms involving group (Fs < 1.693, NS).

Locomotor activity in a novel environment

The mPFC group were not significantly hyperactive (Figure 87). Following square-root transformation, analysis of the total number of infrared beam interruptions using the model group_2 × (bin_{12} × S) revealed an effect of bin ($F_{6.048,114.909}$ = 16.046, $\tilde{\varepsilon}$ = .55, p < .001), reflecting habituation, but no other significant term (group: $F_{1,19}$ = 2.168, NS; group × bin: F < 1, NS).

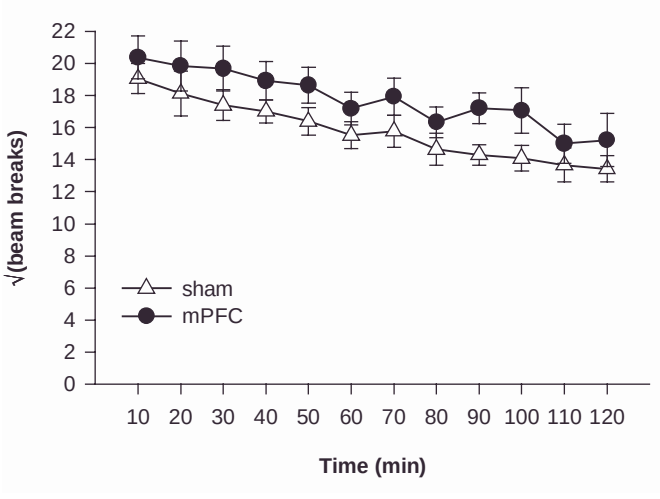

Figure 87. Locomotor activity in a novel environment (120-min session scored in 10-min bins). There were no significant differences between the groups.

Summary

Lesions of the mPFC induced a 'flattening' of the normal within-session shift in preference from the large to the small reward, though lesioned subjects still exhibited this shift and remained sensitive to removal of the delays. They were also generally slower to respond on the levers.

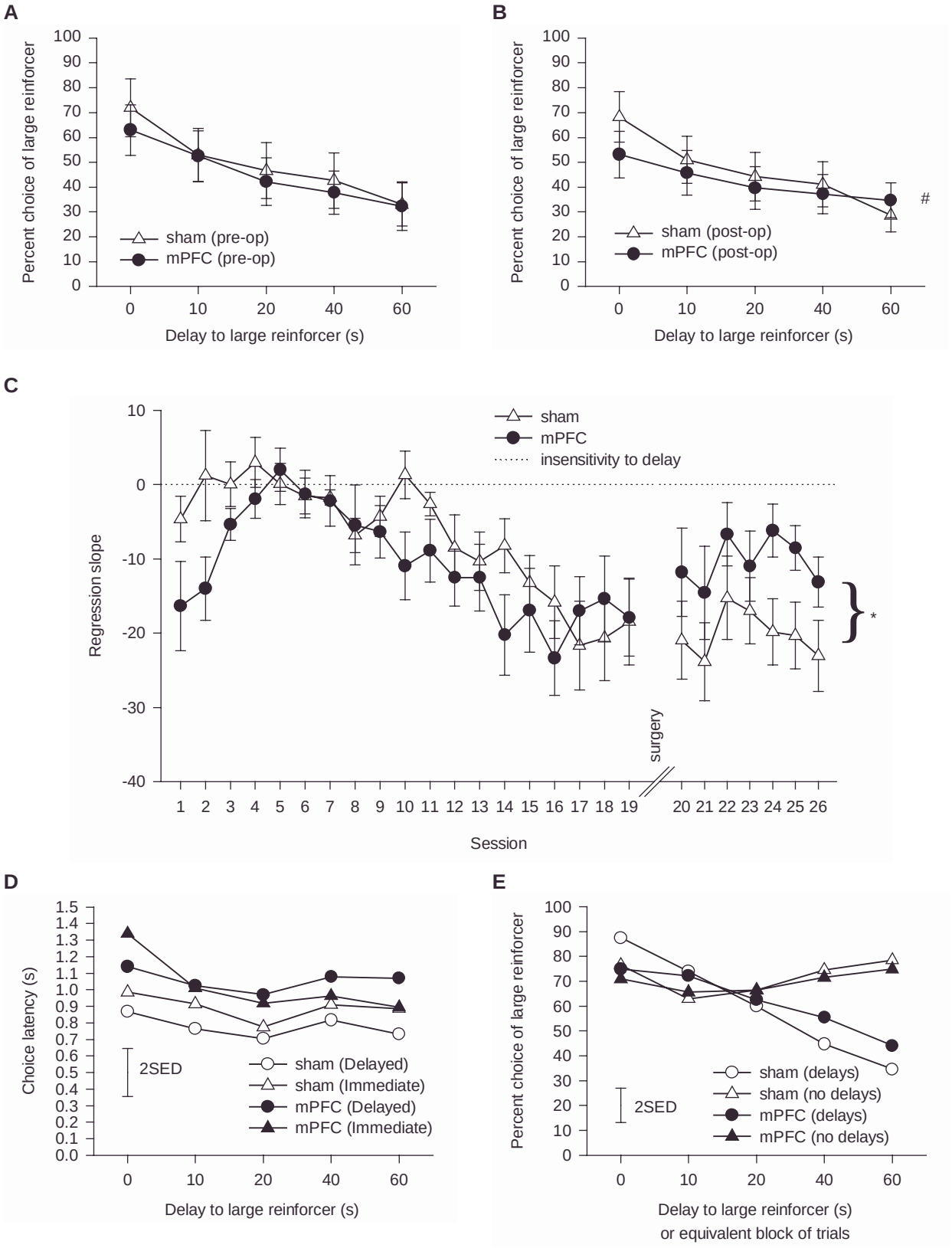

Figure 88. Performance of rats with lesions of the mPFC on the delayed-reinforcement choice task. **A:** Pre-operative performance — data from the last 3 sessions preceding surgery. **B:** Post-operative performance — data from the first 7 sessions following surgery (# $p < .05$, group × delay interaction). **C:** Slope measures before and after surgery (* $p < .05$, post-operative difference between groups). **D:** Latencies to choose a lever; the mPFC group were significantly slower to respond. **E:** Effect of omitting all delays in alternating sessions. (*2SED*, twice the standard error of the difference for the relevant three-way interaction.)

EXPERIMENT 3. EFFECTS OF LESIONS OF THE NUCLEUS ACCUMBENS CORE

Methods

Twenty-four naïve subjects were trained and assigned to two groups as in Experiment 1 (p. 197). They then received lesions of the AcbC (n = 14) or sham lesions (n = 10). At the time of surgery, they weighed 315–372 g. Following recovery, they were retested on the basic task for 7 sessions, and given 4 sessions in which all delays were omitted in alternate sessions (ABAB design), as in Experiment 1.

As a deficit was observed during testing (before histological data were available), further behavioural tests were given to elucidate the nature of the deficit. First, the delay-omission test was repeated over 6 sessions, using an AAABBB design (three sessions with delays present, followed by three sessions with no delays, or vice versa). This test gave subjects longer to respond to the new contingencies. As before, half of the subjects began this test with the delays present, and half with no delays (counterbalanced across groups). Secondly, all animals were given a further 6 sessions with no delays, in an attempt to re-equalize the two groups' performance and ensure that all animals would come to prefer the lever producing the large reinforcer. Finally, the delays were re-introduced for a further 6 sessions.

Following completion of delayed reinforcement testing, subjects were given a 2-h locomotor test (methodologically identical to that used in Chapter 3, p. 78). After this, a pellet / chow consumption test was administered, as described below, before the animals were killed and perfused.

Food consumption tests

Food consumption was assessed using four tests, conducted in subjects' home cages (always with only one rat present) on separate days under conditions of food deprivation.

(1) Subjects were given free access to 45-mg sucrose pellets (Rodent Diet Formula P, Noyes, Lancaster, NH) for 30 minutes; the amount eaten was recorded.
(2) This test was repeated with the chow used as the maintenance diet.
(3) The time taken to consume 50 sucrose pellets was recorded.
(4) The time taken to consume an equivalent mass of chow (2.25 g) was recorded.

Results

Histology

There were no postoperative deaths. Histological analysis revealed that one subject in the core group (J23) had no damage to the Acb, one subject (J5) had an extensive lesion involving the septum, and two other subjects (J6, J19) had lesions encompassing a significant proportion of the AcbSh. These animals were excluded, leaving 10 subjects in the core group (J1, J11, J12, J13, J15, J16, J17, J18, J21, J24) and 10 in the sham group (J2, J3, J4, J7, J8, J9, J10, J14, J20, J22). 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 anterior 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 AcbSh was restricted to the lateral edge of the dorsal shell. Schematics of the lesions are shown in Figure 89; representative photomicrographs of AcbC lesions were shown in Chapter 4 (p. 132).

Nucleus accumbens core: schematic of lesions

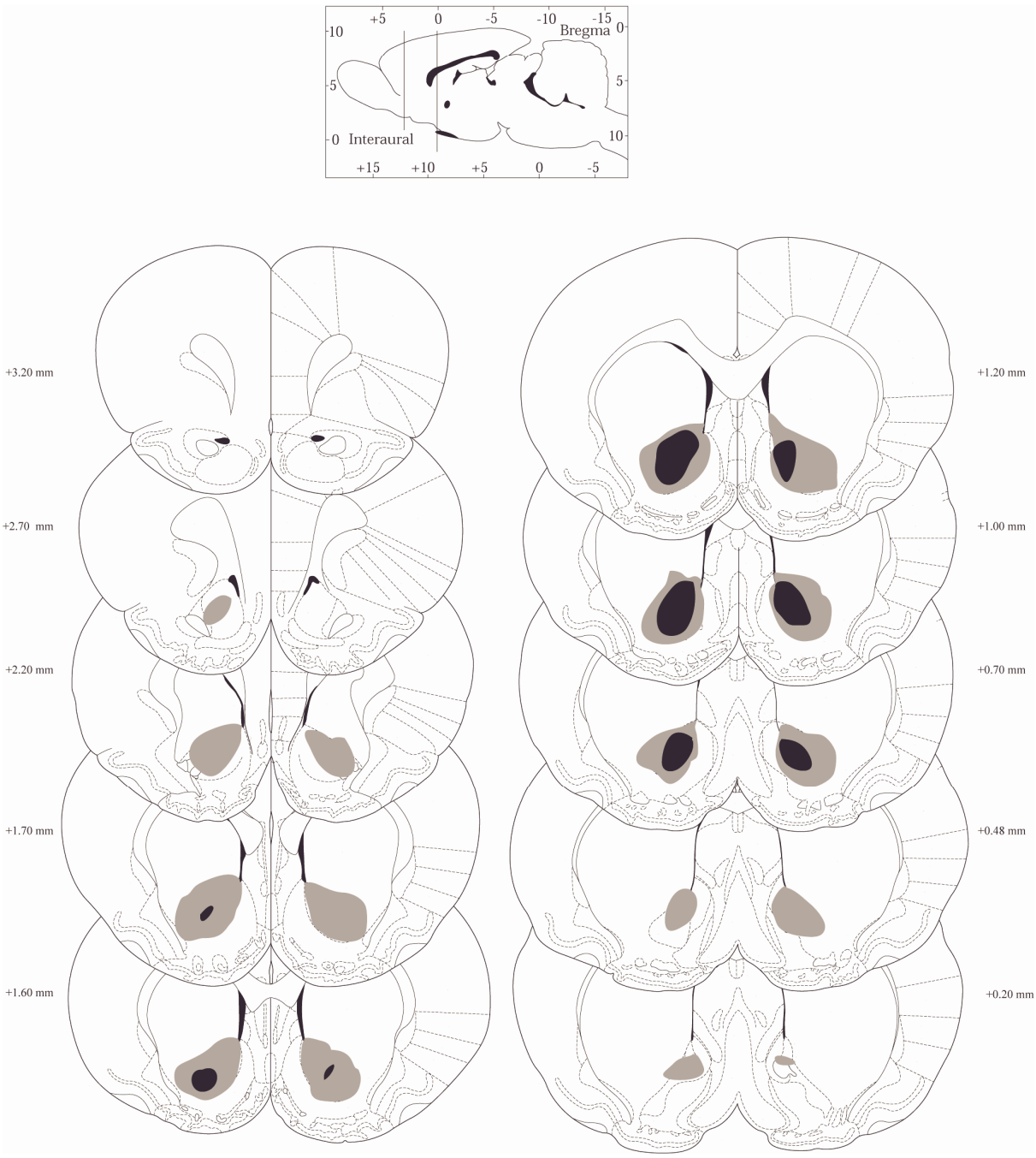

Figure 89. Lesions of the AcbC (subjects J1, J11, J12, J13, J15, J16, J17, J18, J21, J24). Grey shading indicates the extent of the largest area of neuronal loss, and black the smallest. Diagrams are taken from Paxinos & Watson (1998).

Body mass

The core-lesioned group appeared lighter than the control group, and this observation was confirmed. The groups did not differ in body mass at the time of surgery (means ± SEMs: sham 356.0 ± 5.5 g, core 357.9 ± 8.0 g; one-way ANOVA: $F < 1$, NS). However, the core group were underweight thereafter, at around 90% of the mass of the control group — when feeding freely 6–13 days after surgery (sham 407.0 ± 5.6 g, core 359.2 ± 11.9 g; $F_{1,18} = 13.253$, $p = .002$), at the completion of the first delay-omission test (sham 371.8 ± 7.0 g, core 336.8 ± 7.9 g; $F_{1,18} = 10.941$, $p = .004$), and after all delayed reinforcement testing, at the start of the food consumption tests (sham 367.8 ± 7.1 g, core 324.4 ± 10.3 g; $F_{1,18} = 12.055$, $p = .003$).

Pre-operative acquisition

The groups remained matched after histological selection: there were no differences in the pre-operative pattern of choice (Figure 90A, p. 212). Choice ratios from the last 3 pre-operative sessions were analysed using the model group × (delay × S). While there was a highly significant effect of delay ($F_{3.124,56.234} = 20.542$, $\tilde{\varepsilon} = .781$, $p < .001$), there was no effect of group, and no group × delay interaction (Fs < 1, NS).

Baseline post-operative performance

Food was reliably collected and consumed, with the exception of a single occasion on which one core-lesioned subject (J13) was discovered to have left ~9 pellets uneaten at the end of the session.

Choice. The core-lesioned group were dramatically impaired in their ability to choose the large, delayed reward (Figure 90B, p. 212). An analysis of choice ratios from the 7 baseline sessions revealed a significant main effect of group ($F_{1,18} = 13.859$, $p = .002$) and a group × delay interaction ($F_{4,72} = 2.964$, $p = .025$) in addition to a main effect of delay ($F_{4,72} = 37.28$, $p < .001$). However, subgroup analyses showed that both groups still exhibited a within-session shift in preference from the large to the small reward (sham group, effect of delay: $F_{4,36} = 23.668$, $p < .001$; core group: $F_{4,36} = 14.57$, $p < .001$).

As Figure 90B shows, the variance in the core group was substantially less that that in the sham group; the core group's preference for the immediate, smaller reinforcer was very consistent. As this heterogeneity of variance affected the ANOVA in which the two groups were compared (though not those analyses considering each group separately), non-parametric analyses were also conducted. Mann-Whitney U tests confirmed that the core group chose the delayed reinforcer significantly less often than shams at every single delay ($p < .023$ in each case). Surprisingly, the core group chose the large reinforcer less often than the small reinforcer at zero delay (comparison to 50%, $t_9 = –5.147$, $p = .001$).

To confirm that this change reflected a change in the performance of the core group, and not of the shams, choice ratios from the last 3 pre-operative sessions were compared with those from the first 3 post-operative sessions using the model $\text{group}_2 \times (\text{pre}/\text{post}_2 \times \text{delay}_5 \times \text{S})$. This revealed a significant pre / post × group interaction ($F_{1,18} = 10.302$, $p = .005$). Separate analyses of the core and sham groups showed that the choice behaviour of the sham group did not alter following surgery ($F_{1,9} = 3.199$, $p = .107$), while that of the core group did ($F_{1,9} = 7.437$, $p = .023$).

Although Figure 90B suggests that the core-lesioned group exhibited a slightly reduced within-session shift in preference, because they rapidly approached reached a 'floor' at which the delayed reinforcer was seldom chosen, the rapidity of this shift (as assessed by the regression slope measure) did not differ between groups and did not alter across the post-operative sessions (Figure 90C). Analysis of the slope measures using the model $\text{group}_2 \times (\text{session}_7 \times \text{S})$ revealed a non-significant trend towards less steep (less negative / numerically greater) slopes in the core group (effect of group: $F_{1,18} = 3.624$, $p = .073$), with no effect of session and no interaction (Fs < 1, NS).

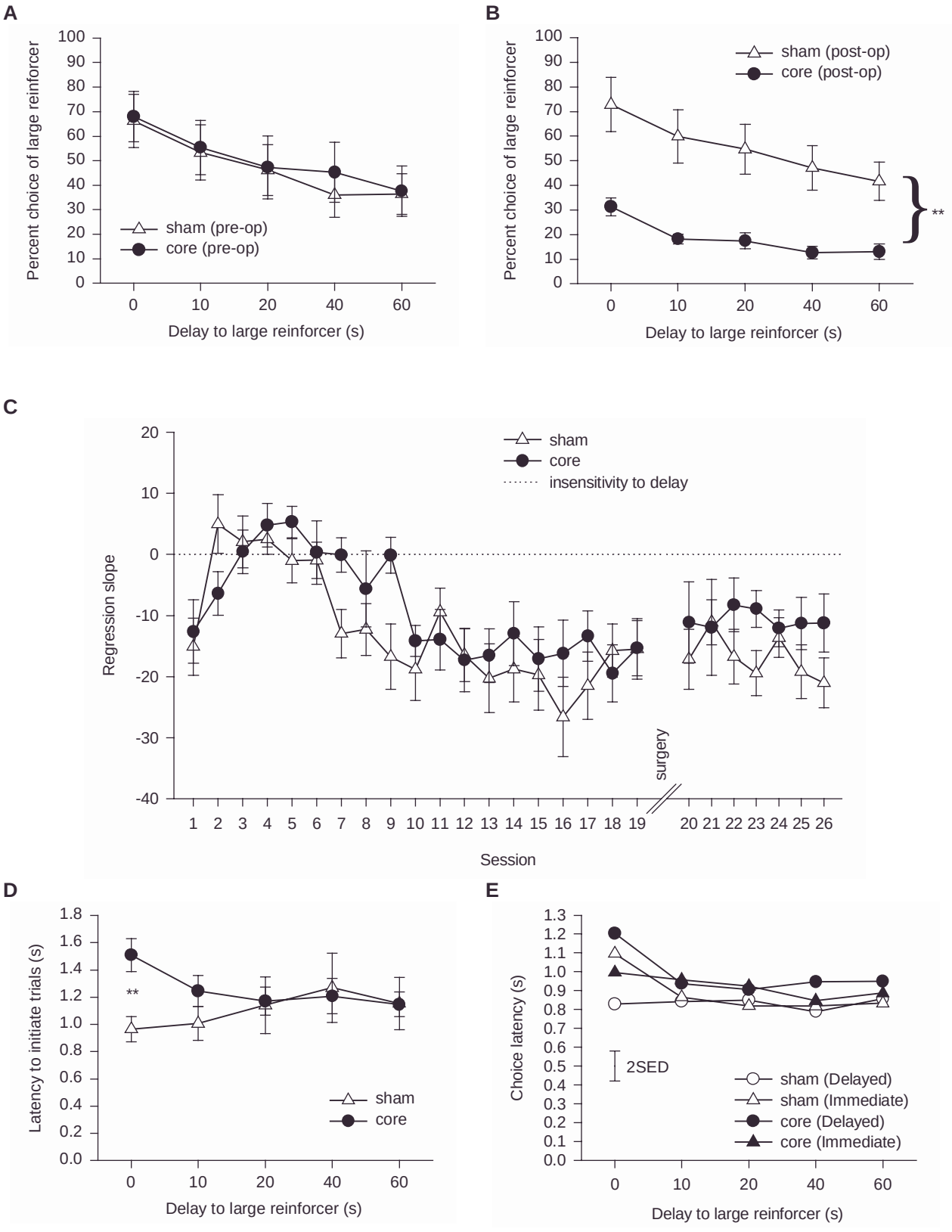

Figure 90. Performance of rats with lesions of the AcbC on the delayed-reinforcement choice task: baseline sessions. **A:** Pre-operative performance — data from the last 3 sessions preceding surgery. **B:** Post-operative performance — data from the first 7 sessions following surgery. Core-lesioned rats were significantly impaired in their ability to choose the larger, delayed reward. **C:** Slope measures before and after surgery. **D:** Latencies to initiate trials. **E:** Choice latencies. (*2SED*, twice the standard error of the difference for the three-way interaction; ** $p < .01$.)

Omissions. Responding was reliable, with all animals regularly sampling both levers, and the two groups did not differ in the number of omissions made. An analysis of the percentage of trials on which an omission occurred, across all delays, revealed no effect of group ($F_{1,18}$ = 2.665, NS).

Initiation latency. The core-lesioned subjects were slower to initiate trials at zero delay (Figure 90D). Analysis of initiation latencies revealed a group × delay interaction ($F_{2.548,45.868}$ = 5.274, $\tilde{\varepsilon}$ = .637, p = .005). As Figure 90D suggests, this was due to slower initiation by the core group at zero delay (one-way ANOVA: $F_{1,18}$ = 12.903, p = .002); between-group differences at non-zero delays were not significant (p > .177).

Choice latency. There was a complex but small difference between groups in choice latency (Figure 90E). Analysis of choice latencies using the model $group_2$ × ($response_2$ × $delay_5$ × S) revealed a group × response × delay interaction ($F_{2.779,38.905}$ = 3.436, $\tilde{\varepsilon}$ = .695, p = .029) in addition to a main effect of delay ($F_{1.636,22.897}$ = 4.344, $\tilde{\varepsilon}$ = .409, p = .032). Analyses of the two group separately showed that there was a response × delay interaction in the sham group ($F_{4,24}$ = 3.624, p = .019), probably due to slower responding on the Immediate lever at zero delay (though the latency difference between the two levers at zero delay was not significant in its own right by *post hoc* testing; $F_{1,8}$ = 3.332, p = .105), though this interaction was not significant in the core group (F < 1, NS).

Collection latency. The two groups did not differ in the speed with which they collected the rewards. An analysis of collection latencies using the model $group_2$ × ($response_2$ × $delay_5$ × S) revealed no significant terms (maximum F was for the three-way interaction: $F_{2.987,41.82}$ = 2.504, $\tilde{\varepsilon}$ = .747, p = .072).

Nosepoking during the delay. There were no differences between the two groups in the rate of nosepoking in the food alcove during the delay. An analysis using the model $group_2$ × ($delay_4$ × S) revealed no significant terms (maximum $F_{1,15}$ = 2.101, NS).

Effect of omitting all delays (ABAB design)

Both groups remained sensitive to the delay. Removing the delays in alternating sessions increased both the sham- and core-lesioned groups' preference for the larger reward (Figure 91A, p. 214). Analysis of choice ratios using the model $group_2$ × ({Delays versus No Delays}$_2$ × trial $block_5$ × S) revealed a revealed a highly significant interaction between the Delay/No Delay factor and the trial block ($F_{1.802,32.444}$ = 16.391, $\tilde{\varepsilon}$ = .451, p < .001) in addition to main effects of group ($F_{1,18}$ = 8.238, p = .01), the Delay/No Delay factor ($F_{1,18}$ = 15.622, p = .001), and trial block ($F_{2.989,53.802}$ = 15.996, $\tilde{\varepsilon}$ = .747, p < .001). The group × {Delay/No Delay} interaction escaped significance ($F_{1,18}$ = 4.182, p = .056), as did the three-way interaction (F < 1, NS). Heterogeneity of variance was not significant.

Confirming this statistical picture, a {Delay/No Delay} × trial block interaction was detectable for both the sham group ($F_{4,36}$ = 13.768, p < .001) and the core group ($F_{1.407,12.659}$ = 5.717, $\tilde{\varepsilon}$ = .352, p = .025) when analysed separately.

The core group's preference for the larger reward remained significantly below that of the sham group in the No Delay condition (main effect of group in the No Delay condition: $F_{1,18}$ = 9.422, p = .007).

Effect of omitting all delays (AAABBB design)

A further delay-omission test was conducted using three consecutive delay or no-delay sessions (AAABBB design). Although this more prolonged experience with the No Delay condition succeeded in increasing the core group's preference for the larger reward, the basic pattern remained the same as for the previous delay-omission test (Figure 91B).

Analysis identical to that for the previous test again detected a highly significant {Delay/No Delay} × trial block interaction, and main effects of the {Delay/No Delay factor} and of trial block; however, in

this test, surprisingly, no group differences were significant (group: $F_{1,18} = 2.97$, $p = .102$; other terms involving group: $F < 1$, NS). Subgroup analyses demonstrated significant {Delay / No Delay} × trial block interactions in both the sham and the core groups. In this test, however, the difference between the sham and core groups in the No Delay condition was not significant ($F_{1,18} = 2.567$, $p = .127$).

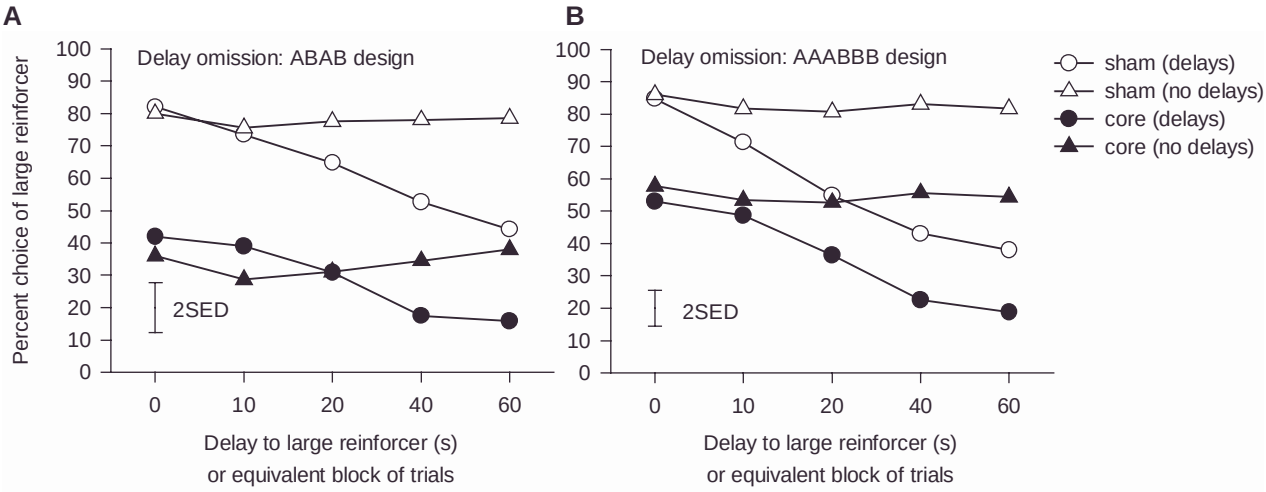

Figure 91. Effect of removing all delays on the performance of sham- and core-lesioned rats. **A:** Effect of omitting all delays in alternating sessions (ABAB design). **B:** Effect of omitting all delays with three consecutive sessions in each condition (AAABBB design). (*2SED*, twice the standard error of the difference for the three-way interaction. Note that this error term is *not* appropriate for the simple between-group comparison.)

Prolonged training without delays, and subsequent reintroduction of delays

Despite the lack of a statistical difference between the groups in the final delay omission test (Figure 91B), the core group's absolute level of preference for the larger reward was not as high as that of the sham group. In an attempt to equalize the groups, further training was given with no delays present (see *Methods*). Data from the last of these sessions are shown in Figure 92A. Though the two groups were not equalized by this training, all tendency to exhibit a within-session shift in preference was removed (Figure 92A). Subsequent reintroduction of delays caused preference for the larger reinforcer to collapse; Figure 92(B–D) shows consecutive blocks of 3 sessions. As the sessions proceeded, the core group's preference for the delayed reinforcer declined first at long delays, and then at progressively shorter delays.

Nevertheless, as clear preference for the large reinforcer had not been re-established in the core group as a whole, one further analysis was conducted. From the last day of no-delay training (session 42; Figure 92A), those rats were selected that met a criterion of ≥90% choice of the large reinforcer in every trial block. This selection eliminated 3 rats from the sham group, leaving 7, and eliminated 5 rats from the core group, leaving 5 (Table 20). Having selected those rats that clearly discriminated between the two reinforcers and were not in the least biased away from the large-reinforcer lever as a result of their experience with delays, Figure 92 was replotted; the results are shown in Figure 93. It can be seen clearly that even those core-lesioned rats that exhibited a strong preference for the large reinforcer when it was delivered immediately (Figure 93A) were extremely intolerant of delay compared to the sham group (Figure 93D). The fact that these core-lesioned rats strongly preferred the large reinforcer in a task when no delays were present at all, but that their preference for the large reinforcer at zero delay declined when delays were reintroduced (compare Figure 93A and Figure 93D at zero delay) suggests that the severe deficit in the efficacy of delayed reward affected responding at non-zero delays, and then generalized to affect their

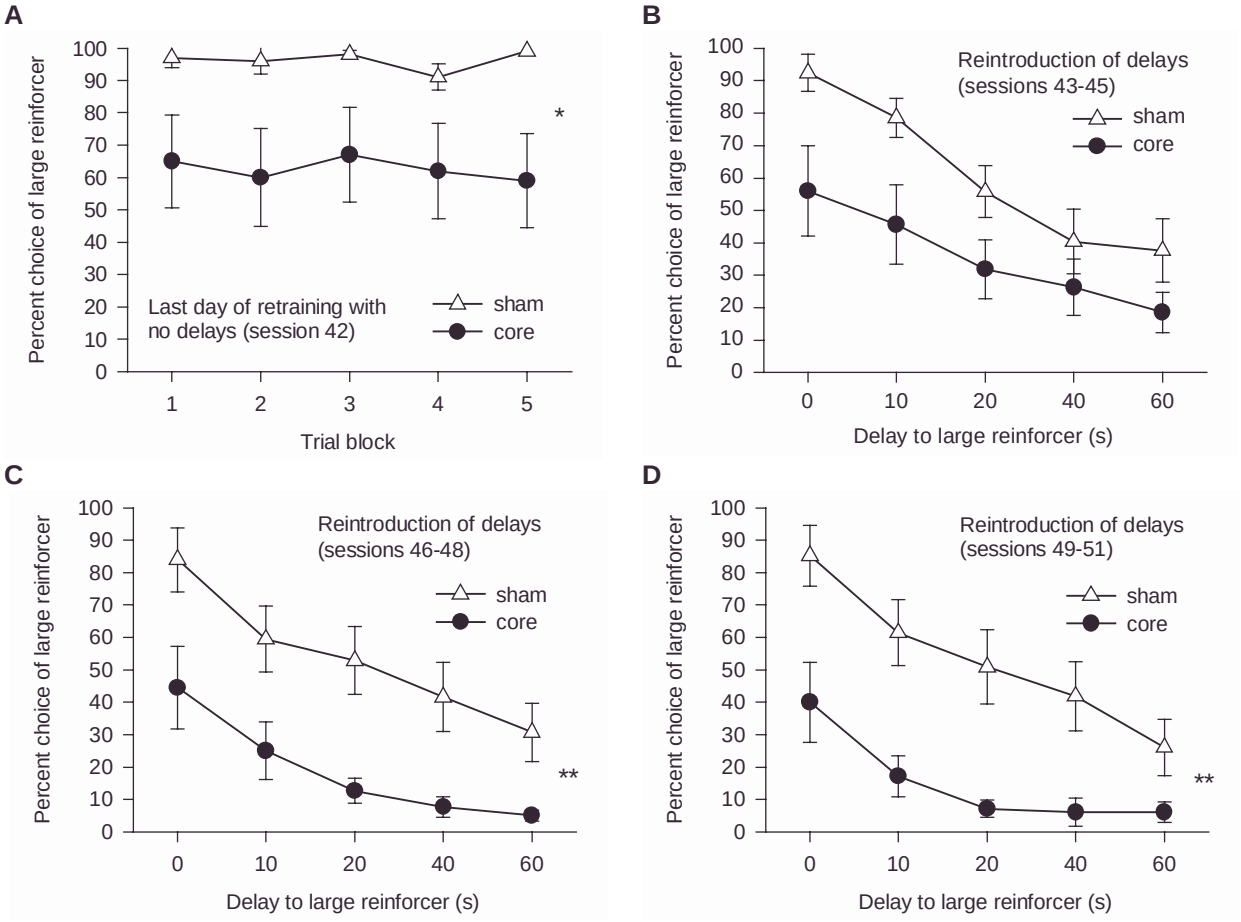

Figure 92. A: Preference following extended training in the absence of any delays (full data set shown in Table 20). **B–D:** Performance over consecutive blocks of sessions upon the reintroduction of delays. As these data exhibit significant heterogeneity of variance, the highly conservative correction of Box (1954) was applied (see Howell, 1997, pp. 322/457/464); * $p < .05$, ** $p < .01$ for the corrected between-group difference.

Table 20. Performance of sham-operated and core-lesioned subjects on the final day of extended training in the absence of delays (session 42, Figure 92A and Figure 93A). The percentage of trials on which the large reinforcer was chosen is shown, for each of the five blocks of ten choice trials. All sham-operated controls and the majority of AcbC-lesioned rats showed a preference for the large reinforcer (>50%) in all trial blocks. Rats that met the more stringent criterion of ≥90% choice of the large reinforcer in every trial block were used for a further analysis (Figure 93). No omissions were made in this session.

Rat	J2	J3	J4	J7	J8	J9	J10	J14	J20	J22	J1	J11	J12	J13	J15	J16	J17	J18	J21	J24
Group	sham	sham	sham	sham	sham	sham	sham	sham	sham	sham	core	core	core	core	core	core	core	core	core	core
Trial block 1	100	100	100	100	100	100	100	70	100	100	90	0	100	100	0	10	100	100	100	50
Trial block 2	100	100	100	100	100	100	100	60	100	100	90	0	100	100	0	0	100	90	100	20
Trial block 3	100	100	100	100	100	90	100	90	100	100	90	0	100	100	0	0	100	90	100	90
Trial block 4	70	100	100	100	70	100	90	80	100	100	100	0	100	90	0	0	100	90	100	40
Trial block 5	100	100	100	100	90	100	100	100	100	100	100	0	100	70	0	0	100	100	90	30
>50% throughout?	√	√	√	√	√	√	√	√	√	√	√	×	√	√	×	×	√	√	√	×
≥90% throughout?	×	√	√	√	×	√	√	×	√	√	√	×	√	×	×	×	√	√	√	×

preference even in the zero-delay condition. This may explain why the core group demonstrated a deficit in responding for the large reinforcer even at zero delay during baseline testing sessions (Figure 90B, p. 212).

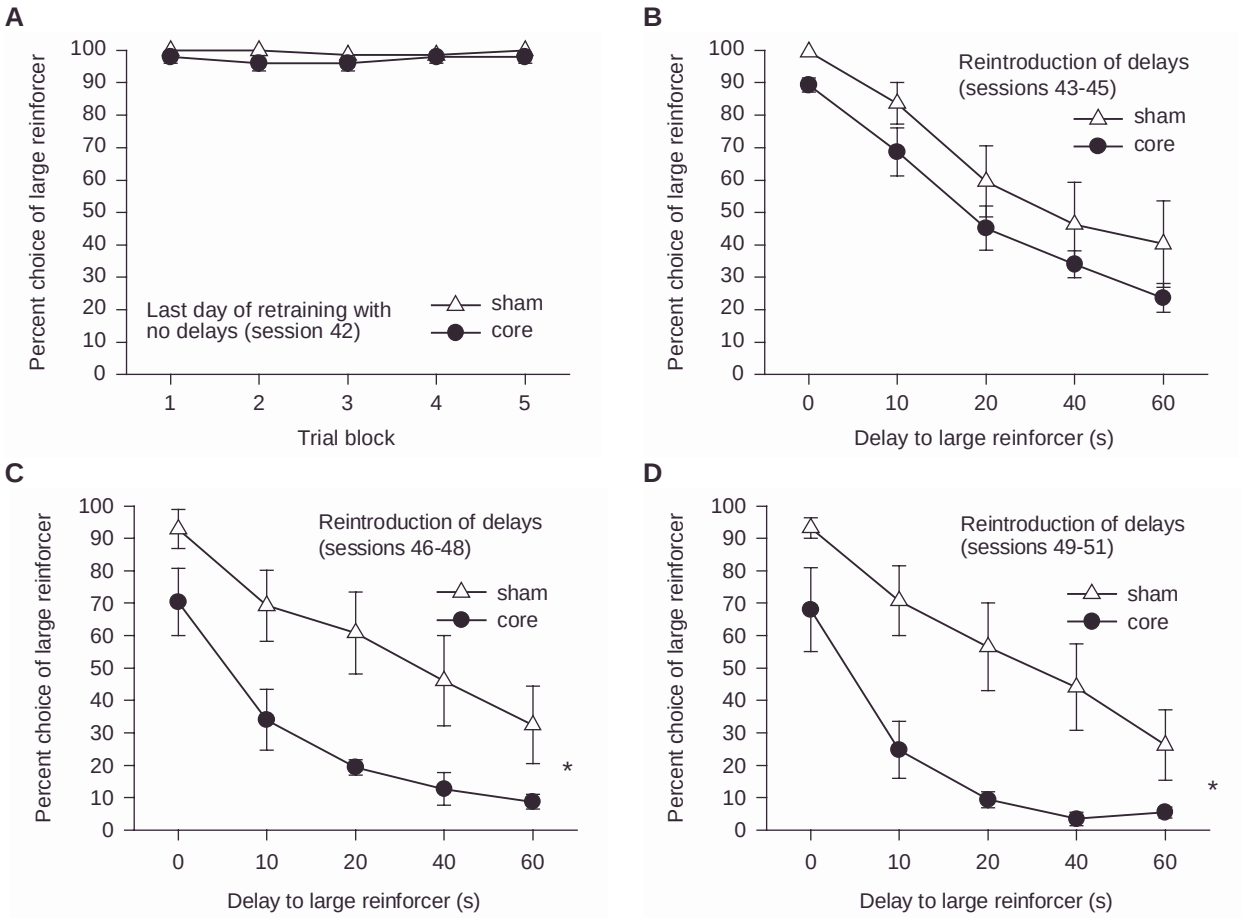

Figure 93. This figure is identical in form to Figure 92, but only includes data from those rats selected on the basis of a criterion of ≥90% preference for the large reinforcer on the last day of training with no delays (see Table 20). The groups were therefore matched in panel **A.** In panels **B–D,** upon reintroduction of the delays, preference for the large reinforcer collapsed in the core group. As these data exhibit significant heterogeneity of variance, the highly conservative correction of Box (1954) was applied (see Howell, 1997, pp. 322/457/464); * $p < .05$ for the corrected between-group difference.

Locomotor activity in a novel environment

Core-lesioned subjects were hyperactive, and slower to habituate to the novel environment of the locomotor testing apparatus (Figure 25). Following square-root transformation, analysis of the total number of infrared beam interruptions using the model $group_2 \times (bin_{12} \times S)$ revealed an effect of bin ($F_{7.777,139.994} = 12.079$, $\tilde{\varepsilon} = .707$, $p < .001$), reflecting habituation, but also an effect of group ($F_{1,18} = 12.057$, $p = .003$), and a group × bin interaction ($F_{7.777,139.994} = 2.279$, $\tilde{\varepsilon} = .707$, $p = .027$).

Food consumption tests

The core-lesioned subjects ate more slowly than the sham-operated controls, at least when consuming the chow used as their maintenance diet; differences in food consumption were not significant for the sucrose pellets used in the delayed reinforcement task.

Mass of chow consumed in 30 min. There was a small but significant difference in the amount of chow consumed: the core group ate less. The mean ± SEM amounts consumed were 8.0 ± 0.4 g (sham) and 6.5 ± 0.5 g (core); one-way ANOVA demonstrated these to be significantly different ($F_{1,18} = 5.777$, $p = .027$).

Time to consume 2.25 g chow. The core group at the fixed amount of chow more slowly (501 ± 39 s) than the shams (375 ± 6 s). Inhomogeneity of variance necessitated a nonparametric test; the difference between the two groups was significant by a Mann-Whitney U test ($p = .005$).

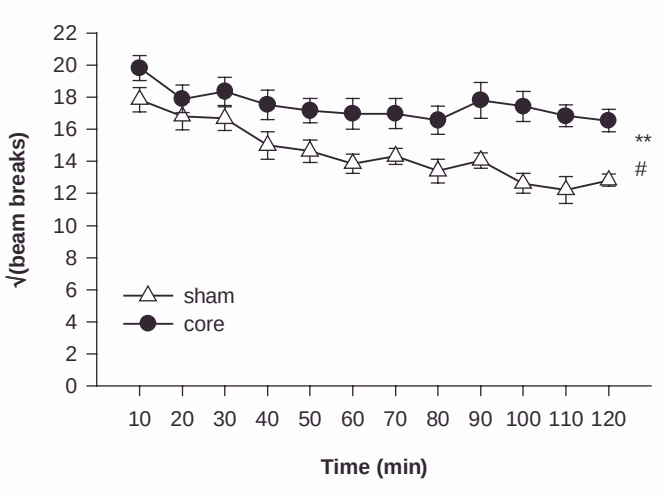

Figure 94. Locomotor activity in a novel environment (120-min session scored in 10-min bins). The core group were hyperactive and habituated more slowly (** $p < .01$, main effect of group; # $p < .05$, group × bin interaction).

Mass of sucrose pellets consumed in 30 min. The core group ate less (9.1 ± 0.5 g) than the shams (11.4 ± 0.9 g); however, this difference was not significant (inhomogeneity of variance necessitated a non-parametric test; Mann-Whitney U test, $p = .063$).

Time to consume 50 sucrose pellets (2.25 g). Though the core group ate the fixed mass of sucrose pellets more slowly (250 ± 30 s) than the shams (199 ± 20 s), this difference was not significant ($F_{1,18} = 1.964$, $p = .178$).

Summary

Lesions of the AcbC induced a profound and lasting deficit in subjects' preference for the large reward when it was delayed. Subjects remained sensitive to removal of the delay and discriminated the two reinforcers. In baseline testing sessions, AcbC-lesioned subjects also failed to choose the large reward as often as shams when it was not delayed; however, prolonged training in the absence of delays re-established preference for the large reinforcer in a majority of lesioned subjects, and these subjects remained hypersensitive to the effects of reintroducing the delays subsequently. In addition, AcbC-lesioned rats were hyperactive, ate less of the food used as their maintenance diet (but showed normal consumption of the reinforcer used in the task), and were approximately 10% lighter than shams.

EXPERIMENT 4. EFFECTS OF INTRA-ACCUMBENS AMPHETAMINE ON CHOICE OF SIGNALLED AND UNSIGNALLED DELAYED REINFORCEMENT

Methods

Twenty-four naïve subjects were trained to press levers for sucrose pellets as before, and to nosepoke in order to initiate discrete-trial presentations of the levers, before being trained a variant of the delayed-reinforcement task adapted for intracranial infusions.

Abbreviated delayed-reinforcement task for intracranial infusions

This task was identical to the delayed-reinforcement choice procedure in Chapter 6, except that only three blocks of trials were used (each comprising two forced and ten free-choice trials), with a descending order of delays. This modification was made in an attempt to ensure that high Acb levels of drug coincided with responding at non-zero delays. The delays used were 60 s, 20 s and 0 s (in order). Trials began every 100 s, as before, for a total session length of 60 min.

Half the subjects were trained in the Cue condition (n = 12), and half in the No Cue condition (n = 12).

A stability criterion was defined as follows: after excluding single-lever trials, choice ratios (delayed lever responses ÷ total responses) were calculated for each rat using the summed responses for three consecutive sessions, and subjected to ANOVA with delay as a within-subjects factor. When the effect of delay was significant at the α = .01 level, the rats were considered to have criterion performance from the first session of the three. (Note that this criterion is not exactly comparable to that used in Chapter 6, in light of the different group sizes used.) Following the triplet of sessions in which the criterion was attained, subjects were given 5 more baseline sessions on the task before surgery.

All subjects then received cannulae aimed at the Acb (see *Methods*). Following recovery, they were retrained on the basic task for 3 sessions, and given a single preliminary infusion of saline to accustom them to the infusion procedure (as described in Chapter 3, p. 77). The preliminary infusion was given in the testing room containing the operant chambers, but the subjects were returned to their home cages following infusion.

Intra-accumbens amphetamine. Four doses of *d*-amphetamine sulphate (0, 3, 10, 20 µg) were given in a volume of 1 µl bilaterally in a digram-balanced Latin square, immediately before each test session. The infusion procedure was described in detail in Chapter 3 (p. 77). The Latin square was then repeated, in order to accumulate data from two sessions per dose per rat.

Results

Regrettably, three rats in the Cue group (N17, N18, N19) died post-operatively, as did one rat (N5) in the No Cue group. One other rat in the No Cue group (N6) died during behavioural testing, and its data were discarded.

Histology

On the whole, the cannula tips were located more ventrally than in previous experiments; they were positioned predominantly in the inferior shell, or at the core–shell boundary. Two rats with tip locations in the ventral pallidum (subjects N4, N24) were excluded, leaving 9 subjects in the No Cue group (rats N1, N2, N3, N7, N8, N9, N10, N11, N12) and 8 in the Cue group (rats N13, N14, N15, N16, N20, N21, N22, N23). Representative photomicrographs of Acb cannulae tracks and injector tip locations were shown in Chapter 3 (p. 82); schematics of the tip locations in the two groups are shown in Figure 95.

Schematic of cannula locations

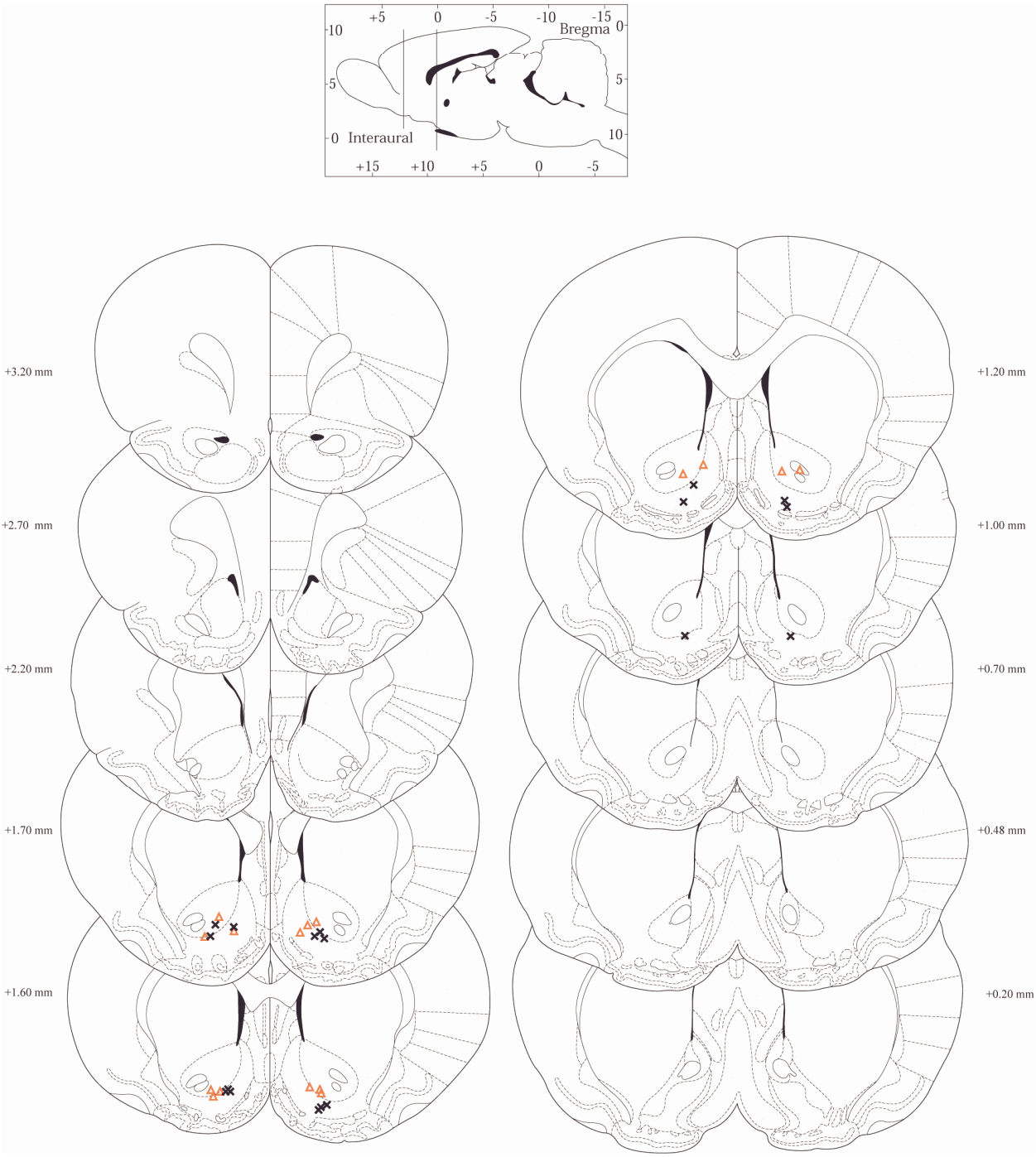

Figure 95. Location of the tips of injection cannulae within the Acb. *Black crosses* indicate subjects in the No Cue group (subjects N1, N2, N3, N7, N8, N9, N10, N11, N12). *Red triangles* indicate subjects in the Cue group (subjects N13, N14, N15, N16, N20, N21, N22, N23). Diagrams are taken from the atlas of Paxinos & Watson (1998).

Acquisition and baseline performance

The Cue group acquired sensitivity to the programmed delay earlier than the No Cue group. The Cue group first met the α = .01 delay-sensitivity criterion for sessions 7–9 and were operated following session 14, while the No Cue group met the criterion for sessions 24–26, and were operated following session 31.

The earlier acquisition in the Cue group was not apparent from an analysis of regression slopes during acquisition (Figure 96A, p. 221). Analysis of regression slope measures for the first 14 sessions (when both groups were in the pre-operative acquisition phase) using the model group × (session × S) revealed an effect of session ($F_{10.849,162.73}$ = 4.144, $\tilde{\varepsilon}$ = .835, p < .001), but no group effect and no group × session interaction (Fs < 1, NS).

However, consideration of choice behaviour did establish that the two groups showed different levels of performance at an equivalent time in the course of acquisition (Figure 96B); at this time, the absolute, session-wide level of preference for the delayed reinforcer was greater in the Cue group than the No Cue group even though the rapidity of the within-session shift in preference did not differ substantially. Analysis of choice ratios from sessions 12–14 in each group (at which time the Cue group had reached criterion but the No Cue group had not) revealed a significant difference in choice behaviour: statistically, there was a main effect of group ($F_{1,15}$ = 8.025, p = .013) as well as of delay ($F_{1.566,23.496}$ = 10.327, $\tilde{\varepsilon}$ = .783, p = .001), but no interaction (F < 1, NS).

This early difference between the groups disappeared as a result of further training of the No Cue group (Figure 96C). Comparison of choice ratios from the last 3 pre-operative sessions in each group (namely sessions 12–14 in the Cue group and sessions 29–31 in the No Cue group) yielded no group differences (delay: $F_{1.603,24.039}$ = 13.94, $\tilde{\varepsilon}$ = .801, p < .001; group and group × delay: Fs < 1, NS).

Re-establishment of baseline performance following surgery

Group differences did not re-emerge following surgery, either in choice ratio analysis (group: $F_{1,15}$ = 1.798, NS; group × delay: F < 1, NS; delay: $F_{2,30}$ = 18.592, p < .001) or analysis of the regression slope measure, which was stable post-operatively (analysed using session and group as factors: all Fs < 1, NS).

Effects of intra-accumbens amphetamine on choice

Some doses of amphetamine decreased preference for the large, delayed reinforcer (Figure 97), particularly at the 20-s delay, but a cue-dependent effect was not found. Analysis of choice ratios using the model $\text{group}_2 \times (\text{dose}_4 \times \text{delay}_3 \times \text{S})$ demonstrated main effects of dose ($F_{3,45}$ = 4.338, p = .009) and delay ($F_{1.377,20.66}$ = 37.738, $\tilde{\varepsilon}$ = .689, p < .001). The dose × delay interaction just escaped significance ($F_{6,90}$ = 2.169, p = .053). No other term was significant (Fs ≤ 1.068, NS).

Surprisingly, pairwise comparisons established that the 3 µg dose and the 20 µg dose significantly reduced preference for the large, delayed reinforcer (p = .024 and .037 respectively) relative to vehicle, while 10 µg had no effect (p = .591). The effects of 3 µg and 20 µg did not differ from each other (p = .226).

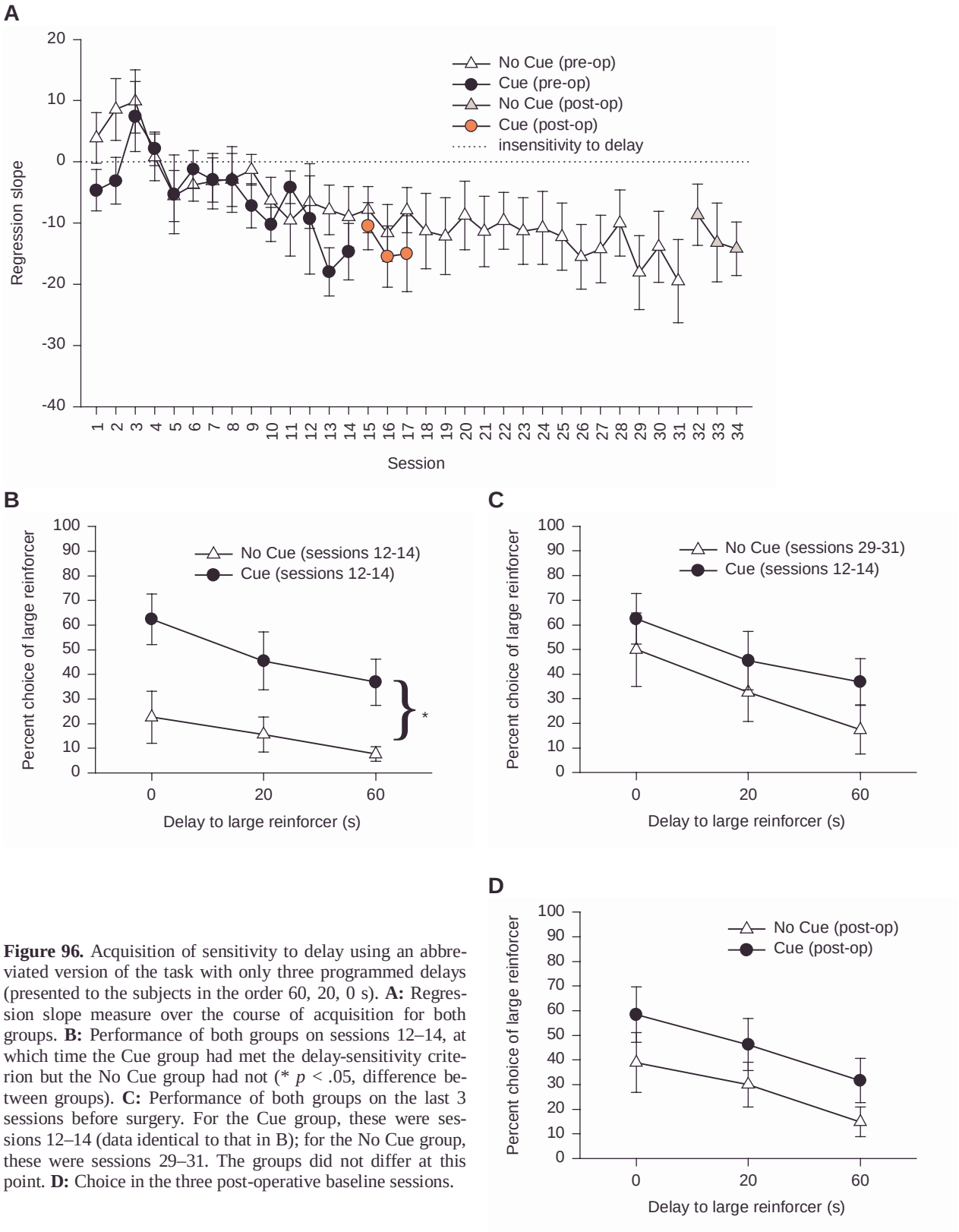

Figure 96. Acquisition of sensitivity to delay using an abbreviated version of the task with only three programmed delays (presented to the subjects in the order 60, 20, 0 s). **A:** Regression slope measure over the course of acquisition for both groups. **B:** Performance of both groups on sessions 12–14, at which time the Cue group had met the delay-sensitivity criterion but the No Cue group had not (* $p < .05$, difference between groups). **C:** Performance of both groups on the last 3 sessions before surgery. For the Cue group, these were sessions 12–14 (data identical to that in B); for the No Cue group, these were sessions 29–31. The groups did not differ at this point. **D:** Choice in the three post-operative baseline sessions.

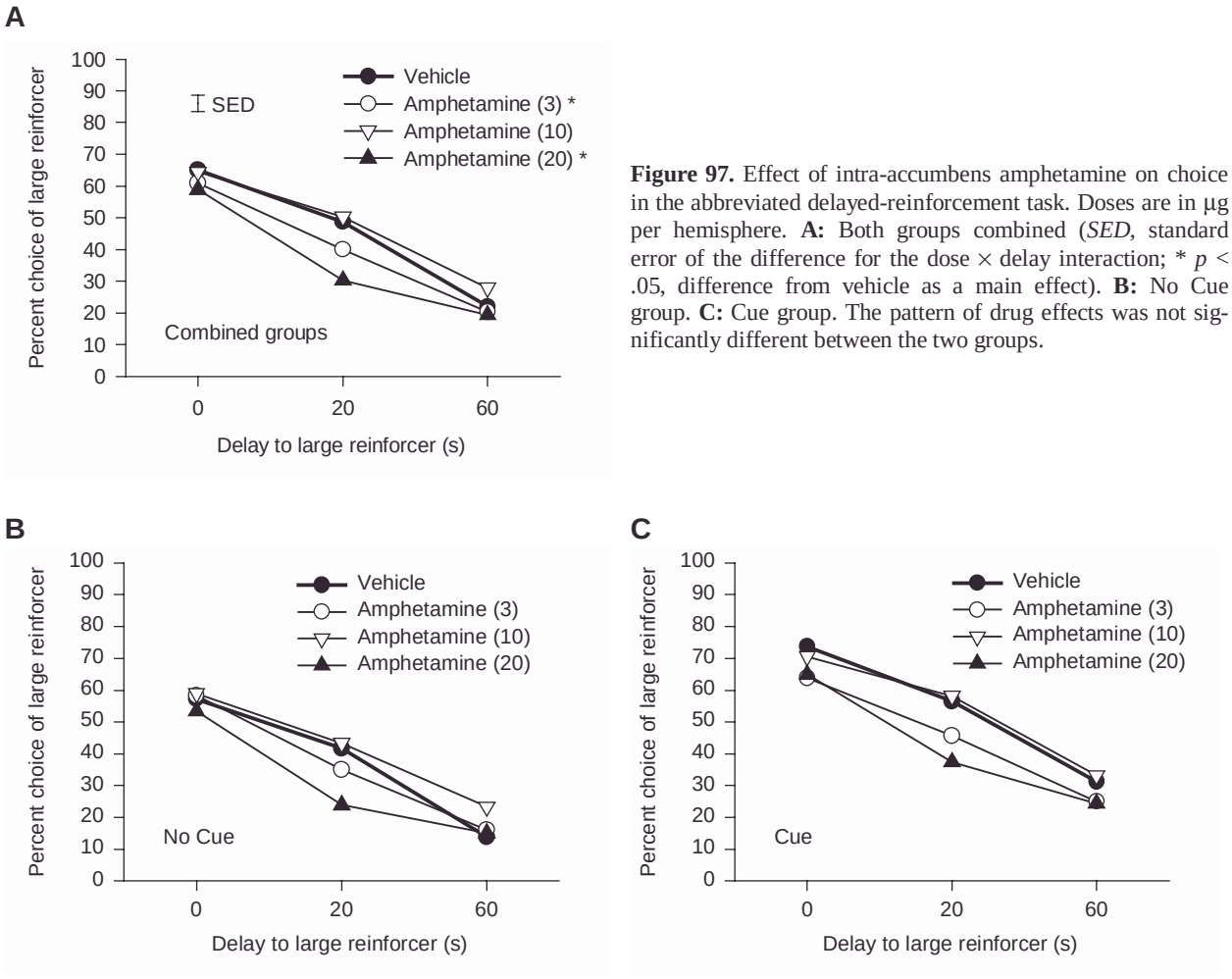

Figure 97. Effect of intra-accumbens amphetamine on choice in the abbreviated delayed-reinforcement task. Doses are in µg per hemisphere. **A:** Both groups combined (*SED*, standard error of the difference for the dose × delay interaction; * $p < .05$, difference from vehicle as a main effect). **B:** No Cue group. **C:** Cue group. The pattern of drug effects was not significantly different between the two groups.

Effects of intra-accumbens amphetamine on latencies and nosepoking behaviour

Initiation latency. Though initiation latencies increased with delay — despite delays decreasing as the session progressed, so that trials were initiated faster at the end of the session — amphetamine did not affect the latency. Initiation latencies were analysed using the model group × (dose × delay × S). There was a main effect of delay ($F_{1.685,25.272} = 8.449$, $\tilde{\varepsilon} = .842$, $p = .002$) but no other term was significant (dose × delay × group: $F_{6,90} = 1.658$, NS; other Fs < 1, NS). The mean initiation latencies (in seconds) were 0.998 ± 0.089 (0 s), 1.019 ± 0.087 (20 s), and 1.235 ± 0.111 (60 s).

Choice latency. Amphetamine did not affect the latency to choose a lever. There were insufficient data to allow a full model to be used, so they were analysed as group × (dose × response × S). This revealed no significant effect of any term (group: $F_{1,14} = 3.531$, $p = .081$, with a slight tendency for faster responding in the Cue group; other terms: $F \leq 2.114$, $p \geq .168$).

Collection latency. Subjects collected the immediate reward faster, and there was a non-significant tendency for amphetamine to slow collection of the large reward dose-dependently. Again, there were insufficient data for a full model, so group × (dose × response × S) was used. This revealed a near-significant dose × response interaction ($F_{1.65,23.106} = 2.904$, $\tilde{\varepsilon} = .55$, $p = .083$) in addition to a main effect of response ($F_{1,14} = 39.006$, $p < .001$).

Nosepoking during the delay. Subjects nosepoked for a greater proportion of the 20-s delay (15%) than of the 60-s delay (12%), and it appeared that the highest dose of amphetamine reduced nosepoking (means across both delays for each dose: vehicle 13.8%, 3 μg 14.9%, 10 μg 14.0%, 20 μg 9.8%). Although the Cue group did nosepoke for more of the delay than the No Cue group (15.2% versus 11.0% respectively), as in Chapter 6, this difference was not significant. Using the proportion of the delay spent nosepoking as the dependent measure, an analysis using the model $group_2 \times (dose_4 \times delay_2 \times S)$ was conducted. This revealed main effects of dose ($F_{3,24} = 4.757$, $p = .01$) and of delay ($F_{1,8} = 11.929$, $p = .009$), with no other significant terms (Fs ≤ 1.07, NS). However, using Sidak-corrected pairwise comparisons, no single dose was found to be significantly different from any other in *post hoc* tests ($p > .12$).

Summary

An abbreviated version of the delayed reinforcement choice task was used for this experiment, with a descending order of delays. Intra-Acb amphetamine reduced subjects' preference for the large, delayed reward slightly, but not in a clear dose-dependent manner (with effects being observed at 3 μg and 20 μg, but not at 10 μg). The effects of amphetamine were not demonstrably different in groups trained with and without a cue present during the delay. The 20-μg dose of amphetamine also appeared to have slight effects to reduce nosepoking in the food alcove during the delay to reinforcement.

DISCUSSION

Lesions of the AcbC induced a profound, long-lasting deficit in the ability to choose a delayed reward; these rats responded reliably but made highly impulsive choices. In contrast, lesions of the mPFC induced a subtle deficit in the pattern of responding while lesions of the ACC had no effect on choice. These experiments represent the first use of focal excitotoxic lesions to study choice of delayed reinforcement, and used a technique of matching corresponding sham and lesioned groups for performance pre-operatively, ensuring high power to detect changes due to the lesions. Intra-accumbens amphetamine injections had somewhat inconsistent effects to reduce preference for the delayed reward, and this effect did not depend on whether the delay was bridged by a signal. The effects of each manipulation will first be discussed separately.

Effects of anterior cingulate cortex lesions

Lesions of the ACC had no effect on choice, establishing that the ACC is not required for rats to choose a delayed reinforcer. Moreover, ACC-lesioned rats remained equally sensitive to unexpected removal of the delays in this task, suggesting that their choices were no more inflexible or 'habitual' than those of shams.

This finding stands in apparent contrast to previous reports of motor impulsivity or disinhibited responding in ACC-lesioned rats. For example, such rats have been found to over-respond to unrewarded stimuli (Bussey *et al.*, 1997a; Parkinson *et al.*, 2000c), and to respond prematurely in situations where they are required to wait (Muir *et al.*, 1996) (though the present lesions may be different from those of Muir *et al.*; see also Chapter 3, p. 119). However, such a dissociation is not in itself unexpected, as motor impulsivity and impulsive choice have been dissociated before ('execution' and 'outcome' impulsivity; Evenden, 1999b).

The ACC-lesioned rats were slower to collect the larger reward, the only behavioural effect of these lesions evident in this task. This deficit resembles very closely the increased latency of ACC-lesioned rats to approach the CS+ predictive of food observed in the autoshaping tasks used in Chapter 3 (and discussed there, p. 97). The slowing might reflect damage to the motor regions of the ACC (Dum & Strick, 1993), but in the present task other measures of response speed (trial initiation and choice latency) were not affected, suggesting perhaps that approach behaviour in ACC-lesioned rats was no longer enhanced by the expectation of a large reward.

The present results also provide a degree of further support for the hypothesis developed in Chapter 3 that the ACC is not critical for instrumental discrimination. Lesioned subjects in the present experiment discriminated between the two levers as well as control subjects did, despite the levers' being visually identical aside from their left / right position. This is in accordance with the view that the ACC is primarily important for the discrimination of similar Pavlovian conditioned stimuli on the basis of their association with reward. As discussed in Chapter 3 (p. 113), there is also evidence to suggest that the ACC may only play a critical role *early* in the learning of some tasks. It is of course possible that this applies to the delay-of-reinforcement task; the lack of a lesion effect does not preclude the involvement of the ACC in task acquisition. However, these results do suggest, despite findings of ACC abnormalities in disorders of impulsivity (e.g. Bush *et al.*, 1999), that ACC dysfunction is not an important contributor to impulsive choice.

Effects of medial prefrontal cortex lesions

Lesion of the mPFC 'flattened' the within-session shift from the large to the small reward; the mean preference for the large reward was *below* that of shams at zero delay, but *above* that of shams at the maximum delay. There is no obvious explanation for this effect within theories of choice of delayed reinforcement; it seems clear that the mPFC lesion resulted in some form of insensitivity to the contingencies or stimuli present in the task.

Contingency perception

Given that Balleine & Dickinson (1998a) demonstrated that lesions encompassing prelimbic cortex impaired rats' sensitivity to instrumental contingencies, it would be reasonable to suggest that a failure of contingency perception was responsible for performance of mPFC-lesioned rats in the present task. However, these rats were just as sensitive as controls to the unexpected removal of all delays; their responding was not inflexible, as might have been expected according to this account. The mPFC group were generally slower to respond on the levers, but this cannot easily be related to a specific cognitive deficit.

Timing ability

A more plausible interpretation is that mPFC lesions disrupted the control over behaviour by the passage of time in each session. There is strong evidence that normal rats learn a session-wide temporal discrimination in this task, and that this temporal discriminative stimulus comes to control responding, and in particular the tendency to shift from the large to the small reward as the session progresses (Chapter 6; Cardinal *et al.*, 2000b). Disruption of such temporal stimulus control might be expected to produce a flattening of the within-session shift of the kind seen.

Indeed, aspirative lesions of the mPFC have previously been shown to induce a general deficit in timing ability in rats (Dietrich & Allen, 1998); lesioned subjects showed a temporal discrimination function that was less steep than normal in the peak procedure, an operant task that assesses the ability to time a discriminative stimulus (Catania, 1970; Roberts, 1981). Indeed, 'temporal organization of behaviour' (albeit an ill-defined term) has been suggested to be a cardinal function of the prefrontal cortex (see e.g. Fuster, 1995). While disruption of timing behaviour on a shorter scale might in principle also affect choice behaviour in a delay-dependent manner (as discussed below, p. 229), there was no evidence for this in mPFC-lesioned subjects.

Effects of nucleus accumbens core lesions

Lesions of the AcbC induced a major deficit in subjects' ability to choose a delayed reward; lesioned subjects made truly impulsive choices. This was not due to an inflexible bias away from the lever producing the delayed reinforcer: AcbC-lesioned rats still chose the large reinforcer more frequently at zero delay than at other delays, and removal of the delays resulted in a rapid and significant increase in the rats' preference for the large reinforcer. Thus, the pattern of choice genuinely reflected a dramatically reduced preference for the large reinforcer when it was delayed, suggesting that delays reduced the effectiveness or value of rewards much more in AcbC-lesioned rats than in controls.

In the initial set of post-operative sessions, the AcbC-lesioned rats preferred the small reinforcer even at zero delay, avoiding the large reinforcer. Prolonged training in the absence of delays did not overcome the tendency to avoid the lever previously associated with delayed reinforcement in all lesioned subjects. Given the pre-operative performance of the same animals (i.e. equal to that of controls), this suggests that the post-operative experience of delayed reinforcement may have been highly aversive for AcbC-lesioned rats (or at least, much less preferable than immediate small reinforcement), inducing them to avoid that

lever permanently. However, the majority of core-lesioned subjects (6 out of 10) showed a consistent preference for the large reinforcer after prolonged training without delays (Table 20, p. 215). Even when sham and AcbC-lesioned subjects were selected who showed near-exclusive preference for the large reinforcer under these conditions, reintroduction of delays caused a dramatic and selective fall in preference for the large, delayed reinforcer in the AcbC-lesioned group (accompanied by a small decline in preference at zero delay; Figure 93, p. 216). These results suggest that the AcbC-lesioned rats' low preference for the large reinforcer at zero delay in the baseline post-operative sessions (Figure 90B, p. 212) was *not* due to a genuine preference for the small reward over the larger reward. Instead, it suggests that this result reflected the marked effects of the delays present later in the session (discussed further below, p. 228).

Primary motivational changes

AcbC-lesioned rats were underweight, and at least two possible contributing factors were observed: these rats exhibited locomotor hyperactivity and ate less of the chow used as their maintenance diet. These changes have been observed before following AcbC lesions (Parkinson, 1998). It is therefore possible that the lesioned rats' motivation to earn food was lower. However, it is unlikely that these changes contributed to their impulsive choice. First, there were no significant differences in the rate at which these subjects consumed the sucrose pellets used as the reinforcer in the task. Second, explicit manipulation of deprivation state has been shown not to affect choice on this task (Chapter 6; Cardinal *et al.*, 2000b). Third, performance of Acb-lesioned animals was not comparable in other respects to that of sated rats (Chapter 6; Cardinal *et al.*, 2000b); for example, they did not make more omissions than sham-operated controls.

Altered sensitivity to reinforcer magnitude or delay?

The core group showed at least some discrimination between the large and the small reinforcer. This is consistent with the observation that the expectancy of reward magnitude continues to have normal effects upon rats' reaction time following excitotoxic Acb lesions, with a smaller reaction time when a large reward is expected (Brown & Bowman, 1995) (though intra-Acb NMDA antagonists do impair this effect; Hauber *et al.*, 2000). A large proportion of the core group showed a preference, sometimes absolute, for the large reward when prolonged training was given with no delays. Five out of ten core-lesioned rats met a very stringent criterion for preference of the large reward under these conditions. These same rats were exquisitely sensitive to delays, preferring the large reinforcer much less than shams when it was delayed. Nevertheless, it remains a possibility that the other rats in the core group did not discriminate between the two reward magnitudes post-operatively, and that the history of delayed reinforcement on one lever permanently reduced their preference for that alternative.

It is also possible that the core group discriminated between the reinforcer magnitudes, but to a lesser extent than normal rats. In this scenario, core-lesioned rats still exhibit impulsive choice behaviourally — that much is clear — but because the perceived value of the large reinforcer is insufficient to overcome the normal effects of delay discounting. The multiplicative hyperbolic model of choice (see Ho *et al.*, 1999) postulates that the value of an immediate reinforcer of physical magnitude q is determined by the equation

$$V_{immediate} = \frac{q}{q+Q}, \text{ also expressed as } V_{immediate} = \frac{1}{1+Q/q} \quad (1)$$

and that the value of this reinforcer when delayed by a time d is given by

$$V_{delayed} = \frac{V_{immediate}}{1 + K \cdot d} \quad (2)$$

where K and Q are 'delay discounting' and 'magnitude discounting' parameters that reflect intrinsic properties of the animal. In this theory, when one assesses an animal's relative preference between two reinforcers of different magnitudes, one of which is delayed, changes in both K and Q may affect choice, as illustrated in Figure 98 and Figure 99. It can immediately be seen from these figures that both hypothetical kinds of manipulation can reduce preference for delayed rewards, inducing impulsive choice, though only one manipulation varies the effects of delay.

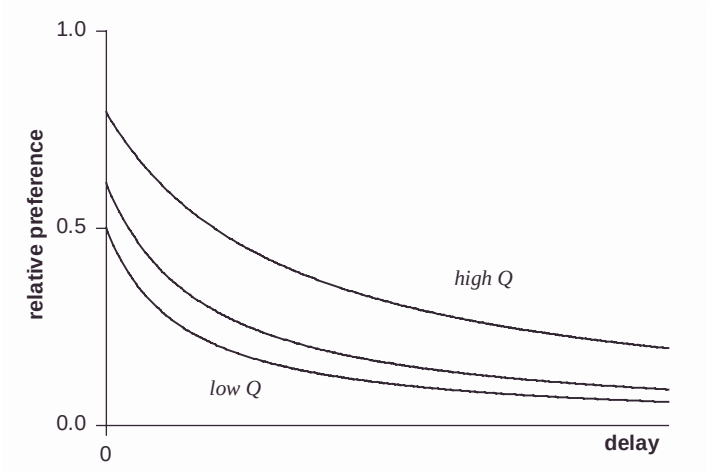

Figure 98. Hypothetical choice / delay curves for three individuals whose sensitivity to delay *per se* is identical, but across whom sensitivity to reinforcer magnitude varies. These curves were generated by assuming that the individual are offered an immediate reinforcer of magnitude 1, and a delayed reinforcer of magnitude 4. The absolute value to the animal of each reinforcer (V_1 and V_4) is calculated separately according to the hyperbolic discounting equations given in the text, and the relative preference is calculated as V_4 / (V_1 + V_4). The delay sensitivity parameter K is identical in all three subjects, but the magnitude sensitivity parameter Q takes the values 0.01, 1, and 100. As $Q \rightarrow 0$, the animal becomes indifferent between the two reinforcers at zero delay; as $Q \rightarrow \infty$, relative valuation of reinforcers at zero delay approaches the relative proportion of their physical magnitudes (in this case, 4 / (1+4) or 0.8).

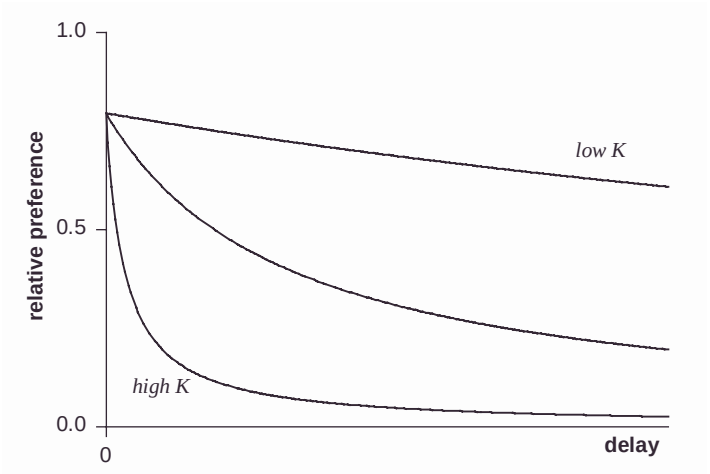

Figure 99. Hypothetical choice / delay curves for three individuals whose sensitivity to reinforcer magnitude is identical, but across whom sensitivity to reinforcer delay varies. These curves were generated as for Figure 98, but Q is held constant (at 100) and K is varied (taking values of 0.1, 1, and 10, though the units are arbitrary).

Theoretically, a critical test of whether a given manipulation affects delay (K) or magnitude (Q) discounting, in this model, is to examine preference at zero delay, which manipulations of K cannot affect. Inspection of choice-by-delay plots (Figure 90 to Figure 93, pp. 212–216) suggests that lesions of the AcbC affected the perception of reinforcer magnitude, as preference for the large reinforcer at zero delay was not as high as that of shams. (A different interpretation is offered below.) Another, more direct test would be to obtain estimates of Q and K for each rat directly, and compare these across groups. In the present task, this might be attempted by assuming

$$\textit{relative preference at delay } d = \frac{V_{large}}{V_{large} + V_{small}} \qquad (3)$$

$$= \frac{\frac{V_{large-immediate}}{1 + K \cdot d}}{\frac{V_{large-immediate}}{1 + K \cdot d} + V_{small-immediate}}$$

This equation might be solved for physical reinforcer magnitudes (q) of 1 and 4 pellets and fitted to individual rats' data using non-linear programming techniques. However, this attempt is doomed to failure — not only because of the variability in rats' preferences, and by the poorly-constrained curve-fitting problem, but because it is clear that rats' preferences in the present task do not conform to this model. If choice ratios are interpreted as *relative preference* according to equations (1) and (3), a contradiction is apparent from Figure 92A (p. 215). Without delays, sham subjects' preferences approached 100% choice of the large reinforcer, whereas in the model, relative preference between a 1-pellet and a 4-pellet reinforcer cannot exceed 80%. The behavioural result comes as no surprise, for it is the well-known phenomenon of maximization on discrete-trial schedules (see Mackintosh, 1974, pp. 190–195).

Thus, behaviour on this task cannot be quantified according to the hyperbolic discounting model. A far more likely interpretation of the failure of core-lesioned rats to choose the large reinforcer as much as shams at zero delay is that their tendency to avoid the delayed reinforcer generalized from trial blocks on which delays were present to the first trial block. Indeed, Figure 92 and Figure 93 show this phenomenon developing.

The task used in the present experiments does not allow the two explanations of impulsive choice (variations in sensitivity to reinforcer magnitude or delay) to be distinguished conclusively. While this may be possible in delay-of-reinforcement choice tasks using indifference-point methodology (Ho *et al.*, 1999, but see Chapter 5), there may be a simpler alternative. Relative preference for two reinforcers is often inferred from the distribution of responses on concurrent VI schedules of reinforcement (see Chapter 1, p. 54). While such an approach is complex when delayed reinforcement is used (see Chapter 1), it is simpler to interpret with immediate reinforcement. If core-lesioned rats were trained on two concurrent VI schedules with identical parameters, with one schedule producing a 1-pellet reward and the other producing a 4-pellet reward, relative preference between the two could be assessed. The matching law (Herrnstein, 1961; 1970) predicts that a subject for whom 4 pellets are worth 4 times as much as 1 pellet would allocate 80% of its responses to the 4-pellet schedule. Normal rats would be expected to perform close to this level, even if they did not 'match' exactly. If core-lesioned subjects exhibited *relative indifference* compared to shams, this would provide independent evidence for reduced reinforcer magnitude discrimination following AcbC lesions (or an abnormality of the matching process itself). If they performed normally, this explanation would become far less likely, in which case the impulsive choice observed in the present experiment could be attributed more specifically to a steeper delay-of-reinforcement gradient.

Published data and the present thesis do not allow this question to be answered directly. However, in Chapter 4, core-lesioned rats were trained on a concurrent VI schedule, albeit for two different reinforcers intended to be of similar value. If anything, these subjects exhibited more pronounced relative preferences than shams (p. 138), indirectly supporting the view that impulsive choice in core-lesioned rats is due to a delay-dependent deficit. However, this issue will require further investigation.

Finally, an explanation in terms of temporal perception might also be offered for the effects of AcbC lesions. The basal ganglia have been suggested to be a component of an 'internal clock', based on the effects of dopaminergic manipulations on timing tasks (see Gibbon *et al.*, 1997). Similarly, forebrain serotonin depletion that affects Acb, among many other structures, impairs timing ability (Morrissey *et al*., 1993; Wogar *et al*., 1993a; Morrissey *et al*., 1994; Al-Zahrani *et al*., 1997), though these impairments sometimes reflect enhanced behavioural switching rather than a true timing deficit (Ho *et al*., 1995; Al-Zahrani *et al*., 1996; Al-Ruwaitea *et al*., 1997a); see Al-Ruwaitea *et al.* (1997b) for a review. A lesion that increased the speed of an 'internal clock' might (following the distinctions of Killeen & Fetterman, 1988) affect choice prospectively (i.e. the lesioned subject perceives itself to be at a later time-point in the session than it actually is, hastening the within-session shift towards the Immediate lever), or might affect retrospective choice (i.e. the lesioned subject experiences a given delay as longer than it remembered, causing a decrease in its preference for the Delayed lever). Unfortunately, there is at present no evidence to address the question of whether excitotoxic AcbC lesions affect behavioural timing.

Hyperactivity and impulsivity: behavioural comparison to models of ADHD

AcbC-lesioned animals exhibited at least two signs of ADHD: locomotor hyperactivity and impulsive choice (Sagvolden & Sergeant, 1998). However, attentional deficits are not evident in such animals: neither 6-OHDA-induced dopamine depletion of the Acb (Cole & Robbins, 1989) nor excitotoxic lesions of the AcbC (A. Christakou, unpublished observations) affect accuracy in the 5CSRTT test of attentional function.

As discussed above, one possible explanation for the impulsive choices of the AcbC-lesioned group is that these rats were hyposensitive to delayed reinforcement (hypersensitive to the effects of delays). This hypothesis may make predictions about performance on free-operant schedules, discussed below, but first it should be noted that reduced preference for a delayed reward as a *goal* of behaviour in choice experiments is not necessarily the same as reduced ability of delayed reinforcement to strengthen behaviour by 'stamping in' a stimulus–response habit (Thorndike, 1911; Grindley, 1932; Guthrie, 1935; Hull, 1943); goal-directed actions and stimulus–response habits are dissociable (Dickinson, 1994).

Sagvolden *et al.* (1998) suggested that reduced efficacy of delayed reinforcement should lead to hyperactivity (increased responding) on free-operant schedules. For example, subjects responding on FI schedules exhibit a typical 'scallop', in which responding increases as the reinforcer is approached in time; this may be because responses at the end of the interval incur a shorter delay to reinforcement (see Mackintosh, 1974, pp. 170–177). According to this logic, subjects who exhibit a steeper delay-of-reinforcement gradient should show a more pronounced FI scallop, as has been observed for the SHR rat (Sagvolden *et al*., 1992). However, there are alternative explanations of FI performance (Mackintosh, 1974, pp. 170–171) — indeed, the smooth scallop is only observed when many intervals are averaged, and is not typical of an individual interval (Gentry *et al*., 1983). Sagvolden *et al.* (1998, p. 62) appear to suggest that the more pronounced scallop is partly a consequence of differential reinforcement of short IRTs; however, it is not clear that this is the case. Ratio schedules do not reinforce particular IRTs with different probabilities, but do reward high rates of responding (short IRTs) with higher local rates of reinforcement, while interval schedules preferentially reinforce *long* IRTs (the longer a subject waits to make the next response, the more likely it is to be reinforced) (see Mackintosh, 1974, p. 177; Dawson & Dickinson, 1990; Tarpy, 1997, pp. 257–258). Regardless of the subject's delay-of-reinforcement gradient, if IRTs represent a basic unit of behaviour to be reinforced (as suggested by Shimp, 1967; 1969), then interval schedules reinforce long IRTs. For the FI scallop to be a consequence of reinforcement of short IRTs, short IRTs would have to occur closer in time to the reinforcer than long IRTs — the scallop phenomenon

intended to be explained. On the other hand, the development of a more pronounced FI scallop is explicable in terms of a steeper delay-of-reinforcement gradient if responses are considered individually.

This issue is of some importance, as it determines whether hyperactivity should follow directly from a steep delay-of-reinforcement gradient. Reduced efficacy of delayed reinforcement does not necessarily imply increased efficacy of immediate reinforcement — Figure 99 illustrates this (compare Figure 1 of Sagvolden *et al.*, 1998). If a steep delay-of-reinforcement gradient does preferentially reinforce short IRTs, it is clear how hyperactivity might emerge (Sagvolden *et al.*, 1998; Sagvolden & Sergeant, 1998). However, if responses are considered individually, the average response would be *less* likely to be reinforced, leading to hypoactivity. Finally, it might be argued that activity levels determine reinforcement efficacy, rather than the other way around. In models such as that of Killeen & Fetterman (1988, p. 288), reinforcement only acts on the behaviour the subject is currently engaged in; delaying the reinforcer simply reduces the probability that the animal has remained in the state associated with that behaviour. In this form of model, changing the rate at which the animal shifts between behaviours — a plausible description of hyperactivity — would be expected to steepen the apparent delay-of-reinforcement gradient.

Thus, there is no clear theoretical compulsion to think that a steep delay-of-reinforcement gradient should produce either hypoactivity or hyperactivity on free-operant schedules. The experimental evidence concerning rats with excitotoxic AcbC lesions indicates that although they exhibit choice behaviour compatible with a steep delay-of-reinforcement gradient (present experiments), and locomotor hyperactivity (present experiments; Maldonado-Irizarry & Kelley, 1995; Parkinson, 1998; Parkinson *et al.*, 1999b), they respond at normal rates on free-operant schedules (e.g. Chapter 4, concurrent VI schedules; Parkinson *et al.*, 1999b, random ratio schedules with conditioned reinforcement).

Implications for theories of nucleus accumbens function

At the least, the present experiments show that the Acb contributes significantly to animals' ability to choose a delayed reward. If further experiments show that it does so specifically by maintaining the value of a reinforcer over a delay, a new avenue of inquiry into Acb function might open up. It has previously been shown in primates that neuronal activity related to the expectation of reward across a delay can be found in the ventral striatum (Schultz *et al.*, 1992; Schultz *et al.*, 1995a; Schultz *et al.*, 1998; Schultz *et al.*, 2000); such activity is a candidate representation of the goals of activity (Schultz *et al.*, 2000). Additionally, striatal neurons may respond to past events, maintaining a form of memory that might assist the association of past acts with reinforcement (Schultz *et al.*, 1995a). These findings represent important data on the forms of information that the AcbC may use to promote actions leading to delayed rewards, and a future challenge will be discover the manner in which these neural signals influence overt behaviour, and the psychological processes they govern. Given the involvement of the Acb in aversive motivation (see Salamone, 1994; Parkinson *et al.*, 1999c), it would also be of great interest to determine whether lesions of Acb induce impulsive choice in an aversive context, impairing the ability to choose a small immediate penalty in preference to a large delayed penalty.

Although the manner in which delayed reinforcement affects free-operant behaviour may be extremely complex, as discussed above, the finding that AcbC lesions reduce subjects' preference for delayed rewards may be useful in interpreting the results of some studies that are at present not clearly understood. For example, Salamone and colleagues have found that dopamine depletion of the Acb leads rats to forgo the opportunity to work for a preferred food, instead consuming more of a less-preferred but freely available food (Salamone *et al.*, 1991; Cousins *et al.*, 1993; Salamone *et al.*, 1994; Cousins *et al.*, 1996), even though reinforcer magnitude discrimination is not overtly impaired by these lesions (Salamone *et al.*, 1994). Similarly, Acb dopamine depletion impairs responding on high-rate but not on low-rate schedules

(McCullough *et al.*, 1993; Salamone *et al.*, 1993; Sokolowski & Salamone, 1998; Aberman & Salamone, 1999). These results have been interpreted as indicating that Acb dopamine depletion impairs the ability of animals to overcome response costs (Salamone, 1994). Although excitotoxic AcbC lesions are clearly not the same as whole-Acb dopamine depletion, two alternative views of these studies are possible. Firstly, as suggested by Parkinson *et al.* (2000a), the impairments may have been due to the loss of a Pavlovian motivational process that normally contributes to instrumental responding (see Chapter 1, p. 50). An interpretation in terms of response costs is certainly inadequate to describe all the data; for example, Acb DA depletion has previously been shown to disrupt displacement behaviours that cannot easily be described as carrying a response cost (Robbins & Koob, 1980). The present results, based on excitotoxic AcbC lesions, provide an even clearer demonstration of the role of the Acb in choice behaviour and the selection of actions, even when those actions do not differ in response effort or cost (in support of Reading *et al.*, 1991; Parkinson *et al.*, 2000a). A second interesting interpretation of the results of Salamone and colleagues, based on the present data, is that the lesions reduced the subjects' inclination to respond for food, particularly on high-rate schedules, because that reward was significantly *delayed*. Instead, the lesioned rats preferred an immediately-available but smaller reward.

Effects of intra-accumbens amphetamine

Theories that attribute impulsive choice to hypofunctional Acb DA systems (see Sagvolden & Sergeant, 1998) thereby suggest that Acb DA normally contributes to the effectiveness of delayed reinforcement (and thereby to self-controlled choice), and would predict that intra-Acb amphetamine would increase preference for delayed reward. Yet the opposite was observed. Injections of amphetamine into the Acb reduced subjects' preference for the large, delayed reward slightly, but not in a clear dose-dependent manner; over all subjects, the 3-µg and 20-µg doses had this effect, but the 10-µg dose did not differ from saline. Furthermore, despite the prediction made in Chapter 6 (p. 192) that intra-Acb amphetamine might enhance the effects of cue stimuli present during a delay to reinforcement to promote 'self-controlled' choice, no cue-dependent effects of amphetamine were observed, and the effects of amphetamine were reasonably consistent across the two groups (Figure 97, p. 222).

A new version of the task was used for this experiment. The sessions were shorter and the delays were arranged in reverse order (with the longest delay presented at the start of the session), in an attempt to ensure that high Acb levels of drug coincided with responding at non-zero delays. However, this new task may have produced methodological problems. The task appeared more difficult for subjects to acquire than the version used in other experiments, with lower levels of preference for the large reinforcer at zero delay (compare acquisition in Figure 96, p. 221, with that in Experiments 1 / 2 / 3 and Chapter 6) and more pronounced differences in absolute preference levels between the Cue and No Cue groups during acquisition (though not in the slope of the within-session shift in preference). If subjects are to reach the same levels of preference at each delay as in the 'standard' version of the task, their within-session shift in preference must be more rapid; possibly this is harder to learn. Both this and the more pronounced group differences in responding may have rendered the abbreviated task less sensitive to pharmacological manipulations.

One other methodological issue is the order in which the delays were given. When drug effects are tested with only an ascending, or only a descending, series of delays, any delay-dependent effects of the drug are confounded with the pattern of responding across a session. It was therefore hoped that the use of a descending series of delays would allow some comparison with the effects of systemic amphetamine observed in Chapter 6, with the potential to distinguish (for example) a tendency to complete the within-

session shift in preference more rapidly from a true effect on preference for delayed reward. However, this training technique meant that the first time the subjects experienced a choice of the two levers, one lever delivered a larger reinforcer but after a 60-s delay. For a subject accustomed to continuous reinforcement, this may have induced rapid extinction on that lever, an effect that might have contributed to poor learning in the present experiment.

In the absence of a clear dose-dependent effect of amphetamine on choice, or on other measures of performance, it is difficult to draw firm conclusions. Taken at face value, the present results indicate that intra-Acb amphetamine causes a slight reduction in preference for delayed rewards, without affecting motoric aspects of task performance, and that signals present during the delay do not contribute to its action at this site. The Acb might therefore be a neural locus of the cue-independent effects of systemic amphetamine (see Chapter 6), but the locus of the cue-dependent effects remains uncertain. However, not only was it possible that the new task was relatively insensitive to the effects of amphetamine (discussed above), but the injector tips in this experiment were on the whole located more ventrally than was intended (Figure 95, p. 219); amphetamine was injected into ventral AcbSh and / or underlying structures, and this fact may also account for the lack of a systematic effect of amphetamine on choice. It will probably prove worthwhile to replicate this experiment comparing the effects of amphetamine injections in the ventromedial AcbSh with injections in the AcbC, particularly given the newly-discovered role of the AcbC in preference for delayed reward (Experiment 3). In doing so, it may also help to adjust the task parameters in an attempt to avoid some of the pitfalls discussed here (ensuring that the abbreviated task is sensitive to the behavioural and systemic pharmacological manipulations used in Chapter 6), or simply to use intra-Acb amphetamine with the full, 100-min task.

Finally, it is interesting to note that rats reared in isolation have recently been found to be less impulsive than socially-reared controls on the delayed reinforcement choice task used in the present experiments (full version, without signals during the delay), and this difference was exaggerated by systemic *d*-amphetamine (Y.-P. Liu, L.S. Wilkinson and T.W. Robbins, unpublished observations; L.S. Wilkinson, personal communication, 4 January 2001). Isolation-reared rats exhibit augmented Acb DA release in response to psychostimulant drugs (Jones *et al.*, 1992; Howes *et al.*, 2000), with some studies showing elevated basal levels of Acb DA (Hall *et al.*, 1998), but they also exhibit other neurochemical abnormalities, including differences in 5-HT levels in the Acb and DA levels in the mPFC and amygdala (Jones *et al.*, 1992; Heidbreder *et al.*, 2000). These differences represent other candidate systems where anatomically- and neurochemically-specific drug infusions might affect impulsive choice.

Autoshaping and impulsivity

Autoshaping itself has been suggested to reflect impulsive behaviour, in that subjects are unable to withhold responses to the CS (Tomie, 1996). Subjects' propensity to autoshape has previously been shown to predict sensitivity to delays in a similar delay-of-reinforcement procedure to that used here (Tomie *et al.*, 1998). Individuals that autoshape readily have been suggested to be more vulnerable to drugs of abuse (Tomie, 1996), while impulsive choice behaviour predicts alcohol self-administration in rats (Poulos *et al.*, 1995). Rats that autoshape readily have higher levels of dopamine and dopamine metabolites in the Acb than rats that do not (Tomie *et al.*, 2000), while dopamine depletion of the Acb and excitotoxic lesions of the AcbC both impair autoshaping (Everitt *et al.*, 1999; Everitt *et al.*, 2000b; Parkinson *et al.*, 2000c; Parkinson *et al.*, submitted).

However, the relationship between impulsivity and autoshaping has not been clearly established. Autoshaping is suggested to represent impulsivity in that the subject is unable to suppress the involuntary

tendency to approach the CS — 'motor impulsivity', or failure of inhibitory control (Tomie, 1996; Tomie *et al.*, 1998). Motor impulsivity has been doubly dissociated from impulsive choice by pharmacological means (summarized by Evenden, 1999b). The correlation between the two suggested by Tomie *et al.* (1998) is therefore not a trivial result. Unfortunately, in the study of Tomie *et al.* (1998), which used a task very similar to that of Evenden & Ryan (1996), over 50% of the subjects showed exclusive preference, choosing the large delayed reward or the small immediate reward at all delays. These subjects scored zero on the measure of impulsive choice used by Tomie *et al.* for correlation with autoshaping CR frequency. Furthermore, the autoshaping stimulus was almost identical to one of the levers used subsequently in the delayed reinforcement choice task; thus, autoshaping and impulsive choice may have been correlated not because of an underlying common cause (impulsivity), but because differences in subjects' experience with the autoshaping stimulus affected choice directly. Finally, the autoshaping task used included no control stimulus (CS–) unpaired with reward; therefore, autoshaping performance in their study was potentially confounded with differences in unconditioned behaviour. (Different autoshaping tasks may also generate different views of what constitutes motor impulsivity: if a subject responds to the CS+ but not to a similar CS–, is it showing good impulse control by suppressing responses to the CS–, or poor impulse control by responding to the CS+ in the first place? One view is that total CS responding is an index of impulsivity, in which case an absence of responding indicates good impulse control, selective CS+ responding indicates mild impulsivity, and responding to both the CS+ and the CS– indicates grossly impaired impulse control.) While Tomie *et al.*'s (1998) result appears to indicate that *sensitivity* to the delays in the choice task correlate with either the propensity to autoshape, general activity, or exploratory tendencies, it is not clear that simple sensitivity to delays is the same as impulsive choice. In particular, it is not obvious that subjects who always chose the small immediate reward in this task exhibited 'zero impulsivity', and the proportion of rats exhibiting this insensitivity to delay may reflect procedural differences (such as the method of training, as suggested in Chapter 6, p. 190). Future investigations of this important area will need to pay particular attention to the definitions of impulsivity used.

The present study raises two further dissociations between autoshaping and impulsive choice. First, lesions of the ACC are known to impair autoshaping, generally in the 'disinhibited' fashion of increasing approaches to a neutral CS– (Chapter 3; Bussey *et al.*, 1997a; Parkinson *et al.*, 2000c). Of course, this may represent a different idea of the relationship between motor impulsivity and autoshaping than that of Tomie *et al.* (1998); ACC lesions have also been suggested to increase motor impulsivity via disinhibition in other tasks (Muir *et al.*, 1996) (though these lesions may differ slightly; see p. 119 and Figure 14, p. 72). However, ACC lesions did not affect impulsive choice in the present experiments. Second, lesions of the AcbC, which abolish the development and performance of autoshaping by reducing approaches to the CS+ (Everitt *et al.*, 2000b; Parkinson *et al.*, 2000c), rendered rats dramatically *more* likely to make impulsive choices.

The possible role of other structures connected to the nucleus accumbens core

It has been shown that while lesions of the AcbC impair rats' capacity to choose a delayed reward, lesions of two of its afferents did not (mPFC lesions produced a deficit but this was qualitatively different). An important task for further investigations is to specify which afferents to the AcbC contribute to its ability to promote the choice of delayed rewards, and through what efferent pathways it does this.

One obvious afferent structure that may provide specific information concerning reinforcer value to the Acb is the BLA, while the CeA might affect preference by modulating the dopamine innervation of the Acb. Another direct afferent is the orbitofrontal cortex, also implicated in the assessment of reward

value and probability (Rogers *et al.*, 1999) (see also Chapter 1 for a discussion of amygdala and orbitofrontal cortex function, and Öngür & Price, 2000 for the delineation of the orbitofrontal cortex in the rat). The orbitofrontal cortex may also be an important efferent target of information travelling through Acb, as this 'limbic loop' of the basal ganglia projects back (through the ventral pallidum) to medial orbitofrontal cortex (Alexander *et al.*, 1986). In addition, it remains to be seen whether the AcbSh also plays a role in the choice of delayed rewards. This is another interesting target of investigation, given the abnormalities of dopamine receptor function detected in the AcbSh of the SHR (Papa *et al.*, 1996; Carey *et al.*, 1998; Papa *et al.*, 1998; Sadile, 2000).

Finally, the limbic corticostriatal circuit may not be the only system involved in delayed reinforcement. In principle, any structure that represents *future reinforcers* across a delay may contribute to the choice of future reinforcers, and exert conditioned reinforcing effects on current behaviour, while any structure that maintains a 'memory trace' of responses across a delay may support the reinforcement of *past responses*. The ventral striatum and orbitofrontal cortex exhibit such activity (Schultz *et al.*, 1995a; Schultz *et al.*, 1998; Schultz *et al.*, 2000), but so do other structures including the dorsal striatum (e.g. Schultz *et al.*, 1995a), implicated in the reinforcement of stimulus–response habits (see Chapter 1, p. 46).

Conclusions

The present results provide direct evidence to support previous conjectures that the Acb is involved in the pathogenesis of impulsive choice. Hitherto, these conjectures have been based on correlational data, including findings of neurochemical abnormalities in the Acb of animal models of ADHD (see Sagvolden & Sergeant, 1998); the present study demonstrates a causal role for Acb dysfunction in impulsive choice. No evidence was found for similar involvement of the ACC or mPFC. It remains to be seen whether failure of Acb dopamine function can also contribute to impulsive choice. The remainder of the neural circuit underlying the efficacy of delayed reinforcers remains to be elucidated, but the present methodology holds promise as a means of identifying it.