# Effects of anterior cingulate cortex lesions on responding for conditioned reinforcement, discrete fear conditioning, autoshaping performance and Pavlovian–instrumental transfer

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**Abstract.** The anterior cingulate cortex (Ant Cing) in the rat has previously been shown to be critical for the acquisition of autoshaping, a measure of appetitive Pavlovian conditioning in which animals come to approach a stimulus (CS+) that predicts food delivery and not to approach a second stimulus (CS–) that does not. Here we demonstrate that Ant Cing lesions do not impair the acquisition of temporally discriminated approach to a CS that predicts reward (US) and is located in the same place as the US. Lesioned animals were able to respond instrumentally for this CS, now acting as a conditioned reinforcer, and the potentiation of responding by intra-accumbens amphetamine was unaffected. The Ant Cing-lesioned rats also acquired a freezing response to a discrete CS paired with footshock at the same level as controls. However, the same subjects were impaired at the acquisition of autoshaping, a task involving two stimuli distinguishable only by spatial location and located away from the source of food. A second group of Ant Cing-lesioned animals were impaired on the performance of the autoshaping task when the lesions were made after training to a criterion. These rats were tested on a Pavlovian–instrumental transfer task, in which an appetitive CS potentiates instrumental responding when it is presented noncontingently, and no impairment was found. A third group were tested on a novel task involving approach to a food magazine on the basis of a discrimination between two similar stimuli located elsewhere; Ant Cing-lesioned rats were impaired. These results suggest that the Ant Cing is critical for the normal expression of appetitive conditioning when multiple stimuli must be discriminated.

# Introduction

- The anterior cingulate cortex (Ant Cing) is a major cortical component of the 'limbic loop' of the basal ganglia. It has previously been implicated in stimulus–reward learning in the rat (Bussey et al., 1996; Bussey et al., 1997a; Bussey et al., 1997b) and other rodents (e.g. Gabriel & Orona, 1982; Gabriel et al., 1991).

- However, the exact contribution that the Ant Cing makes to processes of stimulus–reward learning and Pavlovian conditioning is not well understood.

- Therefore, rats with lesions of the Ant Cing were tested on a range of tasks to which some aspect of stimulus–reinforcer learning was expected to contribute, namely:

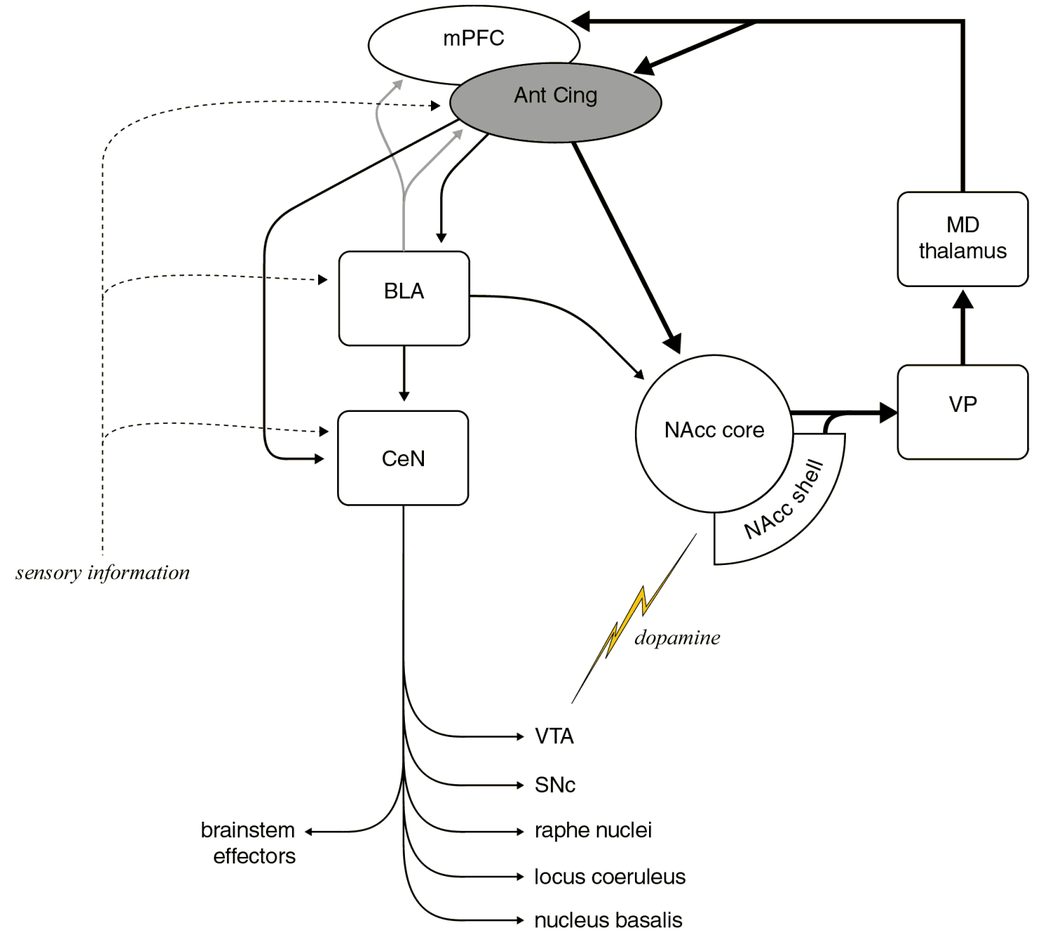
**approach to an appetitive conditioned stimulus (CS);**

**conditioned reinforcement,** in which subjects work for a Pavlovian CS previously paired with reward;

**autoshaping,** in which subjects approach a CS predictive of food, despite this taking them away from the food source itself;

**fear conditioning,** as assessed by the conditioned freezing response, which is a species-specific defence reaction to a CS that has gained aversive properties;

**Pavlovian–instrumental transfer,** in which an appetitive CS presented noncontingently enhances ongoing instrumental responding.




A simplified schematic of part of the 'limbic loop' of the basal ganglia. (*Abbreviations: NAcc – nucleus accumbens; Ant Cing – anterior cingulate cortex; mPFC – medial prefrontal cortex; BLA – basolateral amygdala; CeN – central nucleus of the amygdala; VTA – ventral tegmental area; VP – ventral pallidum.*)

# 1. Autoshaping (acquisition)

In the autoshaping procedure used, two stimuli are presented, a CS+ and a CS–; only the CS+ predicts food. Normal subjects come to approach the CS+, even though this takes them away from the food source, but they do not approach the CS–. Anterior cingulate lesions have previously been shown to impair this behaviour (Bussey et al., 1997a).

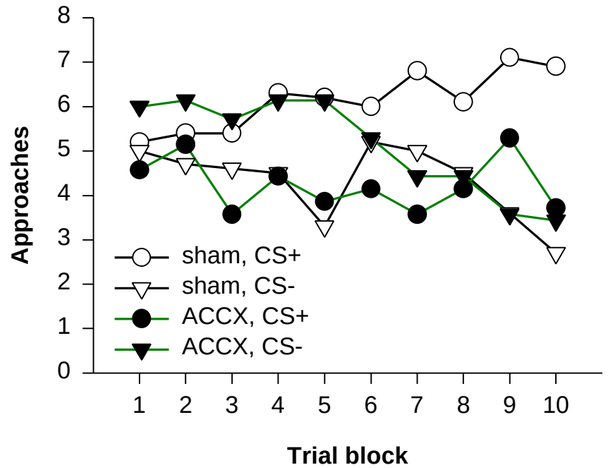
**Methods**

- Twenty-two male hooded Lister rats received lesions of perigenual anterior cingulate cortex (ACCX group, *n* = 12) or sham lesions (*n* = 10), with all animals additionally receiving cannulae aimed at the nucleus accumbens.
- Lesion coordinates from 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.
- Following recovery, subjects were maintained at 85% of their free-feeding mass.

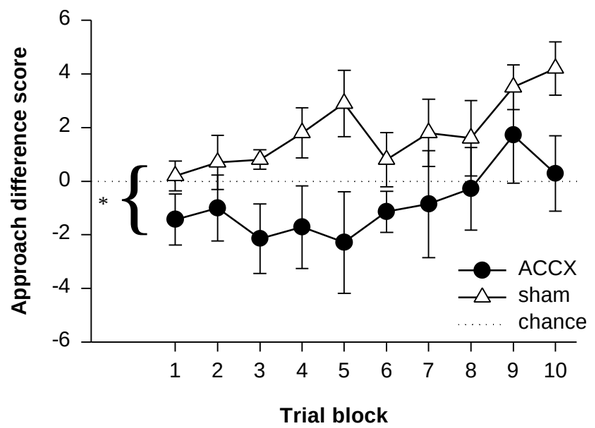
- Autoshaping was assessed in a testing chamber with a computer monitor on one wall and a centrally-located pellet dispenser. In front of the monitor, and at the back of the chamber, there were pressure-sensitive areas of floor to detect the subject's position.
- Rats were first given one session to habituate to the test chamber and learn to collect food pellets from the dispenser.
- After this, they were trained to associate stimuli with the delivery of 45-mg sucrose pellets. The stimuli were 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 across subjects.
- The controlling program waited for the rat to be centrally located at the rear of the chamber, and then presented a stimulus. The CS+ was always followed by delivery of one pellet; the CS– was never followed by food.
- Activation of one of the floor panels in front of a stimulus was scored as approach to the stimulus, and its latency noted.
- A trial consisted of a presentation of the CS+ and one of the CS–, separated by a variable interval of at least 10 s. Rats were trained over two days with 50 trials per day.

**Anterior cingulate cortex lesions impaired the acquisition of autoshaping.**

Final group sizes were *n* = 8 (ACCX) and 10 (sham). Shams came to approach the CS+ more than the CS–. This discriminated approach response was significantly impaired in lesioned rats.




Approach to the stimuli. Each block represents 10 trials (10 presentations of each of the CS+ and CS–). Sham controls show greater discrimination than the ACCX group.




The data shown above are replotted as difference scores (CS+ approaches minus CS– approaches); there is a significant impairment in the ACCX group. (* $p < .05$. A difference score of 0 implies no discrimination.)

# 2. Autoshaping (performance)

Though it has previously been reported that anterior cingulate lesions impair the acquisition of an autoshaped approach response (Bussey et al., 1997a), as confirmed again above, it is unclear whether the anterior cingulate cortex contributes only to learning, or is necessary for performance of a previously-learned response. A new group of subjects received lesions after training and were retested.

**Methods**

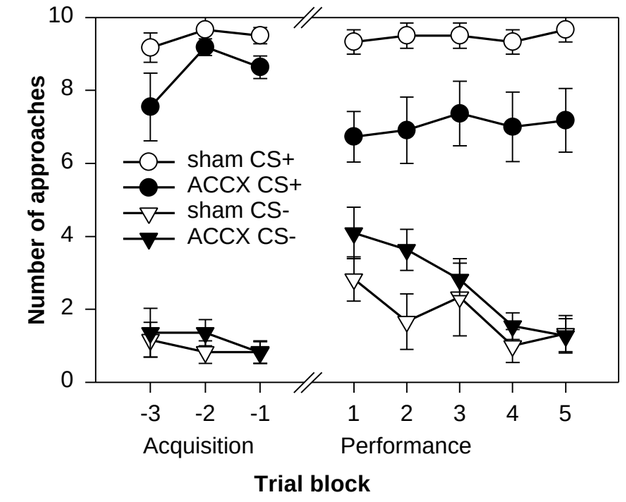
- Twenty-eight naïve male hooded Lister rats were maintained at 90% of their body weight and trained for 100 trials on the autoshaping task described above. Subjects that failed to approach the CS+ on at least 70% of the last 30 trials were given a further 50 remedial trials; if they failed to meet this criterion on the last 30 remedial trials, they were then excluded from the experiment.
- The successful subjects were given free access to food and randomly assigned to groups that received lesions of perigenual anterior cingulate cortex or sham lesions (with the same coordinates as described above).
- Following recovery, they were returned to the food deprivation regimen.
- Their performance on the same autoshaping task was tested for a further 50 trials.

Twenty subjects reached the initial performance criterion. Following histological analysis, the final group sizes were 11 (ACCX) and 6 (sham).

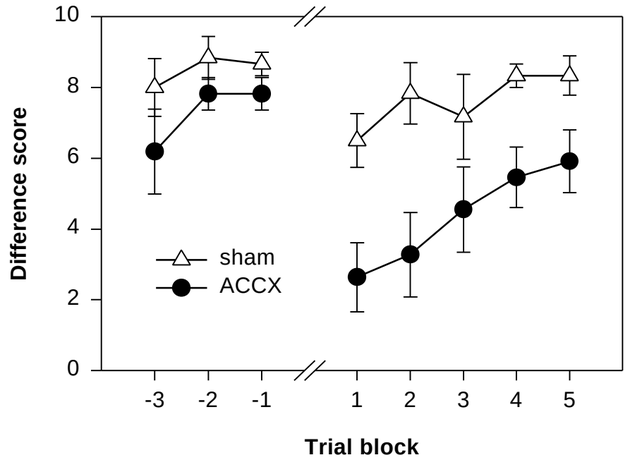
Pre-operatively, the groups did not differ.

Post-operatively, the ACCX group was significantly impaired relative to the sham group. Both groups showed an equivalent post-operative decline in CS– responding, but the ACCX group showed a persistent deficit in CS+ approaches.

**Anterior cingulate cortex lesions impaired the performance of autoshaping.**




Raw approach scores, pre- and post-operatively. Anterior cingulate lesions caused a persistent deficit in CS+ approach.




Difference scores (CS+ approaches minus CS– approaches) calculated for the same data. The ACCX group is significantly impaired.

# 3. Temporally discriminated approach to an appetitive CS

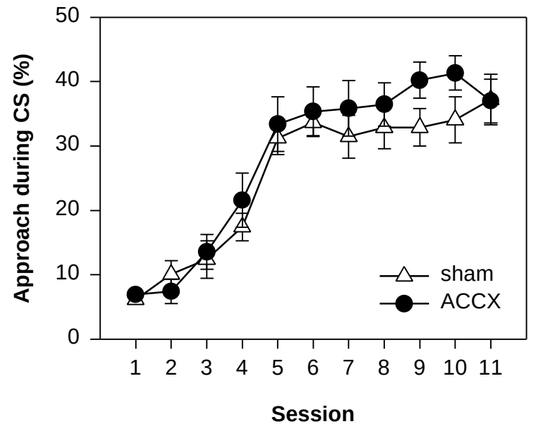
Rats with lesions of the anterior cingulate cortex were conditioned to approach a single CS that predicted food reward.

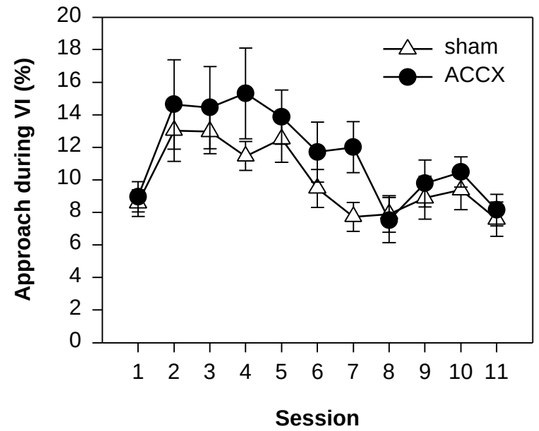
**Methods**

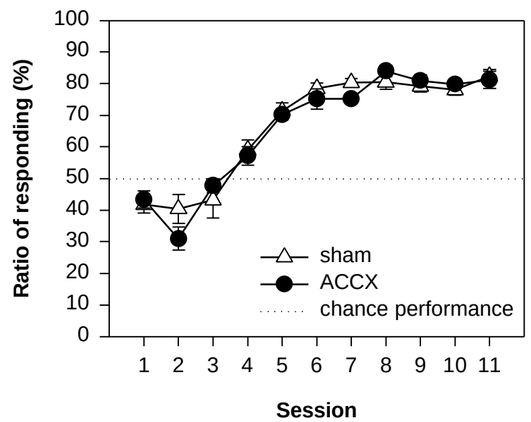
- The subjects were those used in the autoshaping acquisition experiment (*n* = 8 ACCX, 10 sham).
- The task was conducted in operant chambers equipped with a houselight and a food alcove. The alcove contained a traylight, a dipper (which could be elevated to deliver 0.05 ml of 10% sucrose solution), and an infrared detector to record nosepoking behaviour.
- In the variable interval (VI) phase, lasting 30–90 s, the houselight was on and the traylight was off. The dipper was not elevated.
- In the conditioned stimulus (CS) phase, lasting 5 s, the houselight was turned off and the traylight illuminated.
- Immediately following the CS, the unconditioned stimulus (US) was delivered: the houselight was turned back on, the traylight was turned off, and the dipper was elevated for 5 s to deliver food reward.
- Subjects received 30 CS–US pairings per session, with one session per day.

**Anterior cingulate cortex lesions did not impair approach to the CS.**

Both groups learned to approach the alcove during presentation of the CS. There were no differences between lesioned and sham groups. Thus, the same animals that were impaired at autoshaping performed normally in this task.




*Top left:* Approach to the food alcove during CS presentation.

*Top right:* Approach during the interval (VI).

*Bottom left:* A discrimination ratio – the proportion of approach behaviour occurring during the CS. Sham and ACCX groups did not differ in any respect.

# 4. A novel two-stimulus discriminated approach task

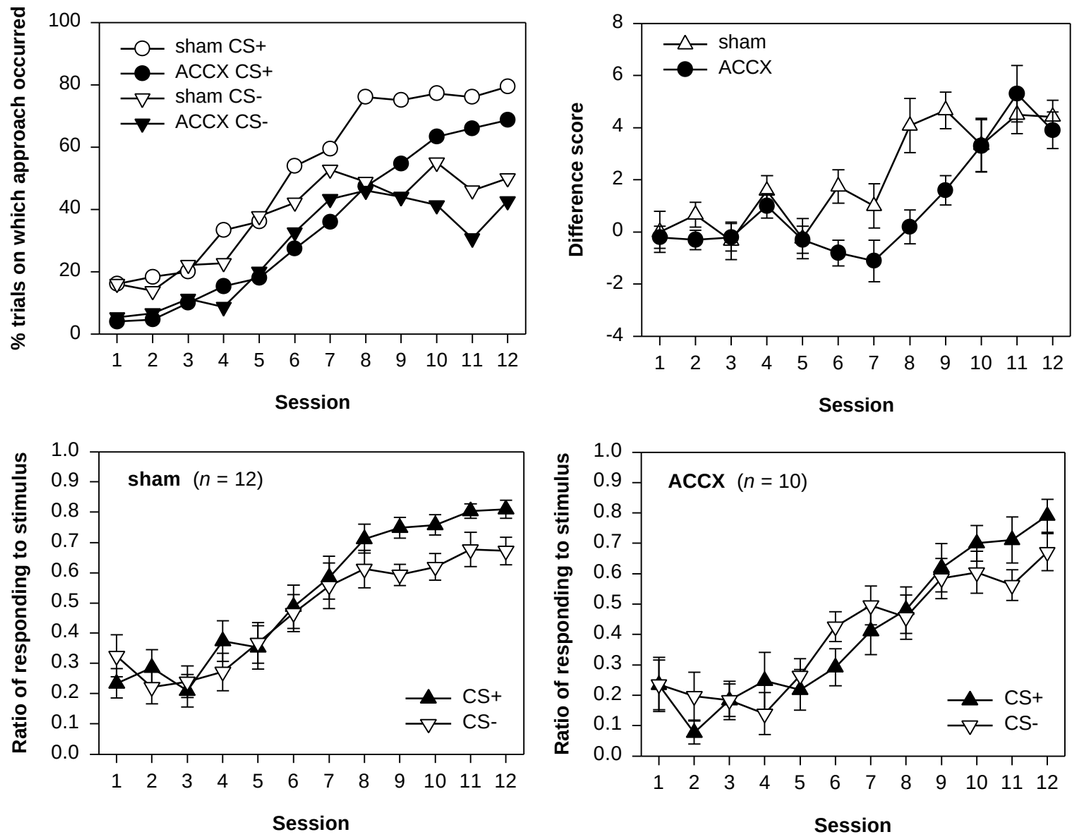
It was shown above that anterior cingulate lesions left some measures of stimulus–reward learning intact – strikingly, temporally discriminated approach to an appetitive CS was learned normally, even though autoshaping (an apparently similar task) was impaired in the same animals. These tasks differ in the number of controlling stimuli, and in the behaviour being measured. To establish the critical behavioural difference, a new task was designed:

| *Task* | *Number of stimuli* | *Approach behaviour* |
|---|---|---|
| temporally discriminated approach | 1 | approach to CS and food source |
| autoshaping | 2 | approach to CS, not food source |
| **(novel) two-stimulus discriminated approach** | **2** | **approach to food source, not CS** |

**Methods**

- Naïve rats received lesions of the Ant Cing ($n$ = 12) or sham lesions ($n$ = 12). Final group sizes were 10 (ACCX) and 12 (sham).
- The task was conducted in the operant chambers. Lights on the wall to the left and right of the food alcove were designated the CS+ and CS–, counterbalanced across rats.
- Initially, the houselight was on and the dipper was lowered. Following a VI of 30–90 s, the houselight was extinguished and a stimulus light 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 (the US). Following the CS–, the houselight was illuminated but the dipper was not raised, and a brief click was generated. Regardless of the stimulus, the chamber was then in the VI state and remained so until the next stimulus.
- One trial consisted of a presentation of the CS+ and one of the CS–, in randomized order. A session consisted of 15 trials, after which the houselight was extinguished. Subjects were trained for 12 sessions on the task, with one session per day.

**Anterior cingulate cortex lesions impaired approach behaviour based on a discrimination between two stimuli.**




*Top left:* Number of stimulus presentations during which the food alcove was approached at least once. *Top right:* Difference scores (CS+ minus CS–) for the data shown in A, showing the significant, though impermanent, impairment in the ACCX group. *Bottom left/right:* Proportion of time spent approaching the food alcove during CS+ and CS–. The sham group develop discrimination, but the ACCX group do not.

# 5. Simple Pavlovian–instrumental transfer

Pavlovian–instrumental transfer is the phenomenon by which Pavlovian conditioned stimuli, presented noncontingently, alter the rate of ongoing instrumental responding. In its simplest form, the Pavlovian CS is associated with the same outcome as the instrumental response. Rats with anterior cingulate lesions were tested on such a task.

**Methods**

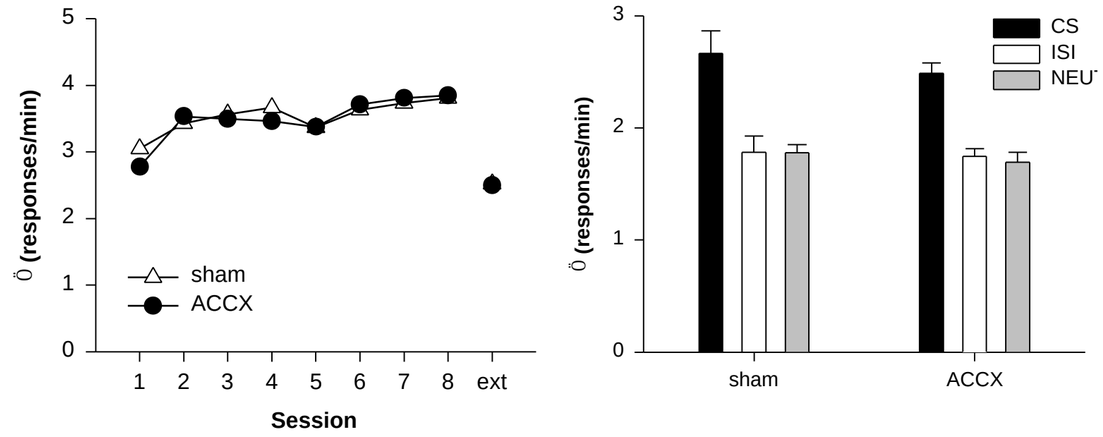
- Subjects were those from the autoshaping performance study, except for two that fell ill (final *n* = 9 ACCX, 6 sham).
- The task was conducted in the same apparatus used for the temporally discriminated approach and conditioned reinforcement tasks, which was new to the subjects. The method is based on Balleine (1994).

- Two stimuli (a 3-Hz flashing light and a 10-Hz relay clicker) were designated CS+ and NEUT, counterbalanced across rats. A houselight was illuminated throughout.
- *Pavlovian training.* Eight training sessions were given. Each session contained six 2-min presentations of the CS, during which reinforcement (45-mg sucrose pellet) was delivered on a random time (RT) 30-s schedule. Stimulus presentations were separated by an interstimulus interval (ISI) of 2–4 min, during which no reinforcement was given. In the final session, two 2-min presentations of the NEUT stimulus were also given unreinforced, to reduce unconditioned suppression when this stimulus was subsequently presented during the test phase.
- *Instrumental training.* Instrumental training was conducted in eight 30-min sessions with a single lever present. Responding was reinforced on a random interval (RI) schedule, whose parameter in subsequent sessions was 2, 15, 30, and thereafter 60 s.
- *Instrumental extinction.* A single 30-min session was given in which the lever was available but unreinforced.
- *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; they all lasted 2 min and were randomised in triplets.

**Anterior cingulate cortex lesions did not impair Pavlovian to instrumental transfer.**

Both groups acquired the lever-press response at the same rate, and no difference emerged in the extinction session.

On test, the CS (but not the neutral stimulus) reliably elevated responding; this is the Pavlovian–instrumental transfer effect. This effect was seen in both the sham and the ACCX groups, which did not differ.




*Left:* Instrumental training and extinction.
*Right:* Pavlovian to instrumental transfer test.

# 6. Conditioned reinforcement

Subjects acquired a new response with conditioned reinforcement, using the CS from the temporally discriminated approach task as the conditioned reinforcer. Amphetamine or vehicle was injected into the nucleus accumbens during the test.

**Methods**

- Subjects were those from the temporally discriminated approach and autoshaping acquisition experiments.
- Accumbens cannulae had final target coordinates of AP +1.6, ML ±1.5, DV –7.0 from dura.

- The conditioned reinforcement test was conducted in the same operant chambers used for the temporally discriminated approach task.
- A session began when the subject nosepoked in the food alcove and lasted 30 minutes.
- Two levers were introduced into the chamber. Responding on one (the CRf lever) produced an abbreviated version of the CS from the previous task, with probability 0.5. The abbreviated CS consisted of extinguishing the houselight and illuminating the traylight for 0.5 s, together with raising the empty dipper. No primary reinforcement was ever given in this task. Responding on the other (NCRf) lever had no programmed consequence and was used as a control for general activity.

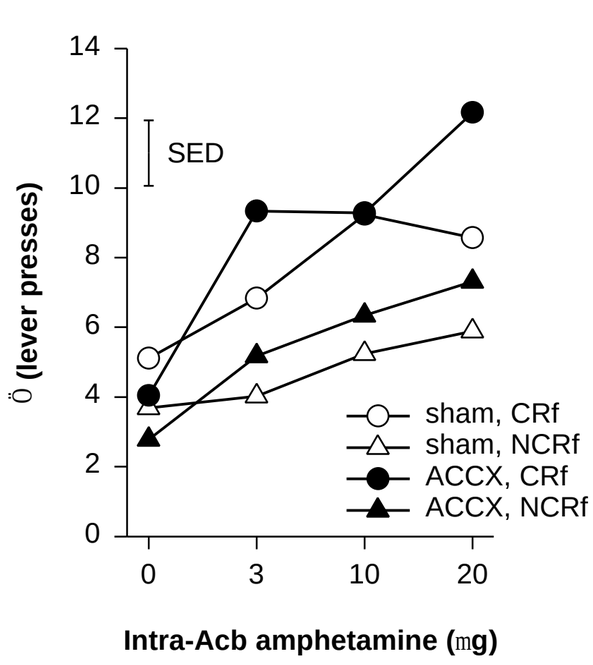
- The conditioned reinforcement test was conducted with intra-accumbens amphetamine. Immediately before each session began, one of 4 doses of D-amphetamine sulphate (0, 3, 10 and 20 µg in a 1-µl volume of 0.1 M sterile phosphate buffer, pH 7.4) was administered bilaterally. Doses were counterbalanced in a Latin square design.

**Anterior cingulate cortex lesions did not impair conditioned reinforcement, or its potentiation by intra-accumbens amphetamine.**

Six rats in the ACCX group and ten in the sham group had cannulae correctly sited in the nucleus accumbens.

Subjects responded more on the CRf lever than on the NCRf lever, indicating that the CS had conditioned reinforcing properties. Responding for the conditioned reinforcer was selectively potentiated by intra-accumbens amphetamine, which increased responding on the CRf lever more than it did on the NCRf lever.

However, there were no differences in responding between ACCX and sham groups.




(SED, standard error of the difference between means.)

# 7. Discrete fear conditioning

The subjects used in the conditioned reinforcement study were given a final test: they were trained to associate a clicker CS with footshock. In a different context, the CS was again played, and the conditioned freezing response was measured.

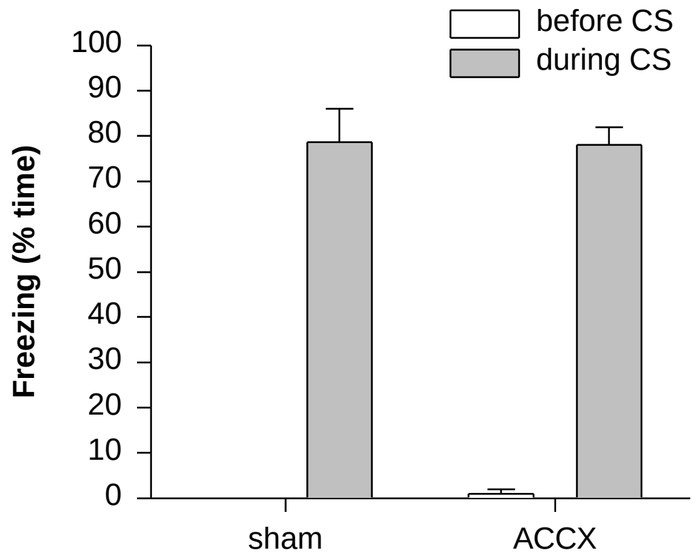
**Methods**

- Subjects were those from the previous study (*n* = 8 ACCX, 10 sham).

- Fear conditioning was carried out using two distinctive experimental contexts, termed Light and Dark, which differed in chamber construction, floor type, visual appearance, lighting conditions and subjective circadian time.
- The Dark context was equipped with a steel grid floor through which electric shock could be delivered.
- On days 1–3 of the experiment, subjects were pre-exposed by being placed for 25 min in each context.
- On day 4, they were placed in the Dark context, where they received 5 presentations of a 10-s, 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 behaviour was videotaped. After 5 min of CS absence, the clicker CS was played continuously for 10 min. Freezing activity was assessed by scoring the tapes in 5-s activity bins, using a stringent criterion: if and only if the animal was motionless apart from respiratory movements for the full 5 s, the bin was scored as “freezing”. The calculated measure was the percentage of bins spent freezing; the 2 minutes preceding CS onset were compared with the 8 minutes following CS onset.

**Anterior cingulate cortex lesions did not impair conditioned freezing.**

Subjects showed no freezing behaviour when placed in the testing chamber, but exhibited robust freezing when the aversive CS was played. There were no differences between sham and ACCX groups.



# Summary of results

In rats, lesions of the anterior cingulate cortex had highly specific effects. They

- **impaired** the acquisition of autoshaping, a task in which stimuli are not located at the source of food, and in which subjects approach a CS+ that predicts food (but do not approach a perceptually similar CS– that does not);

- **impaired** the performance of the same autoshaping task when the lesion was made after training;

- **did not impair** the acquisition of a temporally discriminated approach response to a single appetitive CS that was located at the source of food;

- **impaired** the acquisition of a two-stimulus discriminated approach task, in which subjects approached the food source when stimuli were presented at another location; a CS+ and a CS– were used, and they differed only in location;

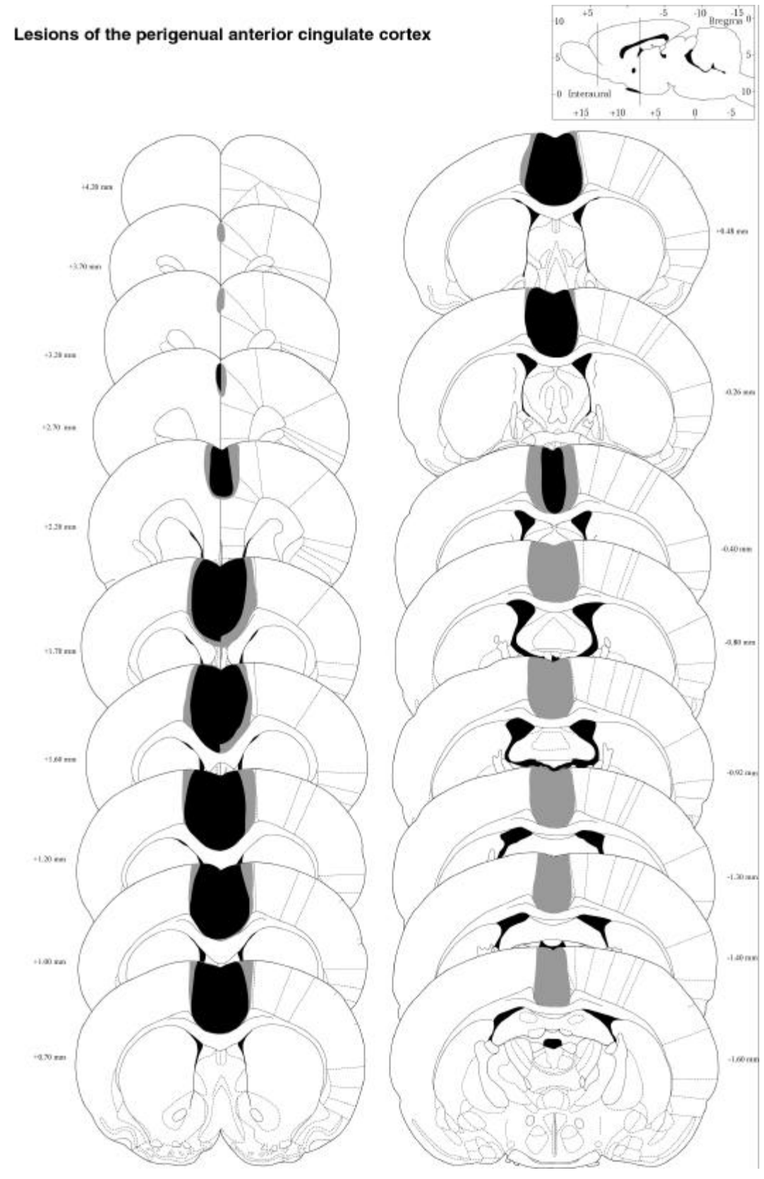
In addition, the lesions **did not impair**

- simple Pavlovian–instrumental transfer.

- the acquisition of a new response with conditioned reinforcement (using a single conditioned reinforcing stimulus);

- the potentiation of responding for conditioned reinforcement by intra-accumbens amphetamine;

- conditioned freezing to an aversive discrete CS;



# Discussion and conclusions

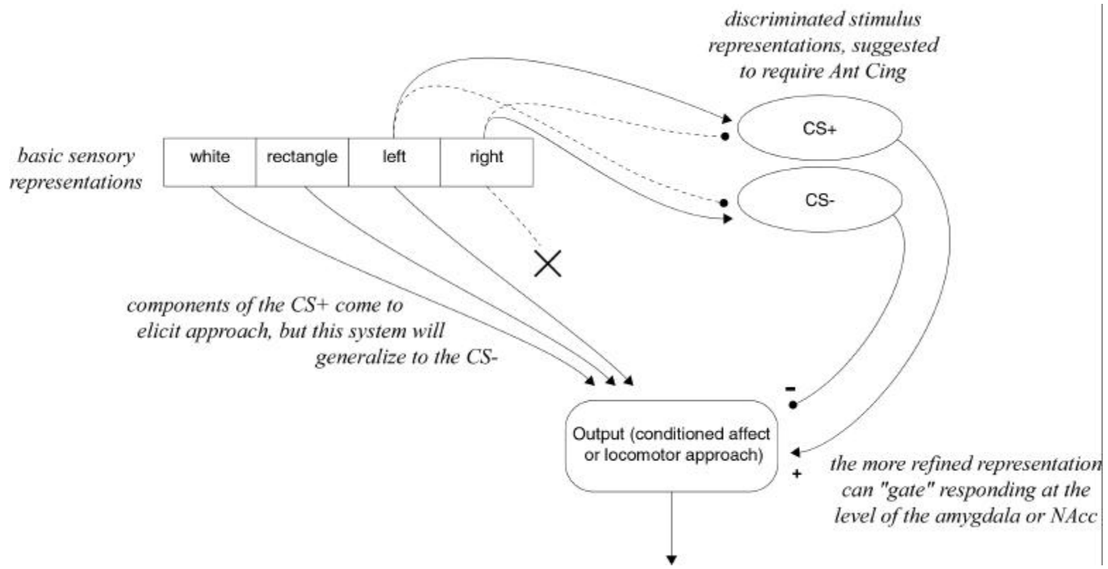
- The present results replicate the finding that autoshaping is impaired following anterior cingulate lesions (Bussey et al., 1997a; Parkinson et al., 1996) and extend the involvement of the Ant Cing to the performance of a previously-learned autoshaped approach response.

- However, the present results also indicate that the Ant Cing is *not* required for the development of appetitive or aversive Pavlovian conditioning *per se*, as lesioned subjects were unimpaired on a range of other tasks assessing conditioning.

- Therefore, the deficit induced by Ant Cing lesions must be more specific. We suggest that **the Ant Cing is required for discriminating multiple stimuli on the basis of their association with reward.**

- According to this hypothesis (Parkinson et al., in press), Ant Cing-lesioned rats can form an affective response to conditioned stimuli, discriminating CS presence from absence; they can also call up an affective representation of the US, and so acquire new responses with conditioned reinforcement. However, CS specificity of the representations is impaired; as a result, tasks that depend upon stimulus–reinforcer associations when those stimuli are difficult to discriminate require the Ant Cing (including autoshaping, and 8-pair concurrent visual discrimination; Bussey et al., 1997b).

- A functional connection between the Ant Cing and the nucleus accumbens core is required for Pavlovian conditioned approach behaviour (Parkinson et al., 2000). According to the present hypothesis, the Ant Cing disambiguates the stimulus for the rest of the limbic circuit described, involving the amygdala and nucleus accumbens (see figure).



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