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Role of the Anterior Cingulate Cortex in the Control Over Behavior by Pavlovian Conditioned Stimuli in Rats

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To investigate the contribution of the anterior cingulate cortex (ACC) to stimulus–reward learning, rats with lesions of peri- and postgenual ACC were tested on a variety of Pavlovian conditioning tasks. Lesioned rats learned to approach a food alcove during a stimulus predicting food, and responded normally for conditioned reinforcement. They also exhibited normal conditioned freezing and Pavlovian–instrumental transfer, yet were impaired at autoshaping. To resolve this apparent discrepancy, a further task was developed in which approach to the food alcove was under the control of 2 stimuli, only 1 of which was followed by reward. Lesioned rats were impaired, approaching during both stimuli. It is suggested that the ACC is not critical for stimulus–reward learning per se, but is required to discriminate multiple stimuli on the basis of their association with reward.

The rodent anterior cingulate cortex (ACC) has been extensively implicated in stimulus–reinforcer learning, in aversive and appetitive situations. The ACC receives nociceptive information and is involved in the coordination of autonomic responses (Fisk & Wyss, 1997; Hsu & Shyu, 1997; Neafsey, Terreberry, Hurley, Ruit, & Frysztak, 1993); early studies found that aspirative lesions of the ACC attenuated classically conditioned bradycardia in the rabbit (Buchanan & Powell, 1982). The rabbit ACC is also involved in active avoidance behavior. Using a task in which rabbits must learn to step in response to a tone (conditioned stimulus, CS+) 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 within the ACC early in avoidance training (Gabriel, Foster, Orona, Saltwick, & Stanton, 1980; Gabriel & Orona, 1982; Gabriel, Orona, Foster, & Lambert, 1980; Gabriel, Vogt, Kubota, Poremba, & Kang, 1991). Lesions of the ACC impair the avoidance response (Gabriel, 1993; Gabriel, Kubota, Sparenborg, Straube, & Vogt, 1991), attributed to the loss of associative information about the significance of a discrete CS (Gabriel, Foster, et al., 1980, 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. The ACC is defined here as cingulate area Cg2 together with overlying Cg1 (Bussey, Muir, Everitt, & Robbins, 1997; Paxinos & Watson, 1998); it encompasses pre-/perigenual and postgenual regions and is shown in Figure 1. For example, Bussey, Muir, et al. (1997) 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 to obtain reward. Furthermore, ACC lesions impair the acquisition of stimulus–reward associations in a selective test of Pavlovian conditioning, namely autoshaping (Bussey, Everitt, & Robbins, 1997; Parkinson, Willoughby, Robbins, & Everitt, 2000). Autoshaping (Brown & Jenkins, 1968) is a measure of Pavlovian stimulus–reward learning in which subjects approach a CS that predicts reward. In a typical autoshaping task designed for use with rats (Bussey, Everitt, & Robbins, 1997), 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 behavior 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. This autoshaped conditioned approach response is generally held to be under the control of Pavlovian, not instrumental, contingencies (Browne, 1976; Jenkins & Moore, 1973; Mackintosh, 1974; D. R. Williams & Williams, 1969), and this has been confirmed for the rat autoshaping task described (Bussey, Everitt, & Robbins, 1997). In contrast to normal rats, ACC-lesioned rats fail to discriminate, approaching the CS+ and CS– equally (Bussey, Everitt, & Robbins, 1997). However, it is intriguing that the lack of discrimination in ACC-lesioned rats often takes the form of increased responding to the CS– rather than decreased responding to the CS+ (Bussey, Everitt, & Robbins, 1997; see also Cardinal et al., 2002; Parkinson, Willoughby, et al., 2000). A comparable result has been

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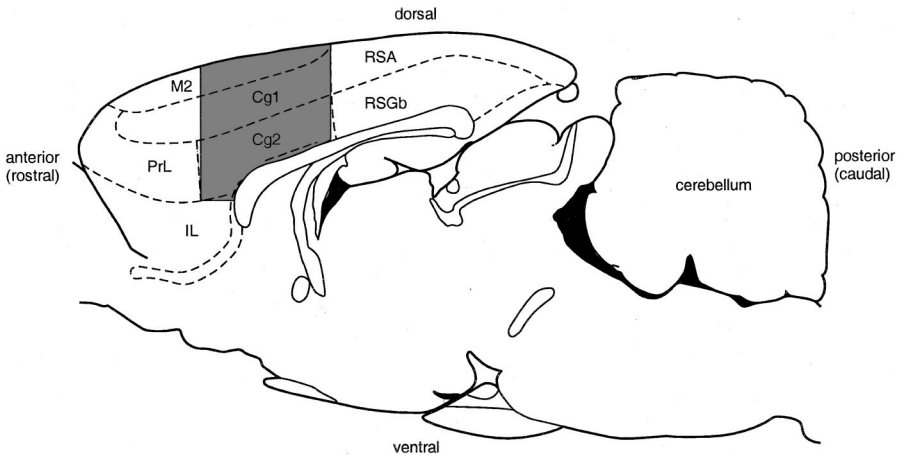

Figure 1. Sagittal paramedian view of the rat brain illustrating the definition of the anterior cingulate cortex used here and the region targeted in the present experiments (gray shading). Cg1, Cg2 = cingulate areas 1 and 2; PrL = prelimbic cortex; IL = infralimbic cortex; M2 = secondary motor cortex; RSA = retrosplenial agranular cortex; RSGb = retrosplenial granular *b* cortex. Reprinted from *The Rat Brain in Stereotaxic Coordinates,* 4th ed., G. Paxinos and C. Watson, Copyright 1998, with permission from Elsevier Science.

observed in a different task, which is nevertheless theoretically similar: Powell, Watson, and Maxwell (1994) found that ACC lesions do not prevent conditioned bradycardia to a CS predictive of shock, but impair discrimination between a CS+ and a CS–. 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, Everitt, & Robbins, 1996), it is unclear whether their impairment in the autoshaping task was due to a failure to learn CS–unconditioned stimulus (US) associations entirely (coupled with a tendency to overrespond to both the CS+ and the CS–) or a specific failure to inhibit responding to unrewarded stimuli.

The ACC, as defined above, projects to the nucleus accumbens core (AcbC; Brog, Salyapongse, Deutch, & Zahm, 1993; Heimer, Zahm, & Alheid, 1995, pp. 600–601; McGeorge & Faull, 1989; Parkinson, 1998; Zahm & Brog, 1992), primarily from the perigenual ACC (Parkinson & Everitt, 1998). This projection, and the AcbC itself, is also critical for the development of autoshaping (Parkinson, Robbins, & Everitt, 1996), suggesting that information stored in or retrieved by the ACC gains access to locomotor response systems via the AcbC (Cardinal et al., 2002; Parkinson, Cardinal, & Everitt, 2000; Parkinson et al., 1996; Parkinson, Willoughby, et al., 2000). In addition, the nucleus accumbens (Acb) is involved in another aspect of Pavlovian conditioning: conditioned reinforcement, in which subjects make an instrumental response to gain access to a CS. Following the discovery that intra-Acb injection of the psychostimulant *d*-amphetamine selectively enhances responding for conditioned reinforcement in a dose-dependent manner (Taylor & Robbins, 1984), attention 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 medial prefrontal cortex (mPFC), and the ACC (Brog et al., 1993; Parkinson, 1998; Zahm & Brog, 1992). Whereas lesions of the ventral subiculum and mPFC do not impair responding for conditioned reinforcement (Burns, Robbins, & Everitt, 1993), lesions of the BLA do so dramatically (Burns et al., 1993; Cador, Robbins, & Everitt, 1989). Though the ACC projects to both the BLA and the Acb and has been implicated in stimulus–reward association, it is not presently known whether the ACC plays a role in the ability of neutral stimuli to gain conditioned reinforcing properties.

To address these questions, Experiments 1 and 2 investigated the effects of excitotoxic lesions of the ACC (see Figures 1 and 2) on a number of tasks requiring subjects to form stimulus–reinforcer associations. In the first such task, a simple, temporally discriminated approach task, a single stimulus predicted the delivery of food at the same location, and approach to this stimulus was measured. Following establishment of the 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 (CRf). At the same time, the effects of intra-Acb amphetamine injections were examined in sham-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 CRf 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 itself a pure measure of Pavlovian conditioning. Although the CS predicted the arrival of food, allowing approach behavior to be classically conditioned to the CS, the CS might also have served as a discriminative stimulus (SD), signaling that an instrumental contingency existed between approach behavior and food acquisition. Therefore, the effects of ACC lesions were also tested using a number of purer measures of appetitive and aversive Pavlovian conditioning: autoshaping, Pavlovian–instrumental transfer (Estes, 1948; Lovibond, 1983), and conditioned freezing. Primary consummatory behavior was also assessed. As Experiments 1 and 2 revealed a dissociation between simple measures of Pavlovian conditioning, which were intact in ACC-lesioned rats, and autoshaping, which was impaired, Experiment 3 used a hybrid task to establish which psychological differences between the two tasks

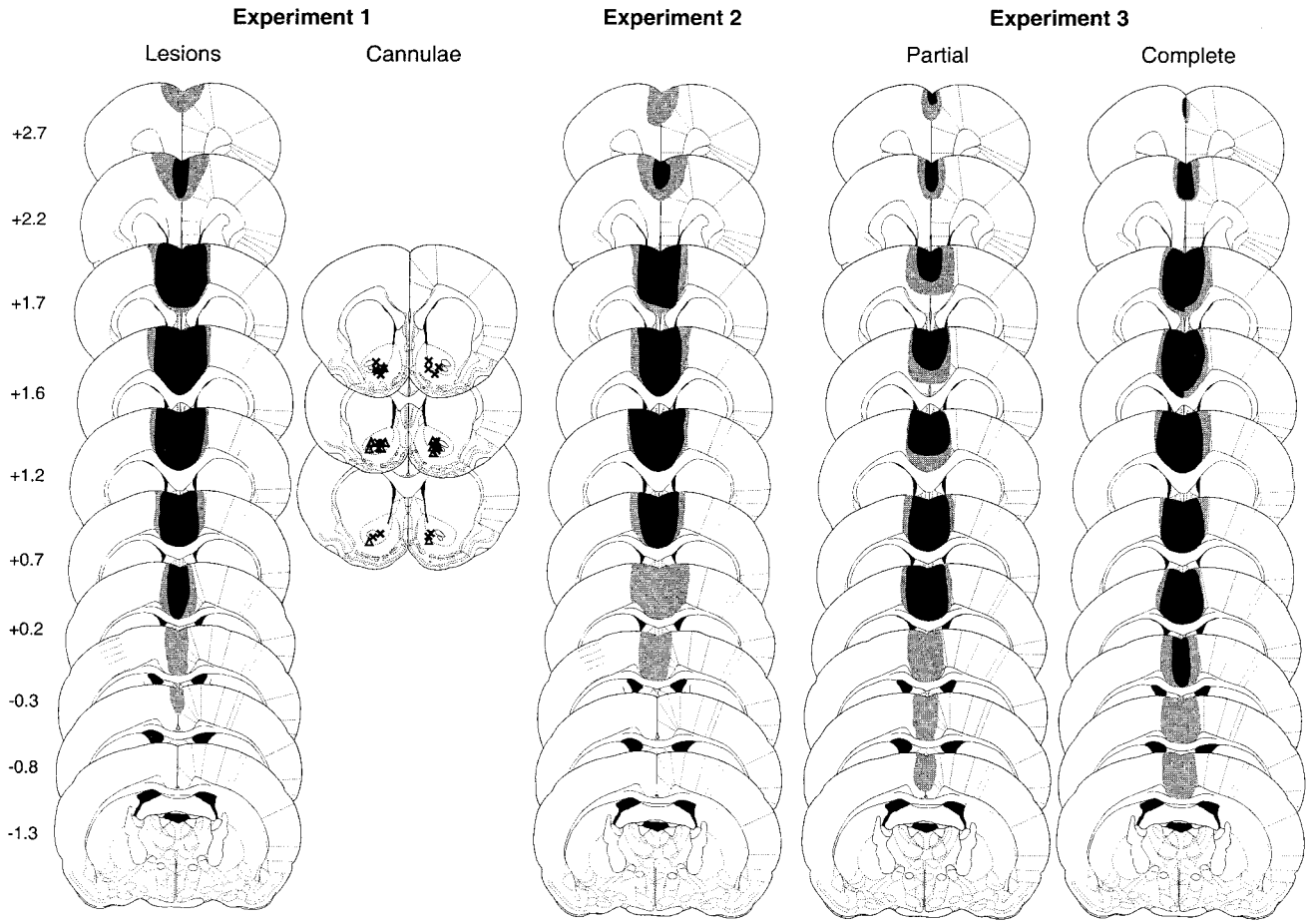

Figure 2. Schematics of lesions and cannula locations. Black shading indicates the extent of neuronal loss common to all subjects; gray shading indicates the area lesioned in at least 1 subject. There are two columns for Experiment 1; the first shows lesion schematics, and the second shows the location of the tips of injection cannulas within the nucleus accumbens (triangles indicate subjects with lesions of the anterior cingulate cortex [ACC]; crosses indicate sham-operated control subjects). There are also two columns for Experiment 3, showing subjects whose lesions included or excluded the ventral perigenual region. 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 in sections +1.6 and +1.7 mm from bregma). Reprinted from *The Rat Brain in Stereotaxic Coordinates,* 4th ed., G. Paxinos and C. Watson, Figures 9–13, 15, 17, 19, 21, and 23, Copyright 1998, with permission from Elsevier Science.

accounted for this dissociation. Preliminary reports of this work have appeared in abstract form (Cardinal, Lachenal, Parkinson, Robbins, & Everitt, 2000; Cardinal, Parkinson, et al., 2000).

Experiment 1: Temporally Discriminated Approach, Conditioned Reinforcement, Autoshaping, Sucrose Consumption, Locomotor Activity, and Conditioned Freezing

Method

Overview

Twenty-two male hooded Lister rats (Harlan-Olac, Ltd, UK) received lesions of perigenual ACC (ACCX group, $n = 12$) or sham lesions (sham group, $n = 10$), with all animals also receiving bilateral cannulas 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 behavioral procedures, in this order: (a) temporally discriminated approach to a stimulus predictive of sucrose; (b) acquisition of a new response with conditioned reinforcement, with intra-Acb amphetamine injections; (c) autoshaping; (d) a sucrose consumption test in the home cages; (e) locomotor activity testing in a novel environment; and (f) 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.

Subjects and Housing Conditions

Subjects were housed in a temperature-controlled room (minimum 22 °C) under a 12-hr reversed light–dark cycle. Subjects were approximately 15 weeks old on arrival at the laboratory and were given a minimum of a week to acclimatize, with free access to food, before experiments began. Experiments took place between 0900 and 2300, with individual

subjects being tested at a consistent time of day. Unless otherwise stated, subjects were experimentally naive, housed in pairs, provided with free access to water, and maintained throughout the experiment at 85–90% of their free-feeding mass using a restricted feeding regimen. Feeding occurred in the home cages at the end of the experimental day. All experimental procedures were subject to United Kingdom Home Office approval (Project Licenses PPL 80/00684 and PPL 80/1324).

Surgery

Animals were anesthetized with Avertin (2% wt/vol 2,2,2-tribromoethanol, 1% wt/vol 2-methylbutan-2-ol, and 8% vol/vol ethanol in phosphate-buffered saline [PBS], 10 ml/kg intraperitoneally) and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA). The skull was exposed and a dental drill was used to remove the bone directly above the injection and cannulation sites. The dura mater was broken with the tip of a needle, avoiding damage to the superior sagittal sinus. Lesions and cannulation were accomplished according to the atlas of Paxinos and Watson (1998), using bregma as the origin and with the incisor bar set at 3.3 mm below the interaural line.

Fiber-sparing excitotoxic lesions were made with 0.09 M quinolinic acid (Sigma, UK) dissolved in 0.1 M phosphate buffer (final pH 7.2–7.4). Toxin was infused through a 28-gauge stainless steel cannula (Semat Technical Ltd, St Albans, UK) attached via polyethylene tubing to a 10-μl syringe (Hamilton Bonaduz AG, Bonaduz, Switzerland) mounted on a Harvard Apparatus (Edenbridge, UK) infusion pump. Lesion coordinates (in millimeters from the skull surface at bregma) were AP +1.2, ML ±0.5, DV –3.0 and –2.2; AP +0.5, ML ±0.5, DV –2.8 and –2.0; AP –0.2, ML ±0.5, DV –2.5 and –2.0. At each site, 0.5 μl was infused over 1 min, after which 1 min (lower sites) or 2 min (upper sites) was allowed for diffusion before the injector was removed. Sham lesions were made in the same manner except that the vehicle was infused.

Intracranial cannulas were implanted by drilling holes in the skull as described above. Four stainless steel screws were placed on each side around the burr holes, and a pair of 22-gauge, beveled stainless steel guide cannulas (13.0 mm long; Coopers Needle Works, Birmingham, UK) were simultaneously lowered to the target position (coordinates AP +1.6, ML ±1.5, DV –5.0 from the dural surface, so that the injectors, cut to protrude 2 mm beyond the cannulas, would be at the final target during experimentation, DV –7.0 from dura). The cannulas were cemented to the screws with dental cement; the inserters were then removed and the guide cannulas were closed with stainless steel wire occluders (diameter 0.36 mm). Postoperatively, animals were given 15 ml/kg of sterile 5% (wt/vol) glucose, 0.9% (wt/vol) sodium chloride intraperitoneally. They were then left to recover for 7 days, with free access to food. At the end of this period, food restriction was resumed.

Histological Assessment

At the end of the experiment, animals were deeply anesthetized with Euthatal (pentobarbital sodium) and perfused transcardially with 0.01 M PBS followed by 4% paraformaldehyde in PBS. Their brains were removed and postfixed in paraformaldehyde before being dehydrated in 20% sucrose for cryoprotection. The brains were sectioned coronally at 60 μm thickness on a freezing microtome and every third section mounted on chrome alum (chromium potassium sulfate)/gelatin-coated glass microscope slides and allowed to dry. Sections were passed through a series of ethanol solutions of descending concentration (3 min in each of 100%, 95%, and 70% vol/vol ethanol in water) and were stained for approximately 5 min with Cresyl violet (0.05% wt/vol aqueous Cresyl violet, 2 mM acetic acid, and 5 mM formic acid in water). Following staining, sections were rinsed in water and 70% ethanol before being differentiated in 95% ethanol. Finally, they were dehydrated and delipidated in 100% ethanol and Histoclear (National Diagnostics, UK) before being coverslipped using DePeX mounting medium (BDH, UK) and allowed to dry. The sections were used to verify cannula and lesion placement. Lesions were detectable as the absence of visible neurons, often associated with a degree of tissue collapse and gliosis.

Behavioral Apparatus

Unless otherwise specified, behavioral testing was conducted in eight identical operant chambers (30 × 24 × 30 cm; MED Instruments Inc., Georgia, VT; Modular Test Cage model ENV-007CT). Each chamber was fitted with a 2.8-W overhead houselight and two retractable levers, 16 cm apart and 7 cm above the grid floor, with a 2.8-W stimulus light above each lever and one located centrally (all 15 cm above the floor). The levers measured 4.5 cm wide × 1.5 cm deep and required a force of approximately 0.3 N to operate. In between the two levers was an alcove fitted with a 2.8-W lightbulb ("traylight," replaced in some experiments by a 60-mcd diffused green LED; RS Components Ltd, UK), an infrared photodiode, a dipper that delivered 0.04 ml when elevated through a hole in the magazine floor, and a tray into which food pellets could be delivered. The chambers were enclosed within sound-attenuating boxes fitted with fans to provide air circulation. The apparatus was controlled by software written by Rudolf N. Cardinal in Arachnid (Paul Fray Ltd, Cambridge, UK), a real-time extension to BBC BASIC V running on an Acorn Archimedes series computer.

Data Analysis

Data collected by the chamber control programs were imported into a relational database (Microsoft Access 97) and analyzed with SPSS 8.01 (SPSS, Chicago, IL) using principles based on Howell (1997). Graphical output was provided by SigmaPlot 5.0. All graphs show group means, and error bars are ±1 *SEM* unless otherwise stated.

Skewed data, which violate the distribution requirement of analysis of variance (ANOVA), were subjected to appropriate transformations (Howell, 1997, section 11.9). Count data (lever presses and locomotor activity counts), for which variance increases with the mean, were subjected to a square-root transformation. Homogeneity of variance was verified using Levene's test.

Behavioral data were subjected to ANOVA with a general linear model. Missing values were not estimated but excluded from analysis. All tests of significance were performed at $\alpha = .05$; full factorial models were used unless otherwise stated. ANOVA models are described using a form of Keppel's (1982) notation; that is, dependent variable = $A \times (B \times S)$, where A is a between-subjects factor and B is a within-subjects factor; S denotes subjects. For repeated measures analyses, Mauchly's test of sphericity of the covariance matrix was applied and the degrees of freedom corrected to more conservative values using the Huynh–Feldt epsilon, $\tilde{\varepsilon}$ (Huynh & Feldt, 1970), for any terms involving factors in which the sphericity assumption was violated. Corrected degrees of freedom are reported to 1 decimal place.

Significant main effects of interest were investigated using post hoc pairwise comparisons with a Sidak correction. Where main effects were found for between-subjects factors with three or more levels, post hoc comparisons were performed with the REGWQ range test (familywise $\alpha = .05$), or Dunnett's test in situations in which several experimental treatments were compared with a single control group. These tests do not require the overall F for groups to be significant, as they control the familywise error rate independently and test different hypotheses from the overall ANOVA, with different power (Howell, 1997, p. 351).

Where significant interactions were found following factorial analysis of variance, simple effects of a priori interest were calculated by one-way ANOVA and tested by hand against the pooled error term. Multiple comparisons for simple effects were performed as described above but using the pooled error term. Where significant interactions were found following repeated measures analysis, a pooled error term was used to test between-subjects simple effects of a priori interest, but separate error terms

(i.e., plain one-way ANOVA) were used for within-subjects factors, as sphericity corrections are inadequate if a pooled error term is used (Howell, 1997, p. 468).

Temporally Discriminated Approach

Four of the operant chambers were used for the acquisition of discriminated approach behavior and conditioned reinforcement tests; for these tasks 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 s, 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% (wt/vol) 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 1 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 [CS proportion ÷ (CS proportion + VI proportion)], 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. Test sessions were conducted in extinction, and immediately followed bilateral administration of one of four doses of intra-Acb D-amphetamine sulfate (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 were separated by 24 hr. 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, Bjijou, & Stinus, 1995), so further spacing of doses was not required.

A session began when the subject nosepoked in the central alcove and lasted 30 min. 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 CS 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 CRf (Burns et al., 1993). Responding on the other (NCRf) lever had no programmed consequence. The lever assignment (left or right) was counterbalanced across rats. Alcove approach frequency and duration were recorded, together with all lever-pressing activity. All measures of behavior were recorded in six 5-min bins.

Intracranial Infusion During Conditioned Reinforcement Test

Before the 1st 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 nonspecific effects of inserting the infusion cannulas during subsequent test sessions. Intra-Acb infusions were performed by inserting two 28-gauge infusion cannulas (diameter 0.36 mm external, 0.18 mm internal; Model C313I, Plastics One, Roanoke, VA; supplied by Semat Technical Ltd, St Albans, UK) through the chronically implanted 22-gauge guide cannulas of gently hand-restrained subjects. The infusion cannulas were 15.0 mm long so as to allow them to protrude 2.0 mm beyond the tips of the guide cannulas; 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-min period. After this, 2 min were allowed for diffusion away from the site of the cannulas to occur, before the cannulas were removed and replaced by occluders and behavioral testing began. Animals were held during the infusion but otherwise allowed to move freely.

Autoshaping

The apparatus used for autoshaping was described fully in Bussey, Everitt, and Robbins (1997). Briefly, the apparatus consisted 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 Timothy 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 four to five pellets placed in and around the dispenser. After this, pellets were delivered on a variable-time (VT) 0–40-s schedule for 15 min.

Acquisition. 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 10–40 s, 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 subject could 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; the latter two were not analyzed. Rats were trained for a total of 100 trials (2 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, Everitt, & Robbins, 1997). Data were analyzed as CS+/CS– approach scores; as difference scores (CS+ approaches minus CS– approaches, Bussey, Everitt, & Robbins, 1997); and as the ratio (CS+ approaches) ÷ (CS+ approaches plus CS– approaches), a measure of stimulus discrimination that is relatively independent of absolute approach activity (Cardinal et al., 2002).

Probe trials. 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+, whereas 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–.

Sucrose Consumption

To assess primary motivation, all animals were given a sucrose consumption test while food deprived. Intake of 10% sucrose solution was measured during 1 hr 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 cm wide × 40 cm deep × 18 cm high, 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 hr. 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* (after Hall, Thomas, & Everitt, 2001). The light context consisted of a 20 cm wide × 21 cm deep × 21 cm high 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 center 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 cm wide × 25 cm deep × 40 cm high 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 preexposed by being placed for 25 min in each context. On Day 4, they were placed in the dark context, in which they received five presentations of a 10-s, 10-Hz clicker CS 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 behavior 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 min preceding CS onset were compared with the 8 min following CS onset.

Results

One subject in the ACCX group lost its cannulas and was killed. There were 3 other postoperative deaths. After histological analysis, all lesions were found to be complete, leaving 8 animals in the ACCX group and 10 in the sham group, of which, respectively, 6 and 10 also had injection sites correctly located within the Acb. Data from all animals with valid lesions were analyzed, except for the conditioned reinforcement test, for which only data from animals with valid lesions and valid cannula placements were used.

Histology

Neuronal loss and associated gliosis extended from approximately 2.5 mm anterior to bregma to approximately 0.3 mm posterior to bregma, destroying perigenual Cg1 and Cg2; there was minimal damage to prelimbic cortex (PrL; a few subjects exhibited a small degree of neuronal loss in the most dorsal aspect of PrL). Infralimbic cortex (IL) and posterior cingulate cortex (PCC) were undamaged, as was the corpus callosum. Figure 2 presents schematics showing the largest and smallest extent of the lesions and the location of the cannula tips; photomicrographs of such lesions have been presented previously (Bussey, Muir, et al., 1997; Bussey, Muir, Everitt, & Robbins, 1996; Parkinson, Willoughby, et al., 2000).

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 3. All dependent variables were analyzed using the model Group × (Session × S). Analysis of the approach ratios revealed a main effect of session, $F(6.9, 110.2) = 92.8$, $\tilde{\varepsilon} = .689$, $p < .001$, reflecting a selective increase in approach during the CS; but there was no effect of group, $F < 1$, *ns*, and no Group × Session interaction, $F(6.9, 110.2) = 1.25$, $\tilde{\varepsilon} = .689$, *ns*. A similar pattern was observed for the proportion of the CS spent nosepoking: session, $F(6.8, 108.5) = 42.1$, $\tilde{\varepsilon} = .678$, $p < .001$; group, $F(1, 16) = 1.29$, *ns*; Group × Session, $F < 1$, *ns;* for the percentage of trials on which the CS was approached at least once: session, $F(10, 160) = 76.9$, $p < .001$; group, $F < 1$, *ns*; Group × Session, $F < 1$, *ns*; and for the time spent approaching the food alcove during the VI: session, $F(6.0, 96.7) = 6.56$, $\tilde{\varepsilon} = .604$, $p < .001$; group, $F(1, 16) = 1.70$, *ns*; Group × Session, $F < 1$, *ns*. It was clear that the learning resulted in dramatically improved access to the US (see Figure 3E), and again there was no effect of the lesion on this measure: session, $F(6.2, 98.8) = 90.7$, $\tilde{\varepsilon} = .618$, $p < .001$; group, $F < 1$, *ns*; Group × Session, $F < 1$, *ns.*

Responding for Conditioned Reinforcement

Animals responded more on the lever producing the CRf (CRf lever) than the control (NCRf) lever, and responding for the CRf was dose-dependently and selectively potentiated by intra-Acb amphetamine, but lesioned and sham groups did not differ (see Figure 4A). Lever-press data were subjected to a square-root transformation and analyzed using the model Group × (Lever × Dose × S). Subjects responded more on the CRf than the NCRf lever: effect of lever, $F(1, 14) = 29.4$, $p < .001$. Amphetamine selectively potentiated responding on the CRf lever: Lever × Dose, $F(3, 42) = 2.84$, $p = .049$; there was also a main effect of dose, $F(3, 42) = 13.5$, $p < .001$. ACC-lesioned animals were not different from controls in any respect—group, $F(1, 14) = 1.66$,

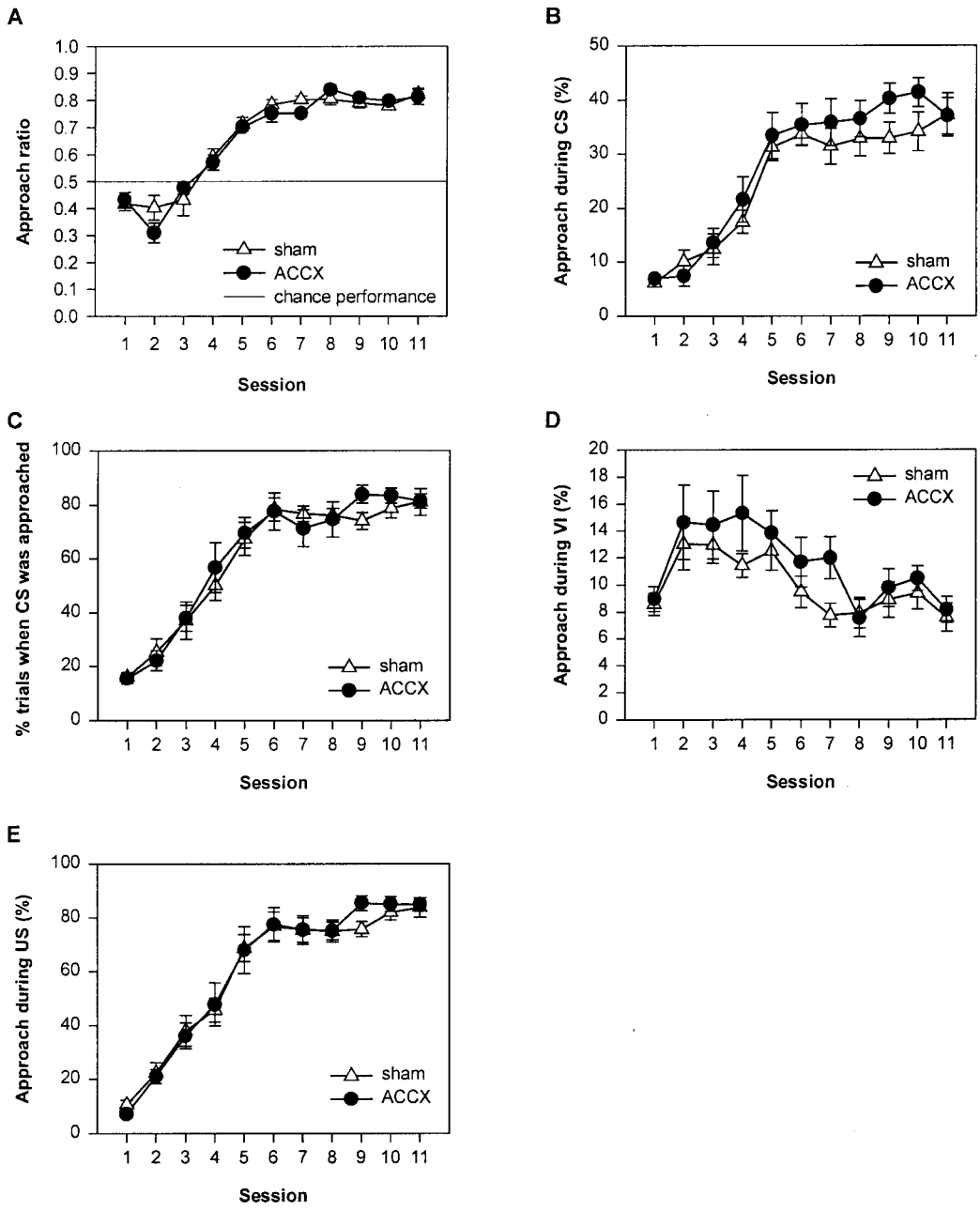

Figure 3. Temporally discriminated approach behavior was unaffected by lesions of the anterior cingulate cortex (ACC). A: Approach ratio. This ratio is calculated as the proportion of the conditioned stimulus (CS) time spent nosepoking divided by the sum of the proportions of CS and variable interval (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, whereas a ratio of 1.0 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: Approach during the VI, as a proportion of VI duration. E: Approach during the unconditioned stimulus (US), as a proportion of US duration. sham = sham-operated controls; ACCX = ACC-lesioned group. Error bars represent ±1 *SEM*.

$p = .218$; Lever × Group, $F < 1$, *ns*; Dose × Group, $F(3, 42) = 2.04$, $p = .122$; Lever × Dose × Group, $F(3, 42) = 1.2$, *ns*—even when the saline dose was considered on its own: lever, $F(1, 14) = 5.71$, $p = .032$; group, $F(1, 14) = 1.59$, *ns*; Lever × Group, $F < 1$, *ns*.

Nosepoking in the food alcove was dose-dependently reduced by intra-Acb amphetamine, but this effect did not differ between groups (see Figure 4B). 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*).

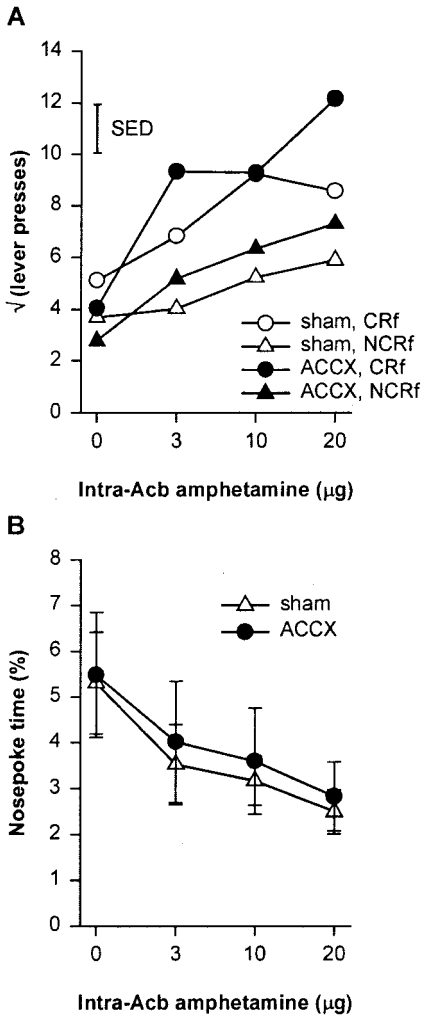

Figure 4. Responding for conditioned reinforcement, with intra-accumbens amphetamine. Lesions of the anterior cingulate cortex (ACC) had no effect on this task. A: Lever pressing (square-root transformed number of lever presses). B: Proportion of time spent nosepoking. Nosepokes during presentation of a conditioned reinforcer were very few and were not included. SED = 1 *SE* of the difference between means for the Lever × Dose × Group term; CRf = responses on the lever producing the conditioned reinforcer; NCRf = responses on the control lever; sham = sham-operated controls; ACCX = ACC-lesioned group; Acb = nucleus accumbens. Error bars in Panel B represent ±1 *SEM*.

Autoshaping

Data from 1 subject in the ACCX group were lost because of a malfunction, leaving 7 lesioned subjects and 10 sham-operated controls.

Acquisition. Lesioned animals were impaired at the acquisition of autoshaping (see Figure 5). An analysis of difference scores revealed a significant impairment in the ACCX group—main effect of group, $F(1, 15) = 6.61$, $p = .021$—together with an effect of trial block, $F(5.4, 81.5) = 2.42$, $\tilde{\varepsilon} = .604$, $p = .038$. The interaction was not significant ($F < 1$, *ns*). Analysis of ratio scores also demonstrated a significant impairment: group, $F(1, 15) = 8.97$, $p = .009$; trial block, $F(5.1, 76.0) = 1.48$, $\tilde{\varepsilon} = .563$, *ns*; Group × Trial Block, $F < 1$, *ns*.

Although sham subjects approached the CS+ faster than the CS–, lesioned rats approached the CS– faster than the CS+ (see Figure 5D). Mean latencies to approach each stimulus were calculated across all trial blocks and were analyzed using the model Group × (Stimulus × S), revealing a Stimulus × Group interaction, $F(1, 15) = 7.30$, $p = .016$.

Probe test. In the probe test (see Figure 5E), there was a nonsignificant trend toward 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 analyzed by one-way ANOVA, revealing no effect of group—$F(1, 15) = 3.93$, $p = .066$—even though the sham group discriminated between the stimuli: sham group compared to 50% discrimination ratio using a one-sample *t* test, $t(9) = 5.67$, $p < .001$, and the ACCX group did not, $t(6) = 1.69$, $p = .142$.

Sucrose Consumption

Primary consummatory behavior 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), $F(1, 16) = 1.06$, *ns*.

Locomotor Activity in a Novel Environment

There was a trend toward hypoactivity in the ACC-lesioned group, but this failed to reach significance (see Figure 6). Following square-root transformation, an analysis of beam breaks by Group × (Bin × S) revealed an effect of group that was close to significance, $F(1, 16) = 4.28$, $p = .055$, together with an effect of time bin, $F(9.0, 144.6) = 15.7$, $\tilde{\varepsilon} = .822$, $p < .001$, reflecting habituation to the novel environment, with no interaction ($F < 1$, *ns*).

Freezing to an Aversive CS

ACC-lesioned subjects did not differ from controls in their ability to freeze to a discrete CS predictive of footshock (see Figure 7). 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, $F(1, 12) = 430.00$, $p < .001$.

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 CRf in ACC-lesioned rats, and they showed normal potentiation of responding for conditioned reinforcement when given intra-Acb 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,

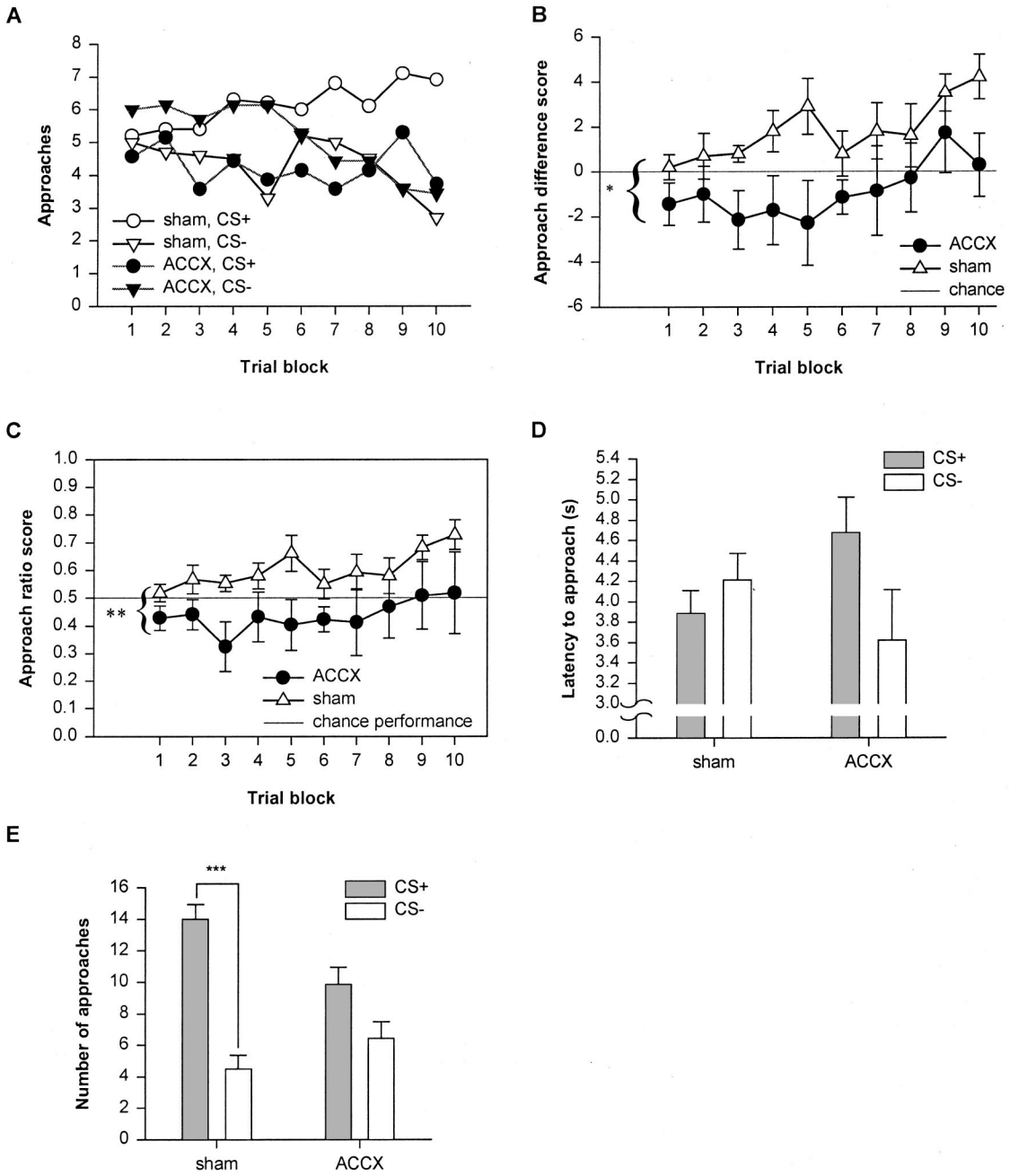

Figure 5. Autoshaping was impaired by lesions of the anterior cingulate cortex (ACC). A: Approaches to the conditioned stimuli (CS+ and CS–) for each group. B: Approach data expressed as a difference score (CS+ approaches − CS– approaches). C: Approach data expressed as a discrimination ratio (CS+ approaches ÷ [CS+ approaches + CS– approaches]). D: Latencies to approach each stimulus, calculated across all trial blocks. E: Autoshaping probe test. Sham-operated controls (sham) 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%). Though no such discrimination was detectable in the ACC-lesioned animals (ACCX), the difference between groups did not reach significance ($p = .066$). Error bars represent ±1 *SEM.* * $p < .05$; ** $p < .01$; *** $p < .001$.

although an autoshaping deficit was observed in the same animals (as observed by Bussey, Everitt, & Robbins, 1997; Parkinson, Willoughby, et al., 2000). The implications are 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

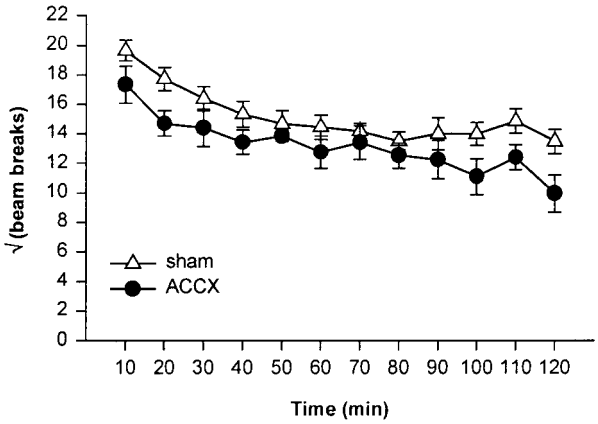

Figure 6. Locomotor response to novelty in sham-operated controls (sham) and anterior cingulate cortex-lesioned rats (ACCX), shown as square-root transformed number of beam breaks. Error bars represent ±1 *SE.*

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 for the performance of an instrumental approach response (for there is an ambiguity as to whether this task measures Pavlovian or instrumental behavior, as discussed in the introduction). Figure 3 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 was only available for a brief time (5 s) in this task, it was beneficial for the subjects to be nosepoking when the US began; this illustrates the unavoidable discriminative stimulus 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 had never had an instrumental relationship to food, so acquisition of discriminated lever pressing demonstrates that the animals had acquired a Pavlovian association between the CS and some aspect of the food (Mackintosh, 1974). In addition to leaving the efficacy of the CRf intact, the lesion did not impair the ability of intra-Acb 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 or CS alcove (as observed by Parkinson, Olmstead, Burns, Robbins, & Everitt, 1999), perhaps because it potentiated the competing response of lever pressing.

Strictly, 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 and Koob (1978) demonstrated that a systemic dopamine indirect agonist, pipradrol, potentiated responding only for a CS explicitly paired with a primary reinforcer; this behavioral specificity has also been demonstrated for intra-Acb amphetamine (Taylor & Robbins, 1984) and dopamine (Cador, Taylor, & Robbins, 1991).

As discussed in the introduction, one suggested function of the ACC is to inhibit unrewarded responding (Muir et al., 1996). 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 behavior in these animals. Thus, the autoshaping deficit cannot be attributed to differences in general activity levels; furthermore, 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 despite absolute levels of responding in ACC-lesioned animals being comparable to those of sham-operated controls in the autoshaping apparatus (see Figure 5A).

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. Two procedural variables seem most likely to account for the difference: the location of the reward relative to that of the CS (these were in the same location for the temporally discriminated approach task but in a different location for autoshaping) and the number of CSs used (one vs. two). Therefore, it is possible either that the ACC is critical for conditioned approach to a stimulus when that stimulus is not located at a source of reward or that the ACC is necessary for discriminating between multiple CSs that are differentially associated with reward. (These two possibilities are examined directly in Experiment 3.)

ACC-lesioned subjects also showed abnormal latencies to respond to the stimuli (as found by Bussey, Everitt, & Robbins, 1997) and reduced discrimination in a probe test (though this

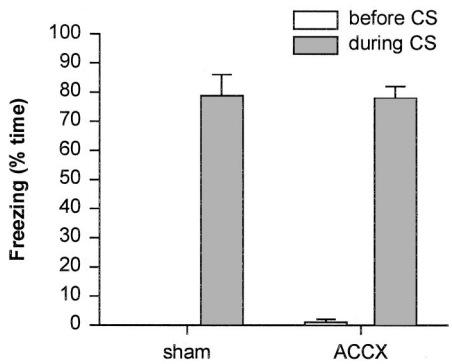

Figure 7. Freezing to an aversive conditioned stimulus (CS+) was not affected by lesions of the anterior cingulate cortex (ACC). The dependent variable is the percentage of time spent freezing, judged from video footage in 5-s bins. The 2 min preceding CS onset are compared with the 8 min following CS onset. sham = sham-operated controls; ACCX = ACC-lesioned animals. Error bars represent ±1 *SEM.*

difference was not significant). Though CS+/CS– discrimination was reduced in ACC-lesioned rats throughout training, the 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 predominated later on (see Figure 5). Though an impairment was clearly demonstrated, the present study measured autoshaping in rats that already had experience of CS–food pairings and of lateralized responding (in the conditioned reinforcement test); for defining the autoshaping impairment more accurately, previous studies using naive rats (Bussey, Everitt, & Robbins, 1997; Parkinson, Willoughby, et al., 2000) may be more reliable.

Unconditioned Measures of Behavior

Lesions of the ACC did not affect primary motivation or consummatory behavior, as assessed by a sucrose consumption test. Similarly, the lesions did not significantly affect locomotor activity in a novel environment. There was a trend toward hypoactivity in the ACCX group, however, which is surprising given that Weissenborn, Robbins, and Everitt (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., 1997, used a postgenual lesion).

Freezing to an Aversive CS

ACC-lesioned rats exhibited normal conditioned freezing behavior. 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 approximately 20% when the clicker has been presented unpaired with shock and 80% or greater when paired (Hall, 1999; Hall et al., 2001).

These results may be contrasted to the demonstrations by Buchanan and Powell (1982) and Gabriel et al. (Gabriel, 1993; Gabriel, Kubota, et al., 1991) 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, 1982), the discrepancy may be explained through differences in the tasks used. First, Buchanan and Powell observed normal eyeblink conditioning in their subjects, though heart rate conditioning was impaired. Aversive eyeblink conditioning is dependent on the cerebellum (see Steinmetz, 2000; Thompson, Swain, Clark, & Shinkman, 2000); as Buchanan and Powell pointed out, even complete decortication does not prevent the acquisition of this conditioned response (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 (Gabriel et al., 1996; Steinmetz, Sears, Gabriel, Kubota, & Poremba, 1991). It may be that freezing is another response that the ACC does not govern. Second, Buchanan and Powell (1982) found at least some heart rate conditioning in ACC-lesioned rabbits, though the magnitude of cardiac deceleration was reduced compared with controls; Gabriel et al. have also reported acquisition of avoidance responding in rabbits with ACC lesions, though acquisition was retarded (Gabriel, Kubota, et al., 1991). 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 bradycardic 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 clearly cause impairments in several appetitive tasks that depend on stimulus–reward associations (Bussey, Everitt, & Robbins, 1997; Bussey, Muir, et al., 1997; Cardinal et al., 2002; Parkinson, Willoughby, et al., 2000).

Experiment 2: Instrumental Conditioning and Pavlovian–Instrumental Transfer

Pavlovian CSs may elicit autonomic and skeletomotor conditioned responses and serve as behavioral goals (as CRfs), but they may also elicit conditioned motivation. A good example is Pavlovian–instrumental transfer (PIT), in which an appetitive Pavlovian CS potentiates ongoing instrumental responding (Estes, 1948; Lovibond, 1983). In the simplest version of 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. In the present experiment, ACC-lesioned rats were tested on such a task.

One auditory and one visual stimulus were used, for a number of reasons. First, demonstration of PIT requires that conditioned and unconditioned effects on instrumental responding be distinguished; therefore, responses to a CS and a neutral (unpaired) stimulus must be compared. So that any potential deficits in stimulus discrimination in ACC-lesioned subjects did not mask the detection of a PIT effect, the CS and neutral stimulus were made as discriminable as possible by choosing two stimuli from different sensory modalities. Second, when a well-localized visual stimulus serves as a Pavlovian CS, it can engender autoshaped approach as well as PIT; if the stimulus is located near or at the instrumental manipulandum, autoshaping and PIT can be confounded. In contrast, a poorly localizable sound (such as the clicker used in the present experiment) cannot easily support autoshaping and can therefore provide a very clear demonstration of PIT. In addition, however, these design constraints allowed the assessment of (a) whether the two stimuli were equally effective at supporting PIT in control animals and, if so, (b) whether ACC-lesioned subjects differed in their basic ability to condition to visual and auditory stimuli.

Method

Subjects

The subjects had previously served in an autoshaping performance study but were naive to the apparatus and stimuli used in the present experiment. Group numbers were 6 (sham) and 9 (ACCX).

Simple PIT

The method was based on that of Balleine (1994). The task was conducted in the operant chambers; a 2.8-W houselight was illuminated throughout. 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 as the CS and a neutral stimulus (NEUT) in counterbalanced fashion.

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 30-s schedule. Stimulus presentations were separated by an interstimulus interval (ISI) of 2–4 min, during which no reinforcement was given. 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 schedule, in which the 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, Smith, & Mirenowicz, 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

Histology

Neuronal loss and associated gliosis extended from approximately 2.5 mm anterior to bregma to approximately 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. All lesions were correctly sited, so the final group sizes were 9 (ACCX) and 6 (sham). Figure 2 shows the largest and smallest extent of the lesions.

Pavlovian Training

The sham and ACCX groups did not differ in their stimulus-related behavior during Pavlovian training (see Figure 8A). 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

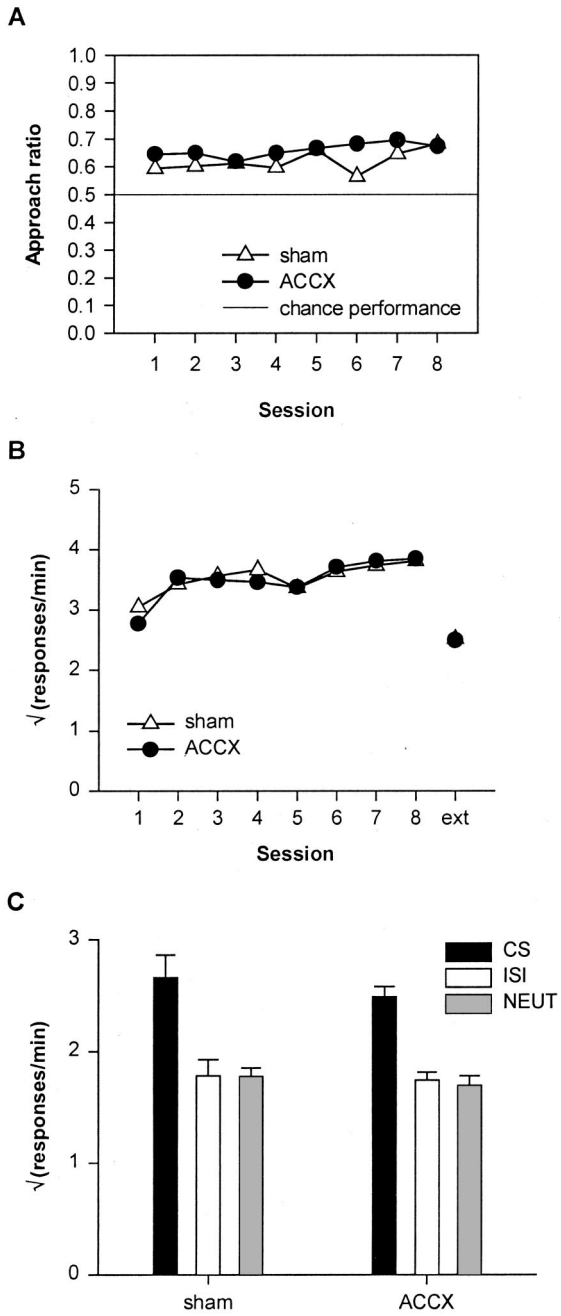

Figure 8. Pavlovian–instrumental transfer. A: Pavlovian training. The approach ratio is the proportion of total nosepoking behavior occurring at times when the conditioned stimulus (CS) was presented (see text). Both groups approached the alcove more during the CS than during the interstimulus interval (ISI), with no group differences. As food was delivered during the CS, the approach behavior partly reflects unconditioned responding. B: Anterior cingulate cortex (ACC) lesions did not impair the acquisition of a free-operant instrumental response or affect responding in extinction. C: Transfer test. ACC lesions did not affect Pavlovian–instrumental transfer; the CS elevated responding relative to the ISI and a neutral stimulus (NEUT). ext = extinction session; sham = sham-operated controls; ACCX = ACC-lesioned animals. Error bars represent ±1 *SEM*.

the model Group × Counterbalancing × (Session × S) revealed no effect of group, $F(1, 11) = 2.023$, *ns*, and no Group × Session interaction, $F(7, 77) = 1.05$, *ns*, with the main effect of session approaching significance, $F(7, 77) = 1.98$, $p = .068$. Subjects nosepoked more during the clicker than the light CS (mean approach ratios were 0.681 and 0.603, respectively): main effect of counterbalancing, $F(1, 11) = 6.56$, $p = .027$, but there were no other effects of the counterbalancing condition (Fs < 1, *ns*).

Instrumental Training

Both groups acquired the instrumental response at the same rate (see Figure 8B). Lever-press data from instrumental acquisition sessions were subjected to a square-root transformation and analyzed 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, $F(4.3, 56.1) = 11.5$, $\tilde{\varepsilon} = .617$, $p < .001$. Similarly, responding did not differ between the groups during the extinction session (univariate ANOVA, $F < 1$, *ns*).

Transfer Test

The CS reliably elevated responding relative to the ISI and the neutral stimulus, and this effect did not differ between groups (see Figure 8C). Response rates for the two test sessions were square-root transformed and analyzed using the model Group × Counterbalancing × (Session × Stimulus × S), in which 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 on the second—effect of session, $F(1, 11) = 75.0$, $p < .001$—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 behavior: stimulus, $F(2, 22) = 72.8$, $p < .001$. 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 $F(2, 22) = 1.55$, *ns*.

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 behavior. In addition, ACC-lesioned rats exhibited normal free-operant instrumental acquisition.

Experiment 3: Two-Stimulus Temporally Discriminated Approach and Conditioned Reinforcement Tasks

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, a neural dissociation of these tasks is not unprecedented (Parkinson, Robbins, & Everitt, 2000; Robledo, Robbins, & Everitt, 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, whereas 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 vs. goal tracking, or preparatory vs. consummatory behavior).

This might also reflect the differential contribution of Pavlovian and instrumental responding. Autoshaping is most probably a Pavlovian response (Browne, 1976; Jenkins & Moore, 1973; Mackintosh, 1974; D. R. Williams & Williams, 1969)—an alternative explanation, that it reflects instrumental approach to a conditioned reinforcer (B. A. Williams, 1994), cannot easily explain the impairment observed in ACC-lesioned rats, as Experiment 1 showed that ACC-lesioned animals work normally for a CRf. However, 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.

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 behavior 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 that are identical except for 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+) signaled the imminent delivery of sucrose solution to a food alcove, whereas the other (CS–) did not. Essentially, this task was identical to autoshaping except that approach was measured to the food alcove rather than to the stimuli. Finding an impairment in ACC-lesioned rats with this task would support Hypothesis 2, and normal performance would support Hypothesis 1. In addition, a conditioned reinforcement test was given using the two stimuli.

Method

Overview

Naive 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 2 sessions.

Two-Stimulus Temporally Discriminated Approach Task

This task was conducted in the operant chambers. The levers were not extended during training. The stimulus lights located above the levers were

designated the CS+ and CS–, counterbalanced left or 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 apparatus. 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, whereas 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 reilluminated, 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 was located 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 were excluded. Neuronal loss and associated gliosis extended from approximately 2.7 mm anterior to bregma to approximately 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, whereas 6 animals had lesions that did not extend this far ventrally. As retrograde tracing studies (Parkinson, 1998; Parkinson & Everitt, 1998) have indicated that this region of the ACC projects most strongly to the AcbC, strongly implicated in appetitive approach behavior (Parkinson, 1998; Parkinson, Robbins, & Everitt, 1999; Parkinson, Willoughby, et al., 2000), a priori 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, $n = 4$). Figure 2 shows the largest and smallest extent of the lesions for the two subgroups. No sham animal was excluded.

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.

First, for direct comparison with autoshaping, the number of trials was calculated in 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.)

Second, 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 following 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 in which approach occurred. The ACCX group were impaired in their ability to discriminate between the two stimuli (see Figure 9A–9C). Analysis of absolute approach scores using the model Group × (Stimulus × Session × 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, $F(1, 20) = 57.6$, $p < .001$; of session, $F(5.5, 110.3) = 53.5$, $\tilde{\varepsilon} = .501$, $p < .001$; and a Stimulus × Session interaction, $F(11, 220) = 14.4$, $p < .001$. In addition, there were Stimulus × Group, $F(1, 20) = 6.83$, $p = .017$, and Stimulus × Session × Group, $F(11, 220) = 2.18$, $p = .017$, interactions. The Session × Group interaction was not significant ($F < 1$, *ns*).

This 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 did the sham group—group, $F(1, 20) = 9.57$, $p = .006$—across all sessions—session, $F(8.0, 159.0) = 60.1$, $\tilde{\varepsilon} = .723$, $p < .001$; Session × Group, $F(8.0, 159.0) = 1.024$, $\tilde{\varepsilon} = .723$, $p = .42$. The ACCX group also responded less to the CS– than did shams: group, $F(1, 20) = 4.46$, $p = .048$, again in a session-independent manner: session, $F(6.7, 133.2) = 24.5$, $\tilde{\varepsilon} = .605$, $p < .001$; Session × Group, $F < 1$, *ns*. Second, the ACCX and sham groups were considered separately. The sham group learned to discriminate between the stimuli: stimulus, $F(1, 11) = 56.9$, $p < .001$; session, $F(5.0, 54.8) = 31.4$, $\tilde{\varepsilon} = .453$, $p < .001$; Stimulus × Session, $F(10.6, 111.1) = 7.08$, $\tilde{\varepsilon} = .96$, $p < .001$. The ACCX group also learned to discriminate eventually: stimulus, $F(1, 9) = 11.5$, $p = .008$; session, $F(5.1, 46.2) = 23.1$, $\tilde{\varepsilon} = .467$, $p < .001$; Stimulus × Session, $F(11, 99) = 11.1$, $p < .001$. Third, the groups' performance was considered for each session. The ACCX group showed discrimination between CS+ and CS– ($p < .05$) from Session 9 on, whereas 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+ minus approaches during the CS–) using the model Group × (Session × S) revealed a significant main effect of group, $F(1,$

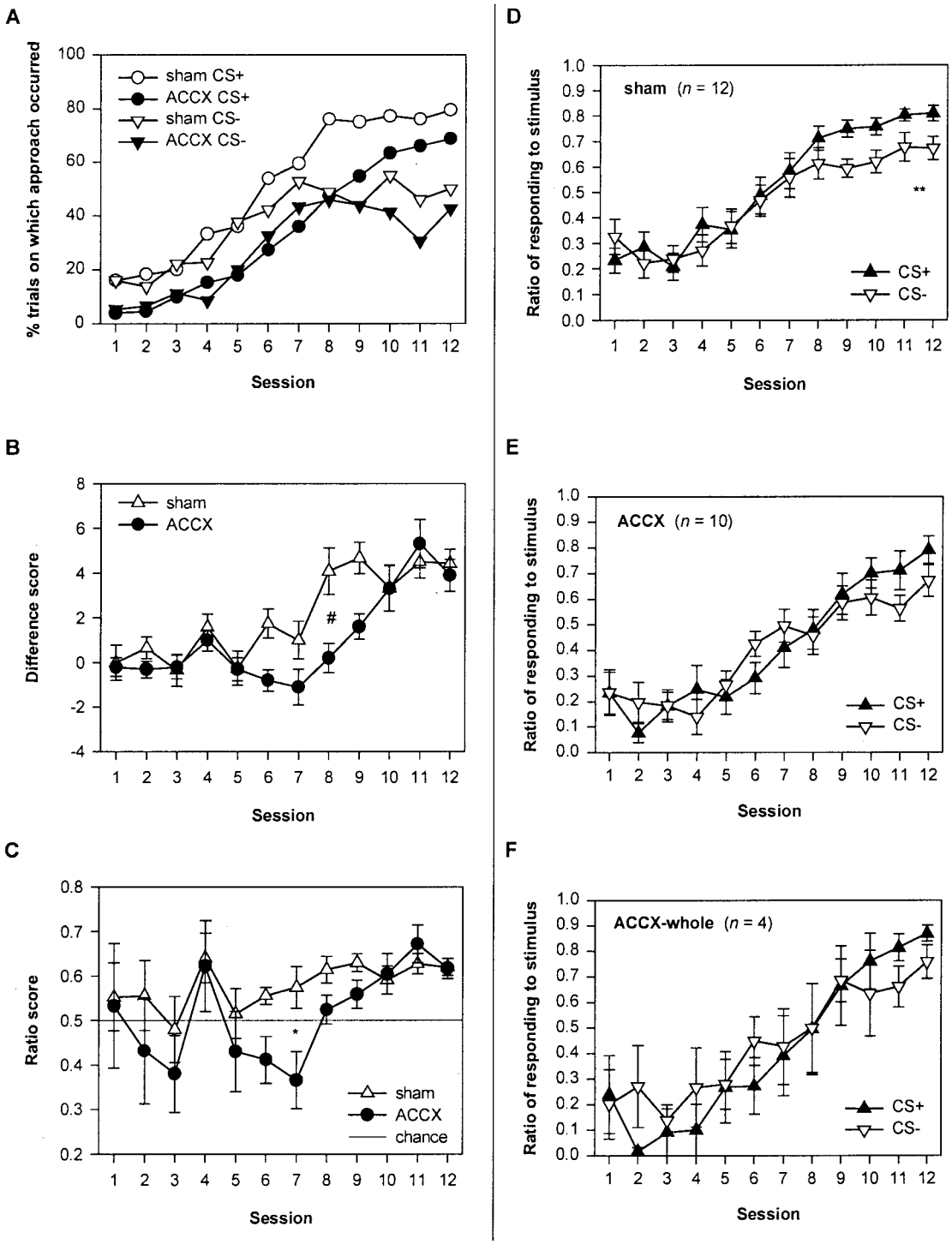

Figure 9. Two-stimulus discriminated approach task (sham-operated controls [sham], $n = 12$; anterior cingulate cortex-lesioned animals [ACCX], $n = 10$) showing impaired discrimination in the ACC-lesioned rats. Left-hand panels are based on the number of stimulus presentations during which a subject approached the food alcove, for comparison with the autoshaping task (see Figure 5). 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 (# $p < .05$, Group × Session interaction). C: Discrimination ratio scores (CS+ approaches ÷ [CS+ approaches + CS– approaches]); * $p < .05$, group difference. Right-hand panels 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 (see Figure 3). D: Performance of sham-operated controls, which successfully discriminated between the CS+ and the CS– (** $p < .01$). E: Performance of animals with lesions of the ACC (ACCX group, $n = 10$), which did not discriminate between CS+ and CS–. F: Performance of that subset of animals with ACC lesions encompassing the ventral perigenual region (ACCX-whole group, $n = 4$), which did not discriminate. Error bars represent ±1 *SEM*.

20) = 6.83, $p = .017$. In addition, there was a main effect of session, $F(11, 220) = 14.4$, $p < .001$, reflecting learning, and a Group × Session interaction, $F(11, 220) = 2.18$, $p = .017$. This interaction was due to slower learning in the ACCX group; they were impaired at the early stages of learning (the simple effect of group was significant for Sessions 6, 8, and 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 analyzed. Again, the ACCX group showed significantly poorer discrimination: effect of group, $F(1, 20) = 7.00$, $p = .016$. Assessed by this measure, the discrimination was poorer across all sessions (Group × Session: $F < 1$, *ns*), though ratio scores increased during training: session, $F(7.5, 150.6) = 2.71$, $\tilde{\varepsilon} = .685$, $p = .009$.

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 less sensitive than the number of trials on which approach occurred. By this measure, the ACCX group did not discriminate between CS+ and CS–, and the sham group did; however, this between-group difference did not reach significance. Those animals with ACC lesions encompassing the ventral perigenual region were significantly impaired compared with shams (Figure 9D–9F).

The approach scores from all subjects were analyzed using the model Group × (Session × Stimulus × S). This showed a nonsignificant trend toward lower levels of stimulus-directed approach in the ACCX group: effect of group, $F(1, 20) = 3.57$, $p = .073$. There were main effects of stimulus, $F(1, 20) = 7.01$, $p = .015$, and of session, $F(5.9, 117.5) = 48.9$, $\tilde{\varepsilon} = .534$, $p < .001$; there was also a Stimulus × Session interaction, $F(7.2, 114.7) = 2.78$, $\tilde{\varepsilon} = .658$, $p = .009$, reflecting the acquisition of differential approach to the two stimuli. The Stimulus × Group interaction did not reach significance, $F(1, 11) = 3.18$, $p = .09$, and no other terms involving group were significant (*F*s < 1, *ns*). However, it is interesting that analysis of the sham and ACCX groups separately demonstrated significant stimulus discrimination in the shams: stimulus, $F(1, 11) = 13.5$, $p = .004$; session, $F(4.2, 45.9) = 24.8$, $\tilde{\varepsilon} = .546$, $p = .051$. There was no evidence of discrimination in the ACCX group: stimulus, $F < 1$, *ns*; session, $F(7.0, 63.0) = 25.4$, $\tilde{\varepsilon} = .636$, $p < .001$; Stimulus × Session, $F(6.3, 56.8) = 1.44$, $\tilde{\varepsilon} = .574$, *ns*, despite similar group sizes (and therefore statistical power).

However, when the ACCX-whole subgroup was compared to shams, they were found to be significantly impaired. Despite the smaller number of animals, there was a Stimulus × Group interaction, $F(1, 14) = 7.28$, $p = .017$, in addition to a main effect of session, $F(5.4, 75.6) = 30.6$, $\tilde{\varepsilon} = .491$, $p < .001$, and a Stimulus × Session interaction, $F(7.2, 100.2) = 2.11$, $\tilde{\varepsilon} = .65$, $p = .048$. No other terms were significant (*F*s < 1.38, *ns*). To explore the nature of the Stimulus × Group interaction, data from each group were analyzed using the model (Session × Stimulus × S). This demonstrated significant discrimination in the sham group, which approached more during the CS+ than during the CS–: stimulus, $F(1, 11) = 13.5$, $p = .004$; session, $F(4.2, 45.9) = 24.8$, $\tilde{\varepsilon} = .38$, $p < .001$; Stimulus × Session, $F(6.0, 66.1) = 2.23$, $\tilde{\varepsilon} = .546$, $p = .051$. No such discrimination was demonstrated in the ACCX-whole group: stimulus, $F(1, 3) = 1.31$, *ns*; session, $F(3.6, 10.8) = 12.5$, $\tilde{\varepsilon} = .327$, $p = .001$; Stimulus × Session, $F(11, 33) = 1.13$, *ns*.

Conditioned Reinforcement

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– (see Figure 10). Square-root-transformed lever-press data were subjected to ANOVA using the model Group × (Lever × Session × S). Considering both groups together, there was a main effect of session, $F(1, 20) = 5.71$, $p = .027$, reflecting extinction. Subjects responded more on the CRf lever, $F(1, 20) = 10.8$, $p = .004$. There was also a Session × Lever interaction, $F(1, 20) = 12.8$, $p = .002$. It is interesting that this was due to improved discrimination on the 2nd test day—simple effects analyses, effect of lever on Day 1, $F(1, 20) = 3.48$, $p = .077$; effect of lever on Day 2, $F(1, 20) = 19.5$, $p < .001$—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*, effect on NCRf lever responding, $F(1, 20) = 18.3$, $p < .001$.

Animals in the ACCX group responded less on test: main effect of group, $F(1, 20) = 14.1$, $p = .001$. There were no other interactions involving group: Group × Session, $F(1, 20) = 2.95$, *ns*; Group × Lever, $F(1, 20) = 1.66$, *ns*; three-way interaction, $F(1, 20) = 1.62$, *ns*. However, it is clear from Figure 10 that discrimination was reduced in the ACCX group, and whereas the sham group on its own demonstrated significant discrimination between the levers—lever, $F(1, 11) = 9.12$, $p = .012$; Lever × Session, $F(1, 11) = 11.1$, $p = .007$—in this analysis, the ACCX group did not: lever, $F(1, 9) = 2.72$, $p = .133$; Lever × Session, $F(1, 9) = 3.09$, $p = .113$.

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, $F(1, 11) = 3.32$, *ns*; in Session 2, *F*(1,

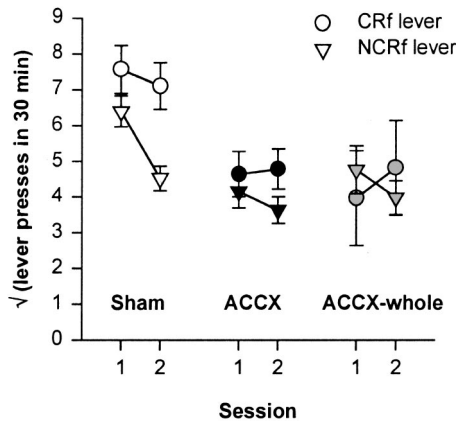

Figure 10. Two-stimulus conditioned reinforcement test. The figure shows the performance of sham animals ($p < .01$, difference between levers in Session 2), animals with lesions of the anterior cingulate cortex (ACCX; $p < .05$, difference on Session 2), and the subgroup of those animals with lesions encompassing the ventral perigenual region of this cortex (ACCX-whole). CRf = responses on the lever producing the conditioned reinforcer; NCRf = responses on the control lever. Error bars represent ±1 *SEM*.

11) = 15.0, $p = .003$. The ACCX group showed a similar pattern: simple effect of lever in Session 1, $F < 1$, *ns*; in Session 2, $F(1, 9) = 6.18$, $p = .035$. 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, $F(1, 3) = 10.7$, $p = .047$. Although this might be interpreted as evidence of lever discrimination, Figure 10 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 2. 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*).

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. On at least some measures, they eventually acquired the discrimination but took longer to learn it than shams. Whereas 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 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 were simply poorer at discriminating between the sensory stimuli (in a manner irrespective of their association with reward): ACC-lesioned rats have been shown to be normal (Bussey, Muir, et al., 1997) 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 in their location. Similarly, ACC-lesioned rats have previously been shown to succeed in learning a conditional visual discrimination using stimuli with which they failed to learn an eight-pair concurrent discrimination (Bussey, Muir, et al., 1997), 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.

Finally, it is not plausible that the ACC is required simply when tasks become difficult in some general sense. In the context of a lesion study, task difficulty can only be defined empirically on the basis of sham performance (a more difficult condition being one in which shams perform worse). Manipulations expected to reduce ACC function have been shown to impair the more difficult condition of a number of tasks, often when multiple stimuli are introduced to a task (discussed in detail below), but sometimes in other conditions, such as when stimulus unpredictability increases in a detection task (impaired by infusion of a muscarinic acetylcholine receptor antagonist into the ACC; J. M. Williams, Mohler, & Givens, 1999). However, ACC lesions do not impair performance on all tasks when they are made more difficult. For example, when subjects must choose between a small, immediate reward and a large, delayed reward, an increase in the delay to the large reward produces a progressive decline in normal subjects' success at obtaining food—that is, the task becomes more difficult. ACC lesions do not impair performance on this task (Cardinal, Pennicott, Sugathapala, Robbins, & Everitt, 2001) and, in some tasks, ACC lesions improve performance (e.g., Bussey et al., 1996). Therefore, task difficulty cannot fully explain the ACC's contribution to behavior, and a more specific explanation of its role must be sought.

In the approach task used here, the CS+ and CS– may have served as instrumental discriminative stimuli, 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 conditional response; 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. (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.) However, discrimination was much reduced in ACC-lesioned animals. Their poor discrimination was not simply attributable to generally low levels of operant responding (as ACC-lesioned rats acquired a free-operant response normally in Experiment 2), or to failure to respond for conditioned reinforcement (given that they responded normally for the CRf in Experiment 1). Thus, the specific failure of discrimination affected two kinds of behavior, locomotor approach and instrumental responding.

General Discussion

Contribution of the ACC to Instrumental and Pavlovian Behavior

Lesions of the ACC have been shown to impair discrimination of reward- or punishment-associated stimuli in Pavlovian tasks (including autoshaping and autonomic conditioning: present experiments; Bussey, Everitt, & Robbins, 1997; Parkinson, Willoughby, et al., 2000; Powell et al., 1994), in tasks whose Pavlovian or instrumental status is ambiguous (two-stimulus discriminated approach task, above; eight-pair concurrent discrimination, Bussey, Muir, et al., 1997; two-way active avoidance, Gabriel, 1993) and in instrumental tasks that depend on Pavlovian associations (responding for conditioned reinforcement, Experiment 3). In tasks where stimulus–reinforcer learning is a disadvantageous strategy, ACC lesions can improve performance (Bussey et al., 1996). At present, the most parsimonious explanation is that the ACC forms or retrieves stimulus–outcome (Pavlovian) associations that may then influence instrumental behavior, consistent with previous suggestions (Bussey et al., 1996; Bussey, Muir, et al., 1997; Gabriel, Foster, et al., 1980).

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, two-stimulus discriminated approach, and two-stimulus conditioned reinforcement tasks presented here and by Bussey, Everitt, & Robbins, 1997; Bussey, Muir, et al., 1997; Gabriel, Kubota, et al., 1991; Parkinson, Willoughby, et al., 2000; Powell et al., 1994). It is unlikely that these results reflect an attentional deficit (Muir et al., 1996) or a failure of spatial discrimination (Bussey, Muir, et al., 1996, 1997; Gabriel, Vogt, et al., 1991; Powell et al., 1994). Specifically, ACC-lesioned rats have been shown to be capable of making left–right spatial discriminations (of stimuli and responses) in a number of paradigms. Though Ragozzino and Kesner (2001) found a deficit in working memory for egocentric spatial responses (left or right turns) following excitotoxic lesions of PrL and pregenual ACC, their subjects were normal at discriminating these responses at zero and short delays. Furthermore, a number of studies have shown that spatial stimulus discrimination, other forms of spatial working memory, and rats' ability to discriminate left and right responses are normal following ACC lesions (Aggleton, Neave, Nagle, & Sahgal, 1995; Bussey, Muir, et al., 1996, 1997; Cardinal et al., 2001; Ragozzino, Adams, & Kesner, 1998; Ragozzino & Kesner, 1998).

In addition, the deficit observed in the present study is unlikely to be perceptual: ACC-lesioned rats can discriminate between two stimuli of different modalities (Experiment 2) and between two visual stimuli differing in a primary submodality such as color (Bussey, Muir, et al., 1997, 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 3; Gabriel, 1990, 1993; Gabriel, Kubota, et al., 1991; Parkinson, Willoughby, et al., 2000). However, the present results demonstrate that ACC-lesioned rats are capable of Pavlovian conditioning in many forms. No deficits are apparent when lesioned subjects are required merely to discriminate between the presence and absence of a single CS (be it appetitive or aversive), as judged by a wide variety of response systems (Experiments 1 and 2). Thus, ACC-lesioned rats were unimpaired at a single-stimulus discriminated approach task, responding for conditioned reinforcement, conditioned freezing, and PIT.

On the basis of these data, it is suggested that the ACC contributes to a sensorimotor aspect of conditioning (Parkinson, Cardinal, & Everitt, 2000). Without the ACC, animals can learn a motivational conditioned 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 unconditioned stimulus (a role attributed to the BLA; Everitt, Cardinal, Hall, Parkinson, & Robbins, 2000); thus, they can learn a new instrumental response for conditioned reinforcement and acquire conditioned freezing. However, CS specificity of these representations is impaired in ACC-lesioned rats; as a result, tasks that depend on stimulus–reinforcer associations when similar stimuli must be discriminated require the ACC. Such tasks include autoshaping (Bussey, Everitt, & Robbins, 1997; Parkinson, Willoughby, et al., 2000), eight-pair concurrent visual discrimination (Bussey, Muir, et al., 1997), the two-stimulus temporally discriminated approach task used in Experiment 3, the active avoidance task of Gabriel et al. (Gabriel, 1993; Gabriel, Kubota, et al., 1991), and CS discrimination in conditioned bradycardia (Powell et al., 1994). According to this hypothesis, the ACC disambiguates CSs for the rest of the limbic circuit of which it is part (see Figure 11).

In Experiment 2 (PIT), there was no evidence that ACC-lesioned rats generalized from the CS to the neutral stimulus. At first glance, this appears to be evidence against the discrimination hypothesis. However, the two stimuli were of different sensory modalities. Generalization between stimuli requires that stimuli share common elements (e.g., Mackintosh, Kaye, & Bennett, 1991). In Experiment 2, the stimuli had no elements in common, generalization would not be expected in the first place, and therefore the loss of a disambiguating structure would not be expected to impair performance. We hypothesize, based on the present results, that ACC lesions would reduce CS+/CS– discrimination in PIT tasks if the CS+ and CS– (or neutral stimulus) shared common elements (i.e., were in the same sensory modality and were similar). Similarly, if the stimuli used for autoshaping were highly discriminable (did not share common elements), we would hypothesize that ACC lesions would not impair autoshaping.

For at least one class of response—locomotor approach—it seems very likely that the ACC influences behavior through the Acb. The ACC projects to the AcbC, which in turn projects to locomotor control regions of the ventral pallidum; lesions of the AcbC impair both autoshaping (Parkinson, Willoughby, et al., 2000) and single-stimulus discriminated approach (Parkinson, Olmstead, et al., 1999), and a functional connection between the ACC and the AcbC is necessary for autoshaping to develop (Parkinson, Willoughby, et al., 2000). The effects of ACC and AcbC lesions on autoshaping differ, however; whereas ACC lesions typically result in "disinhibited" responding to the CS– (Bussey, Everitt, & Robbins, 1997; Parkinson, Willoughby, et al., 2000), AcbC lesions impair the conditioned approach response itself (Parkinson, Willoughby, et al., 2000), just as they prevent conditioned approach to a single CS (Parkinson, Olmstead, et al., 1999).

For tasks in which the ventral striatum is the output structure for behavior, such as locomotor approach (Parkinson, Cardinal, & Everitt, 2000; Parkinson, Olmstead, et al., 1999; Parkinson, Willoughby, et al., 2000), the extra conditioning circuitry that the ACC is suggested to embody (see Figure 11) may be a necessary refinement, as the striatum is itself anatomically capable only of discriminating among 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–) and may need cortical assistance for this. Furthermore, discrimination of two linearly separable input patterns A and AB, where A→reward and AB→0, requires an inhibitory projection from Unit B. As the direct cortical inputs to the striatum are all glutamatergic (excitatory), the striatum might be thought 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, van den Bos, Ploeger, & Ellenbroek, 1991; Floresco, Yang, Phillips, & Blaha, 1998; Pennartz & Kitai, 1991); thus, the ACC may operate to control the input of affective information to the striatum from other structures, in order to direct motivational responses toward appropriate environmental stimuli. This hypothesis therefore predicts that ACC-lesioned rats would

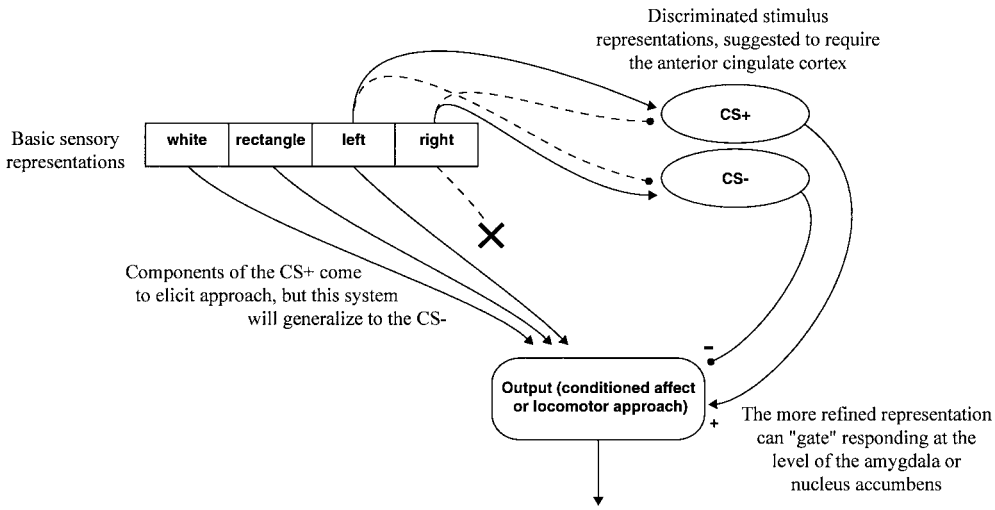

Figure 11. Disambiguation of stimuli, applied to autoshaping. In this example, the CS+ is a white rectangle on the left and the CS– is an identical stimulus on the right. Autoshaping requires the central nucleus of the amygdala, nucleus accumbens core, and nucleus accumbens dopamine (see Cardinal et al., 2002; Parkinson, Cardinal, & Everitt, 2000). In the absence of discriminated activity in the ACC, animals generalize from the CS+ to the CS–, impairing their behavioral discrimination in a disinhibited fashion. However, the animals still discriminate between the presence and the absence of the CS+. From "Limbic Cortical-Ventral Striatal Systems Underlying Appetitive Conditioning," by J. A. Parkinson, R. N. Cardinal, and B. J. Everitt, 2000, *Progress in Brain Research, 126,* p. 272. Copyright 2000 by Elsevier Science. Reprinted with permission.

be impaired at stimulus–reinforcer association tasks involving configural or XOR discriminations.

Note that this account of ACC function does not suggest a primary sensory or perceptual role (ACC-lesioned rats may make the sensory discrimination) but a more specific function—the retrieval of affective information associated with specific stimuli, allowing the generation 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 that share common elements 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 would be unaware as to the correct stimulus toward which appropriate affective responses should be made.

Comparison With Other Interventional Studies in Rodents

The hypothesis of ACC function developed above may be related to studies using very different paradigms. For example, Meunier, Jaffard, and Destrade (1991) have shown that mice with ACC lesions are able to learn a spatial T maze discrimination normally but are impaired when the discrimination is repeatedly reversed. More specifically, Meunier et al. found that ACC lesions did not impair performance on the first, slowly learned reversal. However, sham performance improved over the course of subsequent reversal training, whereas that of ACC-lesioned mice did not. Thus, the presence of the ACC may confer on normal mice an ability to respond rapidly and flexibly to an environment with changing stimulus–reinforcement relationships, withholding responses to unrewarded stimuli. This is consistent with electrophysiological evidence that ACC exhibits reversal of discriminated neuronal activity following behavioral reversal training in rabbits (see Gabriel, 1990, p. 475) and primates (Nishijo et al., 1997). Directly comparable studies using rats are not available. Although Bussey, Muir, et al. (1997) found that ACC-lesioned rats were capable of learning a one-pair visual discrimination and were unimpaired in reversal testing, Bussey, Muir, et al. (1997) did not observe a serial reversal learning effect (an improvement in reversal speed) in shams over the course of three reversals. As sham performance did not improve in this paradigm, the lack of an effect of ACC lesions is consistent with Meunier et al. (1991). 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 overresponding 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 fixed-ratio 1 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 et al.'s (1997) stimulus–reinforcer hypothesis of ACC function 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 above, this may have resulted from differences in lesion site, as the most anterior injection in the present experiments was 0.5 mm anterior to that of Weissenborn et al. Another factor to be considered in Weissenborn et al.'s exper-

iments 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 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. 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 overrespond 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 than those used by Muir et al., and recent results suggest that ACC lesions centered on the perigenual region, similar to those used in the present experiments, do not produce deficits on the 5CSRTT (Christakou, 2001). It should be noted that the psychological basis of premature responding in the 5CSRTT is not well understood; however, it is not clear that these results can be reconciled in terms of a single deficit. At present, the effects of ACC lesions on explicit tests of motor impulsivity (see Evenden, 1999) are not known.

Other studies of the rat ACC have frequently concentrated on the region directly superior to PrL, an area that was not the focus of the present experiments (see Figure 1). For example, Delatour and Gisquet-Verrier (2001) emphasized the role of this more anterior region of pregenual Cg1 (sometimes called dorsal medial prefrontal cortex) in behavioral sequencing. 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, Lloyd, Sahgal, & Aggleton, 1994; Ragozzino et al., 1998) or their ability to switch strategies between the use of visual and spatial cues (Ragozzino, Wilcox, Raso, & Kesner, 1999), yet they produce severe impairments in a number of radial maze tasks (Seamans, Floresco, & Phillips, 1995): Rats with reversible (lidocaine) lesions of the ACC preferentially revisit previously baited arms. This last deficit has clear analogies with the disinhibited, perseverative behavior 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) described the ACC as providing response flexibility by suppressing the effect of simple stimulus–reward associations on behavior, an interpretation clearly compatible with the present results.

Conclusions

The results presented here provide support for the view that the ACC contributes to Pavlovian conditioning. However, it makes a specific contribution to this process: The ACC appears to discriminate similar stimuli (stimuli that share common elements) on the basis of their differential association with reinforcement, providing stimulus specificity to more basic Pavlovian conditioning processes that do not require the ACC. These suggestions are consistent with modern theoretical analyses that view Pavlovian conditioning as resulting from multiple interacting but dissociable processes of learning and associative memory.

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