Research report

Dissociable effects of anterior and posterior cingulate cortex lesions on the acquisition of a conditional visual discrimination: Facilitation of early learning vs. impairment of late learning

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Abstract

Two experiments investigated the effects of quinolinic acid induced lesions of the anterior and posterior cingulate cortices on the acquisition and performance of a conditional visual discrimination (CVD) task, in which rats were required to learn a rule of the type: "If lights are flashing FAST, press the right lever; if SLOW press left". In Experiment 1, animals with lesions of the anterior cingulate cortex (ANT group) demonstrated a significant enhancement in learning during the early stages of task acquisition. Conversely, animals with lesions of the posterior cingulate cortex (POS group) were impaired in learning during the later stages of acquisition. There were no significant differences between the ANT and POS groups on the performance of the task when either variable inter-trial intervals or reduced stimulus durations were imposed. In Experiment 2, the specificity of the lesion effects for processes operative during the early and late stages of learning was tested. Animals were trained to a criterion of 70% correct choices on two consecutive sessions prior to lesioning, and subsequently allowed to continue to acquire the task to the mean asymptotic performance level of 85% correct choices on two consecutive sessions. Animals of the POS group were impaired in learning during this later stage of task acquisition, thus replicating the pattern of results obtained in Experiment 1. The animals in Experiment 2 were then tested following a 30-day retention interval and during extinction (removal of sucrose from the magazine). The extinction test revealed an impairment in the ability of animals in the ANT group to omit lever responses in the absence of reinforcement. These results indicate that the anterior and posterior cingulate cortices are functionally dissociable, and suggest that they may form part of complementary, but competing, learning and memory systems.

Keywords: Discrimination learning; Rule-learning; Conditional; Limbic cortex; Frontal cortex; Cingulate cortex; Extinction; Rat

1. Introduction

The cingulate cortex – a division of medial limbic cortex overlying the full rostrocaudal extent of the corpus callosum – has been little studied relative to other limbic cortical structures such as the hippocampus. It has, nevertheless, been implicated in many functional roles; examples from animal studies include emotional behaviour [30], spatial memory [29,40] aversive Pavlovian conditioning [1], and active avoidance learning [10]. In humans, both cingulectomy and cingulotomy have been performed to treat a variety of conditions, including psychoses and chronic pain [9,46], while inadvertent damage to the cingulate cortex has been reported to induce amnesia and deficits of attention [15,43].

Recently, it has become clear that the cingulate cortex can be divided into anterior and posterior components which are dissociable on anatomical as well as functional grounds. The projection areas of anterior cingulate cortex include mediodorsal thalamus, amygdala and prelimbic cortex, while posterior cingulate cortex connections include the anterior thalamic nuclei, hippocampal formation, and visual cortex (for reviews see [17,44]). In addition, rich reciprocal projections exist between the anterior and posterior cingulate cortices.

Behaviourally, the anterior and posterior cingulate cortices have been dissociated in several studies. For example, anterior but not posterior cingulate lesions have been reported to prevent serial reversal learning in a T-maze, while posterior cingulate lesions disrupted the initial discrimination and first reversal only [23]. Furthermore, Sutherland et al. [40] report that poste-
rior, but not anterior cingulate cortex lesions impair spatial learning in a water maze. In addition, Buchanan and Powell [1] have found that anterior lesions attenuate, but posterior lesions enhance, a classically conditioned response of bradycardia.

Of particular interest with respect to the behavioural dissociation between anterior and posterior cingulate cortex is the series of comprehensive studies by Gabriel and collaborators. Using a multiple-unit recording technique in rabbits learning an active discriminative avoidance task, Gabriel and Orona [13] have shown that discriminative neuronal activity (discharge to CS+ rather than to CS−) develops early in learning in the anterior cingulate and late in learning in the posterior cingulate cortex. Lesion studies provide corroborative evidence: anterior cingulate cortex lesions impair early acquisition of the conditioned response [12], while posterior lesions impair learning during the late stages of task acquisition [14]. These authors conclude that the anterior cingulate cortex, along with the mediodorsal thalamus, constitutes a ‘recency system’ which rapidly encodes the properties of significant stimuli, and will readily abandon this information in order to capture more recent information. In contrast, the posterior cingulate cortex and anterior thalamus are described as components of a ‘primacy system’, which ‘specializes in the relatively long-term retention of plasticity’ [10].

The aim of the present study was to investigate the functions of the cingulate cortex in rats in the acquisition and performance of a conditional visual discrimination (CVD) task. In light of the results of Gabriel et al., we were particularly interested in investigating the role of these structures in the early and late stages of learning. The CVD task requires animals to learn to associate an arbitrary stimulus (SLOW or FAST flashing lights) with an arbitrary response (left or right lever press). It may therefore require the formation of what has been termed a ‘stimulus-response habit’ [25], and has been described by Dudchenko and Sarter [6] as necessitating the use of ‘procedural memory’ for stimulus-response rules. Versions of this task have been used extensively in our laboratory to study, among other things, the functions of different portions of the striatum [33] and the effects of striatal dopamine depletion [36], the effects of isolation rearing [16], and the role of the hippocampus and septo-hippocampal cholinergic system [20,21], the basolateral-thalamic cholinergic projections [7,35], and the caudate-cortical projections [8] in learning, memory and attention.

2. Materials and methods

2.1. Animals

Experimentally naive male Lister hooded rats (Olac, Bicester, UK), weighing between 320 and 350 g at the beginning of the experiment, were housed in pairs in a temperature controlled room (minimum 22°C) under natural daylight. They were provided with free access to water, and by means of a restricted feeding regimen were maintained throughout the experiment at 90% of their free feeding weight.

2.2. Surgical procedures

Animals were anaesthetised with Equithesin (0.3 ml/100 g) administered intraperitoneally, and placed in a Kopf stereotaxic frame fitted with atraumatic carbons. A dental drill was used to remove the bone directly above the injection sites, and the dura mater was broken to the superior sagittal sinus. Quinolinic acid (Sigma, UK) was dissolved in 0.1 M phosphate buffer (pH 7.2–7.4) to a concentration of 0.09 M. A volume of 0.5 μl of this solution was infused through a 30-gauge cannula attached to a 10 μl sampling syringe (SGE, Baton Rouge, USA). When used according to this protocol, quinolinic acid has been found consistently to be effective in destroying cortical neurons. Bilateral lesions of the anterior cingulate cortex (ANT group) were made using the following injection coordinates [31]: (i) AP +0.8, ML +/-0.5, DV -2.0; (ii) AP +0.2, ML +/-0.5, DV -2.9 and -2.0; and (iii) AP -1.0, ML +/-0.5, DV -2.0 and -1.5 (incisor bar set at 2.3 mm below the interaural line) Bilateral posterior cingulate cortex lesions (POS group) were made in the same manner at the following sites: (i) AP -3.2, ML +/-0.5, DV -1.2; (ii) AP -4.0, ML +/-0.5, DV -2.9 and -2.0; and (iii) AP -1.0, ML +/-0.5, DV -2.0. Following each infusion the injection cannula was left in place for a further 2 min. Sham lesions of the anterior cingulate cortex (SHAM-ant group) and of posterior cingulate cortex (SHAM-pos group) were made using the coordinates and procedures above with the exception that no infusion was made following cannula insertion.

2.3. Histology

At the conclusion of behavioural testing the animals were perfused transcardially with 0.9% saline followed by 10% formal saline. After dehydration by immersion in 30% sucrose, the brains were sectioned on a freezing microtome at 60 μm thickness. Every fourth section through the region of the lesions was collected, mounted on glass slides and stained with Cresyl Violet. These sections were used to verify lesion placement and to assess the extent of lesion-induced neuronal loss.

2.4. Apparatus

Preliminary training procedures, CVD acquisition, and task manipulations were carried out in 6 operant
chambers (Campden Instruments, London, Model 4108) fitted with a 2.8 W overhead house light, two retractable levers, three 2.8 W stimulus lights, one located centrally and one above each lever, and a centrally located reinforcement system, consisting of a 0.04 ml dipper within a magazine fitted with a 2.8 W lightbulb, and a hinged Perspex panel through which the animals could gain access to the dipper. Stimulus lights, magazine panel and levers were located on the same wall of the chamber. The chambers were housed within a sound-attenuating box fitted with a fan to provide air circulation. The six chambers were controlled by one Acorn Archimedes series computer, using programmes written by TJB in Arachnid (Paul Fray Ltd., Cambridge), an enriched version of BBC BASIC.

2.5. Behavioural procedures

Preliminary training. Rats were trained during two 15 min sessions to obtain sucrose solution from the magazine. Sucrose was delivered on a FI20 schedule concomitant with illumination of the magazine light. During the first session the magazine panel was fixed open to facilitate access to the magazine. On the third session, a continuous reinforcement schedule (CRF) was introduced, with magazine panel closed. Once the rat opened the panel to obtain reward, the magazine light was extinguished. Throughout this 15 min session, a lever press resulted in the delivery of sucrose solution. During the first CRF session the left lever only was extended into the chamber, in the second session the right lever only was made available, and in the third session both levers were presented alternately for 1 min each. Additional sessions were given as required until all animals were pressing both levers at an equivalent rate, performing at least 50 responses on each lever within a session.

Conditional visual discrimination (CVD) task. The CVD task used in the present study required animals to discriminate between FAST and SLOW flashing lights and to respond appropriately on an assigned lever. For half of the rats in each of the ANT, POS, SHAM-ant and SHAM-pos groups the correct rule was FAST flash = left lever, SLOW flash = right lever; while for the other half, the opposite rule was correct. The FAST and SLOW stimuli had frequencies of ca. 5.0 Hz and 1.0 Hz, respectively, and lasted 2.4 s from onset of first pulse to offset of final pulse. Pulse lengths were adjusted so that for both stimuli the lights were illuminated for the same total amount of time, thus controlling for the possibility that the rats were using luminosity to solve the discrimination (FAST: 12 pulses of 10 cs duration with a 10 cs interpulse interval; SLOW: 3 pulses of 40 cs duration with a 40-cs interpulse interval). Immediately upon termination of a stimulus, both levers were extended into the chamber for a limited hold of 6 s. A correct response resulted in the delivery of reward which was available for a 3-s period; an incorrect response resulted in retraction of the levers and extinction of the house light for a 5-s 'time out' period. Failure to respond during the limited hold similarly lead to a 'time out', and was recorded as an omission. A 5-s inter-trial interval (ITI), with houselight on, preceded each of the 128 trials within the session.

A number of measures were recorded during acquisition of the task:
(i) Percent correct responding (computed from those trials on which responses were made).
(ii) Errors of omission: failure to respond within the limited hold period.
(iii) Latency to respond correctly.
(iv) Latency to collect sucrose reward from the magazine.

2.6. Statistical methods

Data for each variable were subjected to analysis of variance (ANOVA) using the GENSTAT (Rothamsted, UK) statistical package. Further post-hoc comparisons were made using the Newman-Keuls test. Skewed data, which violate the distribution requirement of the ANOVA, were subjected to arcsine, square root or logarithmic transformations [45].

3. Experiment 1: Effects of excitotoxic lesions of the anterior and posterior cingulate cortex on the acquisition of a conditional visual discrimination task

3.1. Behavioral procedures

Following pretraining, 38 rats were allocated, on the basis of their CRF performance, to two lesion and two sham groups: ANT (n = 14), anterior cingulate lesion; POS (n = 14), posterior cingulate lesion; SHAM-ant (n = 5) and SHAM-pos (n = 5), corresponding sham surgeries. Following surgery, animals were given a 14-day post-operative recovery period, and were then retrained on the alternating-lever CRF schedule, until all rats were lever pressing at roughly equivalent rates (approximately two sessions). Animals were then trained on the CVD task to a criterion of 85% correct choices on 2 consecutive days. A calculation was made of the number of sessions required by each animal to progress through three learning stages: (i) chance performance to 59% correct responses (a level of performance just significantly above chance); (ii) 59–70% correct responses; and (iii) 70–85% correct responses (the average level of asymptotic performance for control animals on the CVD task). For each of these criteria, animals were required to attain the criterion score on 2 consecutive sessions.
Following acquisition of the CVD task, animals were challenged with the following manipulations to the basic paradigm:

(i) Variations of the inter-trial interval (0, 2, 5, 10, 20 s).
Equal numbers of trials preceded by each of these inter-trial intervals were randomly presented during a single test session, in order to assess the effect of stimulus unpredictability on task performance.

(ii) A reduction in the duration of the visual discrimination (SLOW stimulus reduced from 3 to 2 pulses; FAST stimulus reduced from 12 to 8 pulses). The frequencies of the two stimuli were therefore the same as for the original acquisition. Luminosity and durations of the 2 stimuli remained equilibrated.

In addition, in order to assess possible motivational changes following cingulate cortex lesions, at the conclusion of testing on the CVD paradigm, all animals were given a 2-h sucrose consumption test within the home cage. During this 2-h period, the water bottles for each cage were replaced with bottles containing 20% sucrose solution. These bottles were weighed prior to and at the conclusion of this consumption test, allowing the quantity of sucrose solution consumed to be calculated in grams/hour.

Finally, for all animals spontaneous locomotor activity was recorded during a 2-h period in novel activity cages equipped with infra-red photocells.

3.2. Results

3.2.1. Histology

Tracings of the outlines of the lesions in individual animals were made, based on the detectable cell loss seen in the Cresyl Violet-stained sections. Histological analysis of the lesions revealed incomplete lesions in one animal in each of the ANT and the POS lesion groups; in the former case, the lesion was unilateral, in the latter case infusion of the excitotoxin was unsuccessful. The posterior cingulate cortex lesions (Figs. 1B and 2B) extended from -1.8 mm from bregma to approx. -4.8 mm and revealed extensive cell loss in areas RSG and RSA according to Zilles [47].

3.2.2. Behavioural results

Post-surgery CRF performance. There was no significant difference (F(3,34)=0.8, P>0.05) between any of the groups in terms of the total number of lever presses during CRF performance 2 weeks following lesion surgery (SHAM-ant = 189; SHAM-pos = 162; ANT = 150; POS = 147 presses). Furthermore, as there was no difference in the CRF performance of the SHAM-ant and SHAM-pos groups on CRF or in subsequent performance of the conditional discrimination task, these animals were treated as a single SHAM group during subsequent analyses.

Acquisition of a conditional visual discrimination. As shown in Fig. 3, where choice accuracy is presented in blocks of 5 sessions, excitotoxic lesions of the anterior cingulate cortex (ANT group) produced a marked facilitation in the acquisition of the conditional task relative to POS and SHAM groups (F(64, 2237)=5.50, P<0.001). However, it is clear from Fig. 3 that there were no differences between the groups in the final asymptotic level of performance on the task.

There were no differences between groups in errors of omission during task acquisition, all groups responding with fewer than 2 omissions within a block of 5 sessions. Furthermore, there was no main effect of the lesion on correct response latency, F(2,35)=1.73, P>0.05, (means: SHAM = 1.23, ANT = 1.06, POS = 1.10 s) or on latency to collect food reward, F(2,35)=1.63, P>0.05, (means: SHAM = 0.41, ANT = 0.39, POS = 0.44 s).

In order to investigate the possibility of differential learning on the two sub-discriminations of the CVD task, data for acquisition were analysed separately in terms of percentage correct responses on the lever associated with the FAST stimulus, and percentage correct responses on the lever associated with the SLOW stimulus. This analysis revealed that the facilitation of acquisition observed in the ANT animals was evident for both sub-discriminations of the task (acquisition of FAST: F(64,2237)=6.61, P<0.001; acquisition of SLOW: F(64,2237)=8.08, P<0.001).

Further analysis of the acquisition data in terms of the number of sessions required to progress between various learning criteria (Stages of Learning) revealed a significant main effect of lesion, F(2,35)=4.04, P<0.05; a significant effect of stages of learning, F(2,35)=20.17, P<0.001; and a significant lesion x stage interaction,
Fig. 1. Photomicrographs showing the extent of representative lesions from the anterior (ANT) cingulate lesion group (A) and in the posterior cingulate (POS) lesion group (B), compared with brain sections from animals of the SHAM-ant (C) and SHAM-pos (D) groups. The material presented in A is typical of animals of the ANT group where marked neuronal loss extends caudally from the genu of the corpus callosum, in most cases producing only minimal loss pre-genu. The material presented in B is typical of lesions observed in the POS group where neuronal loss extends within areas RSA and RSG according to Zilles [47]. Some ventricular enlargement was observed in both lesion groups.

\[ F(4,70) = 2.53, P < 0.05 \] (see Fig. 4). Newman-Keuls post-hoc comparisons revealed that animals of the ANT lesion group took significantly fewer sessions \((P < 0.05)\) to attain the 59% criterion relative to animals of the POS and SHAM groups which did not differ significantly from each other. In contrast, POS animals required significantly more sessions to progress from the 70% to the 85% criterion than animals in either the ANT or SHAM groups which did not differ significantly at this stage.

The data were also analysed in terms of errors of commission during the 3 stages of task learning, revealing a significant main effect of lesion, \(F(2,35) = 5.70, P < 0.01\), and a significant main effect of learning stage, \(F(2,35) = 25.15, P < 0.001\). However, while the lesion \(x\) stage interaction failed to reach significance, \(F(4,70) = 1.98, P = 0.10\), a similar pattern of effects was observed during the early and late stages of learning as that obtained using sessions between criteria as a measure (mean errors to 59%: SHAM = 896, ANT = 516, POS = 855; 70–85%: SHAM = 328, ANT = 342, POS = 625).

In order to evaluate the possibility of response biases in the lesion groups, analyses were performed on the percent overall lever bias (i.e., either to the right or left lever, depending on a particular animal's bias), and percent bias to the lever associated with the FAST stimulus, irrespective of the spatial location of the lever. Data were analysed from the session on which a particular animal first attained the criterion of 59% correct responses for two consecutive sessions. This analysis revealed no significant differences between the lesion groups in terms of spatial response bias, \(F(2,35) = 2.19, P > 0.05\), or bias to respond on the lever associated with the FAST stimulus, \(F(2,35) = 2.77, P > 0.05\).

3.2.3. Behavioural challenges
(i) Effect of variable inter-trial intervals (ITIs). There was no significant effect of the lesions on performance of the task, in terms of choice accuracy, when variable ITIs (0, 2, 5, 10, 20 s) were unpredictably presented within a single test session, \(F(2,35) = 1.51, P > 0.05\), although there was a significant effect of ITI, \(F(4,132) = 5.10, P < 0.001\).

(ii) Effect of reduced stimulus duration. There was no differential effect of the lesion on task performance when the durations of the discriminanda were reduced, \(F(2,35) = 0.46, P > 0.05\), although all three groups showed a reduction in correct responding relative to baseline, \(F(1,33) = 41.0, P < 0.001\).
Fig. 2. Reconstructions of the anterior (left) and posterior (right) cingulate cortex lesions at various anterior-posterior levels (anterior cingulate cortex: +2.15 to -1.78 mm from Bregma; posterior cingulate cortex: -1.78 to -5.65 mm from Bregma). Black shading indicates the extent of the largest lesion within each group; grey shading, the smallest lesion. Drawings of sections are from Swanson [41].

Fig. 3. Effect of quinolinic acid lesions of the anterior (ANT) and posterior (POS) cingulate cortex on acquisition of the conditional visual discrimination task. Mean values for blocks of 5 successive sessions are plotted (mean ± SEM).

3.2.4. Sucrose consumption test

One-way analysis of variance revealed that there was no significant effect of the lesions on consumption of the 20% sucrose solution in the home cage, \( F(2,35) = 3.15, P > 0.05 \), (mean values: SHAM = 23.2; ANT = 27.6; POS = 19.7 g/h).

Fig. 4. Sessions required by anterior cingulate (ANT), posterior cingulate (POS) and sham control (SHAM) lesioned animals to progress between criteria associated with various stages of learning during acquisition of the conditional task (mean ± SEM).

3.2.5. Spontaneous locomotor activity

One-way analysis of variance revealed that there was no significant effect of the lesions on spontaneous locomotor activity in novel activity cages, \( F(2,35) = 0.81, P > 0.05 \), (mean values: SHAM = 1666; ANT = 1967; POS = 1945 crossings).
4. Experiment 2: Effects of lesions of the anterior and posterior cingulate cortices on acquisition of a conditional visual discrimination task during the late stages of learning

In Experiment 1 it was found that animals with lesions of the posterior cingulate cortex were impaired in the late stages of acquisition of the CVD task, suggesting that the lesion affected processes operative during the late stages of learning. An alternative interpretation, however, is that the deficit may have been specific to the late stages of learning due to the animals being required to learn during the early stages under the influence of the lesion: it is conceivable that learning during the late stages of acquisition might be influenced by the manner in which the information was encoded during early stages, which might in turn be influenced by the lesion. This possibility is tested in the present experiment, in which animals were trained to a criterion of 70% correct choices on two sessions consecutively, lesioned, and then allowed to continue acquisition of the task to completion (85% correct responses, on two sessions consecutively). An acquisition deficit following posterior cingulate cortex lesions would suggest that the deficit observed in Experiment 1 was due to the disruption of processes operative specifically during the late stages of learning. The present experiment also tests whether the facilitation of early acquisition in the ANT animals in Experiment 1 was due to the lesion having an effect on processes operative specifically during the early stages of learning. Failure to observe an effect of anterior cingulate cortex lesions in the present experiment would suggest that the facilitation observed in Experiment 1 was due to the lesion having an effect on processes operative specifically during the early stages of learning.

Following acquisition, long-term memory was assessed by subjecting rats to a 30-day retention interval. Finally, animals were allowed to reacquire the task to baseline in order to investigate subsequently the effects of anterior and posterior cingulate lesions on extinction.

4.1. Behavioral procedures

Pretraining and task acquisition proceeded as in Experiment 1. Each of 25 rats, upon attaining a criterion level of performance of 70% correct choices for two consecutive sessions, was assigned to one of four groups as in Experiment 1 (ANT, POS, SHAM-ant and SHAM-pos). Rats were assigned such that as a run of four rats attained criterion, one rat was assigned to each of the four groups. Surgery was performed within 2 days following attainment of the 70% criterion. Thus, the experimental groups were as follows: ANT (n = 8), anterior cingulate lesion; POS (n = 9), posterior cingulate lesion; SHAM-ant (n = 4) and SHAM-pos (n = 4), corresponding sham surgeries. Following surgery, animals were given a 14-day post-operative recovery period, and training on the CVD task was continued to a criterion of 85% correct choices on 2 consecutive days. A calculation was made of the number of sessions required by each animal to attain 85% correct responses.

Following acquisition of the CVD task, animals were challenged with the following manipulations to the basic paradigm which had not been examined in the previous experiment: (i) performance of the basic task following a 30-day retention interval; (ii) performance of the task during extinction (removal of sucrose from the magazine. The empty dipper was raised following a correct response).

4.2. Results

As shown in Fig. 5, the late learning deficit obtained in Experiment 1 was reproduced, animals with posterior cingulate cortex lesions (POS) requiring significantly more sessions to attain criterion performance on the task than sham-operated control animals or animals with lesions of the anterior cingulate cortex, these latter groups not being significantly different from each other, $F(2,77) = 3.71, P < 0.05$.

The results of the 30-day retention interval revealed that all animals were able to perform at approx. 77% following this interval, there being no difference between the groups, $F(2,22) = 0.004, P > 0.05$ (SHAM = 76.94%; ANT = 76.80%; POS = 76.69%).

Following reacquisition of the task to 85% correct responses on 2 consecutive days, performance of animals during a 10-day extinction period was examined (see Fig. 6). The results of this manipulation revealed a significant lesion group x session interaction for response...
omissions, $F(18,198) = 3.005, P < 0.05$. Post-hoc analysis revealed that this effect was due to animals of the ANT group performing significantly fewer response omissions during the first three extinction sessions compared to both SHAM control and POS lesion groups, indicating a resistance to extinction in these animals. For the remaining 7 test days, there were no significant differences between any of the groups in terms of response omissions. There were no differences between the groups in terms of magazine omissions ($F(2,22) = 1.353, P > 0.05$) or percent correct scores ($F(2,22) = 1.634, P > 0.05$).

5. Discussion

The main finding of the present study was that excitotoxic lesions of the anterior cingulate cortex facilitated acquisition of a conditional visual discrimination (CVD) task, specifically during the early stages of learning, whereas lesions of the posterior cingulate cortex impaired learning during the later stages of task acquisition. All animals were, however, eventually able to attain high levels of task performance. Once task acquisition was attained, the groups' performance levels were not differentially affected by various manipulations to the basic paradigm, such as unpredictable presentation of variable inter-trial intervals, reduction of the duration of the visual stimuli, or imposition of a 30-day retention interval. Performance of the task during extinction, however, revealed an impairment in the ability of animals with anterior cingulate cortex lesions to omit lever press responses in the absence of reinforcement.

It is unlikely that these effects were mediated by changes in motivational or motoric function, as there were no differences between any of the ANT, POS or SHAM groups in terms of errors of omission, latency to respond, or latency to collect reward. Furthermore, these groups did not differ in spontaneous locomotor activity as measured by recording in activity cages, or in the amount of sucrose consumed during a sucrose consumption test carried out in the home cage. It is also unlikely that these effects are attributable to disruption of attentional function, for at least two reasons. First, neither reducing the duration of the visual stimuli nor unpredictably varying the inter-trial interval — manipulations thought to increase the attentional load placed upon the animals (see e.g. [8]) — significantly affected task performance. Second, excitotoxic lesions of the anterior cingulate cortex fail to affect choice accuracy or response latency on a continuous performance test of visual attention (the '5-choice serial reaction time task' [26a]). It must be noted, however, that the specific form of attention required for optimal performance in the 5-choice task may be different from that required in the CVD task; specifically, the 5-choice task requires the animal to divide attention across locations in space, while the CVD task requires the animal to attend to discriminative stimuli which are presented consistently in the same spatial location.

5.1. Anterior cingulate cortex

The results of Experiment 1 indicate that ANT animals learned this task more rapidly than did POS or SHAM animals, animals of the ANT lesion group attaining a criterion of 59% on two consecutive sessions in significantly fewer sessions than the POS or SHAM animals. The results of Experiment 2 suggest that recovery of normal functioning prior to the late stages of acquisition was not responsible for the early-learning specificity of the facilitation of acquisition in the ANT animals in Experiment 1. This suggests that anterior cingulate cortex lesions affected processes operative specifically during the early stages of learning.

This result supports those of Gabriel and Orona [13] and Gabriel et al. [12], demonstrating that the anterior cingulate cortex is differentially involved in the early stages of task acquisition. However, whereas Gabriel et al. [12] have shown that anterior cingulate cortex lesions impair early learning, in the present study such lesions facilitated early learning. In the studies by Gabriel and his collaborators, rabbits are required to learn that a tone predicts footshock, and to avoid the shock by stepping in a running wheel when the tone is sounded. A possible explanation for the early learning deficit obtained by Gabriel et al. [12] is that the anterior
The anterior cingulate cortex may mediate the acquisition of stimulus-reinforcer associations, an interpretation which is consistent with a selective deficit in early learning, when it must be learned that the tone predicts shock. The proposal that the anterior cingulate cortex may be involved in stimulus-reinforcer learning receives support from the observation that Pavlovian conditioning is impaired following anterior cingulate cortex lesions [1]. Further evidence is provided by a recent study in which anterior cingulate cortex lesioned animals were impaired in the acquisition of an 8-pair concurrent discrimination task in which the animals were required to learn eight separate stimulus-reward associations concurrently [26]. Finally, rats will learn to lever-press for electrical stimulation to the anterior cingulate cortex, with sites caudal to the genu of the corpus callosum (the area lesioned in the present study) supporting the most rapid acquisition [39]. Indeed, Phillips and Fibiger [32] have suggested that such reward-related activity in the anterior cingulate cortex may be involved in memory storage.

That the anterior cingulate cortex may mediate the formation of stimulus-reinforcer associations provides a possible explanation for the facilitation observed following anterior cingulate cortex lesions in the present study. In the CVD task, rats are pretrained to press the right and left levers for sucrose reward. Following lesioning, the rats learn to press the appropriate lever for reward in the presence of an arbitrary discriminatory stimulus (SLOW or FAST flashing lights). Thus the interaction of associative structures set up during acquisition of the CVD task might be as follows: presentation of a discriminative stimulus, which signals the opportunity for reward, excites a representation of the reward (a stimulus-reinforcer association), which in turn excites a representation of the lever press responses, as a result of previous lever training. However, if these are the only associations learned by the rats, they will be unable to solve the task: presentation of both FAST and SLOW flashing lights will excite a representation of the same reinforcer (sucrose), which, since it is associated with both right and left lever press responses, provides no information as to which lever is correct at that time (for discussion, see [4]). Thus, the acquisition of stimulus-reinforcer associations will not aid in the acquisition of the CVD task. However, the task may be solved by acquiring the FAST-press left, SLOW-press right rules directly, in other words, by the formation of stimulus-response (S-R) 'habits'. It is possible that these two approaches to the solution of the CVD task compete. A possible explanation for the facilitation in the anterior cingulate cortex lesioned animals of the present study, therefore, is that when the initial tendency for the animals to attempt to solve the task in terms of stimulus-reinforcer associations is removed or suppressed following anterior cingulate cortex lesions, a competing process of S-R learning may proceed at a faster rate.

Explanations appealing to the notion of competing multiple memory systems have been offered by other authors in cases where limbic system damage has produced a facilitation in learning (e.g. [28]), although such an interpretation applied to the results of the present study remains speculative.

If this hypothesis is correct then it might be expected that anterior cingulate lesioned animals, whose behaviour becomes more rapidly governed by S-R habit, and who have acquired associative representations involving reward incompletely relative to controls, would be less sensitive to changes in the status of the reinforcer. Indeed, in the present study animals of the ANT lesion group demonstrated persistent responding during extinction, an observation consistent with this prediction. However, in light of recent results demonstrating increased 'anticipatory responding' in anterior cingulate cortex lesioned animals [26a], a more general explanation in terms of behavioural disinhibition cannot be ruled out.

Alternatively, the differential effects of anterior cingulate cortex lesions in the studies of Gabriel et al. and in the present study may be related to the nature of the reinforcer: the former studies required an avoidance response to an aversive stimulus (shock), whereas in the present study an appetitive paradigm was employed. That cingulate cortex ablation can produce deficits in active shock avoidance learning is a reproducible phenomenon [12,18,19,42]. Furthermore, electrical stimulation of the anterior cingulate cortex can produce vocalisations which may be associated with escape responses [37], and it has also been suggested that the anterior cingulate cortex is part of a "medial pain pathway" [44]. Thus, it might appear that the anterior cingulate cortex performs a selective function—mediating behavioural responses to noxious stimuli—which may not be engaged in an appetitive situation. Counter to this notion, however, is the observation that anterior cingulate lesioned animals are impaired in the acquisition of an appetitive 8-pair concurrent discrimination task [26], and also the demonstration by Gabriel et al. [11] that training-induced neuronal activity develops in the anterior cingulate cortex in an appetitive as well as in an aversive paradigm.

A related possibility is that the facilitation of learning in the present study may be attributable to 'anxiolytic' effects of the lesion which might reduce possible disruptive responses to the mildly aversive components of the task (for example, non-reward following errors). However, the animals in the present study were given substantial pre- and post-lesion training in the operant chamber prior to commencement of the CVD task, and there were no differences between groups in terms of lever-pressing during post-lesion CRF training, or on any measure of performance during the CVD task that might have indicated decreased levels of anxiety in the...
ANT animals, such as latency to respond or latency to collect reward. Furthermore, this interpretation is not consistent with the observation that the anterior cingulate lesioned animals did not demonstrate reduced neophobia, as measured by spontaneous locomotor activity on first exposure to unfamiliar cages. Finally, anterior cingulate cortex lesions do not enhance acquisition of other tasks which were carried out in the same operant chambers as those used in the present study and which featured components common to the CVD task, such as lever-press responses and non-reward following an incorrect response (Bussey, Muir, Everitt and Robbins, unpublished results). Nor were they facilitated in tasks carried out in other apparatus; indeed, as mentioned above, anterior cingulate cortex lesioned animals are impaired in these instances. It should be noted, however, that in these studies, as well as in the pretraining carried out in the present study, animals were not exposed to the same stimuli as presented in the CVD task, namely flashing lights, and therefore the possibility of a contribution of non-specific factors related to this aspect of the task cannot definitively be ruled out.

5.2. Posterior cingulate cortex

In marked contrast to anterior cingulate cortex lesions, lesions of the posterior cingulate cortex produced an impairment in learning between the criteria of 70 and 85%. This effect was reproduced in Experiment 2 of the present study where animals were trained to a criterion of 70% prior to lesioning, and subsequently allowed to continue acquisition of the task to a level of 85% correct responses. This latter result suggests that the deficit observed in Experiment 1 was due to the disruption of processes operating specifically during the late stages of learning.

This result is consistent with the work of Gabriel et al. [14], which revealed an impairment late in learning in animals with posterior cingulate cortex lesions. Based on these observations, Gabriel [10] has suggested that the posterior cingulate cortex, along with the anterior thalamus, constitutes a 'primacy system' in which plasticity develops gradually and is not readily abandoned. Such a system might be thought of as a possible substrate for S-R 'habit' formation, which, like the neural activity in the posterior cingulate cortex [13], develops slowly through extended training [4,5,25]. A deficiency in such a system might therefore be expected to be manifested as a deficit late in learning. Thus we speculate that the impairment in late learning observed in the POS animals of the present study may be due to an impairment in the formation of S-R habits.

Importantly, however, animals of the POS group were unimpaired following the imposition of a 30-day retention interval, achieving a mean of >76% correct. Indeed, all groups in the present study performed at this level, thus demonstrating remarkable memory for the conditional rule. Furthermore, the POS animals were eventually able to perform the task at the same level of accuracy as animals of the other groups. This pattern of results suggests that while the posterior cingulate cortex is involved in the acquisition of the conditional rule, the final repository of this information is probably elsewhere.

A possible candidate structure is the dorsal striatum, to which the posterior cingulate cortex strongly projects [22]. Indeed several authors have suggested that the dorsal striatum may be involved in habit formation or procedural learning [24,28,36,38]. Rats with 6-OHDA lesions of the caudate-putamen were found to be markedly impaired in re-acquiring the CVD task when these rats had been trained preoperatively to a criterion of 85% correct choices, thus indicating a possible role for striatal dopamine in 'habit memory' [36]. Whereas excitotoxic lesions of both the ventral and lateral striatum impair initial acquisition of the task, lesions of the mediodorsal striatum, to which the anterior and posterior cingulate cortex both project [22], had no effect on acquisition [33]. This finding is compatible with the idea that the posterior cingulate cortex mediates the acquisition of information used in performing the CVD task, which is at some stage transferred to the striatum for storage.

It might be argued that rather than the posterior cingulate cortex being differentially involved in a fundamentally different kind of learning than the anterior cingulate cortex, it is necessary only in the late stages of learning for reasons related to the executive control of responding, such as the orienting response to the discriminative stimuli, allowing for optimum stimulus sampling, or the capacity to inhibit impulsive response tendencies (for example, responding on the most proximal lever). However, we have recently replicated the late-learning deficit following posterior cingulate cortex lesions in a conditional visual discrimination task in which these variables were tightly controlled (Bussey, Muir, Everitt and Robbins, in preparation). In this task, animals were required to touch computer-generated discriminative stimuli (coloured shapes) in the centre of a touch-sensitive screen [2]. Following this nosepoke, white squares were presented to the left and right of centre. The correct response consisted of a nosepoke to the left or right square, depending on the discriminative stimulus that had been presented. Thus, animals were required to orient directly in front of, and indeed make contact with, the discriminative stimulus before they were presented with the opportunity to respond. Moreover, this method ensured that all rats were located centrally and equidistant from the response areas when the opportunity to respond became available. The posterior cingulate cortex lesions also impaired the late stages of acquisition in this task, providing evidence that stimulus sampling or response control deficits are unlikely to
have been responsible for the impairment found in the present study.

Finally, given the observation that aspirative posterior cingulate cortex lesions impair the acquisition of a spatial navigation task in the water maze [40], it is plausible that the impairment in the posterior cingulate cortex lesioned animals in the present study is due solely to the spatial nature of the required responses, namely left and right lever presses. Although these responses appear to depend on 'egocentric' rather than 'allocentric' spatial information, as required in the water maze task, this does not rule out the possibility that this spatial component of the task may have contributed to the observed deficit. However, the role of the posterior cingulate cortex in the processing of spatial information is by no means well established. For example a recent study has shown that excitotoxic lesions of this area of cortex have no effect on the performance of egocentric or allocentric spatial tasks [27]. Furthermore, we have failed to observe a deficit in water maze learning following excitotoxic lesions of the posterior cingulate cortex (Bussey, Muir, Everitt and Robbins, in preparation). Thus, the issue of the contribution of spatial factors to the impairment in POS animals in the CVD task remains unresolved, and is currently under investigation.

5.3. Neuromodulation of cingulate function

The pattern of results obtained in the present study may provide clues as to the anatomical locus of effects obtained previously on the CVD task following manipulations of various neurotransmitter systems. For example, Everitt et al. [8] found that lesions of the dorsal noradrenergic bundle (DNAB) produced an impairment in learning the CVD task which gradually emerged late in acquisition. It is conceivable that this effect may have been due to noradrenergic denervation of the posterior cingulate cortex, to which the locus caeruleus projects [34]. In another study, forebrain serotonin depletion following intracerebro-ventricular infusions of 5,7-dihydroxytryptamine facilitated acquisition early in learning (Ward et al., in preparation), an effect possibly due to serotonergic denervation of the anterior cingulate cortex. Finally, a very high correlation has been obtained between levels of choline acetyltransferase activity in the anterior cingulate cortex and errors of commission made in acquisition and retention of a conditional discrimination: implications for cholinergic hypotheses of learning and memory, *Behav. Brain Res.*, 42 (1991) 33–42.


