Chapter 3.
Role of the anterior cingulate cortex in the control over behaviour by Pavlovian conditioned stimuli

Abstract. The anterior cingulate cortex (ACC) has been clearly implicated in stimulus–reward learning, but the exact contribution it makes to this process is not well understood. To address this issue, rats with lesions of peri- and postgenual ACC were tested using a variety of tasks to which stimulus–reward learning was expected to contribute. Unexpectedly, rats with ACC lesions learned to approach a food alcove during a stimulus that predicted imminent food delivery (temporally discriminated approach task), and subsequently responded normally for that stimulus in a test of conditioned reinforcement. They also exhibited normal conditioned freezing to an aversive CS that predicted footshock. Yet the same animals were impaired at autoshaping, a deficit observed before in ACC-lesioned animals. Furthermore, an autoshaping deficit was demonstrated when subjects received the lesion *after* training, though some behavioural recovery occurred. Additionally, the phenomenon of simple Pavlovian–instrumental transfer was intact following ACC lesions. In order to resolve the apparent discrepancy between the autoshaping deficit and the lack of a deficit on the temporally discriminated approach task, a new task was developed in which the approach behaviour was identical to that measured during the temporally discriminated approach task, but was under the control of two stimuli, only one of which was followed by reward. ACC-lesioned rats were impaired at the discrimination, approaching during both stimuli. It is suggested that this region of the ACC is not critical for stimulus–reward learning *per se*, but is required when multiple stimuli must be discriminated on the basis of their association with reward. Analogies with primate ACC are discussed.

INTRODUCTION

Delineation and connections of the anterior cingulate cortex (ACC) in the rat

The ACC is one of the three divisions of prefrontal cortex in the rat, the others being the agranular insular and orbitofrontal areas (Zilles & Wree, 1995). Definitions of this region vary. For example, Zilles & Wree (1995) define the ACC as comprising cortical subregions Cg1, Cg2, and Cg3, while Paxinos (1998) refers to Cg3 as prelimbic cortex (PrL). Previous lesion studies from this laboratory have used a definition based on vertical strips of cortex (Bussey *et al.*, 1996; 1997a; 1997b), discussed in detail by Bussey (1997b, p. 920). Figure 14 and Table 9 show these regions and various definitions of the ACC for comparison; the definitions of Bussey (1997b) will be followed in this thesis, except that Bussey's 'medial frontal cortex' will be referred to as medial prefrontal cortex (mPFC).

These cortical regions have an extensive array of connections, summarized in Table 10. The most prominent efferent connections of the mPFC, ACC, and posterior cingulate cortex (PCC) are summarized by Bussey *et al.* (Bussey, 1996; Bussey *et al.*, 1997b) as follows. The mPFC, including anterior Cg1 and PrL, projects to the nucleus accumbens (Acb), mediodorsal nucleus of the thalamus (specifically the me-

dial part thereof: MDM), and the amygdala. The ACC (postgenual Cg1 and Cg2) projects to mediodorsal caudate, the lateral part of the mediodorsal nucleus of the thalamus (MDL), and the amygdala, while PCC projects to anteroventral and anterodorsal thalamic nuclei (AV, AD), the hippocampal formation (subiculum and parahippocampal cortex), visual cortex, and dorsal and mediodorsal striatum (for references, see Bussey *et al.*, 1997b).

Table 9. Definitions of the cingulate cortical divisions vary. See Figure 14.

Term	Definition of Zilles & Wree (1995)	Definition of Bussey *et al.* (1996; 1997a; 1997b), in terms of areas defined by Zilles (1985) and Zilles & Wree (1995)
medial prefrontal cortex	(this term is used descriptively to include infralimbic cortex, Zilles & Wree, 1995, p. 653, but is not defined)	Cg3; Cg1 rostral to genu of the corpus callosum. This is equivalent to PrL plus rostral Cg1 in the atlas of Paxinos & Watson (1998); see Figure 14.
anterior cingulate cortex	Cg1–3	Cg1 and Cg2 caudal to the genu (dorsal and ventral ACC respectively)
posterior cingulate cortex	synonym for retrosplenial cortex	RSA and RSG rostral to the splenium of the corpus callosum
retrosplenial cortex	granular and agranular retrosplenial cortex (RSG, RSA)	RSA and RSG caudal to the splenium of the corpus callosum

Table 10. Connections of the anterior cingulate cortex. From Zilles (1995, p. 654); additional data (*) from Brog *et al.* (1993); abbreviation key also from Price (1995) and Paxinos & Watson (1998).

Area	Afferent input	Efferent output
All areas (Cg1–3)	basal nucleus of Meynert (B); basolateral amygdala (BL); caudal interstitial nucleus of the medial longitudinal fasciculus (CI); dorsal raphé nucleus (DR); median raphé nucleus (MnR); intralaminar thalamic nuclei; locus coeruleus (LC); lateral hypothalamic area (LH); mediodorsal thalamic nucleus (MD); parabrachial pigmented nucleus (PBP); substantia nigra (SN); ventromedial thalamic nucleus (VM); ventral tegmental area (VTA); zona incerta (ZI); centrolateral thalamic nucleus (CL); laterodorsal thalamic nucleus (LDs); periventricular hypothalamic nucleus (Pe); infralimbic cortex (IL); cingulate areas Cg1–3 contralaterally; agranular insular cortical areas	caudal interstitial nucleus of the medial longitudinal fasciculus (CI); intralaminar thalamic nuclei; lateral habenular nucleus (LHb); pontine nuclei (Pn); anterior pretectal nucleus (APT); mediodorsal thalamic nucleus (MD); periventricular hypothalamic nucleus (Pe); median raphé nucleus (MnR); reticular thalamic nucleus (Rt); ventromedial thalamic nucleus (VM); superior colliculus (SC); agranular insular cortex, ventral part (AIV); entorhinal cortex (Ent); presubiculum (PrS); Cg1–3 contralaterally nucleus accumbens core (AcbC)*; nucleus accumbens shell (AcbSh)*
Cg1–2	anteromedial thalamic nucleus (AM); lateral posterior thalamic nucleus (LP)	anteromedial thalamic nucleus (AM); caudate-putamen (CPu); lateral dorsal thalamic nucleus (LD); retrosplenial granular cortex (RSG); retrosplenial agranular cortex (RSA)
Cg1	gigantocellular reticular nucleus, ventral part (GiV); lateral paragigantocellular nucleus (LPG); lateral reticular nucleus (LRt); medial occipital area 2 (Oc2M); occipital cortex, area 1 (Oc1); retrosplenial granular cortex (RSG); retrosplenial agranular cortex (RSA)	agranular insular cortex, dorsal part (AID); agranular insular cortex, ventral part (AIV); pontine reticular nucleus (PnC, PnQ)
Cg2	medial occipital area 2 (Oc2M); retrosplenial granular cortex (RSG); retrosplenial agranular cortex (RSA)	parietal area 2 (Par2); cingulate areas Cg1 and Cg3; retrosplenial granular cortex (RSG)
Cg3 (PrL)	paratenial thalamic nucleus (PT)	amygdaloid nuclei; lateral hypothalamic area (LH); midline thalamic nuclei; paratenial thalamic nucleus (PT); substantia nigra (SN); mesencephalic tegmentum; nucleus of the solitary tract (SoI); olfactory tubercle (Tu); ventral tegmental area (VTA); agranular insular cortical areas; perirhinal cortex (PRh); piriform cortex (Pir); substantia innominata (SI); nucleus of the horizontal limb of the diagonal band (HDB)

In addition to its reciprocal connections with other areas of prefrontal cortex and the basolateral amygdala, the ACC has both direct and indirect connections to the ventral striatum (see Alexander *et al.*, 1986). Not only does the ACC project to the mediodorsal striatum (Zilles & Wree, 1995, p. 654), but both anterior cingulate and prelimbic cortex project to the core and rostral pole of the Acb (McGeorge & Faull, 1989; Zahm & Brog, 1992; Brog *et al.*, 1993; Parkinson, 1998) (see also Heimer *et al.*, 1995, pp. 600–601). The ACC also receives major dopaminergic input from the VTA (Fallon & Loughlin, 1995). Not only does the ACC provide a major input to the ventral striatum but this ‘limbic loop’ of the basal ganglia

projects via the ventral pallidum back to the ACC as well as the mPFC (Alexander *et al.*, 1986). This is the basis of an anatomical argument that the mPFC and ACC are the primary cortical structures whose information content is affected by the Acb (see Heimer *et al.*, 1995, p. 613).

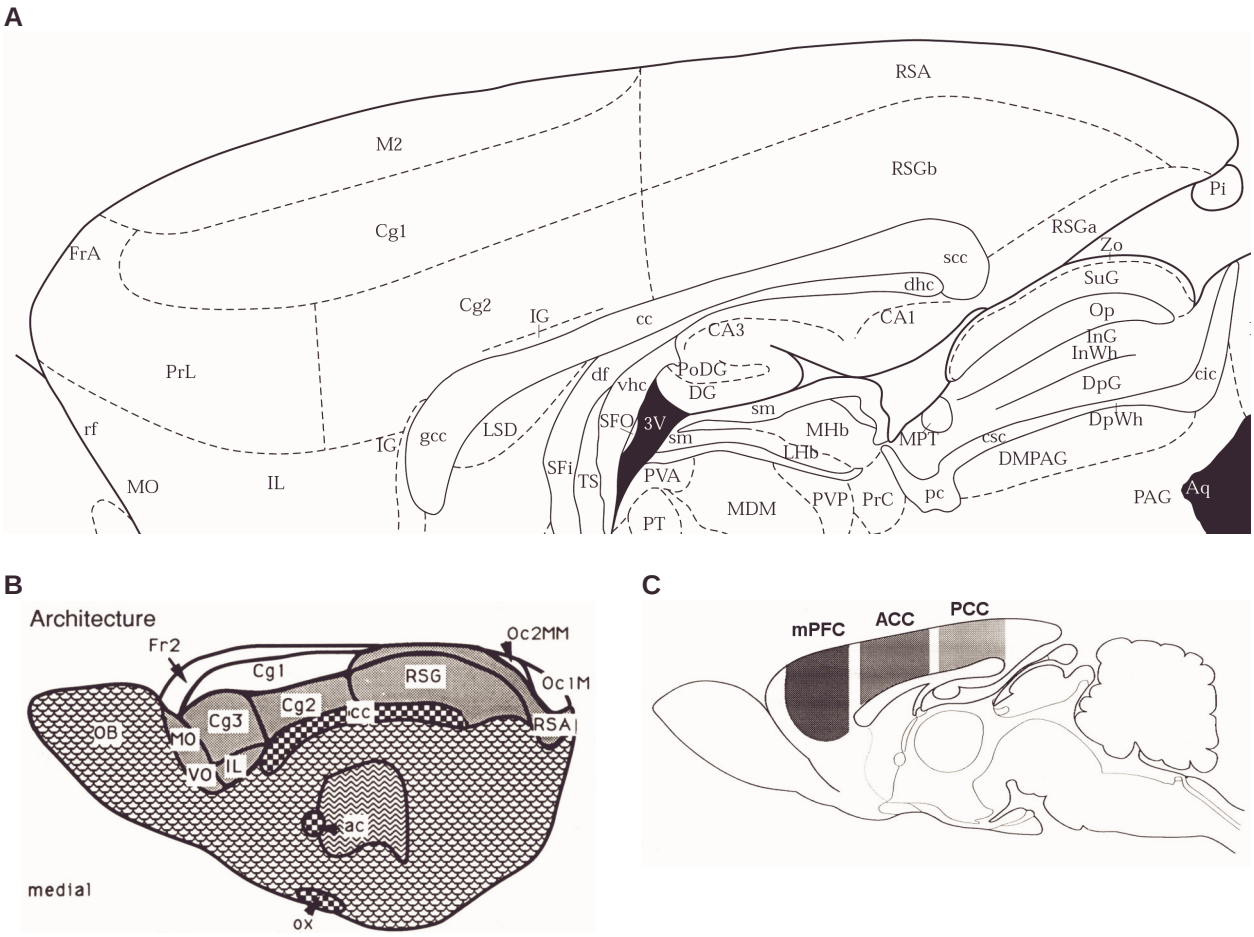

Figure 14. Midline cortex in the rat, including the cingulate areas. **A:** Figure taken from Paxinos (1998); sagittal section at 0.4 mm lateral to the midline. Anterior is to the left. (Selected abbreviations: Cg1 / Cg2, cingulate cortex areas 1 / 2; PrL, prelimbic cortex; IL, infralimbic cortex; M2, secondary motor cortex; FrA, frontal association cortex; MO, medial orbital cortex; cc, corpus callosum; gcc, genu of the corpus callosum; scc, splenium of the corpus callosum.) **B:** Figure taken from Zilles & Wree (1995), p. 650. Unshaded areas, including Cg1, are neocortex; shaded areas, including Cg2–3, are periallo- and proisocortex. (VO, ventral orbital area; OB, olfactory bulb.) **C:** Figure taken from Bussey (1996), indicating the area of the lesions used by Bussey *et al.* (1996; 1997a; 1997b). Vertical strips, from anterior to posterior, represent medial frontal cortex (medial prefrontal cortex, mPFC), anterior cingulate cortex (ACC) and posterior cingulate cortex (PCC). For comparison with other lesion studies of the rat, note that Weissenborn *et al.* (1997) used the same coordinates as Bussey *et al.* for their post-genual ACC lesions, except that the most anterior injection of toxin was 0.1 mm more caudal. Muir *et al.* (1996) used a different ear bar setting but also aimed at post-genual ACC. **D:** Figure indicating the region of the ACC targeted in the present study, encompassing the perigenual area.

Involvement of the rat ACC in stimulus–reinforcer association

A range of studies have implicated the ACC in stimulus–reinforcer association, using both appetitive and aversive tasks. The ACC receives nociceptive information and is involved in the coordination of autonomic responses (Neafsey *et al*., 1993; Fisk & Wyss, 1997; Hsu & Shyu, 1997); early studies found that aspirative lesions of the ACC attenuated classically conditioned bradycardia in the rabbit (Buchanan & Powell, 1982a). In the rabbit, the ACC is also involved in active avoidance behaviour. Using a task in which rabbits must learn to step in response to a tone CS+ in order to avoid a shock, while ignoring a different tone (CS–), Gabriel *et al.* have shown electrophysiologically that discriminated neuronal activity (discharge to the CS+ but not the CS–) occurs early in avoidance training (Gabriel *et al*., 1980a; Gabriel *et al*., 1980b; Gabriel & Orona, 1982; Gabriel *et al*., 1991b). Lesions of the ACC impair the avoidance response (Gabriel *et al*., 1991a; Gabriel, 1993), attributed to the loss of associative information about the significance of a discrete CS (Gabriel *et al.*, 1980a, pp. 158–163/219–221).

In the rat, the ACC has more often been studied using appetitive tasks, which also suggest that it has a role in stimulus–reinforcer association. For example, Bussey *et al.* (1997b) found that lesions of the ACC impaired the acquisition of an eight-pair concurrent discrimination task, in which subjects must learn which stimulus in each of eight pairs of complex visual stimuli must be selected in order to obtain reward. Additionally, Bussey *et al.* have reported that ACC lesions *facilitate* early learning in a conditional visual discrimination (CVD) task (Bussey *et al.*, 1996), though not in all circumstances (Bussey *et al.*, 1997b). In this task, subjects must respond in one way to stimulus A, and in another way to stimulus B; the reward is identical in both situations. This task cannot be solved by the formation of stimulus–reinforcer associations, but is soluble through stimulus–response association. Bussey *et al.* (1996) have suggested that the facilitation they observed with ACC lesions was due to the loss of a stimulus–reward system that normally competes with a stimulus–response system in the PCC during learning or behavioural expression (Bussey *et al.*, 1996; 1997b).

Few of the tasks described so far directly address the question of whether the ACC is involved in classical conditioning. To examine classical conditioning in isolation, it is necessary either to ensure that the animal's behaviour is uncorrelated with the presentation or receipt of the reinforcer, or that the instrumental behaviour that produces the reinforcer is directly opposed to the classically conditioned response elicited by the CS (omission schedules; Sheffield, 1965). Autoshaping, in which animals approach a stimulus that predicts reward, is a relatively selective test of Pavlovian learning. Autoshaping was originally demonstrated in pigeons by Brown & Jenkins (1968), who illuminated a response key and delivered food immediately afterwards. Regardless of the fact that responding had no effect on food delivery, the subjects reliably approached and pecked the key. There is no instrumental contingency specified in the task, and as the autoshaped response is to the stimulus rather than the place of food delivery there is little opportunity for 'implicit' instrumental response–reward associations. Furthermore, the nature of the autoshaped response is specific to the reinforcer (Jenkins & Moore, 1973) and subjects will immediately approach the CS+ following training in which approach has been prevented by a barrier (Browne, 1976). Finally, alteration of the contingencies so that approach prevents reward delivery — an omission schedule — fails to eliminate responding to the CS+ (Williams & Williams, 1969). In the study of Bussey *et al.* (1997a), not only did control rats fail to alter their responding when an omission contingency was introduced, but the ratio of CS+ / CS– approaches *increased* as overall responding extinguished. Thus there is strong evidence that normal animals' behaviour is governed by Pavlovian associations in this procedure.

Bussey *et al.* (1997a) found that lesions of the ACC significantly impaired the acquisition of an autoshaping task. In their task, a visual stimulus (CS+) is presented on a computer screen and followed by

delivery of food at a different location. A second stimulus (CS–) is also presented, but never followed by food. Though the subject's behaviour has no effect on food delivery, normal rats develop a conditioned response in which they selectively approach the CS predictive of food before returning to the food hopper to retrieve the primary reward. In contrast, rats with lesions of the ACC fail to discriminate, approaching the CS+ and CS– equally. It is intriguing to note, however, that the lack of discrimination in ACC-lesioned rats takes the form of increased responding to the CS–, rather than decreased responding to the CS+ (Bussey *et al.*, 1997a; Parkinson *et al.*, 2000c). As ACC-lesioned rats have been shown to be somewhat 'disinhibited', reflected in their tendency to make inappropriate premature responses in a test of sustained attention (Muir *et al.*, 1996), it is unclear whether their impairment in the autoshaping task is due to a failure to learn CS–US associations entirely (coupled with a tendency to over-respond to both the CS+ and the CS–) or a specific failure to inhibit responding to unrewarded stimuli. In fact, it is presently unknown whether the autoshaping impairment represents failure to learn at all, or simply to express learning that occurs in other brain regions. It seems unlikely, however, that the deficit is attentional, as ACC lesions do not impair the accuracy of visual attentional function (Muir *et al.*, 1996).

The ACC projects to the Acb; this projection, and the Acb itself, is also critical for the development of autoshaping, suggesting that information stored in or retrieved by the ACC gains access to locomotor response systems via the Acb (Parkinson *et al.*, 1996; Parkinson *et al.*, 2000c). In addition, the Acb is involved in another aspect of Pavlovian conditioning: conditioned reinforcement. Following the discovery that intra-accumbens injection of the psychostimulant *d*-amphetamine selectively enhances responding for conditioned reinforcement in a dose-dependent manner (Taylor & Robbins, 1984), attention has focused on the neural structures that convey information regarding the value of conditioned reinforcers to the Acb. The major cortical inputs to Acb are the basolateral amygdala (BLA), the entorhinal cortex and hippocampus (largely via the ventral subiculum), the mPFC (including prelimbic cortex, Cg3), and the ACC (Cg1–2) (Zahm & Brog, 1992; Brog *et al.*, 1993; Parkinson, 1998). Lesions of the ventral subiculum and mPFC do not impair responding for conditioned reinforcement (Burns *et al.*, 1993), but lesions of the BLA do so dramatically (Cador *et al.*, 1989; Burns *et al.*, 1993). It is not presently known whether the ACC is also required for conditioned reinforcement. Given that the ACC projects both to the BLA and the Acb, and has been implicated in stimulus–reward association, it is clearly of interest to establish whether it plays a role in the ability of neutral stimuli to gain conditioned reinforcing properties.

In order to address these questions, the present study investigated the effects of excitotoxic lesions of the ACC on the acquisition of a simple, temporally discriminated approach task. In this task, a single stimulus predicted the delivery of food at the same location. Following establishment of this stimulus as an appetitive CS, the subjects were allowed to respond for the same stimulus in the absence of any primary reward, the CS now acting as a conditioned reinforcer. At the same time, the effects of intra-accumbens amphetamine injections were examined in control and ACC-lesioned subjects; in addition to promoting responding in extinction (Robbins, 1976), this technique allowed the establishment of the amphetamine dose–response curve for comparison with previous lesion studies. Although the ability of a stimulus to act as a conditioned reinforcer indicates that it has entered into a Pavlovian association with its US (see Mackintosh, 1983, p. 15), the temporally discriminated approach task used to establish this association was not a pure measure of Pavlovian conditioning. Though the CS predicted the arrival of food, allowing approach behaviour to be classically conditioned to the CS, the CS might also have served as a discriminative stimulus (S^D), signalling that an instrumental contingency existed between approach behaviour and food acquisition. Therefore, the effects of ACC lesions were also tested using a number of purer measures of Pavlovian conditioning: autoshaping, conditioned freezing (a measure of aversive con-

ditioning), and the phenomenon of Pavlovian–instrumental transfer (Estes, 1948; Lovibond, 1983), in which a Pavlovian CS enhances ongoing instrumental responding. As it is unclear whether the autoshaping deficit previously reported in ACC-lesioned rats (Bussey *et al.*, 1997a; Parkinson *et al.*, 2000c) represents a failure of learning or of performance (see Table 6, p. 36), lesions were also made after the acquisition of autoshaped behaviour, to test whether the ACC is required for performance in well-trained animals.

Lesion methods and sites within the ACC

In order to evaluate the function of the cingulate cortex by means of lesion studies, axon-sparing (excitotoxic) lesions must be used, as damage to the underlying cingulum bundle can itself produce significant behavioural impairments (Meunier & Destrade, 1988; Warburton *et al*., 1998). All experiments reported here use the excitotoxic technique.

A pilot study using the same ACC lesion coordinates as Bussey *et al.* (1997a) revealed a non-significant trend towards an impairment in discriminated approach behaviour very early in training (sham $n = 11$, lesion $n = 9$), and a trend towards an impairment in responding for conditioned reinforcement in those animals whose lesions extended anteriorly to the perigenual region of the ACC (sham $n = 10$, lesion subgroup $n = 5$). This same subgroup demonstrated the poorest autoshaping, and the projections from the ACC to the AcbC are known to arise from the perigenual region (McGeorge & Faull, 1989; Brog *et al.*, 1993; Parkinson, 1998). Therefore, all experiments in this thesis used lesions of the ACC centred on the perigenual region.

EXPERIMENT 1: EFFECTS OF ACC LESIONS ON TEMPORALLY DISCRIMINATED APPROACH, RESPONDING FOR CONDITIONED REINFORCEMENT, AND FEAR CONDITIONING TO A DISCRETE CUE

Methods

Overview

Twenty-two male hooded Lister rats received lesions of perigenual ACC (group ACCX, n = 12) or sham lesions (group sham, n = 10), with all animals additionally receiving bilateral cannulae aimed at the Acb. They weighed 295–390 g at the time of surgery. Following recovery, they were maintained at 85% of their free-feeding mass and underwent the following behavioural procedures, in order: (1) temporally discriminated approach to a stimulus predictive of sucrose; (2) acquisition of a new response with conditioned reinforcement, with intra-accumbens amphetamine injections; (3) autoshaping; (4) a sucrose consumption test in the home cages; (5) locomotor activity testing in a novel environment; (6) acquisition of freezing to a stimulus predictive of footshock. During the conditioned freezing test they were allowed free access to food. After this they were killed and perfused for histology.

Housing conditions, operative techniques and stereotaxic coordinates are described fully in the *Methods* chapter.

Temporally discriminated approach

Four operant chambers were used for the acquisition of discriminated approach and instrumental responding phases; for this task they were fitted with a 2.8 W bulb traylight and the pellet tray was not present.

No levers were extended during this task. At the start of any session, the houselight was on, the traylight was off and the dipper was not raised. This phase lasted for a variable interval (VI) of 30–90 seconds, randomly chosen for each cycle of CS–US presentation. This was followed by a CS: the houselight was switched off and the traylight was switched on for a period of 5 s. The CS was immediately followed by the US: the traylight was switched off, the houselight was switched back on, and the dipper was raised for 5 s to deliver 10% w/v sucrose solution. The dipper was then lowered to return the chamber to the starting state and the next VI began.

Animals were trained for 11 sessions with one session per day. In each session, the subjects received 30 presentations of the CS and US. For each period (VI, CS, US), the number of entries into the food alcove and the time spent in the alcove were recorded. The proportions of the CS and VI periods that the subject spent in the alcove were combined to calculate an approach ratio equal to (CSproportion ÷ (CSproportion + VIproportion)), used as a measure of conditioning to the CS.

Acquisition of a new response with conditioned reinforcement

This task was conducted in the same apparatus as the temporally discriminated approach task. Test sessions were conducted in extinction, and immediately followed bilateral administration of one of 4 doses of intra-accumbens D-amphetamine sulphate (Sigma, UK; 0, 3, 10 and 20 µg in 1 µl of 0.1 M sterile phosphate buffer, pH 7.4). Doses were counterbalanced in a Latin square design to eliminate differential carryover effects and separated by 24 h. The Latin square was of a digram-balanced design (Keppel, 1991, p. 339), in which each condition immediately precedes and follows the other conditions once (e.g. 1234, 3142, 2413, 4321). Sensitization to amphetamine does not occur with repeated administration into the Acb (Cador *et al.*, 1995), so further spacing of doses was not required.

A session began when the subject nosepoked in the central alcove, and lasted 30 minutes. Initially, the houselight was switched on, the traylight was off, and both levers were extended. Responding on one of the levers, the CRf lever, resulted in the presentation of an abbreviated version of the previous conditioned stimulus with a probability of 0.5 (a random ratio 2 schedule). To produce this stimulus, the houselight was switched off and the traylight was switched on for 0.5 s, after which the lights were returned to the initial state and the empty dipper was raised for 0.3 s; this stimulus is known to function well as a conditioned reinforcer (Burns *et al.*, 1993). Responding on the other (NCRf) lever had no programmed consequence. The lever assignment (left/right) was counterbalanced across rats.

Alcove approach frequency and duration were recorded, together with all lever-pressing activity. All measures of behaviour were recorded in six 5-minute bins.

Intracranial infusion during conditioned reinforcement test

Before the first test day, all rats were given a preliminary infusion of vehicle and returned to the home cage to familiarize them with the hand-held infusion procedure and to minimize non-specific effects of inserting the infusion cannulae during subsequent test sessions. On the first infusion only, these effects are noticeable; many animals become slightly agitated near the end of the infusion period and a few react briefly as the injector is removed. This has been observed with a variety of intracranial cannula sites (F. Passetti, personal communication, 1998).

Intra-accumbens infusions were performed by inserting two 28-gauge infusion cannulae (∅ 0.36 mm external, 0.18 mm internal; model C313I, Plastics One, Roanoke, Illinois, USA; supplied by Semat Technical Ltd, St Albans, UK) through the chronically implanted 22-gauge guide cannulae of gently hand-restrained subjects. The infusion cannulae were 15.0 mm long so as to allow them to protrude 2.0 mm beyond the tips of the guide cannulae; they were connected by polyethylene (PE50) tubing to two 5-µl syringes (SGE Ltd, Milton Keynes, UK) mounted on a Harvard Apparatus (Edenbridge, UK) infusion pump. Amphetamine was infused in a volume of 1 µl per side over a 2-minute period. After this, 2 minutes were allowed for diffusion away from the site of the cannulae to occur, before the cannulae were removed and replaced by occluders and behavioural testing began. Animals were held during the infusion but otherwise allowed to move freely.

Autoshaping

Apparatus. Autoshaping was assessed in the apparatus shown in Figure 15 and is described fully in Bussey *et al.* (1997a). Briefly, the apparatus consists of a 48 × 30 × 30 cm testing chamber with a display screen on one wall and a pellet dispenser located centrally in front of the display. Pressure-sensitive areas of floor (each 14 × 10 cm) were located directly in front of the display, to the left and right of the dispenser, and also centrally at the rear of the chamber. The apparatus was controlled by software written in BBC BASIC by T.J. Bussey, running on a BBC Master series computer.

Pretraining. Rats were first given one session in order to habituate to the test chamber and to collect 45-mg food pellets (Rodent Diet Formula P, Noyes, Lancaster, NH) from the food receptacle. The houselight was illuminated and subjects were placed in the chamber for 5 min with 4–5 pellets placed in and around the dispenser. After this, pellets were delivered on a VT 0–40 s schedule for 15 min.

Acquisition (CS^+→food, CS^-→0). On the next day, rats were trained to associate stimuli with the delivery of pellets. Stimuli consisted of 8 × 18 cm white vertical rectangles displayed on the left and right of the screen for 10 s. One was designated the CS+ and the other the CS–, counterbalanced between subjects. A trial consisted of presentation of both the CS+ and CS– in a randomized order. Following a VI of 10–40s, the program waited for the rat to be located centrally at the rear of the chamber; this eliminated chance approach to the stimuli, ensured equal stimulus sampling and allowed accurate measurement of approach latency. One stimulus was then presented for 10 s. The CS+ was always followed immediately by the delivery of food; the CS– was never followed by food. After this, another VI followed, the program waited for the rat to return to the rear of the chamber, and the other stimulus was presented. This procedure ensured that the minimum time between CS+ and CS– presentation was 10 s, and that there were never more than two consecutive presentations of either the CS+ or the CS–.

When a stimulus was presented, activation of one of the two floor panels in front of the screen was scored as an approach, and no further approaches were scored during that stimulus presentation. The rat may therefore make four kinds of active response: approach to the CS+, approach to the CS–, approach to the location of the CS+ during CS– presentation, and approach to the location of the CS– during CS+ presentation. Rats were trained for a total of 100 trials (two days with 50 trials per day). Approaches to the CS+ and the CS– were scored in blocks of 10 trials and mean approach latency was calculated over 100 trials (Bussey *et al.*, 1997a). (In some previous studies using this task, trials on which the approach latency was under 10 cs were excluded as representing equipment failure. Covert observation revealed that such latencies were genuinely attainable, because the software took a perceptible fraction

of a second to draw each stimulus, and began timing at the point when drawing was complete; if, while the stimulus was being drawn, the rat ran to the front of the chamber, very short latencies were reported. Therefore, these trials were included in the analysis.) Data were analysed as CS+ / CS– approach scores, as difference scores (CS^+approaches – CS^-approaches) (after Bussey *et al.*, 1997a) and as the ratio (CS^+approaches) ÷ (CS^+approaches + CS^-approaches), a measure of stimulus discrimination that is relatively independent of absolute approach activity.

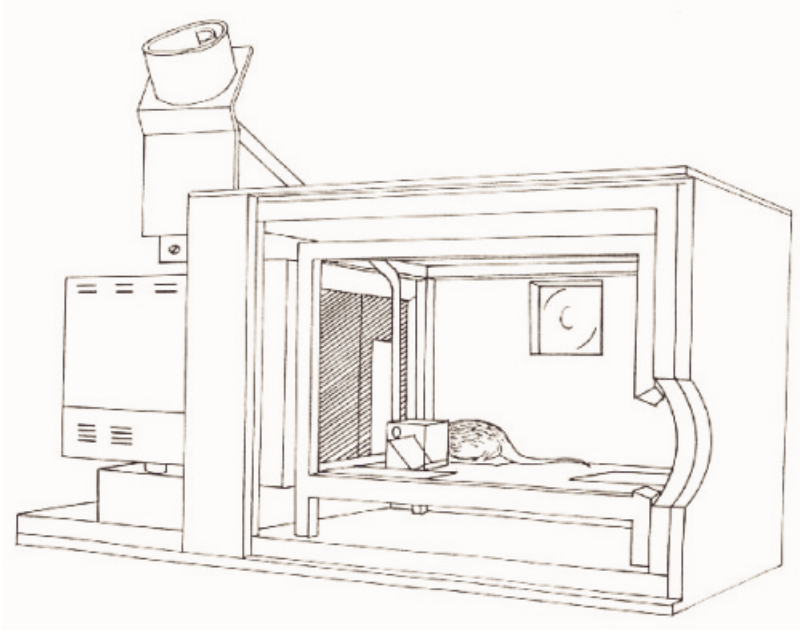

Figure 15. Autoshaping apparatus. (From Bussey *et al.*, 1997a.)

Probe trials (CS+ and CS–). After acquisition, a probe test was performed, consisting of 20 trials in which the CS+ and CS– were presented simultaneously and approaches were measured. Food was not delivered, so this test constituted an extinction trial to the CS+, while the CS– was still a perfect predictor of food absence. The probe test was intended to be a more sensitive test than the acquisition task (in which the subject might form CS–US associations perfectly and yet approach all stimuli), as it forced the subject to make a choice between the CS+ and the CS–.

Omission training. Finally, the contingencies were altered such that approaches to the CS+ prevented the delivery of a food pellet. This manipulation introduced an instrumental contingency directly opposed to the approach response. All other parameters remained the same as in the acquisition phase. There were 50 presentations of the CS+ and of the CS– per session and two sessions were given. As before, only initial approaches were scored; 'successful' omission trials were those in which the CS+ was presented and the subject first approached the CS–, or failed to approach either stimulus. (In fact, the program incorrectly omitted reward even if the rat first approached the CS– and later wandered over to the CS+ side while the stimulus was still present — and, I believe, if contact with the CS– itself was made. However, these were extremely rare events.)

Sucrose consumption

In order to assess alterations in primary motivation, all animals were given a sucrose consumption test while food-deprived. Intake of 10% sucrose solution was measured during 1 h of free access in the home cages with a single subject present.

Locomotor activity in a novel environment

Locomotor activity was measured in wire mesh cages, 25 (W) × 40 (D) × 18 (H) cm, equipped with two horizontal photocell beams situated 1 cm from the floor that enabled movements along the long axis of the cage to be registered. Subjects were placed in these cages, which were initially unfamiliar to them, and their activity was recorded for 2 h. All animals were tested in the food-deprived state.

Fear conditioning to a discrete cue

Fear conditioning was carried out using two distinctive experimental contexts, termed Light and Dark. The Light context consisted of a 20 (W) × 21 (D) × 21 (H) cm chamber fitted with white and steel walls on three sides and a fourth transparent Perspex wall that also served as a door. The floor consisted of a steel grid (bars 0.75 cm apart) on top of which was placed a transparent Perspex sheet; under the grid was a tray of sawdust. There was a white 2.5-W houselight in the centre of the chamber's ceiling. In front of the transparent wall was a Sony VHS-C video camera on a tripod; the room was illuminated by a white fluorescent ceiling lamp at moderate intensity. The Dark context consisted of a 35 (W) × 25 (D) × 40 (H) cm chamber in a room illuminated only by a 40-W red incandescent lamp. The chamber had four black Perspex walls and a transparent ceiling; it had a red 2.5-W houselight and a steel grid floor (bars 1 cm apart), 3 cm above a steel tray scented with a small quantity of apricot-scented oil (Crabtree and Evelyn, UK). A shock scrambler (model 521C, Campden Instruments, Loughborough, UK) could deliver brief electric shock to the grid floor. Both contexts were equipped with identical 80-dB clicker relays.

Contexts were made more discriminable by ensuring a unique time of day was paired with each environment (counterbalanced across rats); for example, half of the rats only ever experienced the Light context in the morning and the Dark context in the afternoon.

On days 1–3 of the experiment, subjects were pre-exposed by being placed for 25 min in each context. On day 4, they were placed in the Dark context, where they received 5 presentations of a 10-s clicker CS (5 Hz cycle for a 10 Hz click rate) terminating in a shock of 0.5 mA lasting 0.5 s. The interval between presentations was 4 ± 1 min and the animals were in the context for 30 min. On day 5, subjects were placed in the Light context and their behaviour was videotaped. After 5 min of CS absence, the clicker CS was played continuously for 10 min. Freezing activity was assessed by an observer scoring the tapes in 5-s activity bins, using a stringent criterion: if and only if the animal was motionless apart from respiratory movements for the full 5 s, the bin was scored as 'freezing'. The calculated measure was the percentage of bins spent freezing; the 2 minutes preceding CS onset were compared with the 8 minutes following CS onset.

Results

One subject in the ACCX group (subject E2) lost its cannulae and was killed. There were 3 other postoperative deaths (E1, E7, E9). After histological analysis, all lesions were found to be complete, leaving 8 animals in the ACCX group (subjects E3, E4, E5, E6, E8, E10, E11, E12) and 10 in the sham group (subjects E13, E14, E15, E16, E17, E18, E19, E20, E21, E22), of which respectively 6 and 10 also had injection sites correctly located within the Acb (all but subjects E5 and E8). Data from all animals with valid lesions were analysed, except for the conditioned reinforcement test, for which only data from animals with valid lesions and valid cannulae placements were used.

Histology

In this group of ACC-lesioned subjects, neuronal loss and associated gliosis extended from ~2.5 mm anterior to bregma to ~0.3 mm posterior to bregma, destroying perigenual Cg1 and Cg2; there was minimal damage to PrL (a few subjects exhibited a small degree of neuronal loss in the most dorsal aspect of PrL). IL and PCC were undamaged, as was the corpus callosum. Photomicrographs of the ACC in a sham-operated and a lesioned rat are shown in Figure 16; this material was typical of lesions in this group. Schematics depicting the largest and smallest extent of the lesions are shown in Figure 17. Photomicrographs of the location of the intra-accumbens guide cannulae and injector tip locations are shown in Figure 18, indicating the minimum and maximum amount of damage done by the guide cannulae, while Figure 19 presents a schematic of the injector tip locations in the two groups.

Anterior cingulate cortex: photomicrographs

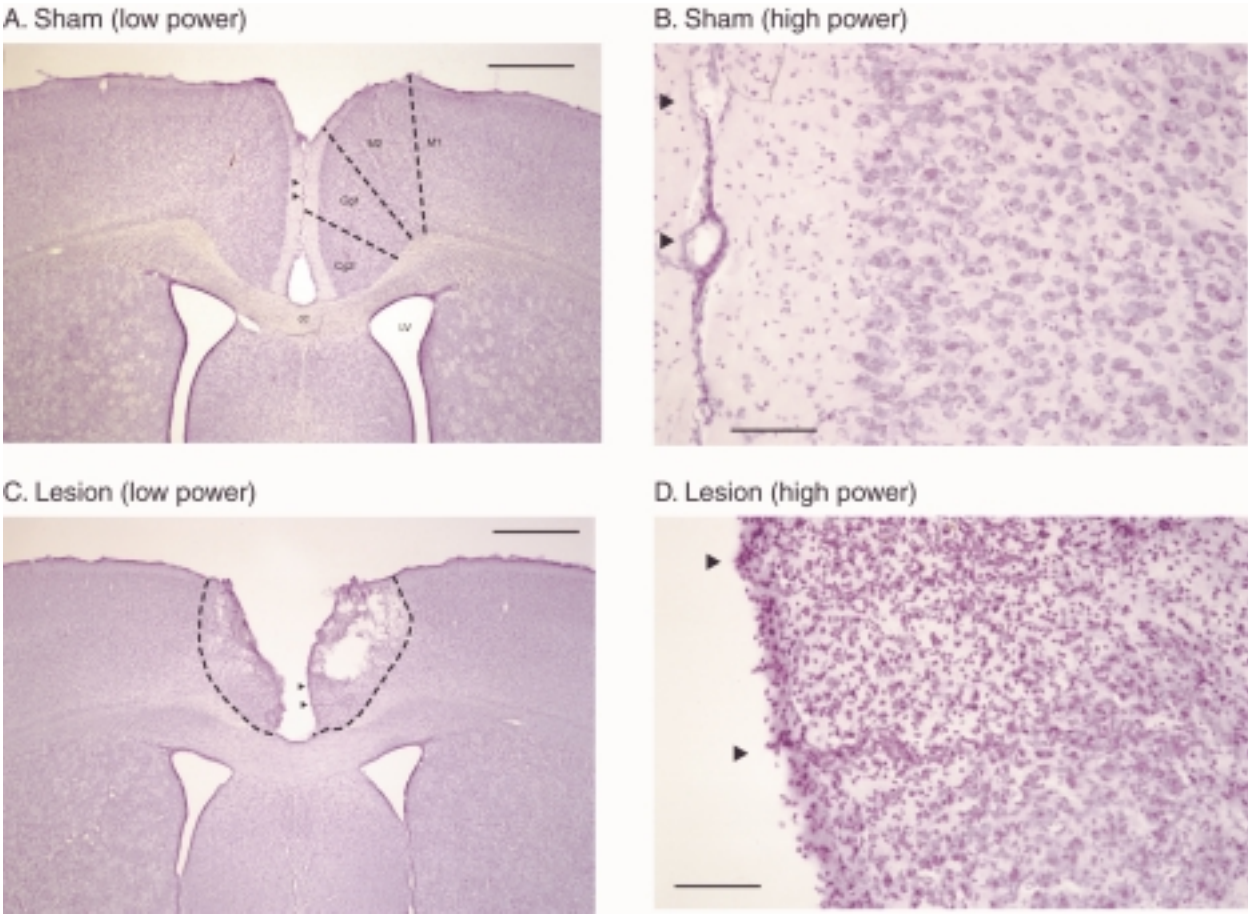

Figure 16. Lesion of the ACC: photomicrographs of coronal brain sections, approximately 0.5 mm anterior to bregma, stained with cresyl violet. **A & B:** sham-operated rat (cc, corpus callosum; LV, lateral ventricle; Cg1 / Cg2, cingulate areas 1 / 2; M2, secondary motor cortex; M1, primary motor cortex). **C & D:** ACC-lesioned rat; dotted lines mark the borders of the lesion. **Left-hand** panels (A & C) are low-magnification view (scale bars are 1 mm); **right-hand** panels are high-magnification views (scale bars are 0.1 mm). Arrowheads mark identical structures in the respective low- and high-power views.

Schematic of lesions

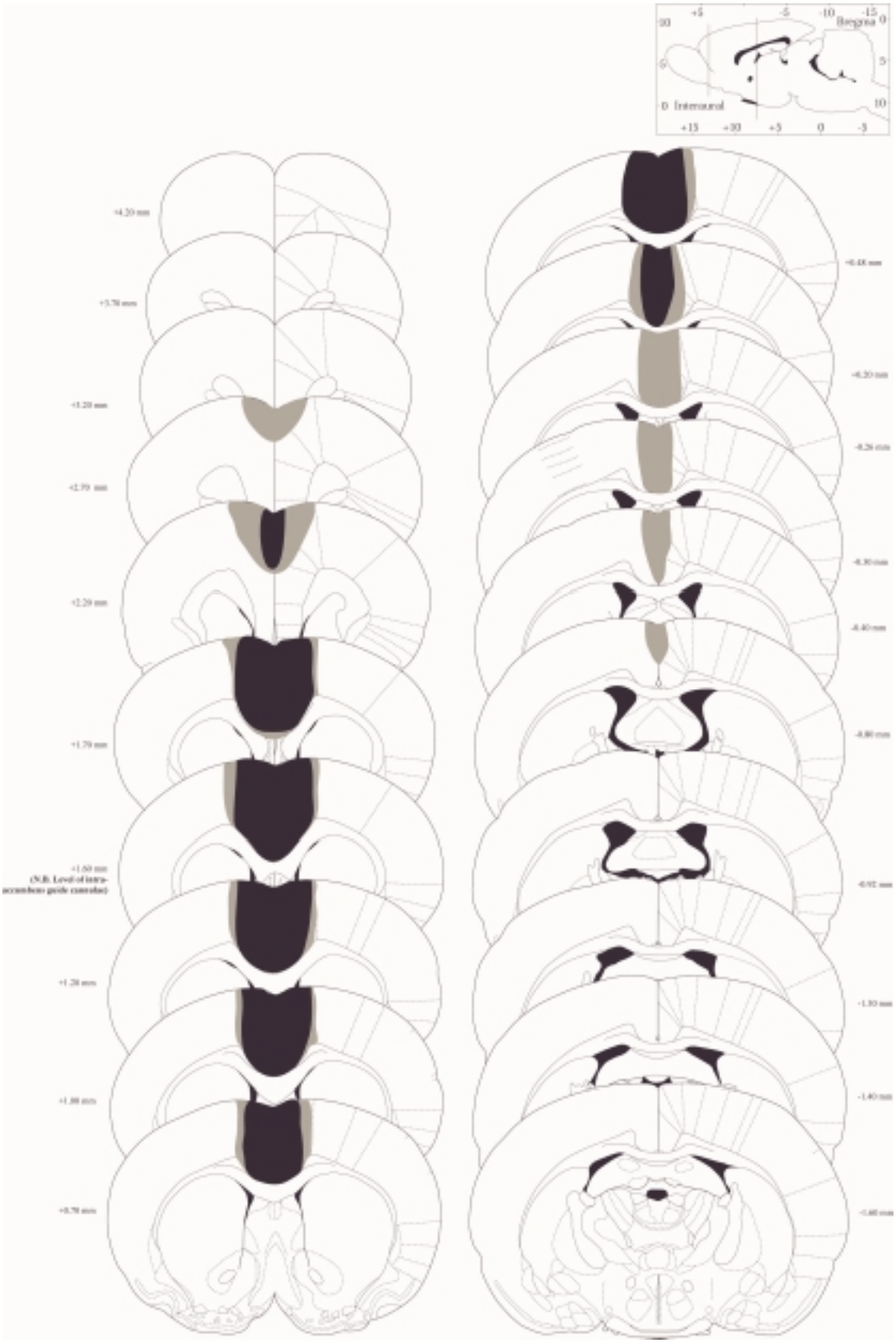

Figure 17. Schematic of lesions of the ACC (subjects E3, E4, E5, E6, E8, E10, E11, E12). Grey shading indicates the extent of the largest area of neuronal loss, and black the smallest. Diagrams are taken from Paxinos & Watson (1998). The pair of vertical lines in the sagittal schematic (top right) indicate the anterior and posterior limits of the series of coronal schematics (main part of figure)

Nucleus accumbens cannulae: photomicrographs of cannula tracks

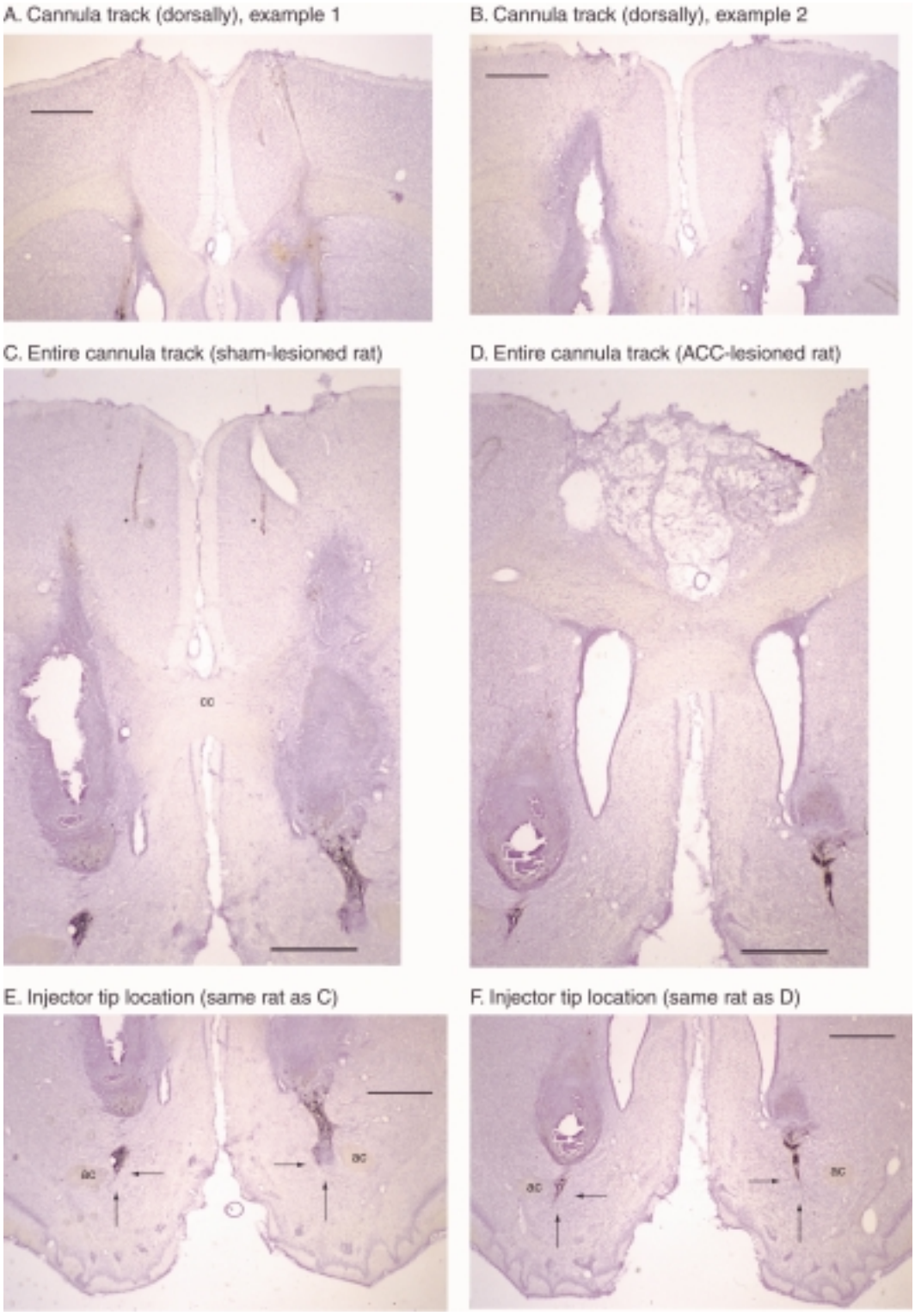

Figure 18. Location of intra-accumbens guide cannulae and injector tips. All sections are at approximately 1.6 mm anterior to bregma. **A:** Dorsal part of cannula tracks in a rat with minimal track damage. **B:** Dorsal track of cannulae in a rat with more pronounced track damage. **C:** View of cannula tracks and location of injector tips within the Acb, in a rat that received sham anterior cingulate surgery. The ACC is intact, and needle tracks are visible where the vehicle was injected (*) (cc, corpus callosum). **D:** Location of injector tips in a cingulate-lesioned rat. The excitotoxic lesion of the ACC is clearly visible (compare Figure 16). **E:** View of the injector tip location in the Acb (same rat as C). Perpendicular arrows point to the tip location in each hemisphere (ac, anterior commissure). **F:** Close-up of the tip location in the Acb (same rat as D). All scale bars are 1 mm.

Nucleus accumbens cannulae: schematic of cannula placements

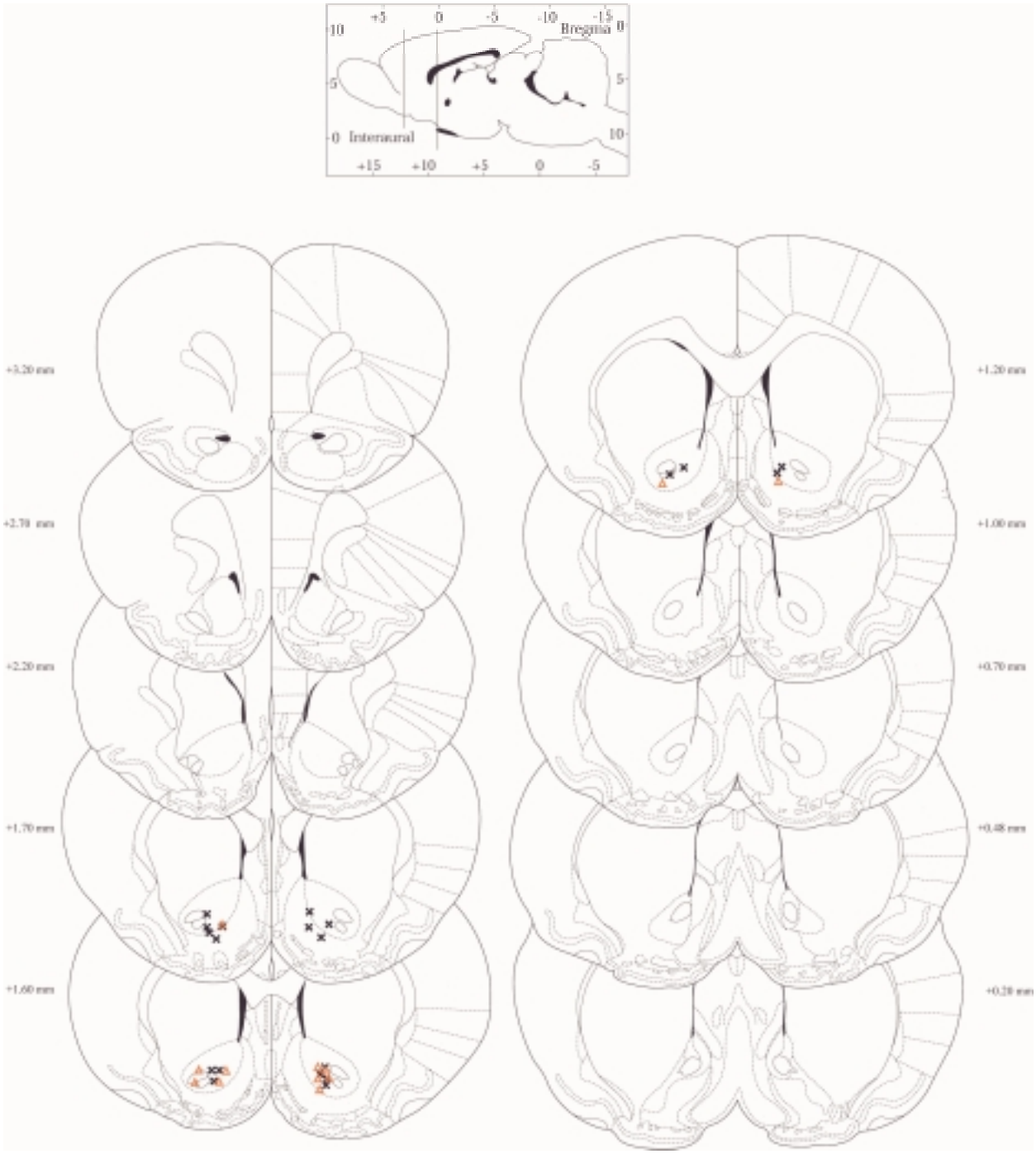

Figure 19. Location of the tips of injection cannulae within the Acb. Red triangles indicate subjects with lesions of the ACC (subjects E3, E4, E6, E10, E11, E12). Black crosses indicate sham-operated control subjects (E13, E14, E15, E16, E17, E18, E19, E20, E21, E22). Diagrams are taken from the atlas of Paxinos & Watson (1998).

Temporally discriminated approach

All animals learned to approach the alcove during the CS selectively; the lesioned and sham groups did not differ in any respect, as shown in Figure 20. All dependent variables were analysed using the model group × (session × S). Analysis of the approach ratios revealed a main effect of session ($F_{6.887,110.187}$ = 92.821, $\tilde{\varepsilon}$ = .689, p < .001), reflecting a selective increase in approach during the CS, but there was no effect of group (F < 1, NS) and no group × session interaction ($F_{6.887,110.187}$ = 1.253, $\tilde{\varepsilon}$ = .689, NS). A similar pattern was observed for the proportion of the CS spent nosepoking (session: $F_{6.781,108.493}$ = 42.108, $\tilde{\varepsilon}$ = .678, p < .001; group: $F_{1,16}$ = 1.289, NS; group × session: F < 1, NS), for the percentage of trials on which the CS was approached at least once (session: $F_{10,160}$ = 76.876, p < .001; group: F < 1, NS; group × session: F < 1, NS) and for the time spent approaching the food alcove during the VI (session: $F_{6.043,96.686}$ = 6.562, $\tilde{\varepsilon}$ = .604, p < .001; group: $F_{1,16}$ = 1.698, NS; group × session: F < 1, NS). It was clear that the learning resulted in dramatically improved access to the US (Figure 20E) and again there was no effect of the lesion on this measure (session: $F_{6.178,98.841}$ = 90.717, $\tilde{\varepsilon}$ = .618, p < .001; group: F < 1, NS; group × session: F < 1, NS).

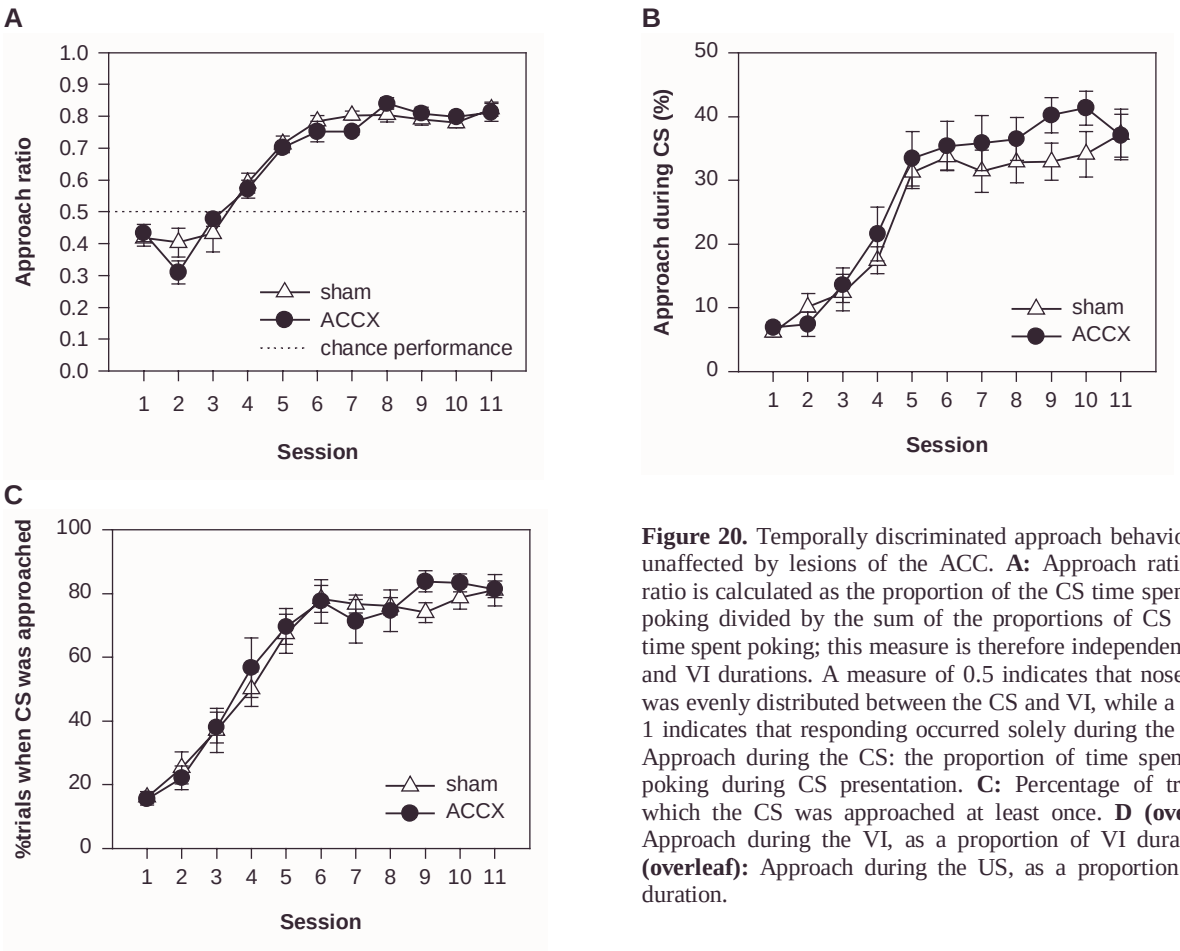

Figure 20. Temporally discriminated approach behaviour was unaffected by lesions of the ACC. **A:** Approach ratio. This ratio is calculated as the proportion of the CS time spent nosepoking divided by the sum of the proportions of CS and VI time spent poking; this measure is therefore independent of CS and VI durations. A measure of 0.5 indicates that nosepoking was evenly distributed between the CS and VI, while a ratio of 1 indicates that responding occurred solely during the CS. **B:** Approach during the CS: the proportion of time spent nosepoking during CS presentation. **C:** Percentage of trials on which the CS was approached at least once. **D (overleaf):** Approach during the VI, as a proportion of VI duration. **E (overleaf):** Approach during the US, as a proportion of US duration.

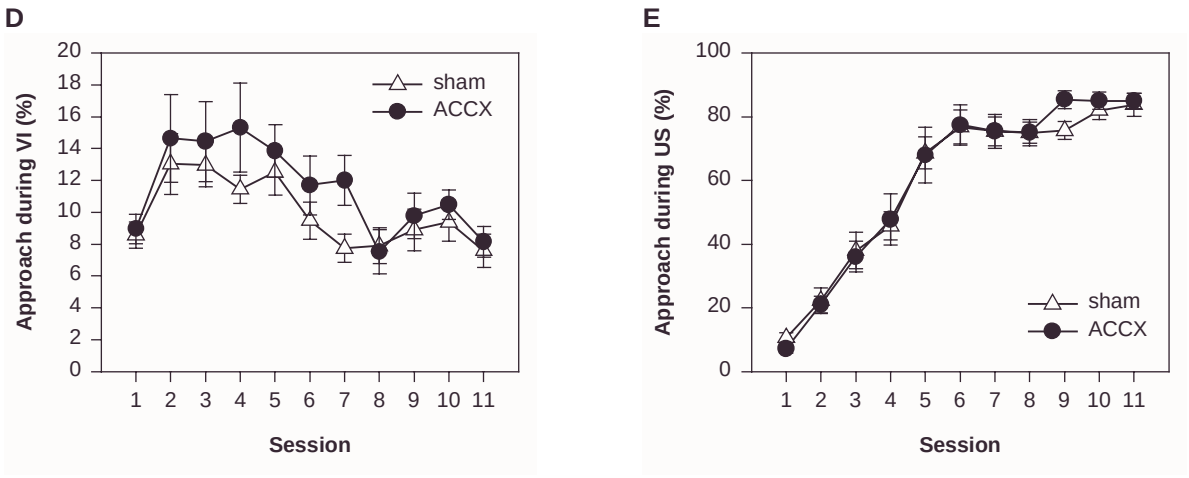

Figure 20 (continued). See previous page for caption.

Responding for conditioned reinforcement

Animals responded more on the lever producing the conditioned reinforcer (CRf lever) than the control (NCRf) lever, and responding for the CRf was dose-dependently and selectively potentiated by intra-accumbens amphetamine, but lesioned and sham groups did not differ (Figure 21A). Lever-press data were subjected to a square-root transformation and analysed using the model group × (lever × dose × S). Subjects responded more on the CRf than the NCRf lever (effect of lever, $F_{1,14} = 29.422$, $p < .001$). Amphetamine selectively potentiated responding on the CRf lever (lever × dose: $F_{3,42} = 2.841$, $p = .049$); there was also a main effect of dose ($F_{3,42} = 13.478$, $p < .001$). ACC-lesioned animals were not different from controls in any respect (group: $F_{1,14} = 1.661$, $p = .218$; lever × group: $F < 1$, NS; dose × group: $F_{3,42} = 2.043$, $p = .122$; lever × dose × group: $F_{3,42} = 1.2$, NS), even when the saline dose was considered on its own (lever: $F_{1,14} = 5.708$, $p = .032$; group: $F_{1,14} = 1.585$, NS; lever × group: $F < 1$, NS).

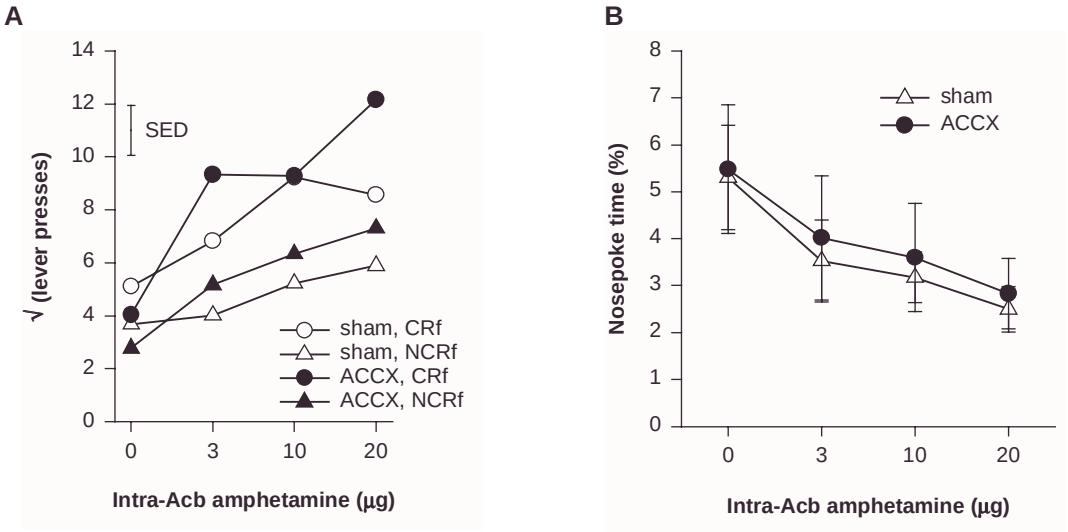

Figure 21. Responding for conditioned reinforcement, with intra-accumbens amphetamine. Lesions of the ACC had no effect on this task. **A:** Lever-pressing. *SED*, one standard error of the difference between means for the lever × dose × group term. **B:** Proportion of time spent nosepoking. Nosepokes during a CRf presentation were very few and were not included.

Nosepoking in the food alcove was dose-dependently reduced by intra-accumbens amphetamine, but this effect did not differ between groups (Figure 21B). An analysis by group × (dose × S) showed an effect of dose ($F_{2.563,35.886} = 9.571$, $\tilde{\varepsilon} = .854$, $p < .001$), but no effect of group and no interaction (Fs < 1, NS).

Autoshaping

Data from one subject in the ACCX group (subject E3) were lost due to a malfunction, leaving 7 lesioned subjects and 10 sham-operated controls.

Acquisition

Lesioned animals were impaired at the acquisition of autoshaping (Figure 22). An analysis of difference scores revealed a significant impairment in the ACCX group (main effect of group, $F_{1,15} = 6.605$, $p = .021$), together with an effect of trial block ($F_{5.433,81.495} = 2.422$, $\tilde{\varepsilon} = .604$, $p = .038$); the interaction was not significant ($F < 1$, NS). Analysis of ratio scores also demonstrated a significant impairment (group: $F_{1,15} = 8.966$, $p = .009$; trial block: $F_{5.066,75.984} = 1.475$, $\tilde{\varepsilon} = .563$, NS; group × trial block, $F < 1$, NS).

While sham subjects approached the CS+ faster than the CS–, lesioned rats approached the CS– faster than the CS+ (Figure 22D). Mean latencies to approach each stimulus were calculated across all trial blocks, and analysed using the model group × (stimulus × S), revealing a stimulus × group interaction ($F_{1,15} = 7.295$, $p = .016$).

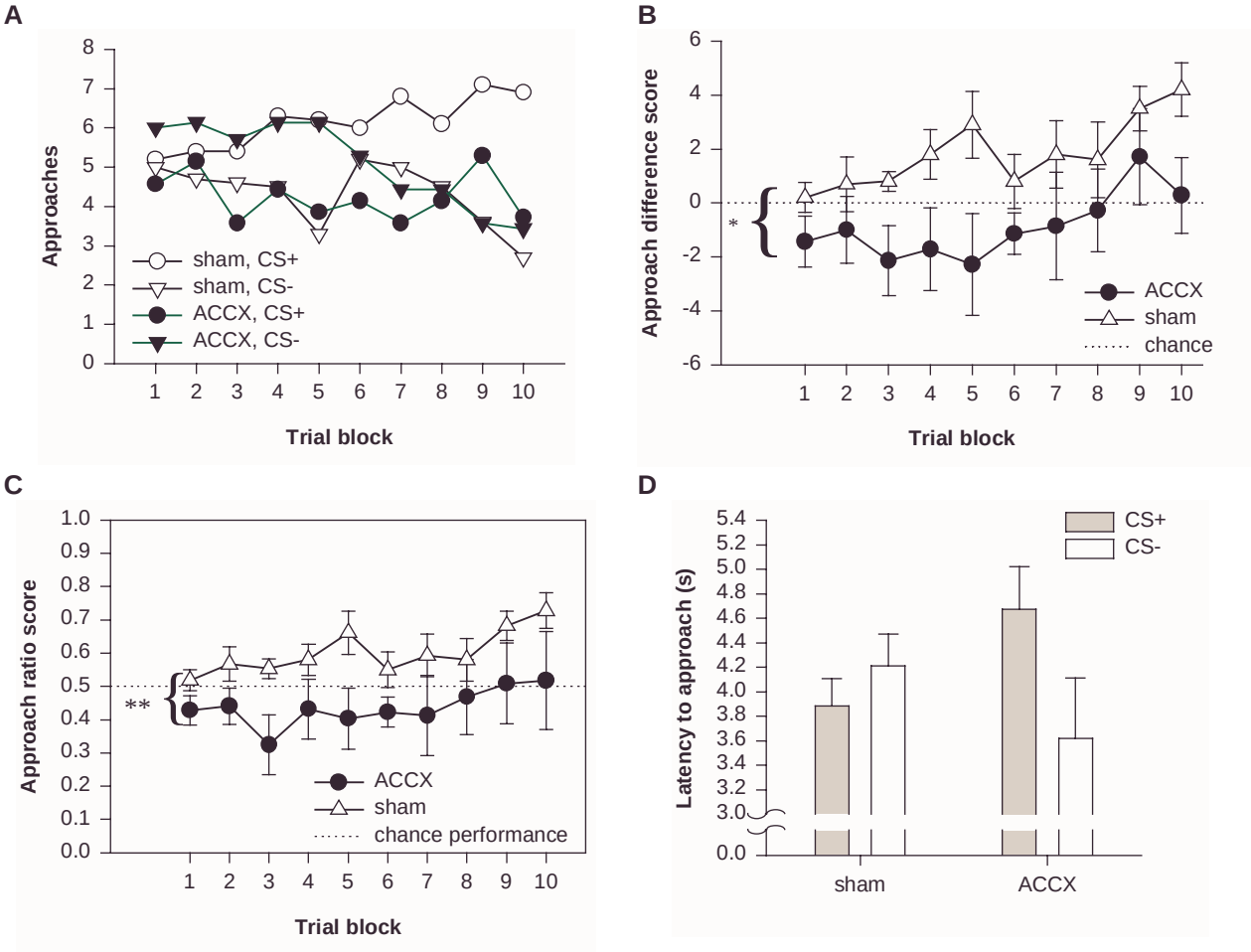

Figure 22. Autoshaping was impaired by lesions of the ACC. **A:** Approaches to the CS+ and CS– for each group. **B:** Approach data, expressed as a difference score (CS^+ approaches – CS^- approaches). **C:** Approach data, expressed as a discrimination ratio (CS^+ approaches ÷ (CS^+ approaches + CS^- approaches)). **D:** Latencies to approach each stimulus, calculated across all trial blocks.

Probe test

In the probe test (Figure 23), there was a non-significant trend towards an impairment in the ACCX group. A discrimination ratio was calculated as the number of trials on which the CS+ was approached divided by the number of trials on which either stimulus was approached. This measure was analysed by one-way ANOVA, revealing no effect of group ($F_{1,15}$ = 3.928, p = .066), even though the sham group discriminated between the stimuli (sham group compared to 50% discrimination ratio by one-sample t test: t_9 = 5.673, p < .001) and the ACCX group did not (t_6 = 1.69, p = .142).

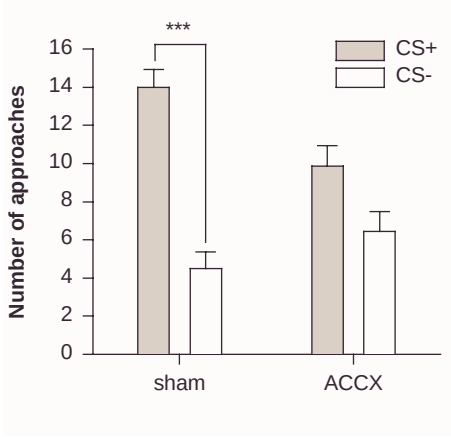

Figure 23. Autoshaping probe test. Sham-operated controls approached the CS+ more than the CS– (as the number of approaches to the two stimuli are not independent, the proportion of trials on which the CS+ was approached was compared to 50%; *** p < .001). Though no such discrimination was detectable in the ACC-lesioned animals, the difference between groups did not reach significance (p = .066).

Omission training

Introduction of the omission contingency resulted in a reduction in the number of CS+ approaches, but the rate of reduction did not differ between groups (Figure 24). An ANOVA of the number of approaches to the CS+ for each trial block revealed a main effect of trial block ($F_{6.163,92.447}$ = 3.332, $\tilde{\varepsilon}$ = .685, p = .005), and a main effect of group ($F_{1,15}$ = 5.06, p = .04), reflecting the different starting points of the two groups, but no interaction ($F_{6.163,92.447}$ = 1.359, NS).

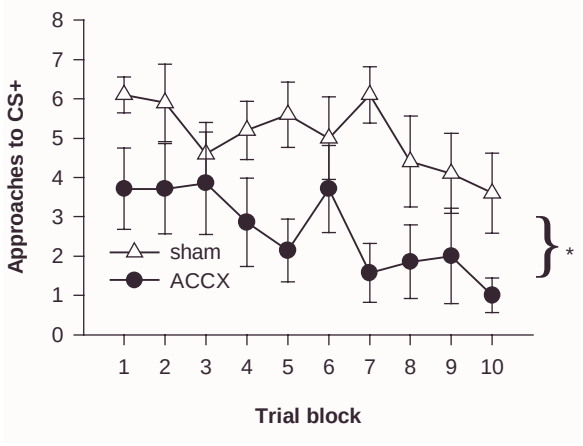

Figure 24. Autoshaping omission test. The ACC-lesioned group approached the CS+ less than the sham group, and both groups' responding declined, but this difference was present from the start and the groups were not *differentially* affected by introduction of an omission contingency.

Sucrose consumption

Primary consummatory behaviour was unaffected by the lesion, with both groups consuming the same amount of sucrose (mean ± SEM: ACCX 25.3 ± 2.1 ml, sham 27.7 ± 1.1 ml; $F_{1,16}$ = 1.056, NS).

Locomotor activity in a novel environment

There was a trend towards hypoactivity in the ACC-lesioned group, but this failed to reach significance (Figure 25). An analysis of √(beam breaks) by group × (bin × S) revealed an effect of group that was close to significance ($F_{1,16}$ = 4.279, p = .055), together with an effect of time bin ($F_{9.039,144.622}$ = 15.704, $\tilde{\varepsilon}$ = .822, p < .001), reflecting habituation to the novel environment, with no interaction (F < 1, NS).

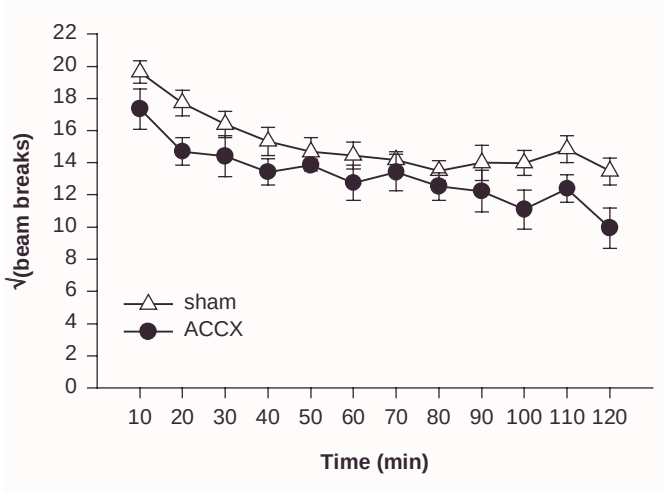

Figure 25. Locomotor response to novelty in sham- and ACC-lesioned rats.

Freezing to an aversive CS

Anterior cingulate-lesioned subjects did not differ from controls in their ability to freeze to a discrete CS predictive of footshock (Figure 26). An analysis of the percentage of time spent freezing, using the model group × (stimulus presence × S), showed no effect of group and no group × stimulus interaction (Fs < 1, NS), despite a robust effect of the stimulus ($F_{1,12}$ = 429.856, p < .001).

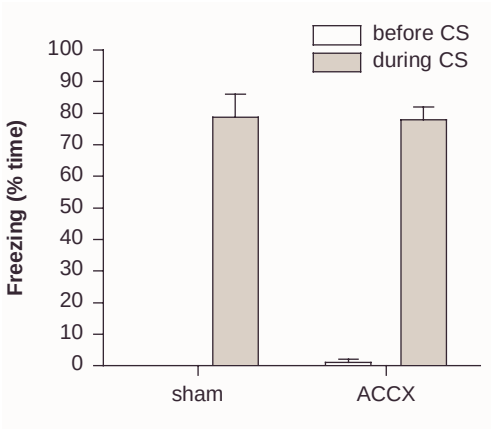

Figure 26. Freezing to an aversive CS+ was not affected by lesions of the ACC. The dependent variable is the percentage of time spent freezing, judged from video footage in 5-s bins. The 2 minutes preceding CS onset are compared with the 8 minutes following CS onset.

Summary

Lesions of the ACC did not affect subjects' ability to show temporally discriminated approach to a CS for food reward. This CS functioned successfully as a conditioned reinforcer in ACC-lesioned rats, and they showed normal potentiation of responding for conditioned reinforcement when given intra-accumbens amphetamine. They were not different from shams in measures of food consumption or locomotor activity, and were also capable of exhibiting conditioned freezing to an aversive CS. However, the same subjects were impaired at autoshaping.

Discussion

The present results establish that a substantial degree of Pavlovian conditioning can occur in rats with lesions of the ACC, although an autoshaping deficit was observed in the same animals, replicating previous findings (Bussey *et al.*, 1997a; Parkinson *et al.*, 2000c). The implications will be discussed for each task used.

Temporally discriminated approach

ACC-lesioned animals were no different from sham-operated controls on any measure of temporally discriminated approach. This implies that, at the least, such animals can either form a Pavlovian association between the CS and the delivery of sucrose and use this representation to approach the CS, or can use the CS as a discriminative stimulus (S^D) for the performance of an instrumental approach response (the ambiguity as to whether this task measures Pavlovian or instrumental behaviour was discussed on p. 74). Inspection of Figure 20 (pp. 84/85) shows that the degree to which animals succeeded in approaching during the US directly paralleled the acquisition of responding to the CS. As the sucrose reward is only available for a brief time (5 s) in this task, it is obviously beneficial for the subjects to be nosepoking when the US begins; this illustrates the unavoidable S^D role of the CS.

Conditioned reinforcement

ACC-lesioned rats acquired an instrumental response with conditioned reinforcement, to the same level as controls. In this task, the response being tested has never had an instrumental relationship to food, so acquisition of discriminated lever-pressing demonstrates that the animals have acquired a Pavlovian association between the CS and some aspect of the food. In addition to leaving the efficacy of the conditioned reinforcer itself intact, the lesion did not impair the ability of intra-accumbens amphetamine to potentiate responding on the CRf lever, dose-dependently and selectively. Amphetamine also dose-dependently reduced the proportion of time the subjects spent nosepoking in the food/CS alcove (replicating a finding of Parkinson *et al.*, 1999b), perhaps because it potentiated the competing response of lever-pressing.

Strictly, of course, the present result is also explicable by a 'novelty-seeking' argument, also known as 'sensory reinforcement' (Kish, 1966) — the suggestion that animals work for the CS simply because it is interesting. However, this question has long since been addressed: Robbins & Koob (1978) demonstrated that a systemic dopamine indirect agonist, pipradrol, potentiated responding only for a CS explicitly paired with a primary reinforcer; this behavioural specificity has also been demonstrated for intra-accumbens amphetamine (Taylor & Robbins, 1984) and dopamine (Cador *et al.*, 1991).

As discussed earlier (p. 74), one suggested function of the ACC is to inhibit unrewarded responding. In the present study, ACC lesions did not increase approach during the unrewarded (VI) phase of the temporally discriminated approach task, or increase responding on the unrewarded (NCRf) lever in the conditioned reinforcement test. These data are therefore not compatible with the simple view that the ACC continuously suppresses responding that (on some occasions) leads to reward, although a role in inhibiting responding to unrewarded stimuli is not ruled out.

Autoshaping

The level of stimulus discrimination exhibited by ACC-lesioned animals in acquisition of the autoshaping task was significantly below that of control subjects, despite normal food consumption and locomotor behaviour in these animals. This result is especially noteworthy as the same animals were found to be unimpaired in the temporally discriminated approach task. At first glance, these tasks are extremely similar: both involve discriminated approach to a CS predictive of food reward. The two procedural vari-

ables that seem most likely to account for the difference are the location of the reward relative to the location of the CS (which are in the same location in the temporally discriminated approach task, and in separate locations in the autoshaping task) and the number of conditioned stimuli used (one versus two).

ACC-lesioned subjects also showed abnormal latencies to respond to the stimuli (as found by Bussey *et al.*, 1997a), and reduced discrimination in a probe test (though this difference was not significant). Though CS+/CS– discrimination was reduced in ACC-lesioned rats throughout training, this deficit was not precisely characterizable as an increase in CS– approaches, or a decrease in CS+ approaches; the former effect predominated early in training and the latter later on (Figure 22A, p. 86). Though clearly demonstrative of an impairment, the present study measured autoshaping in rats that already had considerable experience of CS–food pairings, and of lateralized responding (in the conditioned reinforcement test); for defining the autoshaping impairment more accurately, previous studies using naïve rats (Bussey *et al.*, 1997a; Parkinson *et al.*, 2000c) may be more reliable.

The results of the omission test were not very informative. As the ACCX group approached the CS+ considerably less than the sham group at the end of omission training, and during the probe test, it was not surprising that they also did so at the beginning of the omission test. Both groups' responding declined during this test, but to the same degree. Although the groups were not differentially affected by the introduction of the omission contingency, the observation that their responding to the CS+ declined does not tell us a great deal. It would, of course, be expected that animals sensitive to the instrumental contingency would cease responding. However, a similar decline might be expected of a purely Pavlovian animal. Such an animal would initially respond to the CS, but by virtue of its responding, the US would not be presented and responding would extinguish (eventually to be followed by reinstatement of responding and extinction in a cyclical fashion; see Mackintosh, 1974, pp. 115/127).

Unconditioned measures of behaviour

Lesions of the ACC did not affect primary motivation or consummatory behaviour, as assessed by a sucrose consumption test. Similarly, the lesions did not significantly affect locomotor activity in a novel environment. This is one reason that the autoshaping deficit cannot be attributed to differences in general activity levels, the others being that a deficit was apparent even when considering CS+ approach as a proportion of those trials on which some stimulus was approached (the approach ratio score), and that absolute levels of responding in ACC-lesioned animals were comparable to those of sham-operated controls during the acquisition of autoshaping (Figure 22A, p. 86). There was a trend towards hypoactivity in the ACCX group, however, which is surprising given that Weissenborn *et al.* (1997) found a significant increase in the locomotor response to novelty in animals with ACC lesions. It may be that slight differences in lesion sites across the two experiments account for the difference (Weissenborn *et al.* used a postgenual lesion; see Figure 14, p. 72).

Freezing to an aversive CS

ACC-lesioned rats exhibited normal conditioned freezing behaviour. The criterion used to judge freezing was strict, and it was apparent that following five CS→shock pairings, all animals were immobile for virtually the entire 8-min CS. In this experiment there were no unpaired controls, so it might be suggested that the freezing was an unconditioned response to the clicker CS; however, previous studies using exactly the same apparatus, stimuli, and assessment criterion as the present experiment have shown that freezing occurs at a level of ~20% when the clicker has been presented unpaired with shock, and ≥80% when paired (J. Hall, personal communication, March 1999; Hall, 1999).

These results may be contrasted to the demonstrations by Buchanan & Powell (1982a) and Gabriel *et al.* (Gabriel *et al.*, 1991a; Gabriel, 1993) that — in the rabbit — ACC lesions impair aversive Pavlovian conditioning and avoidance learning. Rather than appeal to procedural differences (the species difference, or the use of an aspirative lesion by Buchanan & Powell), the discrepancy may be explained through differences in the tasks used. Firstly, Buchanan & Powell observed normal eyeblink conditioning in their subjects, though heart-rate conditioning was impaired. As discussed in Chapter 1 (p. 40), aversive eyeblink conditioning is dependent upon the cerebellum; as Buchanan & Powell point out, even complete decortication does not prevent the acquisition of this CR (Oakley & Russell, 1972; 1975; 1976), and Gabriel *et al.* have shown a double dissociation between avoidance learning, which involves the ACC, and eyeblink conditioning, which does not (Steinmetz *et al.*, 1991; Gabriel *et al.*, 1996). It may be that freezing is another response that the ACC does not govern. Secondly, Buchanan & Powell found at least some heart-rate conditioning in ACC-lesioned rabbits, though the magnitude of cardiac deceleration was reduced compared to controls; Gabriel *et al.* have also reported acquisition of avoidance responding in rabbits with ACC lesions, though acquisition was retarded (Gabriel *et al.*, 1991a). Powell *et al.* (1994) found that although lesions of the ACC prevented rabbits from discriminating between a CS+ and a CS–, they did not abolish the conditioned response itself. Given the interesting dissociation in the present series of experiments between autoshaping and temporally discriminated approach tasks, discussed above, the necessity to discriminate between multiple stimuli may be a key factor in determining whether ACC lesions produce observable impairments in Pavlovian conditioning.

Summary

These data suggest that it is incorrect to characterize ACC-lesioned rats as being unable to form stimulus–reward associations. At some level, they are capable of Pavlovian conditioning, both appetitive and aversive. Nevertheless, lesions of the rat ACC have been clearly demonstrated to cause impairments in appetitive tasks that depend upon stimulus–reward associations, both in the autoshaping task used here and in previous studies (Bussey *et al.*, 1997a; Bussey *et al.*, 1997b; Parkinson *et al.*, 2000c). In what circumstances does this impairment in stimulus–reward learning manifest itself? This question will be addressed in Experiment 4.

EXPERIMENT 2: EFFECT OF ACC LESIONS ON AUTOSHAPING PERFORMANCE

Though ACC lesions have been repeatedly shown to disrupt the acquisition of autoshaping, it is presently unclear whether the ACC is involved in the storage of CS–US associations, in their behavioural expression, or in a learning process that regulates their formation. One way of addressing this issue is to examine the effects of lesions made *after* training. If the ACC is specifically involved in the formation of CS–US associations, a lesion should not affect performance in a well-trained animal. In contrast, such a lesion would be expected to disrupt performance if the ACC were critical for storage or retrieval of these associations. In the present study, rats were trained to an asymptotic level of performance on the autoshaping task before receiving lesions of the ACC and being re-tested.

Methods

Twenty-eight male hooded Lister rats were maintained at 90% of their free-feeding body mass and trained for 100 trials on the autoshaping task described earlier (p. 77). Subjects that failed to approach the CS+ on at least 70% of the last 30 trials were given a further 50 remedial trials; if they failed to meet the same criterion on the last 30 remedial trials, they were then excluded from the experiment. The successful subjects were given free access to food and randomly assigned to groups that received lesions of perigenual ACC or sham lesions; at the time of operation, they weighed 274–408 g. Following recovery, they were returned to the food deprivation regimen. Their performance on the same autoshaping task was tested for a further 50 trials; they then received a probe test (as described earlier) and 50 omission trials. After this a 2-h locomotor activity test was conducted in a novel environment with animals food-deprived.

Results

Eight subjects failed to reach the performance criterion (GL2, GL5, GL6, GL10, GL11, GL12, GL15, GL22). Of those that reached the criterion, 11 subjects received ACC lesions (GL1, GL3, GL4, GL9, GL17, GL18, GL19, GL20, GL21, GL23, GL24) and 9 subjects received sham lesions (GL7, GL8, GL13, GL14, GL16, GL25, GL26, GL33, GL34). There were two postoperative deaths in the sham group (GL7, GL14). Exploratory data analysis revealed that one subject in the sham group (GL16) was an outlier (complete absence of approach behaviour on two consecutive sessions with data points consistently >2 SD from the group mean); this subject was excluded from autoshaping and locomotor analysis. One subject in the lesioned group fell ill and was perfused after autoshaping was completed, but before locomotor testing. Histological analysis revealed that all lesions were correctly sited, so the final group sizes for the autoshaping performance test were 11 (ACCX) and 6 (sham).

Histology

In this group of ACC-lesioned subjects, neuronal loss and associated gliosis extended from ~2.5 mm anterior to bregma to ~0.3 mm posterior to bregma, destroying perigenual Cg1 and Cg2; as before, there was very slight damage to dorsal PrL in a few subjects and no damage to IL or PCC. Figure 27 presents schematics showing the largest and smallest extent of the lesions. (Photomicrographs of representative ACC lesions were shown in Figure 16, p. 80.)

Schematic of lesions

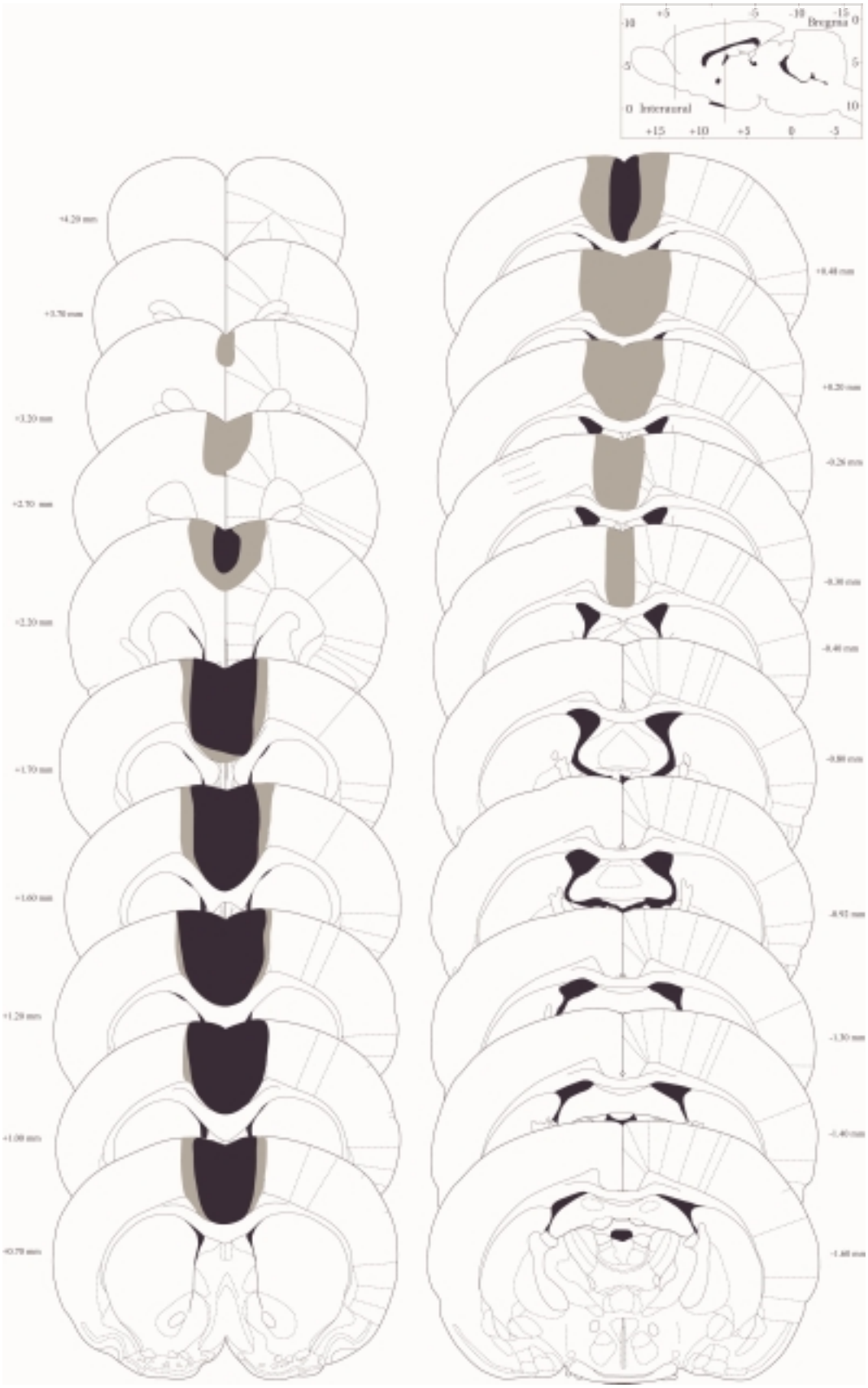

Figure 27. Lesions of the ACC (subjects GL1, GL3, GL4, GL9, GL17, GL18, GL19, GL20, GL21, GL23, GL24). Grey shading indicates the extent of the largest area of neuronal loss, and black the smallest. Diagrams are taken from Paxinos & Watson (1998).

Pre-operative acquisition

Both groups reached the same asymptote of performance pre-operatively (Figure 28). The difference scores for the last three blocks of training (10 trials per block) were analysed using the model group × (block × S); there were no significant effects of group ($F_{1,15}$ = 1.668, NS), block ($F_{1.532,22.98}$ = 2.727, $\tilde{\varepsilon}$ = .766, p = .098) or group × block (F < 1, NS).

Post-operative performance

Lesions of the ACC significantly impaired the performance of the autoshaped response (Figure 28). Analysis of the post-operative difference scores using the model group × (block × S) revealed a significant main effect of group ($F_{1,15}$ = 7.765, p = .014), reflecting poorer discrimination in the ACCX group; there was also a significant effect of block ($F_{4,60}$ = 3.524, p = .012), but no interaction (F < 1, NS). This impairment was also evident following analysis of ratio scores, which also revealed an effect of group ($F_{1,15}$ = 5.73, p = .03) and block ($F_{4,60}$ = 5.144, p = .001). Although Figure 28(B,C) suggests some recovery in the ACCX group, there was no block × group interaction ($F_{4,60}$ = 1.175, NS).

Further analysis demonstrated that this deficit was attributable to a persistent deficit in CS+ approach in the ACCX group. Post-operative CS+ and CS– approach scores were analysed separately, using the model group × (block × S) in each case. These analyses showed that the ACCX group made significantly fewer approaches to the CS+ (main effect of group: $F_{1,15}$ = 5.221, p = .037), an effect that did not alter across testing (terms involving block, Fs < 1, NS). The two groups did not differ in their approaches to the CS– (group: $F_{1,15}$ = 2.149, NS); both groups showed an equivalent decline in CS– responding (block: $F_{4,60}$ = 6.462, p < .001; block × group: $F_{4,60}$ = 1.114, NS). It is this decline in CS– responding that caused a degree of recovery of discriminative performance, evident as an improvement in difference and ratio scores, though the ACCX group remained impaired throughout testing.

Although these analyses did not demonstrate that the groups recovered at different rates, it was certainly the case that the ACCX group recovered to some extent (on all measures of performance), and did discriminate between the two stimuli. An improvement in discrimination scores was apparent for the ACCX group (main effects of block for difference scores, $F_{4,40}$ = 3.831, p = .01; for ratio scores: $F_{4,40}$ = 5.922, p = .001). Similarly, analysis of absolute approach scores in the ACCX group demonstrated a main effect of stimulus ($F_{1,10}$ = 28.495, p < .001) and block ($F_{4,40}$ = 3.978, p = .008) and a stimulus × block interaction ($F_{4,40}$ = 3.831, p = .01). No such improvement was detectable in the sham group, which performed well throughout (no block effects for the discrimination scores: maximum $F_{4,20}$ = 1.681, NS; or stimulus × block interaction for absolute approach scores: $F_{4,20}$ = 1.211, NS).

Lesioned animals were slower to approach both stimuli. Mean latencies to approach each stimulus were calculated across all post-operative trial blocks, and analysed using the model group × (stimulus × S). This revealed a main effect of group ($F_{1,15}$ = 5.636, p = .031), with the ACCX group showing longer approach latencies, and a main effect of stimulus ($F_{1,15}$ = 15.543, p = .001) as subjects approached the CS+ faster than the CS–. There was no stimulus × group interaction ($F_{1,15}$ = 1.413, NS).

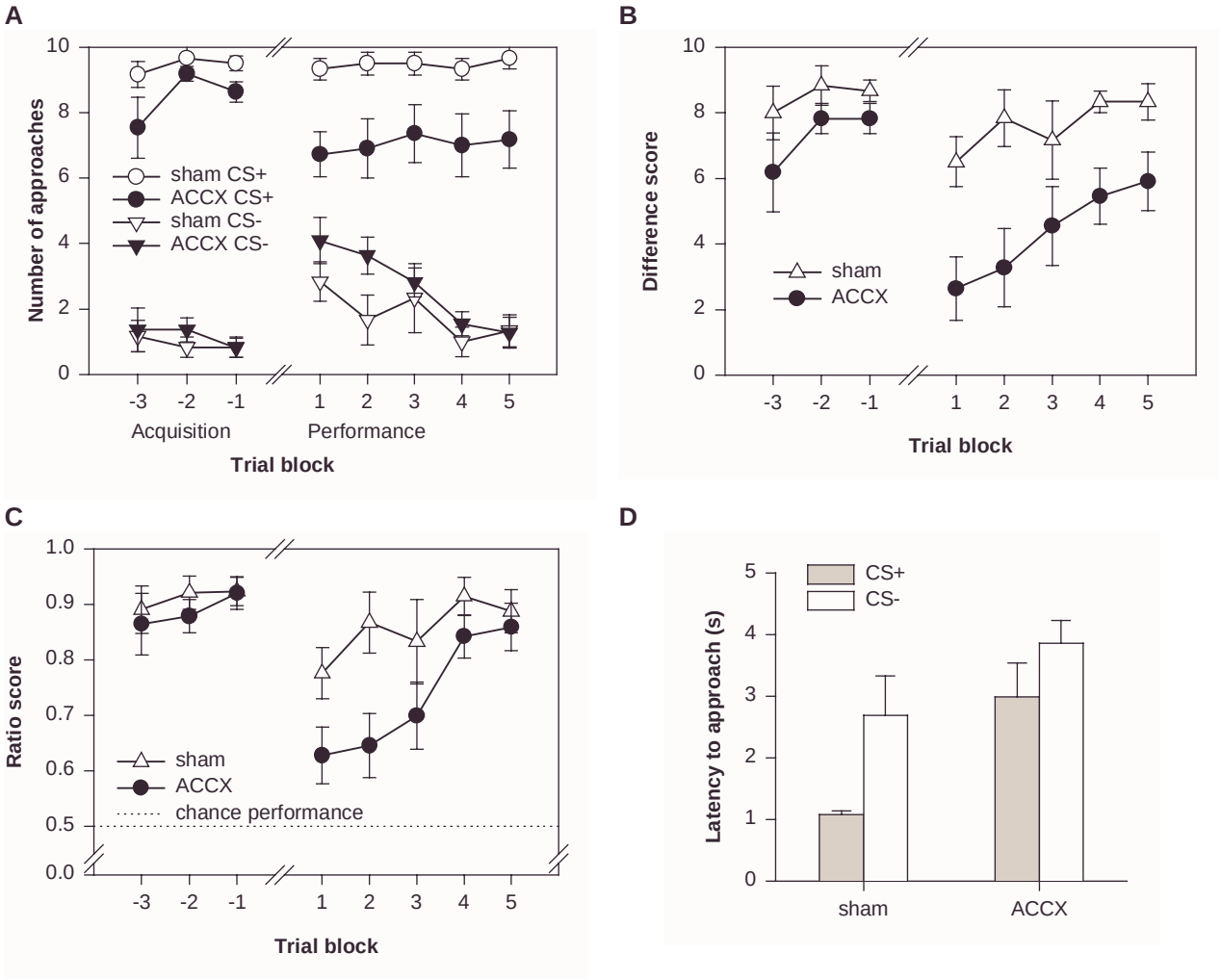

Figure 28. Lesions of the ACC impair the performance of autoshaping when those lesions are made after training. The final three sessions of pre-operative acquisition training are shown, together with post-operative performance. **A:** Approaches to CS+ and CS–. **B:** Difference scores. **C:** Discrimination ratio scores, calculated as for Figure 22 (p. 86). **D:** Latencies to approach each stimulus post-operatively. The ACCX group approached more slowly.

Probe test

ACC-lesioned subjects showed reduced discrimination in the probe test (Figure 29). A discrimination ratio was calculated as the number of trials on which the CS+ was approached divided by the number of trials on which either stimulus was approached. Analysis of this measure by one-way ANOVA revealed an impairment in the ACCX group ($F_{1,15} = 4.566$, $p = .049$). However, both groups discriminated between the CS+ and CS– (sham group compared to 50% discrimination ratio by one-sample t test: $t_5 = 22.077$, $p < .001$; ACCX group: $t_{10} = 9.515$, $p < .001$).

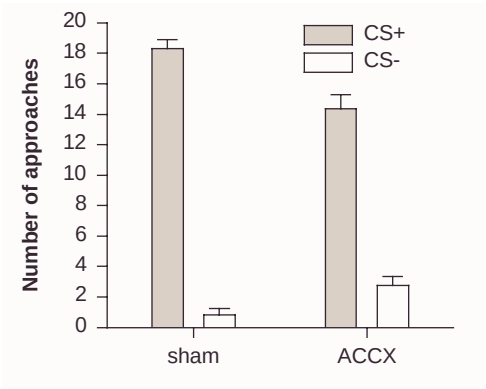

Figure 29. Autoshaping probe test, showing reduced CS+/CS– discrimination in ACC-lesioned rats. (N.B. Approaches to the CS+ and CS– are mutually exclusive on any given trial.)

Omission test

Lesioned subjects did not differ from shams in their response to the introduction of an omission contingency (Figure 30). Analysis of the number of trials on which the CS+ was approached using the model group × (block × S) revealed a near-significant effect of group ($F_{1,15}$ = 4.269, p = .057), reflecting the previously-established lower baseline of CS+ approaches in the ACCX group, but no effect of block ($F_{4,60}$ = 1.226, NS) and, critically, no block × group interaction (F < 1, NS). (It may be worth noting that even if the ACCX group had ceased responding to the CS+ more rapidly, for which there was no statistical proof, it could not be stated with confidence that they were 'more instrumental' animals, better able to inhibit Pavlovian conditioned responding, as more rapid Pavlovian extinction would be an alternative explanation.)

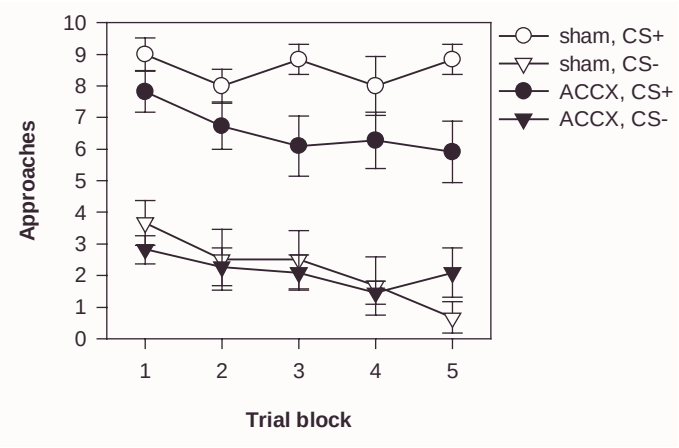

Figure 30. Autoshaping omission test; ACC-lesioned rats did not differ significantly from sham-operated controls.

Locomotor activity in a novel environment

As one animal in the ACCX group (rat GL3) fell ill after the autoshaping tests and was perfused, final group numbers for the locomotor test were 10 (ACCX) and 6 (sham).

There was no clear pattern of difference in locomotor activity between sham and ACCX groups, although there were statistical differences in the pattern of habituation to novelty. Beam-break data were subjected to a square-root transformation and analysed using the model group × (bin_{12} × S). There was no main effect of group (F < 1, NS), but in addition to the main effect of bin ($F_{8.49,118.859}$ = 27.459, $\tilde{\varepsilon}$ = .772, p < .001), reflecting habituation, there was a bin × group interaction ($F_{8.49,118.859}$ = 3.48, $\tilde{\varepsilon}$ = .772, p = .001). The only bin for which a simple effect was significant in its own right was the bin finishing 70 min into the session (simple effect of group for this bin, $F_{1,14}$ = 5.205, p = .039), but elimination of this bin left the interaction term still significant ($F_{7.628,106.793}$ = 2.883, $\tilde{\varepsilon}$ = .763, p = .007). Inspection of Figure 31 suggests that this may have been due to slight hyperactivity in the ACCX group late in the session.

Summary

Lesions of the ACC impaired the performance of an autoshaped response that had been trained to asymptote pre-operatively. This deficit was primarily due to a decrease in CS+ approaches in the lesioned animals, and persisted throughout testing.

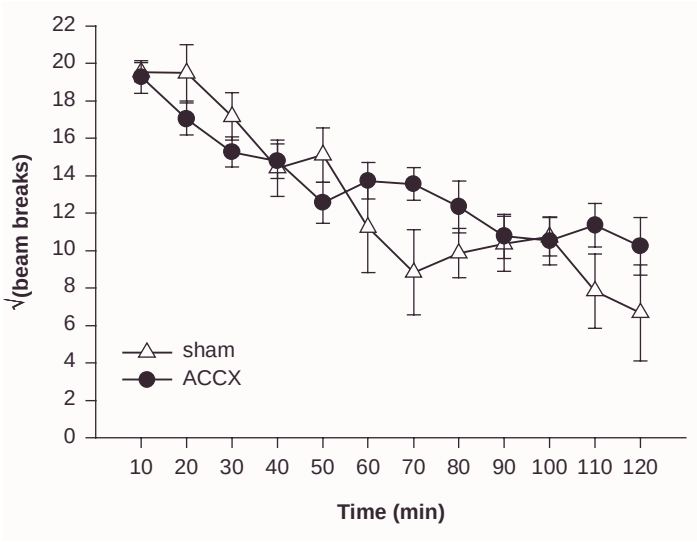

Figure 31. Locomotor response to novelty. 120-min session scored in 10-min bins.

Discussion

Nature of the autoshaping deficit

Previous studies have consistently found ACC-lesioned rats to approach the CS– more than control rats (Bussey *et al.*, 1997a; Parkinson *et al.*, 2000c), whereas the performance deficit in the present experiment was due to a reduction in CS+ approach (the transient post-operative increase in CS– approach was not significantly greater than that observed in sham-operated controls). It may be that a failure to discriminate between the two stimuli may naturally manifest itself as an alteration in either decreased CS+ or increased CS– responding, influenced by the level of general activity of the subjects in the autoshaping apparatus, a factor to which the slight differences in lesion coordinates may have contributed. The most anterior injection in the present experiments was advanced rostrally by 0.4 mm compared to previous autoshaping studies (Bussey *et al.*, 1997a; Parkinson *et al.*, 2000c), and by 0.5 mm compared to Weissenborn *et al.* (1997), the only comparable study for which locomotor data are available. Indeed, while Weissenborn *et al.* (1997) found their ACC-lesioned rats to be clearly hyperactive, this was not obvious in the present experiments (Figure 25, p. 88; Figure 31, p. 97). In further support of this interpretation, reduced CS+ approach, as well as reduced CS– approach, was observed during acquisition in Experiment 1 (Figure 22A). Nevertheless, impairments in CS+/CS– discrimination have been a consistent feature of autoshaping in ACC-lesioned rats, whether assessed by acquisition or performance testing, or by probe tests (Bussey *et al.*, 1997a; Parkinson *et al.*, 2000c; present data).

In the present study, a very high level of CS+ approach was attained pre-operatively, compared to previous studies (Bussey *et al.*, 1997a; Parkinson *et al.*, 2000c); this was entirely due to the use of a selection criterion. Another interpretation supported by the present data, therefore, is that the ACC contributes to CS+ approach especially in those animals who discriminate very well; this effect may usually be masked by variability in normal subjects.

Speed of responding

Lesioned subjects were slower to approach the stimuli. This accords with the observations of Bussey *et al.* (1997a), but not Parkinson *et al.* (2000c), who found that ACC-lesioned rats approached the stimuli faster than control subjects. Again, it is possible that slight differences in lesion location account for these discrepancies. Indeed, Parkinson *et al.* (2000c) suggested that ventral ACC damage was more likely to produce stimulus–reward learning deficits while dorsal ACC damage might affect the speed of respond-

ing, based on the distinction between the dorsal, 'motor' ACC and the ventral, 'emotional' ACC (Dum & Strick, 1993; Neafsey *et al.*, 1993). The present lesions were slightly more anterior than those of Bussey *et al.* (1997b), and damaged dorsal ACC more consistently than those of Parkinson *et al.* (2000c), and, as suggested above, there is some indirect evidence (via Weissenborn *et al.*, 1997) that the ACC-lesioned subjects in the present study were not as active as those in previous autoshaping studies that used more posterior coordinates, though this cannot be established directly. However, if more anterior, more dorsal ACC lesions produce less hyperactivity, it is not clear why Parkinson *et al.* (2000c) found more rapid responding in ACC-lesioned rats, given that their lesions were slightly more anterior on average than those of Bussey *et al.* (1997a).

Inspection of the data from these studies suggests that the approach latencies of ACC-lesioned rats are actually more consistent across studies than those of the sham-operated controls, in terms of absolute magnitude (all these studies used the same apparatus). Another hypothesis, therefore, is that the selection process contributed to the observed results: the selection of subjects with especially high levels of discrimination led to unusually low approach latencies in the sham group. This hypothesis would suggest that the ACC contributes to rapid approach in animals who discriminate well. However, it is not clear how this hypothesis would account for the significant *reduction* of approach latency in ACC-lesioned rats found by Parkinson *et al.* (2000c). It has been suggested that autoshaping exhibits important individual variability (Tomie *et al.*, 1998; 2000); it would be intriguing if the ACC is a source of this variability. If applied to approach latencies, this hypothesis would predict that the variance of ACC-lesioned, unselected rats was smaller than the variance of sham-lesioned, unselected rats. There have been no direct tests of this hypothesis, as variance comparisons have not been published for the present autoshaping task; however, inspection of Figure 22D (based on non-naïve subjects; page 86) does not support this suggestion.

Learning versus performance

The finding that post-training ACC lesions impair the performance of autoshaping strongly suggests that its role is not limited to learning the stimulus–reward associations; instead, the ACC is involved in storage or retrieval of the associations (a mnemonic role), or in the mechanism of behavioural expression.

This is a difficult question to answer conclusively, as it may be argued that further pre-operative training might have rendered performance independent of the ACC, or that recovery might eventually have been observed post-operatively in ACC-lesioned subjects. There is some evidence that the ACC has a time-limited role (that is, the ACC is particularly important *early* in learning). Gabriel *et al.* (1991a) have reported that two-way active avoidance behaviour is eventually acquired in ACC-lesioned rabbits. Furthermore, when ACC lesions, or combined lesions of MD and anteroventral (AV) thalamus, are made after the acquisition of avoidance behaviour, the lesions do not impair performance as much as they do when made before training (Gabriel *et al.*, 1980a, p. 162; Gabriel, 1993; Freeman *et al.*, 1996; Hart *et al.*, 1997). They suggest that the ACC (with the MD thalamus) rapidly acquires the CS+ / CS– discrimination, then 'teaches' the AV and the PCC, 'relegating' the discriminative role and releasing the ACC for further learning; Gabriel *et al.* have even suggested this as an analogue of behavioural automatization (Gabriel *et al.*, 1980a, pp. 143–163 / 220; Freeman *et al.*, 1996). In the words of Hart *et al.* (1997), the engram is nomadic. In accordance with this hypothesis, lesions of AV or PCC did not impair acquisition, but impaired asymptotic performance of this task (Gabriel *et al.*, 1983; Gabriel *et al.*, 1987). To some degree, this would also accord with views of primate ACC as a specialized 'error detector' (to be discussed later); a structure responsible for error detection and correction might become less important as the task is automatized. It is not known whether such an 'automatization' account is applicable to autoshaping, a putative Pavlovian response; this suggestion could be envisaged as the supersession of CS–US associations by

CS–UR associations as the dominant representation controlling behaviour, a hypothesis that would be testable by devaluing the US after brief or extended training.

There is some evidence from rat studies to support a role for the ACC early in learning. Parkinson *et al.* (2000c) found that a degree of discrimination was eventually acquired by ACC-lesioned subjects in the autoshaping task, though they never attained the performance of shams (see Parkinson *et al.*, 2000c, p. 49). Bussey *et al.* (1996) found that ACC lesions facilitated early learning of a CVD, a task that may depend on S–R associations, while PCC lesions impaired late learning. These results and those of Gabriel's group (see Freeman *et al.*, 1996) would be anticipated if the ACC formed stimulus–reward associations early in training, while the PCC formed stimulus–response habits (Bussey *et al.*, 1996; 1997a; 1997b). In tasks where both systems contribute, such as autoshaping and Gabriel's active avoidance task, ACC lesions impair performance early in acquisition (Gabriel, 1990; Gabriel *et al.*, 1991a; Gabriel, 1993; Parkinson *et al.*, 2000c), while PCC lesions impair late performance of active avoidance (Gabriel *et al.*, 1987). In S–R tasks, where the two systems compete, ACC lesions improve performance early in acquisition (Bussey *et al.*, 1996) while PCC lesions impair performance later on (Bussey *et al.*, 1996; 1997b). In tasks only soluble via stimulus–reward associations, PCC lesions may improve performance (Bussey *et al.*, 1997b). A critical prediction of the hypothesis of Gabriel *et al.* (that the ACC teaches the PCC) is that PCC lesions, which do not impair the acquisition of autoshaping (Bussey *et al.*, 1997a), would impair performance if the response were overtrained.

In support of this compelling account, there was partial recovery of the ACCX group in the present experiment, and they did discriminate between the stimuli, both in the performance test and the probe test. However, the ACCX group did not recover fully. The recovery was largely due to a decline in CS– responding; the deficit in CS+ approach was persistent and showed no signs of recovery. As these animals were not hypoactive in a locomotor test, there is no reason to think that the deficit in CS+ responding was due to a general lack of activity. Though it may be that the autoshaping response was not sufficiently overtrained to observe normal function after ACC lesions (compare Hart *et al.*, 1997), the response was behaviourally asymptotic before the lesion.

EXPERIMENT 3: EFFECTS OF ACC LESIONS ON 'SIMPLE' PAVLOVIAN–INSTRUMENTAL TRANSFER

Pavlovian conditioned stimuli may elicit autonomic or skeletomotor conditioned responses, and serve as behavioural goals (conditioned reinforcers), but may also elicit conditioned 'motivational' responses, as discussed in Chapter 1 (p. 26). One example is Pavlovian–instrumental transfer (PIT), in which an appetitive Pavlovian CS potentiates ongoing instrumental responding. The results of Experiment 1 demonstrated that the ACC is not necessary for simple Pavlovian conditioning; accordingly, it was anticipated that normal PIT should be observed in ACC-lesioned rats.

In this task, a Pavlovian association is first established between a CS and reward. Subjects are then trained to respond instrumentally for the same reward (with no CS present), and in an extinction test, responding is assessed in the presence and absence of the CS. This is a 'simple' test of PIT, in that the instrumental reinforcer is the same as the Pavlovian US (see Chapter 1).

Methods

Subjects

The subjects were those that previously served in the autoshaping performance study, except for two animals that fell ill after locomotor testing and were perfused (rats GL4 and GL8). This left $n = 6$ (sham) and 9 (ACCX). These subjects were tested on a Pavlovian–instrumental transfer task of the simple kind, described below.

Simple Pavlovian to instrumental transfer

The task was conducted in the standard operant chambers, which were new to the subjects. The method was based on Balleine (1994).

Throughout the experiment, the reinforcer used was one 45-mg sucrose pellet (Rodent Diet Formula P, Noyes, Lancaster, NH). The task used two stimuli. Stimulus 1 consisted of the left and right stimulus lights (2.8 W bulbs) flashed at 3 Hz. Stimulus 2 was a clicker relay operated at 10 Hz. These stimuli were designated the CS and neutral stimulus (NEUT) in counterbalanced fashion. A 2.8-W houselight was illuminated throughout.

Pavlovian training. Eight training sessions were given. Each session contained six 2-min presentations of the CS, during which reinforcement was delivered on a random time (RT) 30-s schedule. Stimulus presentations were separated by an interstimulus interval (ISI) of 2–4 min, during which no reinforcement was given. Conditioning was assessed as a discrimination ratio: the proportion of total nosepoking time during the CS, corrected for the differences in CS and ISI duration (that is, CS% / {CS% + ISI%}). In the final session, two 2-min presentations of the NEUT stimulus were also given, unreinforced, to reduce unconditioned suppression when this stimulus was subsequently presented during the test phase.

Instrumental training. Instrumental training was conducted in eight 30-min sessions with a single lever present. Responding was reinforced on a random interval (RI) schedule, whose parameter in subsequent sessions was 2, 15, 30, and thereafter 60 s.

Instrumental extinction. A single 30-min session was given in which the lever was available but unreinforced, following the observation that PIT is best observed when the response has been partially extinguished (Dickinson *et al.*, 2000, p. 473). No further Pavlovian sessions were given after instrumental training.

Transfer test. The transfer test was conducted over two sessions with the lever present but never reinforced. In each session, the CS, NEUT, and ISI were presented four times each; the stimuli (including the ISI) all lasted 2 min and were randomized in triplets, with the constraint that the same stimulus was never presented in two consecutive 2-min periods.

Results

Pavlovian training

The sham and ACCX groups did not differ in their stimulus-related behaviour during Pavlovian training (Figure 32). The approach ratio during Pavlovian sessions was calculated from the proportion of the CS spent nosepoking (%CS) and the proportion of the ISI spent nosepoking (%ISI) as follows: approach ratio = (%CS) ÷ (%CS + %ISI). As pellets were being delivered during CS presentation, this measure is not a pure measure of conditioned responding, being contaminated by unconditioned approach to the food. However, the two groups did not differ: an analysis using the model group × counterbalancing × (session × S) revealed no effect of group ($F_{1,11}$ = 2.023, NS) and no group × session interaction ($F_{7,77}$ = 1.045, NS), with the main effect of session approaching significance ($F_{7,77}$ = 1.981, p = .068). Subjects nosepoked more during the clicker than the light CS (mean approach ratios 0.681 and 0.603, respectively; main effect of counterbalancing: $F_{1,11}$ = 6.555, p = .027) but there were no other effects of the counterbalancing condition (Fs < 1, NS).

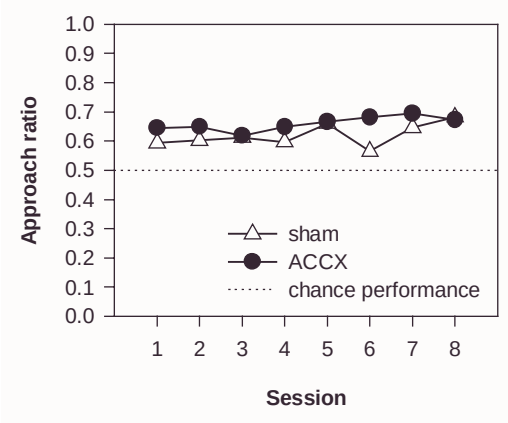

Figure 32. Pavlovian training. The approach ratio is the proportion of total nosepoking behaviour occurring at times when the CS was presented (see text). Both groups approached the alcove more during the CS than during the interstimulus interval, with no group differences. As food was delivered during the CS, the approach behaviour partly reflects unconditioned responding.

Instrumental training

Both groups acquired the instrumental response at the same rate (Figure 33). Lever-press data from instrumental acquisition sessions were subjected to a square-root transformation and analysed using the model group × (session × S). There was no effect of group, and no group × session interaction (Fs < 1, NS), though there was a main effect of session ($F_{4.316,56.114}$ = 11.528, $\tilde{\varepsilon}$ = .617, p < .001). Similarly, responding did not differ between the groups during the extinction session (univariate ANOVA, F < 1, NS).

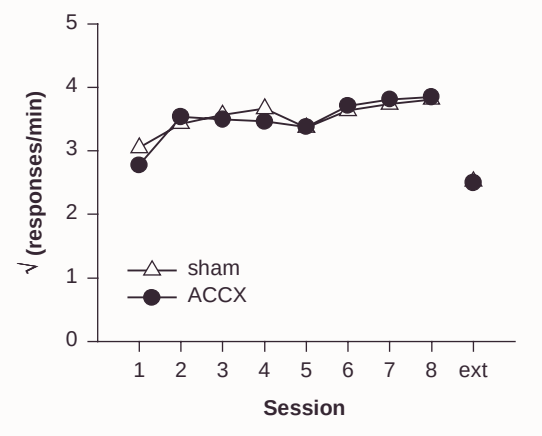

Figure 33. ACC lesions did not impair the acquisition of a free-operant instrumental response, or affect responding in extinction (*ext*, extinction session).

Transfer test

The CS reliably elevated responding relative to the ISI and the neutral stimulus, and this effect did not differ between groups (Figure 34). Response rates for the two test sessions were square-root transformed and analysed using the model $group_2 \times counterbalancing_2 \times (session_2 \times stimulus_3 \times S)$, where stimulus had three levels (CS, ISI and NEUT) and counterbalancing had two (light or clicker CS). Predictably, subjects responded more on the first test session than the second (effect of session: $F_{1,11} = 74.968$, $p < .001$), but there were no other effects of the test session. Similarly, the counterbalancing condition had no effect on responding; thus, the light and clicker were equally effective as CSs. The CS significantly affected behaviour (stimulus: $F_{2,22} = 72.784$, $p < .001$). Pairwise comparisons using a Sidak correction showed that responding during the CS was greater than during the ISI or the NEUT stimulus ($p < .001$), which did not differ from each other ($p = .966$). The sham and ACCX groups did not differ in any respect (maximum $F_{2,22} = 1.549$, NS).

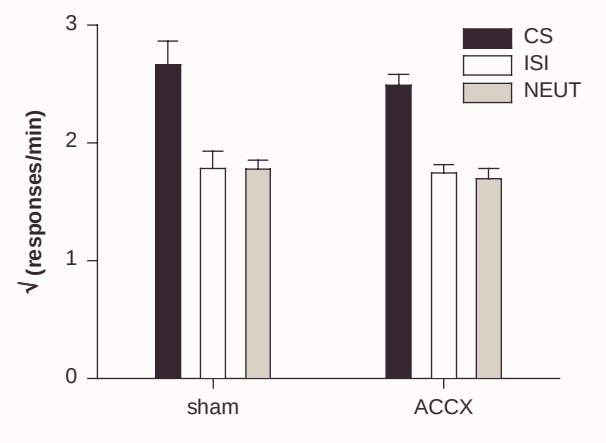

Figure 34. Transfer test. ACC lesions did not affect Pavlovian–instrumental transfer; the CS elevated responding relative to the ISI and a neutral stimulus.

Discussion

These results provide a further demonstration of normal Pavlovian conditioning in ACC-lesioned rats, who exhibited normal PIT, indicating that the conditioned motivational impact of the appetitive CS (see Dickinson, 1994) was intact and able to modulate instrumental behaviour. In addition, it demonstrates normal free-operant instrumental acquisition in ACC-lesioned rats. Finally, there was no evidence that the ACCX group generalized to the neutral stimulus, which was of a different modality to the CS. If the ACC is indeed critical for discriminating between rewarded and unrewarded stimuli, as discussed earlier, it must be assumed that the visual and auditory CSs were too different for generalization to have occurred in the first place. However, the idea that the rat ACC is necessary to discriminate similar stimuli on the basis of their association with reward (but is not required for conditioning *per se*) has not yet been tested directly. Experiment 4 will do so.

EXPERIMENT 4: EFFECTS OF ACC LESIONS ON A TWO-STIMULUS DISCRIMINATED APPROACH TASK

Experiment 1 demonstrated a striking dissociation in which ACC-lesioned rats successfully learned to approach a single appetitive CS in a temporally discriminated approach task but were impaired at autoshaping. Indeed, the neural basis of these tasks has been dissociated before: CeA lesions impair autoshaping (Parkinson *et al.*, 2000b) but not temporally discriminated approach (Robledo *et al.*, 1996). Therefore, a further experiment was designed to explore the difference between the two tasks. As discussed earlier, these two tasks differ in two main ways.

The first is the location of the CS relative to the US. In the temporally discriminated approach task, the CS is presented in the same spatial location as the food, while in the autoshaping task, approach to the CS takes the subject away from the food source. It may be that the ACC is critical for appetitive approach to a CS, but not for approach to a US (literally, sign-tracking versus goal-tracking, or preparatory versus consummatory behaviour).

This might also reflect the differential contribution of Pavlovian and instrumental responding. As discussed in Chapter 1 (p. 41), autoshaping is most probably a Pavlovian response — the alternative explanation, that it reflects instrumental approach to a conditioned reinforcer (Williams, 1994a), cannot easily explain the ACC impairment, as it has now been shown that ACC-lesioned animals can work normally for a CRf. In the temporally discriminated approach task, there is an unavoidable instrumental contingency between approach to the site of the CS and food acquisition: the CS might serve as a discriminative stimulus for instrumental approach. However, a version of the discriminated approach task with an inadvertent instrumental contingency has also been employed; in this version (Burns *et al.*, 1993), nosepokes caused the sucrose dipper to rise (B.J. Everitt, personal communication, 29 January 1999). Different effects of BLA lesions have been observed on the Pavlovian and instrumental versions of this task (Cador *et al.*, 1989; Burns *et al.*, 1993), which weakens the argument that the Pavlovian version used in Experiment 1 differs *further* from autoshaping in the degree of instrumental contingency. Nevertheless, this remains a possibility.

In summary, this difference between the two tasks leads to the hypothesis (Hypothesis 1) that the rat ACC is critical for Pavlovian conditioned approach, not instrumental or consummatory approach behaviour, and not other simple forms of Pavlovian conditioning (such as conditioned freezing or PIT).

The second difference is the number of stimuli used. In the autoshaping task, the subject is required to discriminate two stimuli, identical apart from their location. In the simple discriminated approach task, the discrimination is temporal: the subject is merely required to discriminate the presence of a single stimulus from its absence. The hypothesis that follows from this (Hypothesis 2) is that the rat ACC is necessary for discriminating similar stimuli on the basis of their association with reward.

To distinguish these two possibilities, a task was designed that had features of both the temporally discriminated approach and autoshaping tasks. Approach was to the food source, as in the temporally discriminated approach task, but two similar stimuli governed approach, as in autoshaping. One stimulus (CS+) signalled the imminent delivery of sucrose solution to a food alcove, while the other (CS–) did not. Essentially, this task is identical to autoshaping except that approach is *measured* to the food alcove, rather than to the stimuli. Pilot experiments established that normal rats could discriminate the two stimuli, although with difficulty. Finding an impairment in ACC-lesioned rats with this task would therefore support Hypothesis 2, and normal performance would support Hypothesis 1. In addition, a conditioned reinforcement test was given using the two stimuli.

Methods

Overview

Naïve subjects received lesions of the ACC (n = 12) or sham lesions (n = 12); their body mass at the time of surgery was 333–379 g. Following recovery, they were maintained at 85% of their free-feeding mass. The subjects were subsequently trained for 12 sessions on a two-stimulus discriminated approach task (described below), as pilot studies had determined that significant CS+ / CS– discrimination emerged in normal animals within this time; a conditioned reinforcement test was then conducted for two sessions.

Two-stimulus temporally discriminated approach task

This task was conducted in the operant chambers used for the temporal discriminated approach task described previously (p. 76).

The levers were not extended during training. The stimulus lights located above the levers were designated the CS+ and CS–, counterbalanced left / right across rats. At the start of every session, the houselight was on and the dipper was lowered. This phase lasted for a VI of 30–90 s. Next, the houselight was extinguished and one of the stimulus lights was illuminated for 5 s. Following presentation of the CS+, the houselight was illuminated and the dipper raised for 5 s to deliver 10% sucrose solution; this constituted the US. Following presentation of the CS–, the houselight was similarly illuminated but the dipper was not raised, and a brief click was generated in order that both stimuli had an auditory and a visual component. Regardless of the stimulus, the chamber was then in the starting state and the next VI began.

One trial consisted of a presentation of the CS+ and a presentation of the CS–; the order of the stimuli was randomized within each trial. A session consisted of 15 trials, after which the houselight was extinguished. Subjects received one session per day. For each period (VI, CS+ / CS–, US or a notional 5-s equivalent following the CS–), the number of alcove entries and the time spent nosepoking in the alcove were recorded.

Two-stimulus test of conditioned reinforcement

This task was conducted in the same operant chambers. Two 30-min sessions were given on consecutive days, during which the houselight was illuminated and two levers were available, designated the CRf and NCRf levers. Responding on the CRf lever produced an abbreviated version of the CS+ with probability 0.5, while responding on the NCRf lever produced an abbreviated version of the CS– with probability 0.5. The abbreviated CS+ was produced by extinguishing the houselight and illuminating the CS+ stimulus light for 0.5 s, after which the houselight was re-illuminated, the stimulus light was switched off and the empty dipper was raised for 0.3 s. The corresponding CS– stimulus was identical except that the other stimulus light was used, and a click replaced elevation of the dipper. The levers were assigned so that the CRf lever was located underneath the CS+ stimulus light, and the NCRf lever under the CS– stimulus. Lever-pressing and nosepoking were recorded in 5-min bins.

Results

Histology

Histological analysis determined that two of the lesions in the ACCX group were incomplete, and these subjects (I2, I11) were excluded. Neuronal loss and associated gliosis extended from ~2.7 mm anterior to bregma to ~0.3 mm posterior to bregma. However, the ACCX group was somewhat heterogeneous; 4 animals had lesions including the ventral perigenual portion of Cg2 at 1.6–1.7 mm anterior to bregma (subjects I5, I6, I9, I12; Figure 36 presents a schematic showing the largest and smallest extent of the lesions in these subjects), while 6 animals had lesions that did not extend this far ventrally (subjects I1, I3, I4, I7, I8, I10; schematics of lesions in these subjects are shown in Figure 35). As retrograde tracing studies (Parkinson, 1998) have indicated that this region of the ACC projects most strongly to the AcbC,

strongly implicated in appetitive approach behaviour (Parkinson, 1998; Parkinson *et al.*, 1999c; Parkinson *et al.*, 2000c), analyses were conducted using both the complete lesion group (ACCX group, $n = 10$) and the subgroup with ventral perigenual lesions (designated the ACCX-whole group, $n = 4$). No sham animal was excluded, leaving $n = 12$ for this group (subjects I13, I14, I15, I16, I17, I18, I19, I20, I21, I22, I23, I24). Photomicrographs of representative ACC lesions were shown in Figure 16, p. 80.

Schematic of lesions

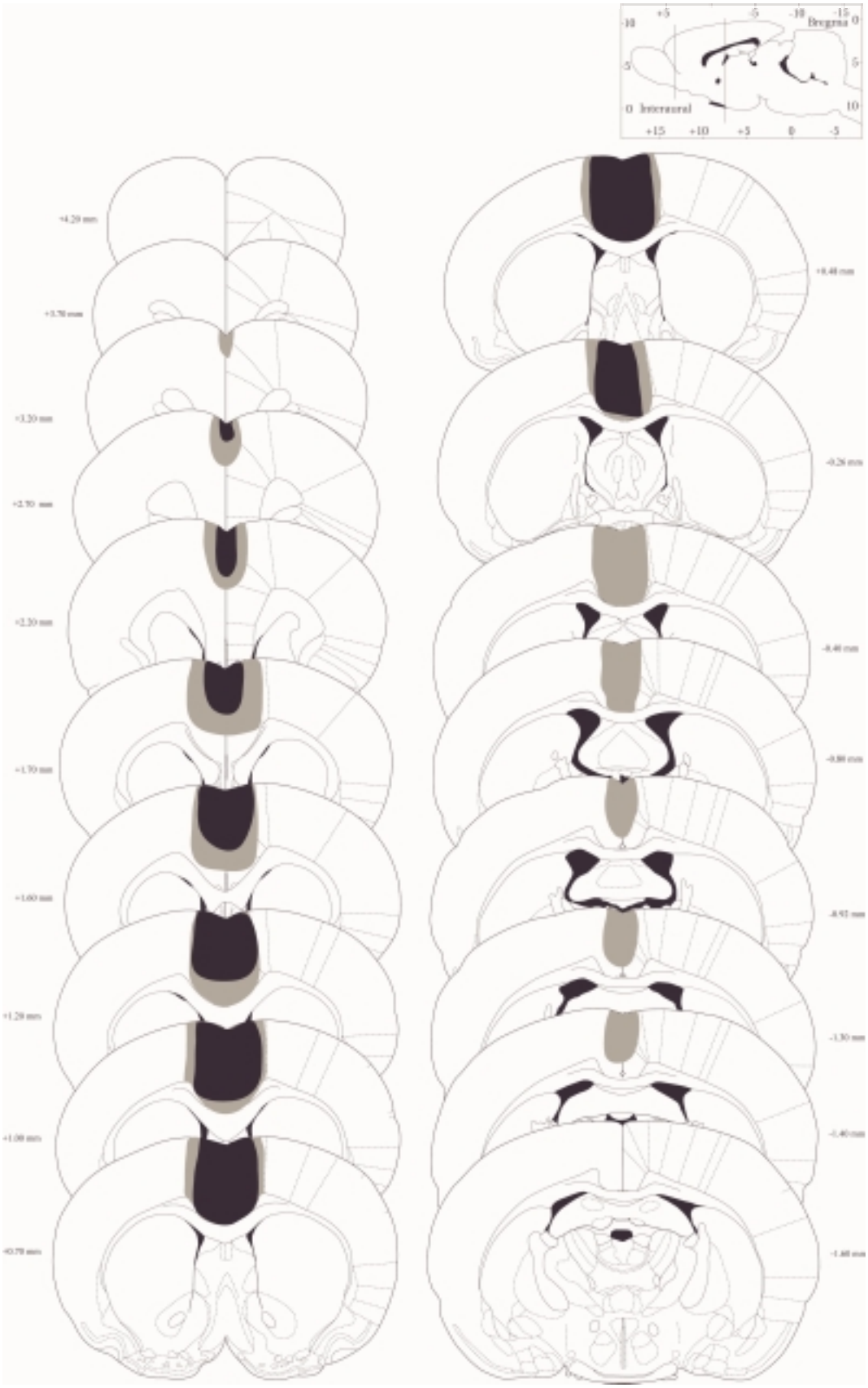

Figure 35. Lesions of the ACC excluding the ventral perigenual region. Grey shading indicates the extent of the largest area of neuronal loss, and black the smallest. Diagrams are taken from Paxinos & Watson (1998). Subjects: I1, I3, I4, I7, I8, I10. Subjects were classified as having whole or partial ACC lesions on 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).

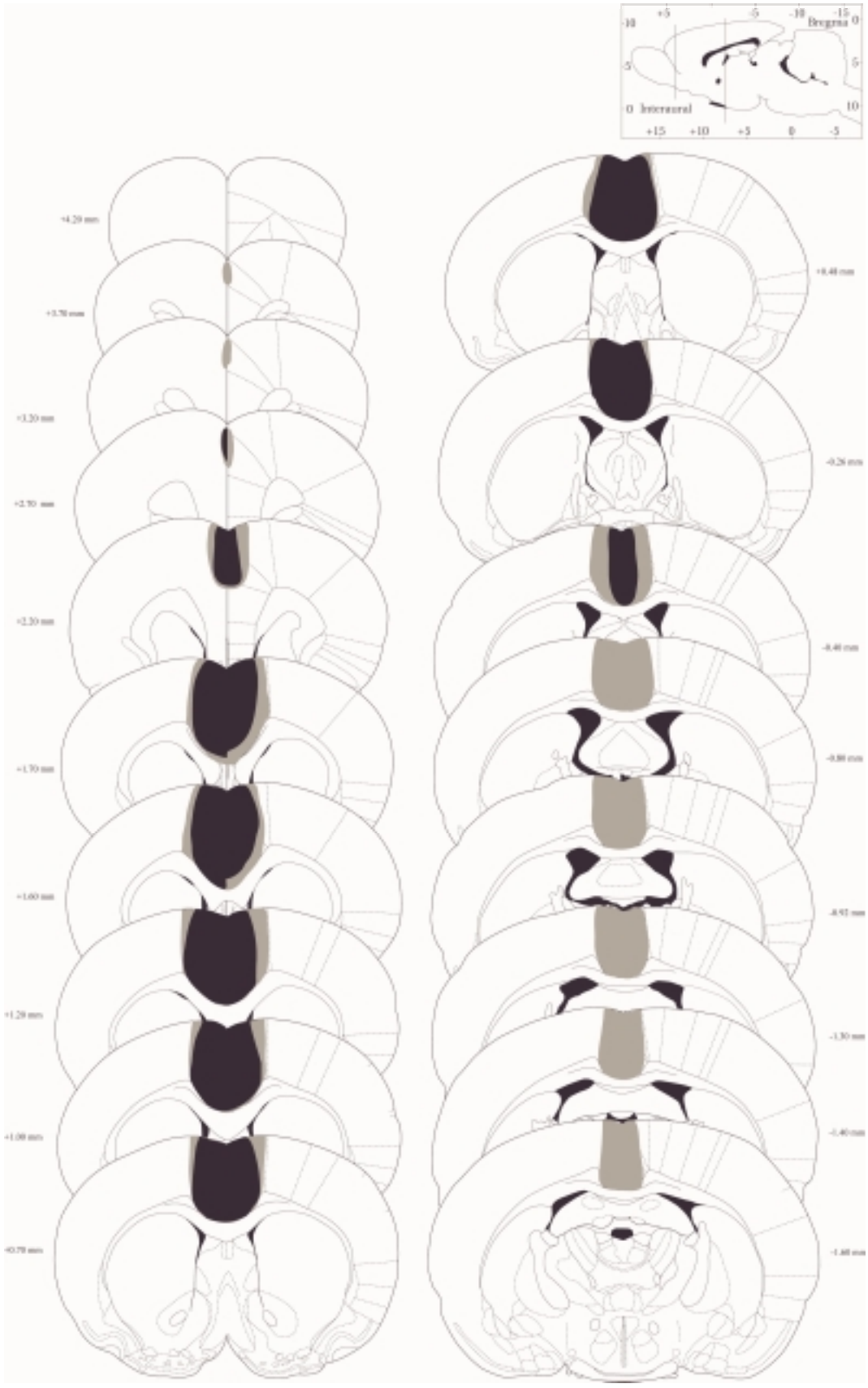

Figure 36. Lesions of the ACC including the ventral perigenual region. Grey shading indicates the extent of the largest area of neuronal loss, and black the smallest. Diagrams are taken from Paxinos & Watson (1998). Subjects: I5, I6, I9, I12. Compare Figure 35.

Two-stimulus discriminated approach task

As this task was designed to be comparable to the autoshaping task used previously, but also to the temporally discriminated approach task, two primary measures of performance were used.

Firstly, for direct comparison with autoshaping, the number of trials was calculated on which at least one nosepoke occurred during stimulus presentation, for both the CS+ and the CS–. From these, difference and ratio scores were calculated, as for the autoshaping task. (If no approach occurred to either stimulus during a session, a ratio score of 0.5 was assigned, though this was a very rare occurrence.)

Secondly, for comparison with previous temporally discriminated approach tasks, an approach discrimination ratio was calculated: the *proportion* of each stimulus period spent nosepoking (%stimulus) was compared to the proportion of the ISI spent nosepoking (%ISI) using the formula *discrimination ratio = %stimulus ÷ (%stimulus + %ISI)*. This ratio was calculated for both the CS+ and CS–, and ISI responding was calculated over both ISI periods in the corresponding trial (including the ISI preceding the CS+ and that preceding the CS–). Therefore, the ratios for CS+ and CS– are directly comparable, as both are calculated relative to the same %ISI.

Analyses based on the number of trials on which approach occurred

The ACCX group were impaired in their ability to discriminate between the two stimuli (Figure 37).

Analysis of absolute approach scores using the model $\text{group}_2 \times (\text{stimulus}_2 \times \text{session}_{12} \times \text{S})$ demonstrated that the ACCX group made fewer approaches overall (main effect of group: $F_{1,20} = 7.48$, $p = .013$). There was a main effect of stimulus ($F_{1,20} = 57.626$, $p < .001$), and of session ($F_{5.516,110.326} = 53.52$, $\tilde{\varepsilon} = .501$, $p < .001$), and a stimulus × session interaction ($F_{11,220} = 14.443$, $p < .001$). In addition, there were stimulus × group ($F_{1,20} = 6.827$, $p = .017$) and stimulus × session × group ($F_{11,220} = 2.178$, $p = .017$) interactions. The session × group interaction was not significant ($F < 1$, NS).

This highly complex pattern of results was investigated using simple effects analyses. First, the CS+ and CS– were considered separately. The ACCX group responded less to the CS+ than the sham group (group: $F_{1,20} = 9.567$, $p = .006$) across all sessions (session: $F_{7.952,159.048} = 60.114$, $\tilde{\varepsilon} = .723$, $p < .001$; session × group, $F = 1.024$, NS). The ACCX group also responded less to the CS– than did shams (group: $F_{1,20} = 4.458$, $p = .048$), again in a session-independent manner (session: $F_{6.658,133.153} = 24.454$, $\tilde{\varepsilon} = .605$, $p < .001$; session × group: $F < 1$, NS). Second, the ACCX and sham groups were considered separately. The sham group learned to discriminate between the stimuli (stimulus: $F_{1,11} = 56.89$, $p < .001$; session: $F_{4.978,54.759} = 31.375$, $\tilde{\varepsilon} = .453$, $p < .001$; stimulus × session: $F_{10.555,111.106} = 7.076$, $\tilde{\varepsilon} = .96$, $p < .001$). The ACCX group also learned to discriminate (stimulus: $F_{1,9} = 11.452$, $p = .008$; session: $F_{5.137,46.233} = 23.142$, $\tilde{\varepsilon} = .467$, $p < .001$; stimulus × session: $F_{11,99} = 11.085$, $p < .001$). Third, the groups' performance was considered for each session. The ACCX group showed discrimination between CS+ and CS– ($p < .05$) from session 9 on, while the sham group first showed discrimination on session 4 (and subsequently on sessions 6 and 8–12).

These analyses indicate that both groups acquired discrimination, with the shams acquiring faster, but do not answer the question of whether the *degree* of discrimination differed between groups. For this, direct measures of discriminative ability were used.

Analysis of difference scores (approaches during the CS^+ – approaches during the CS^-) using the model $\text{group}_2 \times (\text{session}_{12} \times \text{S})$ revealed a significant main effect of group ($F_{1,20} = 6.827$, $p = .017$). In addition, there was a main effect of session ($F_{11,220} = 14.443$, $p < .001$), reflecting learning, and a group × session interaction ($F_{11,220} = 2.178$, $p = .017$). This interaction appeared to be due to slower learning in the

ACCX group, which were impaired at the early stages of learning (simple effect of group significant for sessions 6,8,9 at $p < .01$) but reached the same difference score as shams by the end of session 12.

The impairment did not depend on the use of a difference score as the dependent measure, but was apparent when ratio scores (which are relatively independent of general activity levels) were analysed. Again, the ACCX group showed significantly poorer discrimination (effect of group: $F_{1,20} = 6.995$, $p = .016$). Assessed by this measure, the discrimination was poorer across all sessions (group × session: $F < 1$, NS), though ratio scores increased during training (session: $F_{7.53,150.597} = 2.713$, $\tilde{\varepsilon} = .685$, $p = .009$).

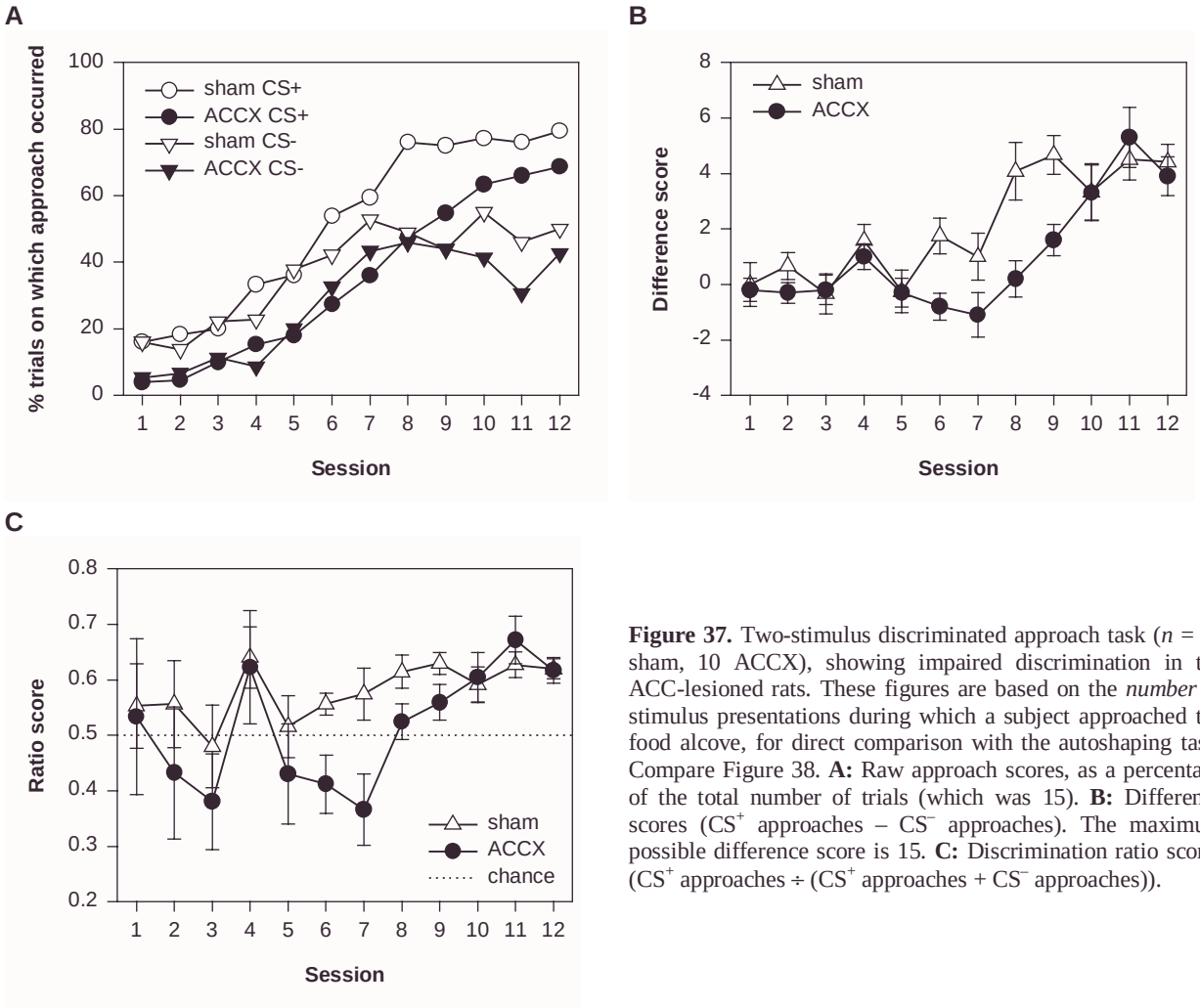

Figure 37. Two-stimulus discriminated approach task ($n = 12$ sham, 10 ACCX), showing impaired discrimination in the ACC-lesioned rats. These figures are based on the *number* of stimulus presentations during which a subject approached the food alcove, for direct comparison with the autoshaping task. Compare Figure 38. **A:** Raw approach scores, as a percentage of the total number of trials (which was 15). **B:** Difference scores (CS^+ approaches – CS^- approaches). The maximum possible difference score is 15. **C:** Discrimination ratio scores (CS^+ approaches ÷ (CS^+ approaches + CS^- approaches)).

Analyses based on the proportion of time spent nosepoking to each stimulus

This approach score measures approach to a stimulus relative to that occurring during the VI. It proved a less sensitive measure than the number of trials on which approach occurred, as analysis of the proportion of time spent nosepoking only revealed an impairment in those animals with anterior cingulate lesions encompassing the ventral perigenual region (Figure 38).

The approach scores from all subjects were analysed using the model $group_2 \times (session_{12} \times stimulus_2 \times S)$. This showed a non-significant trend towards lower levels of stimulus-directed approach in the ACCX group (effect of group: $F_{1,20} = 3.574$, $p = .073$). There were main effects of stimulus ($F_{1,20} = 7.006$, $p = .015$) and session ($F_{5.876,117.53} = 48.928$, $\tilde{\varepsilon} = .534$, $p < .001$), and a stimulus × session interaction ($F_{7.235,114.701} = 2.78$, $\tilde{\varepsilon} = .658$, $p = .009$), reflecting the acquisition of differential approach to the two stim-

uli. However, the stimulus × group interaction did not reach significance ($F_{1,11}$ = 3.178, p = .09), and no other terms involving group were significant (Fs < 1, NS). Interestingly, though, analysis of the sham and ACCX groups separately demonstrated significant stimulus discrimination in the shams (stimulus: $F_{1,11}$ = 13.487, p = .004; session: $F_{4.176,45.936}$ = 24.84, $\tilde{\varepsilon}$ = .38, p < .001; stimulus × session: $F_{6.007,66.079}$ = 2.233, $\tilde{\varepsilon}$ = .546, p = .051) but no evidence of discrimination in the ACCX group (stimulus: F < 1, NS; session: $F_{7.0,63.002}$ = 25.413, $\tilde{\varepsilon}$ = .636, p < .001; stimulus × session $F_{6.314,56.823}$ = 1.438, $\tilde{\varepsilon}$ = .574, NS), despite similar group sizes (and therefore statistical power).

However, when the ACCX-whole subgroup were compared to shams, they were found to be significantly impaired. Despite the smaller number of animals, a stimulus × group interaction was found ($F_{1,14}$ = 7.277, p = .017), in addition to a main effect of session ($F_{5.402,75.621}$ = 30.581, $\tilde{\varepsilon}$ = .491, p < .001) and a stimulus × session interaction ($F_{7.155,100.172}$ = 2.105, $\tilde{\varepsilon}$ = .65, p = .048). No other terms were significant (Fs < 1.381, NS). To explore the nature of the stimulus × group interaction, data from each group were analysed using the model (session × stimulus × S). This demonstrated significant discrimination in the sham group, who approached more during the CS+ than during the CS– (stimulus: $F_{1,11}$ = 13.487, p = .004; session: $F_{4.176,45.936}$ = 24.84, $\tilde{\varepsilon}$ = .38, p < .001; stimulus × session: $F_{6.007,66.079}$ = 2.233, $\tilde{\varepsilon}$ =.546, p = .051), but no such discrimination in the ACCX-whole group (stimulus: $F_{1,3}$ = 1.312, NS; session: $F_{3.594,10.783}$ = 12.534, $\tilde{\varepsilon}$ = .327, p = .001; stimulus × session: $F_{11,33}$ = 1.133, NS).

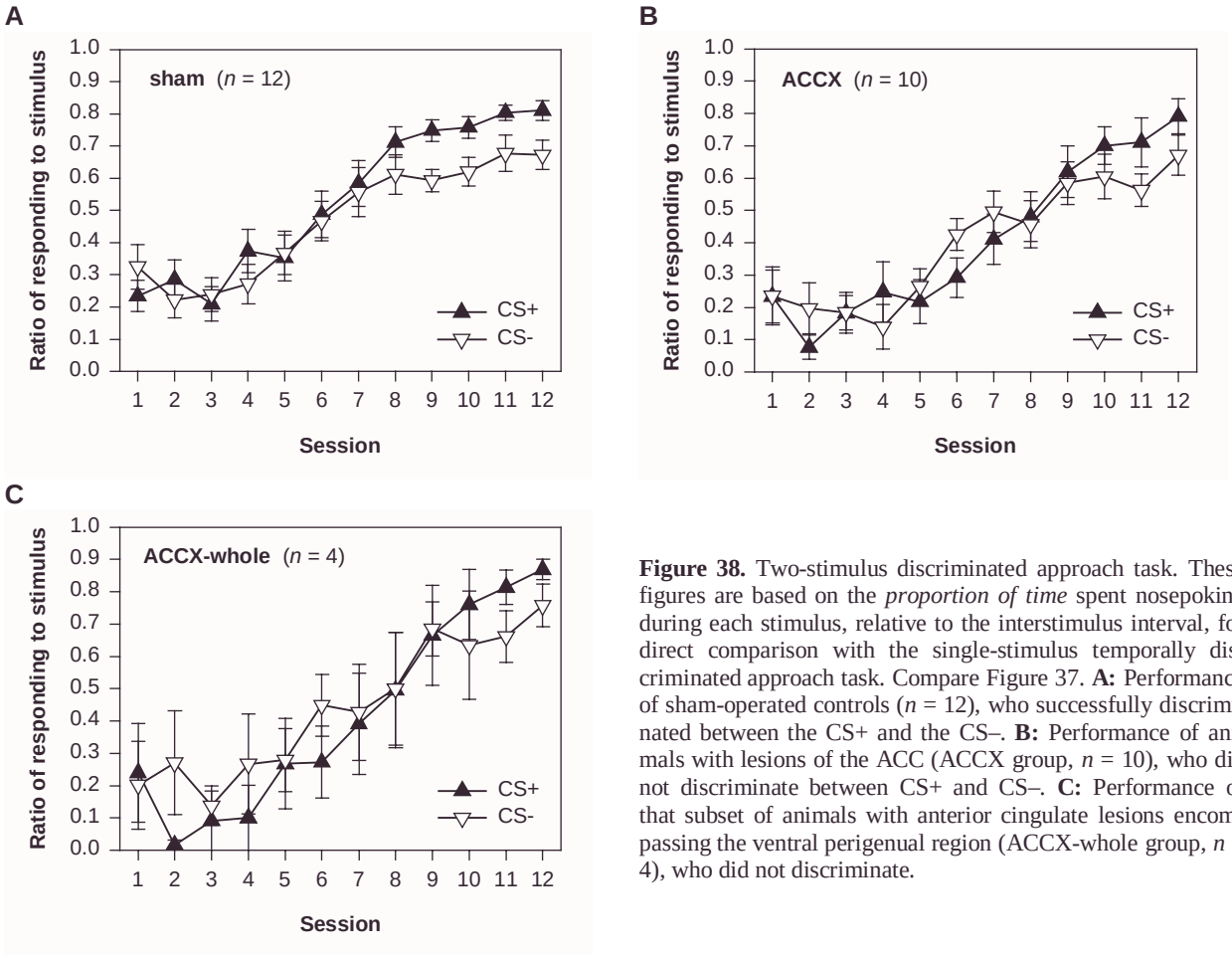

Figure 38. Two-stimulus discriminated approach task. These figures are based on the *proportion of time* spent nosepoking during each stimulus, relative to the interstimulus interval, for direct comparison with the single-stimulus temporally discriminated approach task. Compare Figure 37. **A:** Performance of sham-operated controls (n = 12), who successfully discriminated between the CS+ and the CS–. **B:** Performance of animals with lesions of the ACC (ACCX group, n = 10), who did not discriminate between CS+ and CS–. **C:** Performance of that subset of animals with anterior cingulate lesions encompassing the ventral perigenual region (ACCX-whole group, n = 4), who did not discriminate.

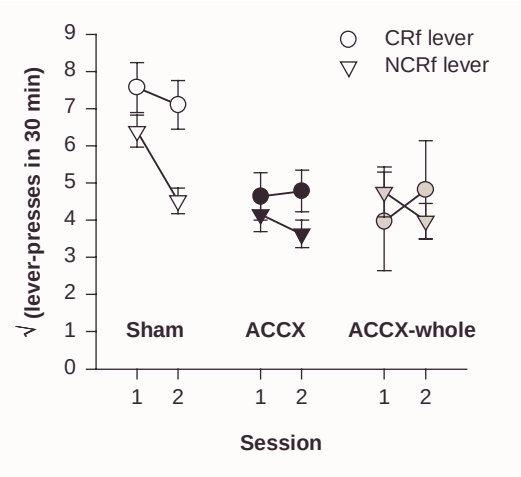

Figure 39. Two-stimulus conditioned reinforcement test. The figure shows the performance of sham animals, animals with lesions of the ACC, and the subgroup of those animals with lesions encompassing the ventral perigenual region of this cortex.

Two-stimulus conditioned reinforcement test

The sham-operated group preferred the CS+ to the CS– when allowed to respond for the two stimuli; thus, the CS+ served as a conditioned reinforcer. The ACCX group responded less, and showed poorer discrimination between CS+ and CS– (Figure 39). Square-root-transformed lever-press data were subjected to ANOVA using the model group$_2$ × (lever$_2$ × session$_2$ × S). Considering both groups together, there was a main effect of session ($F_{1,20}$ = 5.705, p = .027), reflecting extinction. Subjects responded more on the CRf lever ($F_{1,20}$ = 10.832, p = .004). There was also a session × lever interaction ($F_{1,20}$ = 12.872, p = .002); interestingly, this was due to improved discrimination on the *second* test day (simple effects analyses: effect of lever on day 1, $F_{1,20}$ = 3.476, p = .077; effect of lever on day 2: $F_{1,20}$ = 19.484, p < .001), which was due to a reduction responding on the NCRf lever but not on the CRf lever (orthogonal simple effects analyses: effect of session on CRf lever responding, F < 1, NS; on NCRf lever responding, $F_{1,20}$ = 18.276, p < .001).

Animals in the ACCX group responded less on test (main effect of group: $F_{1,20}$ = 14.092, p = .001). However, there were no other interactions involving group (group × session: $F_{1,20}$ = 2.952, NS; group × lever: $F_{1,20}$ = 1.659, NS; three-way interaction, $F_{1,20}$ = 1.615, NS). However, it is clear from Figure 39 that discrimination was reduced in the ACCX group, and while the sham group on its own demonstrated significant discrimination between the levers (lever: $F_{1,11}$ = 9.117, p = .012; lever × session: $F_{1,11}$ = 11.138, p = .007), in this analysis, the ACCX group did not (lever: $F_{1,9}$ = 2.72, p = .133; lever × session: $F_{1,9}$ = 3.085, p = .113).

When the two sessions were considered separately for each group, the shams showed discrimination only on session 2 (simple effect of lever in session 1: $F_{1,11}$ = 3.315, NS; in session 2, $F_{1,11}$ = 14.971, p = .003). The ACCX group showed a similar pattern (simple effect of lever in session 1: F < 1, NS; in session 2, $F_{1,9}$ = 6.179, p = .035). Thus, some discrimination was apparent in ACC-lesioned subjects, but it was much poorer than in sham-operated controls.

These conclusions were not materially altered by consideration of the ACCX-whole subgroup alone, except that these subjects showed a significant lever × session interaction ($F_{1,3}$ = 10.668, p = .047). While this might be interpreted as evidence of lever discrimination, Figure 39 shows that this was not the case: the interaction was due to a 'crossover', with the ACCX-whole subgroup responding more on the NCRf lever in session two. Considering each session separately, the ACCX-whole subgroup never showed discrimination (simple effect of lever in session 1: F < 1, NS; in session 2, F < 1, NS).

Incidentally, these results demonstrate in a within-subjects design that the CS+ was a more effective reinforcer than the CS– in sham-operated animals, eliminating a 'stimulus-seeking' explanation of their preference for the CRf lever in this task.

Summary

ACC-lesioned rats were significantly impaired at acquiring a discriminated approach response governed by two similar stimuli, only one of which was followed by reward. Like shams, they learned to approach during the CS+, but they also approached during the CS–, and exhibited much poorer CS+/CS– discrimination during acquisition. By at least some measures, they eventually acquired the discrimination, but took longer to learn it than shams. While the sham group responded more for the CS+ than the CS– in a test of conditioned reinforcement, the ACCX group responded less and did not discriminate to the same degree as shams.

Discussion

The results of this experiment provide clear support for Hypothesis 2: that the rat ACC contributes to discriminating similar stimuli on the basis of their association with reward, though it is not necessary for stimulus–reward associations *per se*. It is highly unlikely that the lesioned subjects simply failed to discriminate between the stimuli: ACC-lesioned rats have been shown to be normal (Bussey *et al.*, 1997b) or even improved (Bussey *et al.*, 1996) at tasks requiring left–right discrimination, and the stimuli used in the present task (and in the autoshaping experiments) differed in no way except their location. Similarly, ACC-lesioned rats have previously been shown to succeed in learning a CVD using stimuli with which they failed to learn an 8-pair concurrent discrimination (Bussey *et al.*, 1997b), again making a perceptual deficit an unlikely explanation. Nor is it plausible that a failure of response discrimination can account for the present results, as no response discrimination was required in the approach task (the responses measured following the CS+ and CS– were identical).

In the approach task, the CS+ and CS– may have served as instrumental S^Ds, just as in the one-stimulus version of the task used in Experiment 1. Indeed, it is not obvious that approach to a food alcove located *away* from the stimulus is in any sense a Pavlovian CR; thus, performance on the approach task may have been instrumental. Nevertheless, the CS+ predicted food delivery, so it was expected to enter into Pavlovian association with reward; in confirmation of this, the CS+ served as a CRf for both sham and ACC-lesioned rats, though discrimination was again much reduced in ACC-lesioned animals. Their poor discrimination is not simply attributable to generally low levels of operant responding (as ACC-lesioned rats acquired a free-operant response normally in Experiment 3), or failure to respond for conditioned reinforcement (given that they responded normally for CRf in Experiment 1). Thus, the failure of discrimination affected two kinds of measured behaviour, approach and instrumental responding.

GENERAL DISCUSSION

Contribution of the ACC to instrumental and Pavlovian behaviour

Lesions of the ACC have been shown to impair discrimination of reward- or punishment-associated stimuli in Pavlovian tasks, including autoshaping (present experiments; Bussey *et al.*, 1997a; Parkinson *et al.*, 2000c) and autonomic conditioning (Powell *et al.*, 1994); in tasks whose Pavlovian / instrumental status is ambiguous (two-stimulus discriminated approach task, above; two-way active avoidance, Gabriel, 1993; 8-pair concurrent discrimination, Bussey *et al.*, 1997b); and in instrumental tasks depending on Pavlovian associations (responding for conditioned reinforcement, above). At present, the most parsimonious explanation is that the ACC forms or retrieves stimulus–outcome (Pavlovian) associations that may then influence instrumental behaviour, consistent with previous suggestions (Gabriel *et al.*, 1980a; Bussey *et al.*, 1996; Bussey *et al.*, 1997b).

In this section, the hypothesis will be developed that the ACC plays a specific role in *discriminating* stimuli on the basis of their association with reinforcement. However, there is also evidence that the ACC is particularly important *early* in learning (see also pp. 98–99); this will be considered first.

A time-limited role for the ACC?

The results of Experiment 4 also support the view of Gabriel and colleagues, derived from work with aversive conditioning in the rabbit, that the ACC has a time-limited role in learning (see pp. 98–99). Indeed, similar results have been obtained in PET studies of learning in humans (Raichle *et al.*, 1994; Petersen *et al.*, 1998). If the number of CS–US pairings is an important factor in the timecourse of the ACC's contribution to learning (and this may not be the case; see Poremba & Gabriel, 1999), an approximate quantification can be made from the present data. Judging by the difference scores from the two-stimulus approach task (Figure 37B, p. 109), the ACCX group reached the performance of the sham group at around session 10, after 150 CS^{+}–US pairings. This exceeds the number of pairings that have been given in autoshaping acquisition tasks (Experiment 1; Bussey *et al.*, 1997a; Parkinson *et al.*, 2000c), or autonomic conditioning experiments (Powell *et al.*, 1994), in which impairments have been observed. It corresponds roughly to the number of pairings experienced by the subjects in Experiment 2 (the autoshaping performance study) at the point when recovery was observed, as judged by the difference scores (though there was still a clear impairment in CS+ approach at this point). It would be extremely interesting, therefore, to investigate the effects of ACC lesions on autoshaping when made after considerable overtraining, say 250 or 500 CS–US pairings, as this might be beyond the point at which the ACC becomes unnecessary. At this point, behaviour would be expected to depend on other structures, such as the PCC (following Gabriel, 1993).

It is not easy to relate this quantitative estimate to the impairments found in operant discrimination tasks (Gabriel *et al.*, 1991a; Bussey *et al.*, 1996; Bussey *et al.*, 1997b), in which the impairments are typically measured by trials taken to reach a performance criterion. However, 150 US presentations is of the same order as the number of reinforcers required to establish an instrumental habit under a ratio schedule (Adams, 1982). It has been suggested that removal of the ACC leaves rats under the control of a S–R habit system (Bussey *et al.*, 1996), suggesting that experiments measuring the time after which the ACC is not required are actually measuring the speed at which a habit develops. It would be interesting to test an idea related to this hypothesis directly by administering an instrumental contingency test to ACC-lesioned rats (cf. Balleine & Dickinson, 1998a).

As discussed earlier (p. 99), ACC lesions impair autoshaping to a lesser degree when made following training than when made before acquisition (compare Experiment 4 to Bussey *et al.*, 1997a; Parkinson *et al.*, 2000c), and some discrimination may eventually be attained by ACC-lesioned animals even if the lesions are made before acquisition (Parkinson *et al.*, 2000c, p. 49). This raises the possibility that the ACC is not required for *Pavlovian* responding after prolonged training. If this is indeed the case, what kind of representation controls behaviour thereafter? One highly speculative interpretation of the results summarized here is that Pavlovian skeletomotor conditioned responding can become 'habitual' through extended CS–US pairing, in the way that instrumental behaviour does. Psychologically, this would equate to Pavlovian responding being controlled by US-independent representations after prolonged training, more than it is after brief training (a suggestion testable by examining the effects of US devaluation on conditioned responding, as discussed in Chapter 1, pp. 21 / 25, and on pp. 98–99). Available behavioural evidence suggests the opposite (see Hendersen *et al.*, 1980; Mackintosh, 1983, p. 61) but the question has not been extensively studied.

Although conditioned responding may remain sensitive to US devaluation even after prolonged training, there is at least one Pavlovian process whose importance diminishes with overtraining. That is 'mediated learning', defined as the ability of a CS-activated representation of the US to enter into new associations (Holland, 1998). (Using a food US, mediated learning may be demonstrated by giving CS–US pairings, then pairing the CS with LiCl, and finally testing for an aversion conditioned to the US. Holland (1981) suggested that when the CS is paired with LiCl, the CS retrieves a representation of the US that can become associated with the LiCl, even though the food US is not physically present at that time.) Holland (1998) demonstrated that mediated learning occurred after brief amounts of initial training, but not after extended training, even though conditioned responding to the CS remained after extended training. On the basis of these results, Holland (1998) suggested that an *overtrained* CS maintains the ability to elicit a representation of the US for performance of the CR ('mediated performance'), but this US representation cannot enter into new associations (cannot be used for mediated learning), either because it has reduced associability (following the theory of Pearce & Hall, 1980), or because mediated learning and mediated performance are embodied in distinct representational systems. Holland suggests one possibility of the latter kind: that mediated performance (simple conditioned responding) depends on the CS retrieving a *US-specific motivational value*, while mediated learning depends on the CS retrieving *specific sensory attributes of the US* (Holland, 1990b; 1990a; 1998). As discussed in Chapter 1 (p. 37) and by Holland (1998), there is strong evidence that the BLA is required for mediated performance and the retrieval of a US-specific motivational value, while it has been suggested that retrieval of US-specific sensory attributes depends on primary or higher-order sensory cortices, such as gustatory neocortex and rhinal cortex. It is not known whether the ACC is required for the transitory Pavlovian phenomenon of mediated learning. The present experiments and previous data, discussed above, suggest a transitory role for the ACC in learning. The present experiments also suggest a deficit in the CS specificity of Pavlovian associations in ACC-lesioned rats, but US specificity (which, according to this theory, is necessary for mediated learning) has not been tested, and it is not known how the ACC interacts with sensory cortex during learning. However, a deficit in mediated learning would not be sufficient explanation for the relative resilience of overtrained behaviour to ACC lesions observed by Gabriel and colleagues (see pp. 98–99).

The observation that ACC lesions do impair autoshaping performance (Experiment 2), even if transiently, suggests that the ACC does not merely 'supervise' learning in other systems, but stores or retrieves associations itself. The observation that the role of the ACC appears to diminish with time (reviewed above and on pp. 98–99) may be explained in two ways. As suggested above, the ACC might

contribute to a particular *form* of Pavlovian representation, whose importance normally diminishes with extended training. However, it is not necessary to postulate that the balance of representations controlling Pavlovian performance changes in order to explain the involvement of the ACC early in learning. The ACC might simply form a temporary store for the *same* kind of representations that eventually govern performance. It has been suggested that, early in training, associations are set up in the ACC and used to 'teach' other neural systems (Gabriel *et al.*, 1980a). The early and late representations might be of the same kind (be they CS–$US_{sensory}$, CS–US_{value}, CS–affect, etc.), with the ACC serving as a rapid but impermanent associator. On the basis of the evidence available to date, this seems the most likely explanation, but the issue is not settled.

Synthesis: a suggested role for the ACC in 'disambiguating' stimuli for its corticostriatal circuit

The ACC has been shown to be critical in a wide range of appetitive and aversive tasks in which two or more similar stimuli must be discriminated on the basis of their association with reinforcement (in the autoshaping and two-stimulus discriminated approach/conditioned reinforcement tasks presented in this chapter, and by Gabriel *et al.*, 1991a; Powell *et al.*, 1994; Bussey *et al.*, 1997a; Bussey *et al.*, 1997b; Parkinson *et al.*, 2000c). It is unlikely that these results reflect an attentional deficit (Muir *et al.*, 1996) or a failure of spatial discrimination (Gabriel *et al.*, 1991b; Powell *et al.*, 1994; Bussey *et al.*, 1996; Bussey *et al.*, 1997b). However, ACC-lesioned rats can discriminate between two stimuli of different modalities (Experiment 3, this chapter) and between two visual stimuli differing in a primary submodality such as colour (Bussey *et al.*, 1997b, Experiment 3). In at least some studies, ACC-lesioned animals have exhibited an early failure to discriminate between two CSs, but eventually improved or succeeded completely, implying that the early failure to discriminate was not due to a primary perceptual deficit (Experiment 4, this chapter; Gabriel, 1990; Gabriel *et al.*, 1991a; Gabriel, 1993; Parkinson *et al.*, 2000c). No deficits are apparent when ACC animals are required only to discriminate temporally between the presence and absence of a single CS, whether appetitive or aversive, and as judged by a wide variety of response systems; thus, ACC-lesioned rats were unimpaired at a single-stimulus discriminated approach task, responding for conditioned reinforcement, conditioned freezing, and PIT.

For at least one subset of these behaviours — locomotor approach — it seems very likely that the ACC influences behaviour through the Acb. The ACC projects strongly to the AcbC, which in turn projects to locomotor control regions of the ventral pallidum; lesions of the AcbC impair both autoshaping (Parkinson *et al.*, 2000c) and single-stimulus discriminated approach (Parkinson *et al.*, 1999b), and a functional connection between the ACC and the AcbC is necessary for autoshaping to develop (Parkinson *et al.*, 2000c). The effect of ACC and AcbC lesions on autoshaping differ, however; while ACC lesions typically result in 'disinhibited' responding to the CS– (Bussey *et al.*, 1997a; Parkinson *et al.*, 2000c), AcbC lesions impair the conditioned approach response itself (Parkinson *et al.*, 2000c), just as they prevent conditioned approach to a single CS (Parkinson *et al.*, 1999b).

On the basis of these data, it is suggested that the ACC contributes to a sensorimotor aspect of conditioning (Parkinson *et al.*, 2000a). Without the ACC, animals can learn an 'affective' response to CSs; thus, they perform normally in the single-stimulus discriminated approach task, and exhibit PIT. They can also call up a motivational representation of the US (a role attributed to the BLA; Everitt *et al.*, 2000a), and so acquire a new response with conditioned reinforcement, and acquire conditioned freezing. However, CS specificity of these representations is impaired in ACC-lesioned rats; as a result, tasks that depend upon stimulus–reinforcer associations when similar stimuli must be discriminated require the ACC

(including autoshaping, and 8-pair concurrent visual discrimination). According to this hypothesis, the ACC disambiguates the stimuli for the rest of the limbic circuit of which it is a part (as illustrated in Figure 40).

For tasks in which the ventral striatum is the 'output' structure for behaviour, this 'extra' controlling circuitry may be a necessary refinement, as the striatum is itself anatomically capable only of discriminating amongst linearly separable cortical inputs (Wickens & Kötter, 1995, p. 206); on its own, the striatum should therefore be unable to perform an exclusive-or (XOR) discrimination (A^+, B^+, AB^-). Furthermore, discrimination of two linearly separable input patterns A and AB where A→reward, AB→0 requires an inhibitory projection from unit B. As the direct cortical inputs to the striatum are all glutamatergic (excitatory), the striatum would seem unable to solve even this discrimination. However, the different cortical afferents to the Acb have been shown to gate each other's glutamatergic inputs (Cools *et al.*, 1991; Pennartz & Kitai, 1991; Floresco *et al.*, 1998) and in this sense, the ACC may operate to control the input of affective information (perhaps from the BLA) in order to direct motivational responses towards appropriate environmental stimuli. This hypothesis therefore predicts an impairment in configural or XOR discriminations in ACC-lesioned subjects.

Note that this account of ACC function does not suggest a primary sensory or perceptual role — ultimately, ACC-lesioned rats may make the sensory discrimination — but, more specifically, a role in the retrieval of appropriate affective information for specific stimuli that are attended to, and thus in the production of appropriate affective responses to stimuli (see also Turken & Swick, 1999). The concept that even early sensory representations may be neurally dissociated on the basis of the *response* for which the representation is used is not new (Goodale & Milner, 1992); from this perspective, the ACC may be critical for discriminating stimuli *for the purposes of stimulus–reinforcer associations*, but not for other perceptual processes. ACC-lesioned animals would be able to discriminate a CS+ from a CS– perceptually, but be unaware as to the correct stimulus towards which appropriate affective responses should be made.

This hypothesis can be shown to account for the impairment of avoidance learning by ACC lesions in rabbits (Gabriel, 1990; Gabriel *et al.*, 1991a; Gabriel *et al.*, 1991b), for in this task formation of specific stimulus–reinforcer associations confers an advantage. Indeed, in active avoidance behaviour an internally generated expectation of reinforcement may be particularly relevant, as successful behaviour results in the absence of primary reinforcement. As discussed earlier, the ACC is a site where discriminated activity (discharge to the CS+ but not the CS–) occurs early in discriminated avoidance training (Gabriel *et al.*, 1977). More generally, the ACC may provide stimulus–reinforcer information to other response systems. Thus, projections from the ACC to the CeA (see Fisk & Wyss, 1997), ultimately influencing brainstem effector mechanisms, may direct autonomic responses toward appropriate environmental stimuli. This is supported by studies demonstrating a role for the ACC in the coordination of autonomic responses (Buchanan & Powell, 1982b; Neafsey *et al.*, 1993), and more directly by the finding that ACC lesions disrupt discriminated autonomic responses to a CS+ and CS– whilst not impairing the response itself (Powell *et al.*, 1994), much like the effects of ACC lesions on skeletomotor responses in the autoshaping procedure (Bussey *et al.*, 1997a; Parkinson *et al.*, 1999c). Finally, in tasks where stimulus–reinforcer learning is a disadvantageous strategy, ACC lesions can improve performance (Bussey *et al.*, 1996) (see p. 73).

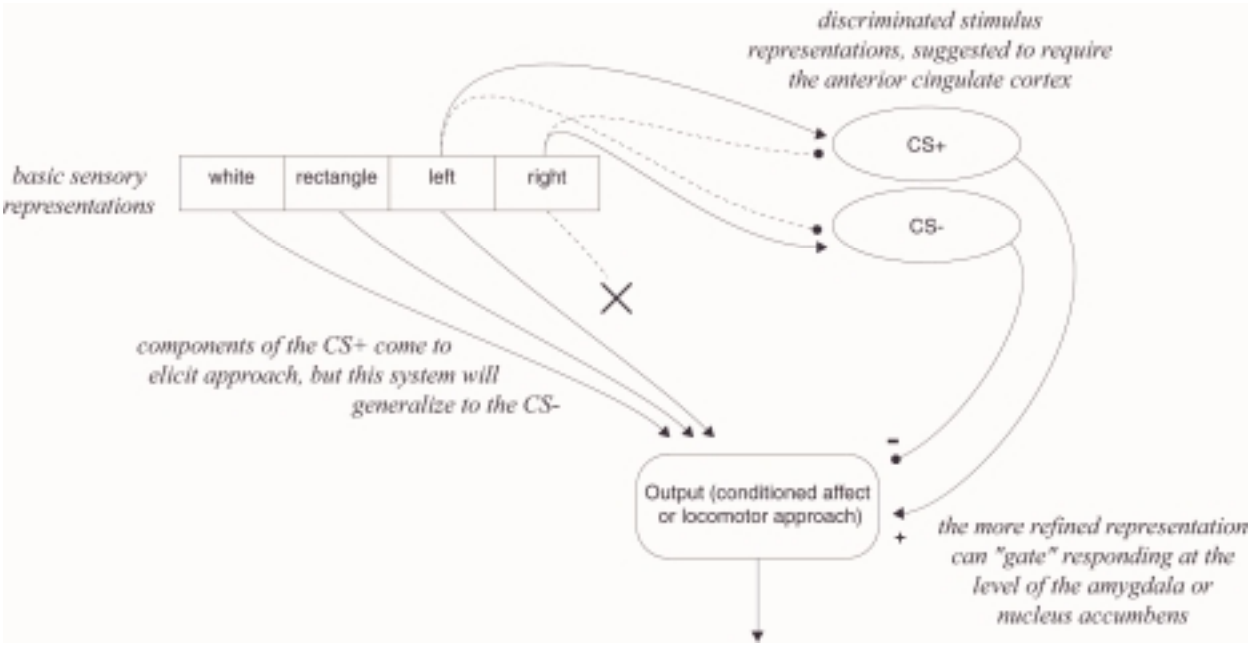

Figure 40 illustrates 'disambiguation' of stimuli, applied to autoshaping. In this example, the CS+ is a white rectangle on the left and the CS– is an identical stimulus on the right. Expression of autoshaping requires the CeA, AcbC, and Acb dopamine. In the absence of discriminated activity in the ACC, animals generalize from the CS+ to the CS–, impairing their behavioural discrimination in a disinhibited fashion. However, the animals will still discriminate between the presence and the absence of the CS+.

Interactions of the ACC with the amygdala and perirhinal cortex

The way in which the ACC interacts with the amygdala is far from clear, and requires further investigation. On the one hand, it has been suggested that discriminated neuronal activity in the ACC depends on the amygdala. Poremba & Gabriel (1999), using rabbits, found that inactivation of the amygdala using the GABA receptor agonist muscimol blocked discriminated avoidance learning and cingulate neuronal plasticity, replicating previous findings with electrolytic lesions (Poremba & Gabriel, 1997). Furthermore, the amygdala-inactivated rabbits showed no savings effect, suggesting that they failed to learn while the amygdala was inactivated, not that they simply failed to perform the response. However, amygdala inactivation had no effect on well-trained animals. (Indeed, further training was not necessary to reduce the amygdalar involvement; simple passage of time was enough!)

On the other hand, studies of autoshaping do not suggest the involvement of the BLA in ACC-dependent tasks: BLA-lesioned rats have been shown to acquire normal discriminated autoshaping behaviour (Parkinson *et al.*, 2000b). Poremba & Gabriel (1999) suggested that the amygdala is critical for learning about 'emergency' situations involving stimuli of extreme motivational valence, which might account for the studies just described, but the BLA is clearly involved in other appetitive tasks (Everitt *et al.*, 1999; Everitt *et al.*, 2000a).

It is possible that these data may be reconciled by closer consideration of the anatomical site concerned. Poremba & Gabriel (1999) aimed their muscimol injections at the BLA, but did not specify the degree to which the CeA was affected. In their earlier study (Poremba & Gabriel, 1997), the CeA was damaged by the electrolytic lesions used, and there was a significant correlation between CeA (and lateral amygdala) damage and performance of the avoidance task, while this correlation was not significant for the BLA. These results are easily reconciled with autoshaping studies showing that the CeA, but not the BLA, is critical for the development of autoshaping (Parkinson *et al.*, 2000b). Given that the amygdala

plays a time-limited role in the acquisition of the avoidance task used by Gabriel and colleagues (Poremba & Gabriel, 1997), it is extremely interesting to note that lesions of the CeA do *not* impair the performance of a well-trained autoshaped response (Everitt *et al.*, 2000b) at a stage of training when ACC lesions do (Experiment 2). These results would be compatible with a role for the CeA in the learning process through which the ACC acquires specific stimulus–reinforcer associations in this task. As the amygdalocortical projections to the ACC arise predominantly from the basal nucleus (see Amaral *et al.*, 1992, pp. 46–47), it may be that the CeA influences cortex through its projections to the chemically defined systems of the brainstem reticular formation. There are precedents for this suggestion (see Everitt *et al.*, 2000a); for example, the CeA has been shown to have a role in upregulating the associability of conditioned stimuli via its projections to the basal forebrain cholinergic systems (Holland & Gallagher, 1993a; Chiba *et al*., 1995; Holland, 1997), which project to wide areas of cortex, including the ACC (Butcher, 1995); in turn, regulation of cortical CS representations has been shown to depend upon the cholinergic innervation of the cortex (Weinberger, 1995).

Finally, while the ACC has been implicated in stimulus–reinforcer learning, investigations of stimulus–*stimulus* learning have implicated the perirhinal cortex (PRh) as a critical site for complex, cross-modal and configural associations (e.g. Saksida & Bussey, 1998; Murray & Bussey, 1999; Murray *et al*., 2000; Nicholson & Freeman, 2000; Saksida *et al*., 2000). It remains to be established whether the ACC and PRh interact when complex stimuli are associated with reinforcement, and what their relative contributions to behaviour are.

Comparison with other interventional studies in rodents

Can the hypothesis of ACC function developed above explain results from studies using very different paradigms?

Mice

Meunier *et al.* (1991) have studied spatial discrimination learning and reversal in a T-maze using mice. Mice with ACC lesions learned the initial acquisition and first reversal at the normal rate, but they were impaired during subsequent reversal sessions, failing to show positive learning transfer when compared to controls. Yet when all they had to do was learn the *same* discrimination over several days (repetition), there was no impairment. In fact, they learned the first repetition more easily than control animals. Meunier *et al.* interpreted the ACC deficit as an inability to remember the temporal order of previously acquired spatial responses, though the integrity of each individual response was maintained.

It is worth noting the timescale implied by this hypothesis. The reversal sessions of Meunier *et al*. (1991) were on consecutive days (in any one session, one arm of the T-maze was baited consistently). A memory for temporal order only helps in the solution of the reversal task if the animal is following a rule of the sort: ‘what was right yesterday? Let me do the other today.’ As justification for their hypothesis, Meunier *et al*. (1986) have shown that mice with ACC lesions can still demonstrate interproblem transfer in a maze task supposed to require the formation of a general rule, but not a rule involving temporal order. But this is insufficient justification to call this phenomenon ‘memory for temporal order’. The general form of the ‘temporal order’ hypothesis also makes clear predictions; for example, rats with ACC lesions should be impaired on a discrimination task using three stimuli presented in a sequence, where ABC→reward and BAC→no reward; this has not yet been tested.

If these findings are re-examined in the light of Bussey’s (1996) hypothesis, it could be that the ACC-lesioned mice in Meunier’s (1991) study successfully learned S–R associations that allowed them to perform normally on the initial acquisition session, first reversal and repetitive tests. PCC lesions impaired

mice on exactly these tests. However, the presence of the ACC might confer an ability to respond rapidly and flexibly to an environment with changing stimulus–reinforcement relationships, withholding responses to unrewarded stimuli, which in normal mice results in improved performance over the course of reversal training.

Rats

Interventional studies using rats have revealed other features of the phenotype of ACC lesions that are not all easy to encompass within the hypothesis outlined above. In particular, they emphasize *disinhibition* and *over-responding* in ACC-lesioned rats. Weissenborn *et al.* (1997) studied the acquisition of responding for intravenous cocaine under second-order schedules of reinforcement. ACC-lesioned rats exhibited greater locomotor activity (both spontaneous and cocaine-induced), they were more likely to self-administer excessive amounts of cocaine during acquisition, and while their dose–response curve was normal on a FR1 schedule, they responded at high rates throughout the fixed-interval phase of the second-order schedule, exhibiting an attenuated fixed-interval 'scallop'. Weissenborn *et al.* related this to Bussey's (1997a) hypothesis by suggesting that the rats had failed to learn the significance of the cocaine-associated stimulus that normally maintains responding on this schedule. Such hyperactivity was not found in the present series of experiments; as discussed on pp. 90 / 97, this may have resulted from differences in lesion site. Another factor to be considered in Weissenborn *et al.*'s (1997) experiments was chronic cocaine experience, which might interact with the effects of ACC lesions.

Muir *et al.* (1996) studied a five-choice serial reaction time task (5CSRTT) in which rats must wait for the presentation of one of five brief visual stimuli, and then respond at the location of the stimulus in order to gain reward. Muir *et al.* found that ACC lesions had no effect on the accuracy of visual attentional performance, either at baseline or with superimposed attentional manipulations (varying the stimulus duration or the ITI, or interpolating bursts of white noise). However, the lesions increased the number of premature, anticipatory responses (in which the animal responds before a stimulus has been presented), increased the number of 'perseverative' responses (in which the animal responds several times to the location where a stimulus was recently presented), and decreased the number of errors of omission. The same animals performed normally on a test of passive avoidance, in which electric shock is delivered in one half of a two-chamber apparatus and the subject subsequently avoids the 'dangerous' chamber.

Clearly, these results may be explained in terms of disinhibited or impulsive motor responding. The results of Muir *et al.* (1996) suggest that the ACC-lesioned rats were unable to withhold responding to locations where rewarded stimuli were intermittently presented. However, there was no evidence of such a deficit in the present series of experiments; locomotor hyperactivity was not apparent, no test of free-operant responding demonstrated hyperactivity, and ACC-lesioned rats did not over-respond to the location of a rewarded CS when that CS was not present. Differences in lesion site may partly be responsible for these discrepancies — the present lesions were more anterior to those used by Muir *et al.* (1996, Figure 1C) (see also Figure 14 caption, p. 72) and recent results suggest that ACC lesions centred on the perigenual region, similar to those used in the present experiments, do not produce deficits on the 5CSRTT (A. Christakou, unpublished observations; personal communication, 10 October 2000). It should be noted that the psychological basis of premature responding in the 5CSRTT is not well understood; however, it is not clear how these results can be reconciled in terms of a single deficit. Investigating the role of the ACC in explicit tests of 'motor impulsivity' (see Evenden, 1999b) such as the go / no-go task (e.g. Harrison *et al.*, 1999) may be well worth while. This task (in which subjects must to respond to one stimulus but withhold responding to another stimulus) has the added advantage that the degree to which one response is prepotent can be varied by altering the relative proportion of 'go' and 'no-go' trials, pro-

viding a further test of inhibitory control. The role of the ACC in impulsive *choice* will be investigated in Chapter 7.

Other studies of the rat ACC have frequently concentrated on the region directly superior to PL (equivalent to dorsal mPFC in Figure 14C, p. 72), an area that was not the focus of the present experiments. Despite the differences in location, there are some commonalities among findings. For example, such lesions have minimal effects on rats' spatial discrimination or working memory (Neave *et al*., 1994; Ragozzino *et al*., 1998) or their ability to switch strategies between the use of visual and spatial cues (Ragozzino *et al*., 1999), yet produce severe impairments in a number of radial maze tasks (Seamans *et al*., 1995): rats with reversible (lidocaine) lesions of the ACC preferentially revisit previously baited arms. This last deficit has clear analogies with the 'disinhibited', perseverative behaviour observed in the 5CSRTT by Muir *et al.* (1996), but might also be explicable in terms of a failure to inhibit responding to unrewarded stimuli (maze arms) in a situation in which there are many stimuli, differentially associated with reward, and in which the rewarded stimulus changes rapidly. Seamans *et al.* (1995, p. 1071) describe the ACC as providing response flexibility by suppressing the effect of simple stimulus–reward associations on behaviour, an interpretation clearly compatible with the present results.

Homology between rodent and primate ACC

In order to examine the present results in the context of primate lesion and imaging studies, it will be vital to consider the homology between rodent and primate ACC. The various terminologies used to describe rat ACC are summarized by Neafsey *et al.* (1993) and reproduced in Table 11. The lesions used in the present series of experiments were of Cg1 and Cg2 situated at, and caudal to, the genu of the corpus callosum, corresponding to area 24a and caudal area 24b in the rat (see Figure 14, p. 72; Table 9, p. 71; and Table 11). In turn, these areas have a homologue in monkey and human ACC, as judged by their pattern of afferent and efferent connections (Öngür & Price, 2000); Figure 41 depicts rat prefrontal cortex and maps of macaque and human PFC that were designed to represent homologous regions with the same area number (Öngür & Price, 2000). A rough equivalence may therefore be drawn across the three species.

Table 11. Cytoarchitechtonic subdivisions of anterior cingulate cortex, from Neafsey (1993); compare Figure 14 (p. 72). (Abbreviations are the same as those in Figure 41, with the addition of Prcm, medial precentral cortex; Cg1–Cg3, cingulate cortex; HP, hippocampal rudiment.)

Source	Dorsal						Ventral
Krettek & Price (1977)	Prcm	ACd	ACv	PL	IL	DPC	TT
Krieg (1946); Vogt & Peters (1981)	4	24b	24a	32	25	25	TT
Zilles & Wree (1985)	Fr2	Cg1	Cg2	Cg3	IL	IL	HP
Uyling & van Eden (1990)	Fr2	ACd	ACv	PL	IL	IL	TT

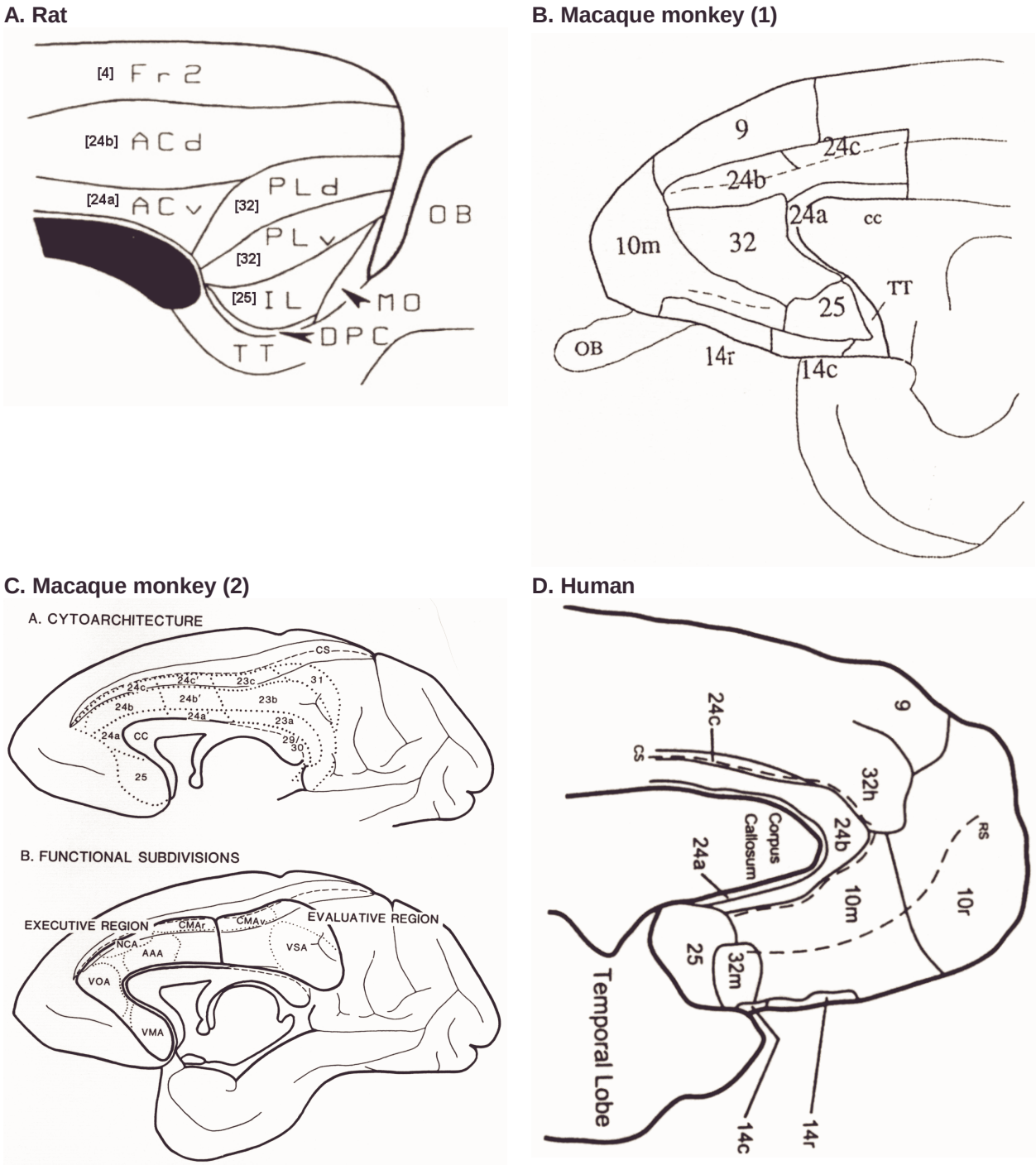

Figure 41. Medial prefrontal cortex in rats, monkeys, and humans (not to the same scale). The top of the diagram is the superior direction in each panel. **A:** Medial frontal cortical regions in the rat, rostral to the right, from Neafsey *et al.* (1993); compare Figure 14 (p. 72). The number-based designations from Table 11 (p. 120) have been superimposed upon the original figure. (Fr2, frontal cortex 2; ACd / ACv, dorsal / ventral anterior cingulate cortex; PLd / PLv, dorsal / ventral prelimbic cortex; IL, infralimbic cortex; MO, medial orbital cortex; DPC, dorsal peduncular cortex; TT, taenia tecta; OB, olfactory bulb.) The corpus callosum is shown in black. **B:** Orbital and medial prefrontal cortex in the macaque monkey, rostral to the left, from Carmichael & Price (1994) via Öngür & Price (2000) (cc, corpus callosum). **C:** Rhesus macaque monkey cingulate cortex, rostral to the left, from Vogt *et al.* (1992), showing functionally specialized regions. (CS, cingulate sulcus; VMA, visceromotor area; VOA, vocalization area; NCA, nociceptive area; CMAr / CMAv, rostral / ventral cingulate motor areas; AAA, attention-to-action area; VSA, visuospatial area.) **D:** Human medial prefrontal cortex, rostral to the right, from Öngür & Price (2000).

Interventional studies in primates

Most studies of the primate ACC are rather unhelpful for comparison to the present studies of the contribution of the ACC to conditioned behaviour. This is for two reasons. Firstly, many primate interventional studies concerned with the ACC have used non-excitotoxic lesion techniques (see Devinsky *et al.*, 1995). The nature of the lesion is critically important in this region; any lesion that destroys the cingulum bundle will disconnect large portions of cortex, for this bundle contains not only all afferent and efferent connections of the cingulate cortex, but also fibres that pass to and from the rest of the prefrontal (including orbitofrontal) cortex, notably the reciprocal connections between the prefrontal cortex and the medial temporal lobe (Vogt, 1993, pp. 24–25). (The functions of the orbitofrontal cortex were briefly reviewed in several contexts in Chapter 1, pp. 39 / 45 / 55, and will not be considered here.) Secondly, most studies have concentrated on unconditioned behaviour. It is clear that regions of the primate ACC are involved in a bewildering range of motivationally-oriented unconditioned behaviour. Devinsky *et al.* (1995), reviewing these studies, consider the ACC to be the 'anterior executive' region of the cingulate cortex (cf. Figure 41C); the ACC is further subdivided into an 'affect' region (area 25 and rostral area 24) and a 'cognition' region (caudal areas 24′ and 32′, nociceptive cortex and the cingulate motor areas).

Functional subdivisions of the rhesus monkey ACC are shown in Figure 41C. As the regional names would suggest, the primate ACC has been implicated in the perception of pain (and by reference to the rodent literature, avoidance learning); as a part of premotor cortex; in visceromotor control (and, again by reference to the rodent literature, classically conditioned autonomic responses); and in vocalization that has social or emotional content (Devinsky *et al.*, 1995), interpretations that are supported by stimulation studies in humans and other primates. In addition, the ACC has been implicated in action selection or 'attention to action' (discussed below). This last concept has the most relevance to the present rat experiments, and has been best studied in the human; therefore, studies of the human ACC will be considered next.

Correlational studies in humans

Isolated destruction of the human ACC is rare (Devinsky *et al.*, 1995), so lesion studies of humans have mostly been of patients with frontal lobe tumours. Lesions of the ACC have produced a wide variety of symptoms, including apathy, inattention, autonomic dysregulation, emotional instability, and akinetic mutism (Devinsky *et al.*, 1995; Bush *et al.*, 2000). However, such studies are often compromised by a lack of anatomical specificity: tumours and epileptic foci do not respect anatomical boundaries, and if these tumours involve the ACC, their resection inevitably compromises the cingulum bundle, and thus orbitofrontal cortex function. Indeed, many of the patients studied by Damasio and colleagues (see Chapter 1, p. 55) have ACC damage in addition to orbitofrontal lesions (Bechara *et al.*, 2000). Some of the best studies of human ACC are therefore correlational, in that they aim to observe differences in ACC activity that are correlated with task performance or mental state, without using interventional techniques to alter ACC function and observe the effect on behaviour. While interventional techniques are required to show that the ACC has a causal role in a particular aspect of behaviour, correlational techniques have provided useful information about ACC function.

Emotional states, emotionally significant stimuli, and mood

The anterior, ventral ACC (Brodmann's areas 24a / b and 25), part of the 'affective' subdivision of the ACC (Devinsky *et al.*, 1995), is now strongly implicated in the pathology of depression in humans (Bench *et al.*, 1992), as well as in the control of normal mood. Drevets *et al.* (1997) observed that this

area of the ACC ('subgenual prefrontal cortex' or subgenual area 24; see Öngür *et al*., 1998) showed decreased blood flow in unmedicated familial bipolar and unipolar depressives using positron emission tomography (PET), though this was in part due to a reduced grey matter volume as assessed by magnetic resonance imaging (MRI); if this is corrected for, blood flow per unit volume was increased (Mayberg, 1997; Drevets, 2000). Mayberg *et al.* (1994; 1996; Mayberg, 1997) have demonstrated similar abnormalities; metabolic activity in rostral ACC (rostral area 24a / b) is also unique in differentiating those depressed patients who eventually respond to pharmacological antidepressant therapy from those that do not (Mayberg *et al*., 1997). Areas 24a / b and 25 are also part of a cortical network whose metabolic activity alters in normal sadness (Mayberg *et al*., 1999). Mayberg *et al.* (1999; Mayberg, 2000), reviewing these data, have suggested that hyperactivity of subgenual area 24 / area 25 is a primary factor in sadness and depression, causing reciprocal suppression of metabolism in adjacent ACC and dorsolateral prefrontal cortex, which may explain the efficacy of surgical destruction of the subgenual cingulate as a therapy for refractory depression.

Imaging studies have also shown that the human ACC responds to emotionally significant stimuli. It is reliably activated by cocaine-associated cues in cocaine users, more so than by neutral stimuli in the same individuals, or by cocaine-associated cues in non-users (Maas *et al*., 1998; Childress *et al*., 1999; Garavan *et al*., 2000); such activation may be associated with cocaine craving (e.g. Volkow *et al*., 1996; Volkow *et al*., 1997; Maas *et al.*, 1998; Childress *et al.*, 1999). While fewer studies have examined the effects of natural reinforcers, it appears that the ACC is similarly activated by emotionally significant non-drug stimuli in normal humans (sexual images; Garavan *et al.*, 2000).

Attention, conflict monitoring, error detection, and action selection

Attention and action

In humans, PET studies have provided evidence that the ACC is involved in executive attention. In attentional target detection tasks, blood flow increases with the number of targets to be detected, while flow to the anterior cingulate gyrus is reduced below baseline during the maintenance of vigilance (reviewed by Posner, 1995, pp. 620–621). These PET studies have also suggested a role for the ACC in 'willed' tasks, perhaps with a motivational role; along with dorsolateral PFC, blood flow to ACC is significantly increased in tasks requiring a voluntary choice of action, compared to routine, well-rehearsed actions (Frith *et al*., 1991).

Detecting errors: the error-related negativity (ERN) and its localization to the ACC

An event-related brain potential (ERP) is an electroencephalographic (EEG) potential that has been time-locked to an event. While studying choice reaction times (RTs) in humans, it was observed that a negative EEG potential was evoked when subjects made an error (Falkenstein *et al*., 1990; Gehring *et al*., 1990; Gehring *et al*., 1993). This potential was named the error-related negativity (ERN).

The literature on the ERN is large and will be summarized briefly (for reviews, see Brown, 1999; Falkenstein *et al*., 2000; Scheffers & Coles, 2000). The ERN begins to develop at around the time of the erroneous response, and peaks ~100 ms later; it is small or non-existent following correct responses. The ERN is hypothesized to reflect part of a process in the brain that monitors ongoing actions, compares them with intended actions, detects any mismatch, flags the presence of an error if mismatch exists, and takes action to correct ongoing or future performance (e.g. Gehring *et al.*, 1993; Bernstein *et al*., 1995; Miltner *et al*., 1997). There is wide support for this general view. ERNs are generated in a variety of tasks, including visual and auditory discriminations, go / no-go tasks, and the Eriksen flankers task, in

which subjects must respond to the identity of a briefly-presented target letter (H or S) that is either flanked by compatible letters (e.g. HHHHH) or by letters associated with the alternative response (e.g. SSHSS) (Gehring *et al.*, 1993). This generality suggests that the ERN is not a reflection of the stimuli used in the tasks. The ERN also occurs regardless of the particular motor response being measured (Holroyd *et al*., 1998). Nor does it depend on a particular type of error: in one go/no-go task, subjects may make errors of choice, in which they respond with the incorrect hand on 'go' trials, or errors of action, in which they respond (with either hand) on 'no go' trials; both these conditions generate an ERN (Scheffers *et al.*, 1996). The error-detection process appears to rely on the same representations that govern task performance: for example, when well-practised subjects perform RT tasks for long periods without sleep, their performance worsens as a result of impaired perceptual processing, and the ERN declines as the representation of the correct response is degraded (Scheffers *et al*., 1999). The ERN also reflects subjects' perception of accuracy. Scheffers & Coles (2000) gave subjects a task in which the visual stimuli governing performance were degraded. Regardless of behavioural accuracy, the ERN at the time of responding correlated with subjects' subsequent reports of how inaccurate the response was — that is, errors perceived as such were associated with large ERNs, but so were correct responses perceived as errors. The ERN was smaller on trials where the subject was unsure whether an error had been made (due to limitations on the available data), and smallest when the subject thought he had responded correctly (even when an error had been committed). Finally, when the subject must learn to respond based on a delayed feedback signal, an ERN is generated in response to feedback indicating incorrect performance (Miltner *et al*., 1997), a moment at which no response is being made.

Much of the controversy about the precise significance of the ERN is attributable to the bidirectional hypothesis of its function stated above: errors are suggested to generate the ERN, and the ERN is suggested to correct errors. Thus, the ERN is associated with conditions of error; for example, it is larger when the task instructions emphasize response accuracy over speed (on trials matched for RT; Gehring *et al.*, 1993) and when responses are late in a task in which speed is emphasized (Luu *et al*., 2000b); greater motor discrepancies between intended and actual responses also generate larger ERNs (Bernstein *et al.*, 1995). However, the ERN is also associated with correction processes: large ERNs are also associated with less forceful errors that are more likely to be followed by correction responses (with longer RTs), and large ERNs are associated with more conservative behaviour in the future (see Scheffers & Coles, 2000).

In support of early speculations (Gehring *et al.*, 1993), equivalent dipole analyses, together with neurophysiological and biophysical considerations, point to the ACC as the likely source of the ERN (Dehaene *et al*., 1994; Coles *et al*., 1998; Bush *et al*., 2000) — indeed, the ERN may have first been noticed by researchers recording directly from the ACC (area 24) in macaque monkeys (Gemba *et al*., 1986). The ACC has thus been likened to a supervisory attentional system (Norman & Shallice, 1986) (see Grossman *et al*., 1992). Given the importance of error signals in many models of learning (famously, that of Rescorla & Wagner, 1972), there has been considerable interest in relating the ERN to learning (see Kopp & Wolff, 2000; Schultz & Dickinson, 2000). Another well-studied candidate for an error signal in the brain is the dopamine system; midbrain dopamine neurons in the SNc/VTA fire in response to primary rewards, but come to respond instead to signals predictive of reward, and signal discrepancies between predicted and experienced rewards (Mirenowicz & Schultz, 1994; Schultz *et al*., 1995b; Mirenowicz & Schultz, 1996; Schultz *et al*., 1997). This has led to the incorporation of the DA signal in models of learning, most notably those based on the algorithm entitled *temporal difference* (TD) learning (Sutton, 1988; Barto, 1995; Houk *et al*., 1995). In an intriguing development, it has been suggested that

the TD error signal conveyed by DA neurons is responsible for the ERN in the ACC (Holroyd *et al*., 1999) — intriguing not least in relation to the suggestion (p. 117) that the CeA, a likely controller of the VTA, may regulate ACC function. However, the data summarized here suggest that the ACC's functions are more to do with response errors than errors of reward prediction (Schultz & Dickinson, 2000).

Finally, the ERN has been shown to be abnormal in psychopathological states to which the ACC has been suggested to contribute. A potential link from the ERN literature to the involvement of the ACC in depression (discussed above, p. 122) has been provided by Luu *et al.* (2000a), who found that the ERN was larger in normal humans who scored highly for the personality dimensions of 'negative emotionality' and 'negative affect' as assessed by questionnaires. Similarly, Gehring *et al.* (2000) found that the ERN was larger in patients suffering from obsessive–compulsive disorder (OCD), a disorder in which self-monitoring and error correction may be pathologically enhanced, in which ACC metabolism is abnormal, and which has been successfully treated surgically by cingulotomy (Baer *et al*., 1995; Devinsky *et al.*, 1995; Gelder *et al*., 1995, pp. 180–185; Breiter *et al*., 1996; Busatto *et al*., 2000).

Activating the ACC: response competition and the Stroop test

Studies of the ERN are supported by an array of functional imaging data implicating the ACC in error-related tasks. The spatial precision of PET and functional MRI (fMRI) far exceeds that of the EEG; thus, the anatomical basis of activation focus can be accurately localized. However, the temporal resolution of PET and fMRI is far poorer than the EEG (while the technique of magnetoencephalography or MEG, which has high spatial and temporal resolution, is presently only suitable for superficial cortical sites). Inevitably, the poorer temporal resolution of functional imaging has led to controversy about the significance of activation foci within the ACC.

The Stroop test (Stroop, 1935) is a prototype of the tasks that increase metabolic activity in the ACC. In the Colour Stroop (Figure 42), the subject must report the colour of a series of words, while ignoring the word itself. The task has congruent and neutral conditions, in which the word itself helps or does not contribute to performance (Figure 42, left and middle columns), but in the incongruent condition, each word is the name of a colour that differs from the colour in which the word is printed (Figure 42, right).The incongruent condition of many variants of the Stroop test strongly activates a focus in the ACC (Pardo *et al*., 1990; Bench *et al*., 1993; Carter *et al*., 1995; Derbyshire *et al*., 1998; MacDonald *et al*., 2000), just as the Stroop test elicits an ERN from the ACC (Liotti *et al*., 2000). The precise locus depends on the nature of the task; thus, while the Counting Stroop (prototype: report the number of words present, even when the words are numbers) activates the 'cognitive', caudal division of the ACC, the Emotional Counting Stroop (prototype: count neutral or emotionally-charged words, such as MURDER) activates the 'affective' division, rostral and inferior to the genu of the corpus callosum (Bush *et al*., 1998; Whalen *et al*., 1998; Bush *et al.*, 2000; MacLeod & MacDonald, 2000).

Evaluation of errors, or evaluation of response competition (conflict)? As the incongruent condition of the Stroop test activates the ACC even when behavioural performance is accurate (see MacLeod & MacDonald, 2000), it has been suggested that the ACC evaluates the degree of response competition or conflict, rather than simply detecting errors. In a different continuous performance task, Carter *et al.* (1998) similarly observed that the ACC is not only activated when incorrect responses are made, but when correct responses are made under situations of high response competition. Similar results have been obtained by Rogers *et al.* (1999), who observed ACC activation that was correlated with response conflict using a decision-making task in which error rates were held constant. Carter *et al.* (1998; 1999) suggest that rather than implement a comparator process (correct versus actual responses), the ACC monitors compe-

blue	willow	red
yellow	trek	green
red	armchair	blue
green	prefect	yellow
blue	felicitous	blue
green	destructive	green
yellow	milk	yellow
blue	bore	blue
red	selection	red
yellow	karyotype	green

Figure 42. Activate your ACC: a version of the Stroop test. The subject is asked to read aloud the colour each word is printed in (ignoring the word itself), as accurately and rapidly as possible. The left-hand column illustrates a congruent condition, the middle column is a neutral condition, and the right-hand column is an incongruent condition. There is a reaction time cost for the incongruent condition and this condition strongly increases metabolic activity in the ACC.

tition between responses. They argue that 'conflict monitoring' is a better description of ACC function than 'error detection'.

Evaluation, or strategic selection of actions? Investigators have also sought to define whether the ACC is primarily evaluative, detecting errors or response conflict, or strategic, implementing 'selection for action' (a term originated by Allport, 1987, defined as 'processes that reduce the competition between potential responses to a stimulus') (see Carter *et al.*, 2000). As Devinsky and colleagues state, 'when a response selection is made, including the decision not to move, area 24′ is engaged' (Devinsky *et al.*, 1995, p. 298). There is experimental evidence both for an evaluative interpretation (e.g. Botvinick *et al.*, 1999; Carter *et al.*, 2000) and a strategic interpretation (e.g. Paus *et al.*, 1993; Awh & Gehring, 1999; Turken & Swick, 1999). When examining action selection, dissociations within the ACC have also been observed for different response modalities, suggesting that the 'executive control' functions of the ACC are separable according to the response system being controlled (Paus *et al.*, 1993; Awh & Gehring, 1999; Turken & Swick, 1999).

Two criticisms can be levelled at this approach, one practical and one functional. The practical problem with this approach is the potential for a bidirectional relationship between errors and ACC activation (for example, one might expect the following sequence: more errors → ACC activation → correction → fewer errors). This possibility complicates many of the studies cited (see MacLeod & MacDonald, 2000), especially when one allows that error correction may occur before the action is made. For example, an error-detector might be involved in the incongruent Stroop test because subjects start to generate internal representations of multiple responses (to the word and to the colour), one of which triggers an internal error signal, leading to on-line correction of behaviour. Response competition and error detection may share features. Part of the reason for the success of ERN research is that the EEG technique allows trial-by-trial monitoring (something that is difficult using PET or fMRI), but part has been due to experimental technique that breaks the bidirectionality described above — for example, by providing an error-related signal in the absence of responding (Miltner *et al.*, 1997), or by measuring the ERN when subjects' belief about the accuracy of their responses differs from the actual accuracy (Scheffers & Coles, 2000).

On a functional level, the distinction between evaluative and strategic functions may be — at least in part — doomed to failure. If an error-detecting process cannot correct errors, what good is it? If a super-

visory action-selection mechanism is not activated when response competition or the likelihood of error is high, then when? In this respect, consideration of the interaction *between* structures is likely to be as helpful as the consideration of the structures themselves. Recent studies are beginning to take this approach, considering, for example, the contribution of the dorsolateral PFC to the function of the human ACC (Cohen *et al*., 2000; Gehring & Knight, 2000).

Relating human and rodent studies

It would be optimistic to be able to relate the entire literature on human ACC function to studies of rats, mice and rabbits. In particular, there is little evidence to address the question of whether the rodent ACC responds to errors or response-conflict situations (though the macaque ACC does; Gemba *et al.*, 1986), and there are few anatomically well-specified human lesion studies investigating the behavioural role of the ACC. However, common themes can be drawn. The rostral division of the human ACC responds to stimuli of affective significance (e.g. Whalen *et al.*, 1998), as does the rabbit ACC (Gabriel *et al.*, 1980a; Gabriel *et al.*, 1980b; Gabriel & Orona, 1982; Gabriel *et al.*, 1991b). The rabbit ACC uses this information to contribute to the selection of actions in instrumental avoidance tasks, a function similar to that attributed to the human ACC, and both the human and the rodent ACC control a wide variety of skeletomotor and autonomic response systems (e.g. Paus *et al.*, 1993; Powell *et al.*, 1994; Devinsky *et al.*, 1995; Bussey *et al.*, 1997a; Awh & Gehring, 1999; Turken & Swick, 1999). The rat ACC contributes to the control of behaviour when faced with two or more similar stimuli predicting different outcomes (present experiments, and Gabriel *et al.*, 1991a; Powell *et al.*, 1994; Bussey *et al.*, 1997a; Bussey *et al.*, 1997b; Parkinson *et al.*, 2000c); analogies may be drawn with human 'response conflict' accounts. The human ACC is suggested to be activated by novelty or errors (Falkenstein *et al.*, 1990; Gehring *et al.*, 1993; Dehaene *et al.*, 1994; Berns *et al*., 1997; Coles *et al.*, 1998) and thus to be involved in learning (Kopp & Wolff, 2000; Schultz & Dickinson, 2000); it is activated early in the acquisition of new tasks (Raichle *et al.*, 1994; Petersen *et al.*, 1998). Similarly, the contribution of rodent ACC is most marked early in training, when most learning might be expected to occur (see pp. 99 / 113); the monkey ACC ERN is present only during learning, when errors are still being made (Gemba *et al.*, 1986), and the mouse ACC appears to contribute to performance when response–outcome contingencies are changing rapidly (Meunier *et al.*, 1991). It is to be hoped that future studies will begin to bridge these two literatures.