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ORIGINAL INVESTIGATION

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# Excitotoxic lesions of the basolateral amygdala impair the acquisition of cocaine-seeking behaviour under a second-order schedule of reinforcement

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**Abstract** In these experiments we sought to establish the intravenous (IV) self-administration of cocaine under a second-order schedule of reinforcement in order: (i) to obtain reliable, drug-free levels of responding with cocaine as a reinforcer, and (ii) to enable investigation of the neural mechanisms by which arbitrary cues gain motivational salience and, as conditioned reinforcers, control over drug-seeking behaviour. Initially, each infusion of cocaine was made contingent upon a response on one of two identical levers and was paired with a 20-s light conditioned stimulus (CS). Responses on the second lever were recorded, but had no programmed consequence. When rats acquired stable rates of self-administration, a second-order schedule of the type FRx(FRy:S) was introduced, with values of "x" being increased progressively to 10 and then "y" from 2 through 8. Priming (i.e. non-contingent) infusions of cocaine were never given. Once the first infusion was obtained under the second-order schedule, further infusions were made contingent on each response (to a maximum of ten infusions/day). Each stage was repeated daily until the first infusion of each session was achieved within a 5-min criterion. Rats with bilateral, excitotoxic lesions of the basolateral amygdala readily acquired the IV self-administration of cocaine under a continuous reinforcement schedule, initially administering more infusions and maintaining a slightly elevated level of self-administration than controls. Despite increased numbers of CS/drug pairings, basolateral amygdala-lesioned rats were severely impaired in the acquisition of the second-order schedule of IV cocaine reinforcement. Lesioned rats showed a cocaine dose-response function that was shifted upwards relative to control subjects. There was no significant difference between drug-naive amygdala-lesioned and control animals in the locomotor response to intraperitoneal injections of cocaine. These experiments indicate the feasibility and utility of second-order schedules in studying the neurobehavioural basis of cocaine-seeking behaviour. They suggest a dissociation in the neural mechanisims underlying cocaine-taking and cocaine seeking behaviour, and demonstrate the potential importance of the basolateral amygdala in the processes by which previously neutral stimuli gain control over drug-seeking behaviour.

**Key words** Cocaine · Second-order schedule · Conditioned reinforcer · Nucleus accumbens · Amygdala · Reward · Dopamine

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## Introduction

The reinforcing (or rewarding) effects of psychomotor stimulant drugs such as amphetamine and cocaine can be investigated using the technique of intravenous drug self-administration. Responding can be maintained by such drugs under a number of schedules of reinforcement, including interval and ratio schedules (Pickens and Thompson 1968; Goldberg 1973; Johanson and Schuster 1981; Corrigal and Coen 1989; McGregor and Roberts 1993). In particular, progressive ratio schedules have been used to obtain breaking point indices of the reinforcing effect of drugs (Loh and Roberts 1990). One problem that arises with such measures of the reinforcing efficacy of drugs is that responding may also be affected by other, acute effects of the intravenously self-administered drug that are unrelated to its reinforcing effect. Thus, after an initial infusion of drug – often given non-contingently in many studies in order to elicit responding – the reinforcing and rate-altering effects of psychomotor stimulant drugs are confounded. This confounding is also problematic when trying to interpret neurochemical or neural correlates of self-administration behaviour, for example the in vivo monitoring of dopamine in the ven-

tral striatum (Hurd et al. 1989; Phillips et al. 1991; Fontana et al. 1993; Wise et al. 1995).

An alternative method for measuring the reinforcing efficacy of drugs is to employ a second-order schedule in which drug infusions are presented contingent upon completion of the schedule requirement (Goldberg 1973; Kelleher 1975; Goldberg et al. 1976; Goldberg and Tang 1977). Such responding, often called "drug-seeking behaviour", can be maintained for quite long periods in the undrugged state by the contingent presentation of secondary, or conditioned, reinforcers which are ultimately paired with each drug infusion. Characteristic patterns of responding can be maintained by such stimuli according to the "second-order" unit schedule. Thus, for example monkeys and rats can be trained to respond under a second-order schedule in which every *n*th response produces a stimulus paired with intravenous drug delivery, with the drug infusion contingent on the final response after a fixed interval has elapsed. Such a procedure may provide a model of drug "craving" (see Markou et al. 1993) as well as a means for investigating the neural and neurochemical basis of the impact of drug-associated cues on drug-seeking behaviour that is independent of the acute effects of the self-administered drug.

Our previous work has demonstrated that the control over behaviour exerted by stimuli paired with primary rewards (i.e. conditioned incentive or secondary reinforcers) is greatly enhanced by dopamine-dependent mechanisms in the ventral striatum (Taylor and Robbins 1986; Robbins et al. 1989; Wolterink et al. 1993). Indeed, amplifying the effects of drug-associated cues may constitute a significant mechanism for the reinforcing effects of psychomotor stimulant drugs such as amphetamine and cocaine (Phillips and Fibiger 1990; Robinson and Berridge 1993). The interaction of drug-induced increases in dopamine transmission with the effects of conditioned reinforcers is also known to depend on glutamatergic afferents to the nucleus accumbens arising especially from the basolateral amygdala (see Burns et al. 1994). Following lesions of the basolateral amygdala, the capacity of conditioned reinforcers to affect behaviour in the presence or absence of drugs such as amphetamine is reduced (Cador et al. 1989; Everitt et al. 1989; Robbins et al. 1989; Everitt and Robbins 1992). For example, under a second-order schedule of sexual reinforcement, excitotoxic lesions of the basolateral amygdala impaired the capacity of a brief light conditioned stimulus (CS+) to maintain responding by male rats for presentation of an oestrous female (Everitt et al. 1989).

Consequently, in the study reported here we have established a second-order schedule of intravenous cocaine self-administration. Subsequently, we have investigated the effects of lesions of the basolateral amygdala on the reinforcing effects of cocaine and on the acquisition of this newly-established second-order schedule of intravenous cocaine reinforcement. In these experiments we therefore sought to test the hypothesis that the ability of drug-associated CSs to elicit and maintain drug-seeking behaviour depends on the integrity of the basolateral amygdala, a limbic forebrain structure that is known from our earlier work to mediate associations between environmental cues and primary reinforcers and thus their impact on instrumental behaviour (Everitt and Robbins 1992).

## Materials and methods

### Animals

Male Lister hooded rats (Olac, Bicester, UK) weighing between 300 and 350 g at the beginning of the experiment, were housed under a 12-h: 12-h reversed light/dark cycle (lights off 0900 hours). Experiments were carried out between 0900 and 1400 hours. Food was made available at 1700 hours and each animal received 20 g Purina lab chow/day sufficient to maintain pre-operative body weight and growth. Water was freely available at all times in the home cage. The experiments were undertaken in accordance with the United Kingdom 1986 Animals (Scientific Procedures) Act (Project Licence PPL 80/00684).

### Surgery, intravenous catheterisation

Rats were anaesthetised with Avertin (10 g 99% 2,2,2-tribromoethanol, (Sigma-Aldrich Company, Dorset, UK) in 5 mg tertiary amyl alcohol and 4.5 ml phosphate buffered saline (Dulbecco "A"; Unipath Ltd, Basingstoke, Hampshire, UK) in 40 ml absolute alcohol; 1 ml/100 g body weight IP). Excitotoxic lesions of the basolateral amygdala were made by infusion of 0.09 M quinolinic acid (Sigma-Aldrich Company, Dorset, UK) buffered to pH 7.0–7.3 in 0.1 M phosphate buffer via a 1 µl SGE syringe (SGE UK, Milton Keynes, UK). Two injections of 0.3 µl each were made in each hemisphere [co-ordinates AP: –2.3, –3.0; L: ±4.6; V: –7.3 from the dural surface; incisor bar: –3.3]. The infusion cannula was left in position for 2 min following each infusion to allow for the complete diffusion of the toxin. Sham lesioned controls underwent identical surgical procedures, but phosphate buffer vehicle was infused instead of the excitotoxin.

Following stereotaxic surgery, animals were allowed to recover with free food for 3 days. On day 4 they were again anaesthetised with Avertin and implanted with chronic indwelling jugular catheters which exited dorsally between the scapulae (see Caine et al. 1992 for detailed procedure). The catheters were made using guide cannulae (C313G 5UP Semat Technical Ltd, St Albans, Herts, UK) and silicon tubing (STHT-C-030–0 and STHT-C-020–0, Osteotec Ltd, UK). They were flushed daily for the first 5 days post-surgery with 0.1 ml of a strong antibiotic solution (Timentin 3.2 g: 200 g potassium clavulanate with 3 g ticarcillin; Beecham Research, Welwyn, Herts, UK; 65 mg of this powder was dissolved in 1 ml 0.9% sterile saline: Animalcare Ltd, Dunnington, UK) and thereafter daily with 0.1 ml heparin solution (CP Pharmaceuticals Ltd, Wrexham, UK; 30 units/ml 0.9% sterile saline).

At the end of the experiment rats were killed under deep pentobarbitone sodium BP anaesthesia (Euthatal™, Rhone Mérieux Ltd, Harlow, Essex, UK) and perfused through the heart with 0.9% saline followed by 4% paraformaldehyde (40 g powdered PFA (Merck, Darmstadt, Germany) in 1 l phosphate buffer). The brains were removed and post-fixed in 4% PFA for 2 h following which they were left overnight in 20% phosphate-buffered sucrose solution. Sections were cut at 60 µm on a freezing microtome (Anglia Scientific, Norwich, UK) and mounted on gelatine-treated slides which were then stained with Cresyl Violet.

### Lesion assessment

Lesions were assessed according to patterns of neuronal loss, with or without associated gliosis. The areas of neuronal loss so-defined within and around the amygdala were mapped individually

onto schematic representations of coronal slices of the brain (Swanson 1992) by an experimenter blind to the behavioural data. Assignment to basolateral amygdala lesion groups was done on the basis of the presence of bilateral basolateral amygdala lesions and no, or only unilateral, damage to other structures, such as the central amygdaloid nucleus, periamygdaloid cortex or the overlying globus pallidus.

## Apparatus

Experiments were carried out using four operant chambers (28×26×28 cm; Gerbrands Corporation, Arlington Mass., USA). Each chamber contained two retractable levers 4.8 cm wide, positioned equidistantly on one wall, 17.5 cm apart and 9 cm from the grid floor. The force required to depress a lever was approximately 12 g. The chamber was illuminated by a red enamel 2.5 W, 24 V house light which was positioned on the top of the chamber. A circular opaque disc (2.5 cm in diameter) positioned 10 cm above each lever could also be illuminated by a 2.5 W, 24 V light bulb as a stimulus light. Each box was equipped with a Razel infusion pump (Semat Technical Ltd, St Albans, Herts, UK) which could be operated via software controlled by a computer. Intravenous infusions of cocaine were delivered through a single channel liquid swivel (Stoelting, Wood Dale, Ill., USA) with connector attachments (Plastics One, Roanoke, Va., USA). Three rapid presses on either lever initiated the session, signalled by illumination of the house light. The initiating lever automatically registered it as the *drug* lever while responses on the other *control* lever had no programmed consequence, and these provided an index of basal levels of activity only. Subsequent depression of the *drug* lever caused the house light to extinguish and the *drug* lever stimulus light to be illuminated. Both levers then simultaneously retracted and the infusion pump was activated for 4 s, delivering a 0.1 ml intravenous infusion of cocaine solution (1.5 m/kg). After a further period of 16 s, the levers were again extended into the chamber, the stimulus light went out (total duration 20 s) and the house light was illuminated. Further depression of the *drug* lever repeated this sequence of events and resulted in further infusions of cocaine.

The operant chambers were housed in a sound-attenuated box and the pumps were positioned outside them. External noise was further masked by ventilating fans mounted on the side of each box. The apparatus was controlled and data was collected by an Acorn Archimedes microcomputer (Acorn Computers Ltd, Cambridge, UK), running the control language Arachnid (an extension of BASIC; Fray 1980). The total number and temporal pattern of responses was recorded for each lever.

Locomotor activity was measured using 16 fixed wire activity cages (25×40×18 cm) each fitted with two parallel infra-red photo beams, 2 cm from the cage floor 20 cm apart on the long axis of the cage. Interruption of either beam was processed by a BBC Master computer (Acorn Computers, Cambridge, UK) situated in an adjacent room, and registered as a single activity count for that cage. Activity counts were recorded in 10-min time bins.

## Drugs

Cocaine hydrochloride (McFarlan-Smith, Edinburgh, UK) was dissolved in sterile 0.9% saline. All doses of cocaine were calculated as the salt. Quinolinic acid (Sigma-Aldrich Company Ltd, Dorset, UK), 0.09 M was dissolved, in sterile phosphate buffered saline and the pH adjusted to 7.0–7.3, with 0.1 M NaOH.

## Procedure

### *Experiment 1: acquisition of cocaine self-administration*

The first 7 days of IV cocaine self-administration were studied in both basolateral amygdala-lesioned and sham-operated rats. Each animal was allowed access to the drug for 2 h on consecutive days, 2 days each week were drug-free. Once responding had stabilised (4–7 days), the animals began the next phase of the experiment.

### *Experiment 2: within-session dose-response function*

Each test began with 30 min IV cocaine self-administration at the training dose (1.5 mg/kg), followed by four separate doses self-administered over 45 min each. In order to reduce the duration of the dose-response test, eight doses were divided into two separate tests, A and B, which were conducted on separate days with an intervening baseline day. The order in which the tests were carried out was also counterbalanced across groups. Dose response A: 1.5, 0.75, 0.188, 0.047, 0.012 mg/kg per infusion. Dose response B: 1.5, 0.375, 0.094, 0.023, 0.00 (saline) mg/kg per infusion. Care was taken to ensure the length of tubing from the pump to the connecting swivel was as short as possible and equal for all boxes, thus guarding against mixing doses as the syringes were changed within session. However, as a precaution against such problems, data from the first 15 min of each 45-min dose period were excluded from the final analysis. This procedure has been validated in our previous work (Phillips et al. 1994a,b). Responding was recorded for both control and drug levers throughout.

### *Experiment 3: second-order schedule of self-administration*

During the acquisition of IV cocaine self-administration, depression of the assigned drug lever resulted in the house light being extinguished and a 1-s delay followed by the presentation of a 20-s light stimulus (CS+) with a 0.1 ml infusion of cocaine (1.5 mg/kg) over 4 s. This continuous reinforcement schedule of IV cocaine self-administration can also formally be described as a second-order schedule of the type FR1(FR1:S); thus each response on the drug lever resulted in a single drug infusion which was paired with a 20-s light stimulus. Once stable responding had been achieved, the response requirements of the second-order schedule were systematically increased by increasing the ratios of responses, first for the IV cocaine infusions and then for presentations of the CS+.

In the first stage, each rat was required to respond on the active lever under an FR10 schedule thereby earning ten presentations of the CS+, each presented under a FR1, for the first IV infusion of cocaine, i.e. a second-order schedule of the type FR10(FR:1S). Subsequently the second-order requirements were made more stringent by progressively increasing the ratio of responses for the CS+ in stages, i.e. FR10(FR:2S), FR10(FR4:S), FR10(FR:8S). Once the first infusion was obtained, nine further infusions were made contingent upon each response (maximum ten infusions/day). Each second-order stage was repeated until the animal completed the response requirement (drug-free) for its first IV cocaine infusion of the day, within a 5-min criterion. To succeed at any stage, the rat had to perform to criterion within five consecutive daily sessions. Those rats that did not achieve this criterion were adjudged to have failed, but received further opportunities on subsequent days to achieve the criterion on successive sessions. Those rats that were successful moved directly onto the next stage of the schedule, on their next test day.

### *Experiment 4: locomotor response to IP cocaine*

Ten drug-naive, basolateral amygdala-lesioned and six sham-operated control animals were randomly allocated to individual photocell cages. These animals were never surgically prepared with chronic IV catheters and at no time had any self-administration experience. Two consecutive 2-h daily sessions were sufficient to habituate the animals to the novel environment, and saline injections were administered in the home cage on the day prior to the start of the test. An initial saline test day was given in a manner identical to that on the following drug test days, where all animals received three doses (10, 20, 30 mg/kg) of cocaine in an ascending order, with a drug free day between each dose. All test ses-

sions lasted for 2 h, with saline or cocaine administered 30 min into each session.

### Statistical analyses

All statistical analyses with the exception of the Fisher Exact Probability test, were carried out using CLR ANOVA (Clear Lake Research, USA) for Apple Mac computers. Data from experiment 1 were initially subjected to an ANOVA with three factors: Group (Basolateral amygdala, Sham); Lever (Drug, Control); Day (1,2,3,4). Statistically significant interactions ($P<0.05$) were analysed further for Simple Main Effects using the pooled error term (Winer 1971, pp. 529–532) and individual means were compared using the Newman-Keuls test. Contingency tables of animals acquiring or failing each stage of the second-order schedules of self-administration were analysed non-parametrically using a 2×2 Fisher Exact Probability test. Locomotor data were collected in 10-min bins over the 2-h session, and subjected to ANOVA with two factors, Group (Basolateral amygdala, Sham)×Time (12×10-min bins).

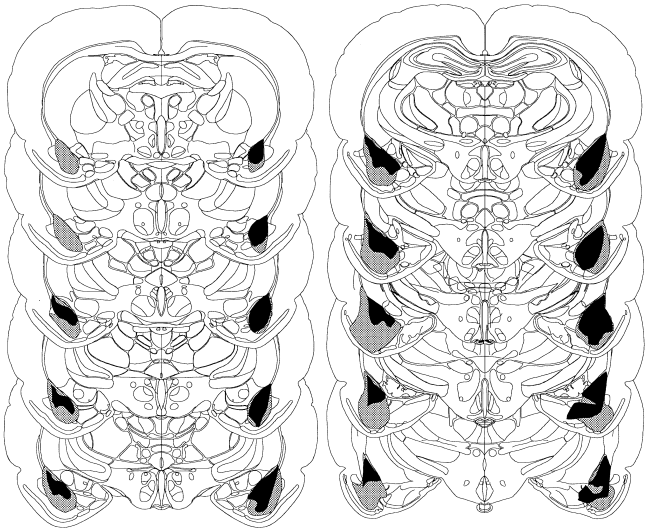

**Fig. 1** Schematic illustration of the largest (*stippled shading*) and smallest (*solid black shading*) lesions of the basolateral amygdala in rats in experiments 1–4). Lesions were defined as areas of neuronal loss by an assessor "blind" to the behavioural data. In general, the central and medial nuclei of the amygdala were not included in the lesions and in the majority of cases the lesions were associated with <90% destruction of the lateral and basal magnocellular nuclei, together with variable damage to the accessory basal and basomedial nuclei. The drawings are adapted from Swanson's Brain Maps (1992) and cover the sections –2.12 to –3.3 from bregma according to the atlas of Paxinos and Watson (1986)

## Results

### Lesion assessment

Microscopic analysis of the brains of animals infused with quinolinic acid into the basolateral amygdala revealed marked neuronal loss and associated, but variable gliosis. Only animals having sustained bilateral lesions of the majority of the antero-posterior extent of the basolateral amygdala were included in each experiment (numbers are indicated in each experiment below). In some cases, there was unilateral damage to the central nucleus of the amygdala, but in general this nucleus was spared bilaterally, as we have observed previously (Everitt and Robbins 1992; Burns et al. 1993). Similarly, medial and cortical parts of the amygdala were not damaged by infusions of quinolinate into the basolateral amygdala. Occasionally, there was cell loss in parts of the piriform or perirhinal cortex, but this was also usually only unilateral. Finally, neuronal loss was sometimes seen in the overlying caudal regions of the dorsal globus pallidus, but this too was usually seen only unilaterally. Assigning animals post hoc to experimental groups of basolateral amygdala lesions was done by an assessor "blind" to the behavioural results. Rats sustaining bilateral damage to structures other than the basolateral amygdala, or that had sustained only unilateral basolateral amygdala damage were excluded from the behavioural analysis. A schematic of the largest and smallest basolateral amygdala lesions included in the studies reported are illustrated in Fig. 1. Representative photomicrographs of lesioned and control brains are shown in Fig. 2.

### Experiment 1: acquisition of intravenous cocaine self-administration

Sixteen basolateral amygdala-lesioned and eight sham-operated rats began this experiment. Of the 16 lesioned animals, five were discarded from the final analysis because histological assessment showed the lesions to be incomplete, either unilateral or not encompassing sufficient of the full antero-posterior extent of the basolateral amygdala. A further two animals from each group were also eliminated due to catheter blockade, making the final grouping nine lesions and six shams. Figure 3 shows active (drug) and inactive lever responses during the first 7 days of acquisition of IV cocaine self-administration. Basolateral amygdala-lesioned rats showed elevated drug lever responding compared to sham lesioned animals on all but the last day of acquisition. Three-way ANOVA revealed a significant effect of Group [$F(1,13)=5.29$, $P=0.04$] and a significant Group×Lever interaction [$F(1,13)=7.81$, $P=0.01$] which reflects the gradual disappearance of a difference in responding on the active and inactive levers due to the progressive increase in responding on the active lever by sham operated controls. Post hoc analysis revealed significant differences between sham-operated and basolateral amygdala lesioned rats in their responding on the active lever [$F(1,25)=12.57$, $P<0.005$], but no difference between the groups in inactive lever responding [$F(1,25)=0.001$, $P=0.971$].

By day 2 of acquisition sham animals clearly differentiated between the drug and control levers, responding at progressively higher levels on the drug versus the control lever. Subsequent two-way ANOVAs by Group revealed a significant Day×Lever interaction [$F(6,30)=3.5$, $P<0.001$] for the sham group, and the basolateral amygdala lesioned group [$F(6,48)=4.48$, $P<0.005$], reflecting the progressive separation of responding on the active (drug) and inactive (control) levers during the 7 days of acquisition.

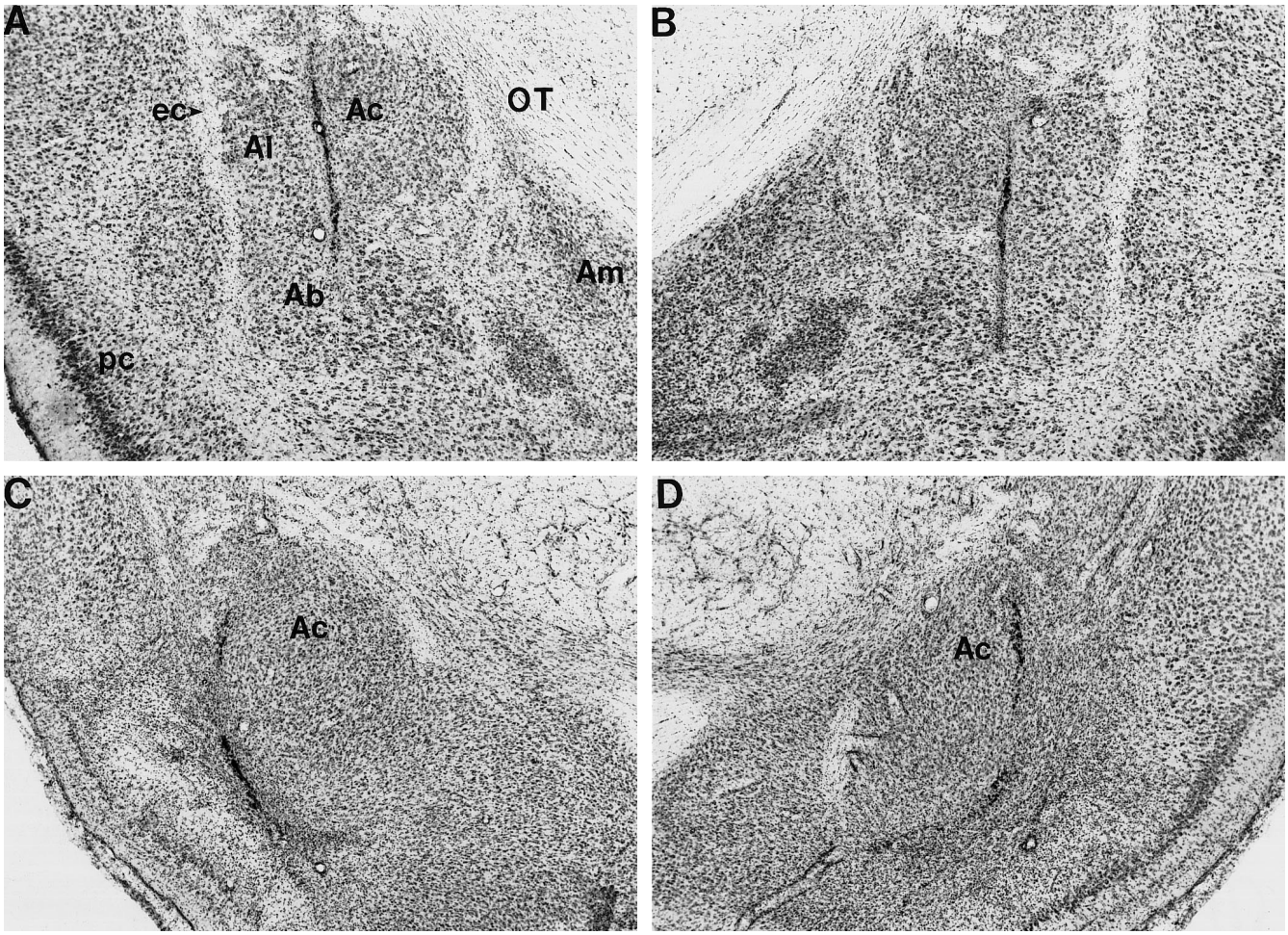


**Fig. 2** **A**–**D** Photomicrographs of the appearance of quinolinate lesions of the basolateral parts of the amygdala in 60 μm sections of Cresyl Violet-stained material from two control and two basolateral amygdala-lesioned rats. **A** and **B** are photomicrographs from sham-operated control subjects who received infusions of phosphate buffer intra-amygdala. **C** and **D** are photomicrographs from rats having received infusions of quinolinate intra-amygdala. The optic tract is labelled *OT*. Intense gliosis and extracellular deposits associated with the paths of the infusion cannulae are seen clearly in all four sections. Note that in **C** and **D**, basal (*Ab*) and lateral (*Al*) nuclei of the amygdala have been more-or-less completely destroyed with the result that, in both cases, this area of the amygdala has collapsed such that the central nucleus (*Ac*), which is generally completely spared, now lies adjacent to the external capsule (*ec*; compare **A** with **C** and **B** with **D**). In **C**, there is obvious damage to a region of the piriform cortex (*pc*) which is seen in a minority of lesioned brain (compare **A** with **C**). In **D** it can be seen that this extra-amygdala damage is restricted to a much smaller segment of the piriform cortex, especially its deeper layers. Most frequently, such damage when it occurs, is seen only unilaterally. The medial nucleus of the amygdala is labelled *Am*

## Experiment 2: within-session dose-response function

Following experiment 1, a further two lesioned animals were lost due to catheter blockade. Therefore, seven basolateral amygdala-lesioned and six sham-operated control animals were used to study the self-administered IV cocaine, dose-response function. Figure 4 shows that animals with lesions of the basolateral amygdala showed an augmented response across the entire dose range of IV cocaine when compared with sham-operated controls. A three-way ANOVA revealed main effects of Group [$F(1,11)=6.24$, $P<0.05$], Dose [$F(9,99)=26.92$, $P<0.001$] and Lever [$F(1,11)=219.72$, $P<0.001$]. There was no significant Group×Dose interaction [$F(9,99)=1.44$, NS]. A subsequent two-way ANOVA of Drug Lever by Dose alone showed there was also no significant difference between the groups' drug lever responses, at any dose of cocaine [$F(9,99)=1.13$, NS].

## Experiment 3: acquisition of a second-order schedule of IV cocaine reinforcement

### *Acquisition of IV cocaine self-administration*

Thirty-four basolateral amygdala-lesioned and 22 sham-operated control animals began this experiment, but numbers were reduced progressively mainly due to catheter failure over the relatively long duration of the experiment. Eight lesioned animals were discarded from the final analysis because of incomplete lesions, and one lesioned animal died post-IV surgery due to infection, making the inital grouping 25 lesions and 22 shams.

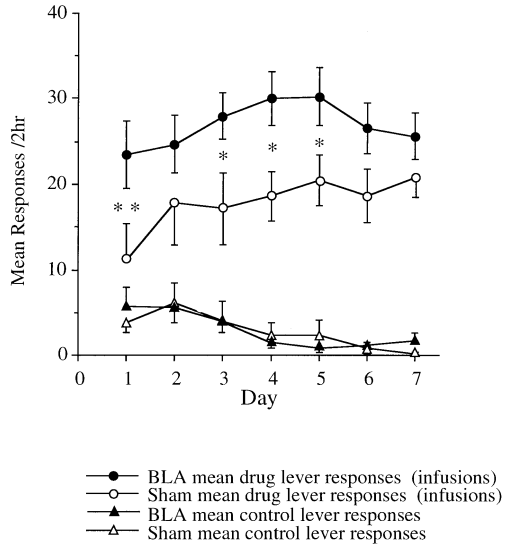


**Fig. 3** Acquisition of IV cocaine self-administration in basolateral amygdala-lesioned (*BLA*) and sham-operated control rats, active and inactive lever responding in both groups across the first 7 days of acquisition. BLA animals are represented by *filled symbols* and the sham operated controls are represented by *open symbols*. *Circles* show active (drug) lever responses, *triangles* show inactive (control) lever responses. Error bars=±SEM. **P*<0.05, ***P*<0.01

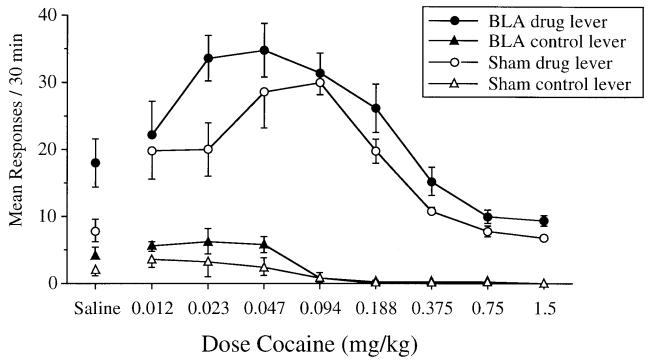


**Fig. 4** Within-session IV cocaine dose-response function for sham-operated and basolateral amygdala-lesioned (*BLA*) rats. BLA animals are represented by *filled symbols* and the sham operated controls are represented by *open symbols*. *Circles* show active (drug) lever responses, *triangles* show inactive (control) lever responses. Error bars=±SEM

During the first 4 days of acquisition of IV cocaine self-administration, basolateral amygdala-lesioned animals responded at significantly higher rates on the active, drug lever compared to controls, thus confirming the results of experiment 1 (data not shown). Three-way ANOVA revealed significant Group×Day [$F(3,135)=5.23$, $P=0.002$] and Group×Lever [$F(1,45)=13.21$, $P<0.001$] interactions. Post-hoc analysis revealed a significant difference between sham-operated and basolateral amygdala-lesioned rats in responding on the active [$F(1,73)=29.46$, $P<0.001$], but not the inactive levers [$F(1,73)=3.01$, $P=0.09$]. Responding on the active lever was greater in basolateral amygdala lesioned rats compared to shams on each of the first 4 days of acquisition ($P<0.05$ on each day); on day 1, basolateral amygdala lesioned animals made an average of 23±2.7 drug lever responses/2 h compared to 8±1.6 drug lever responses/2 h in the sham group, whereas on day 4 the lesioned group self-administered 21±2.1 IV infusions of cocaine in 2 h as opposed to 14±1.6 infusions by shams. The significant difference between groups disappeared thereafter.

Analysis of control and drug lever responding in sham-operated rats revealed a significant Day×Lever interaction [$F(3,66)=6.9$, $P<0.001$], reflecting the progressive increase in responding selectively on the drug lever (between days 1 and 3) and decreased responding on the control lever (between days 1 and 2).

A similar analysis of drug and control lever responding in basolateral amygdala-lesioned rats revealed that they responded at a relatively constant, high rate on the drug lever on each day and which was much greater than responding on the control lever [main effect of Lever $F(1,23)=135.17$ $P<0.0001$ and Day $F(3,69)=2.94$ $P<0.05$].

### *Acquisition of a second-order schedule of IV cocaine self-administration*

Following acquisition of IV cocaine self-administration, nine lesioned animals and ten sham animals were lost due to catheter-related problems. Sixteen basolateral amygdala-lesioned and 12 sham-operated animals entered this phase of the experiment. Figure 5 shows the proportion of basolateral amygdala-lesioned and sham-operated animals reaching criterion for acquisition of the second-order schedule of IV cocaine self-administration at each stage of training. As significantly more basolateral amygdala-lesioned animals failed to reach criterion and therefore repeated each acquisition stage, the size of the lesioned group was necessarily reduced at successive stages, as shown in the percentage data in Fig. 5. Attrition was equivalent for both groups through eventual failure of IV catheter patency which becomes increasingly inevitable with prolonged testing.

Thus, although basolateral amygdala-lesioned animals showed an elevated initial rate of IV cocaine self-administration and consequently increased numbers of CS-cocaine pairings, they were clearly impaired from the very first stage of acquisition of the second-order schedule [FR10 (FR1:S)]. Fisher Exact probability estimations carried out at each second-order stage, independently showed a significant difference in acquisition between the groups in their acquisition of the first ($P=0.01$) and second ($P=0.05$) stages of the second order schedule. Due to diminishing group size further analyses at higher levels of the schedule requirement were not possible. The lower panel of Fig. 5 shows the proportion of rats reaching criterion on each day during the acquisition of each successive stage requirement of the second-order schedule. The marked difference between basolateral amygdala-lesioned animals and shams as the conditioned stimulus-maintained response requirements of each stage

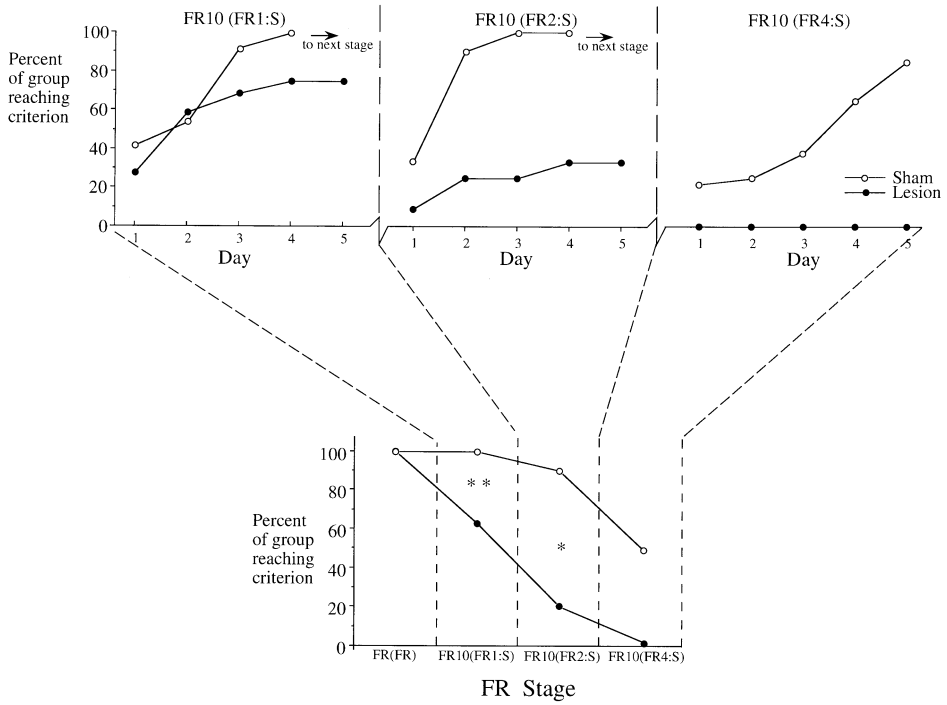


**Fig. 5** Acquisition of a second-order schedule of IV cocaine self-administration in basolateral amygdala (*BLA*) lesioned and sham-operated control rats. The *lower panel* shows the overall summary of the results: the proportion of rats attaining criterion at successive stages of acquisition are shown (BLA-lesioned rats, *closed circles*; Shams, *open circles*). In the *upper three panels*, the performance of lesioned and control rats during each day of acquisition of each stage is shown in more detail. It can be seen that rats with lesions of the basolateral amygdala repeated more sessions at each stage of acquisition and that a progressively smaller group of basolateral amygdala-lesioned rats, compared to controls, moved onto the next stage. They were deemed to have failed a stage if they did not reach the criterion response requirement after five repetitions. **P*< 0.05, ***P*<0.01

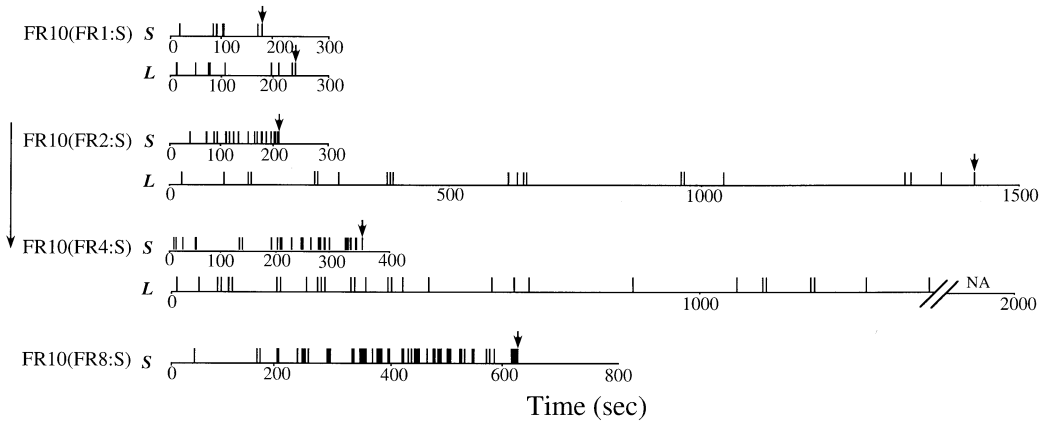


**Fig. 6** Representative event records of responding by BLA-lesioned and sham-operated rats (denoted *L* and *S*, respectively) at each stage of acquisition of the second-order schedule of IV cocaine reinforcement. It can be seen that as the stringency of the stages increases, rats with BLA lesions take significantly longer to complete the fixed ratios of responses and receive the first IV infusion of cocaine. Sham-operated rats also tended to make their responses in bursts. Note that no basolateral amygdala-lesioned rat achieved the FR10(FR8:S) stage. Drug infusions are represented by *arrows*. *NA* indicates that the drug infusion was not achieved. CS presentations are not indicated, because the resolution of print is such that several lever presses may appear superimposed on each other. However, in each case, ten CSs were presented for each drug infusion earned

is clearly seen. Figure 6 shows representative event records of responding in basolateral amygdala-lesioned and control subjects at each stage of the second-order schedule. The increased time taken by rats with basolateral amygdala lesions to complete the response requirements at each stage is illustrated for a typical subject. The rapid, burst-like responding by shams, which depends on the contingent presentation of the CS+, is also evident in the event record.

## Experiment 4: locomotor response to IP cocaine

Ten basolateral amygdala-lesioned animals and six sham-operated animals were used in this experiment. Following histological assessment two lesioned animals were discarded because of incomplete lesions, making a total of eight lesions and six shams. There were no significant differences in spontaneous locomotor activity between basolateral amygdala and sham lesioned animals in sessions 1 [$F(1,12)=0.69$, NS] and 2 [$F(1,12)=0.01$, NS]. Figure 7 shows that there were also no significant differences in the locomotor response to IP cocaine at any dose between basolateral amygdala-lesioned and sham-operated controls [$F(1,12)=1.22$, NS].

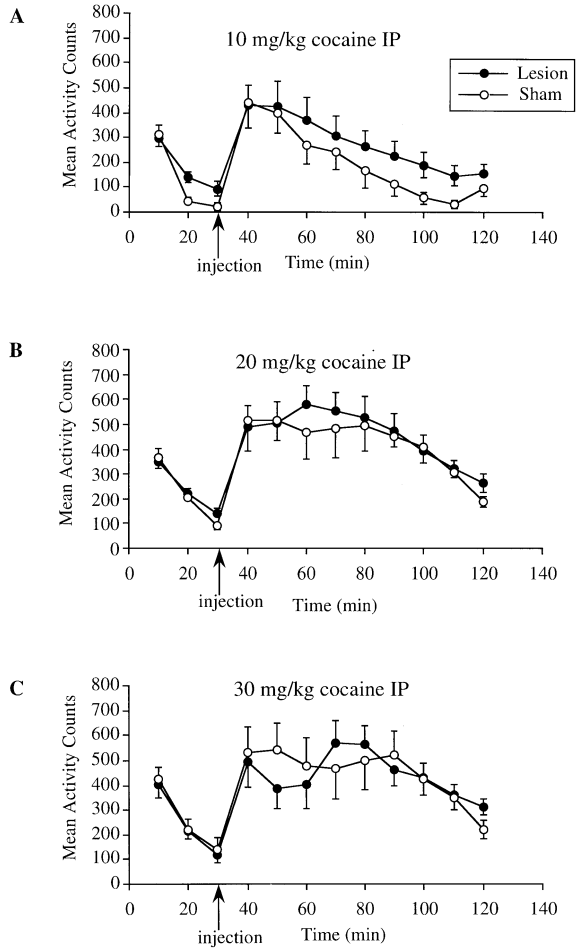


**Fig. 7 A–C** Locomotor response to IP cocaine in sham-operated control and basolateral amygdala-lesioned rats. BLA animals are represented by *filled symbols* and the sham operated controls are represented by *open symbols*

There was a significant main effect of Time at each dose [$F(11,132)$ 16.66, 15.21, and 10.49, $P<0.01$ at 10, 20 and 30 mg/kg cocaine, respectively], but Group×Time interactions were not significant at any dose.

## Discussion

A major finding in this study was that lesions of the basolateral amygdala severely impaired the acquisition of IV cocaine self-administration under a second-order schedule of drug reinforcement. Control animals were significantly faster and required fewer trials at each stage of the second-order schedule to attain their first IV infusion of cocaine. Thus, it would appear that contingent presentation of a light CS+ that had previously been paired with *each* drug infusion during the initial acquisition of IV cocaine self-administration had reduced efficacy in supporting responding in the basolateral amygdala-lesioned animals, compared with sham-operated controls, a result which indicates the importance of this limbic structure in mediating the effects of conditioned reinforcers. Despite the reluctance of lesioned animals to respond for their first cocaine infusion under the second-order schedule, once the required number of responses had been completed to earn the CS+ presentations, animals self-administered their additional nine infusions under a continuous reinforcement schedule *more* quickly than controls, suggesting that the impaired responding under the second-order schedule reflected a change in drug-seeking, not drug-taking behaviour, which may be the result of an impairment in the association between the conditioned stimulus and the effects of IV self-administered cocaine. Thus, lesions of the basolateral amygdala disrupted the acquisition of instrumental responding for an IV infusion of cocaine only under conditions when such responding was critically dependent on the contingent presentation of a drug-associated secondary or conditioned reinforcer.

### Acquisition of cocaine self-administration

The initial finding that basolateral amygdala-lesioned animals self-administered over twice as many infusions of IV cocaine on day 1 of acquisition was somewhat unexpected. Sham-operated controls typically acquired IV cocaine self-administration in four daily sessions, by which time the responding for cocaine had begun to stabilise at a level which remained relatively constant, under a simple fixed-ratio:1 (i.e. continuous) schedule of reinforcement throughout the experiment. During the initial 4 day acquisition period, sham-operated animals significantly differentiated between the drug and inactive levers, preferring the drug lever over the control lever by the second acquisition session and thereafter, active lever responses increased progressively. In contrast, rats with lesions of the basolateral amygdala self-administered IV cocaine at an asymptotic level from day 1 of acquisition. This rate was significantly higher than the rate of self-administration by the control group rats over each of the first 7 days of acquisition, but eventually both groups of rats self-administered cocaine at approximately the same rate during each session.

There are several possible causes of the accelerated acquisition of IV cocaine self-administration shown by the lesioned group. First, lesions of the basolateral amygdala may have altered the rewarding efficacy of cocaine, perhaps because this limbic forebrain region is one site at which cocaine exerts its reinforcing effects. Indeed, self-administered cocaine is associated with elevated extracellular levels of dopamine in the amygdala (McGregor et al. 1995) and infusions of the $D_1$ dopamine receptor antagonist SCH23390 into the amygdala increase cocaine self-administration (McGregor and Roberts 1993b; Caine et al. 1995). Alternatively, lesions of the basolateral amygdala may have changed the reinforcing effects of cocaine indirectly, for example, by altering the response to cocaine within the nucleus accumbens. This structure is widely held to be a most important site mediating the reinforcing efficacy of cocaine (Koob

1992) and it receives a rich glutamatergic projection from the basolateral amygdala (Kelley et al. 1982; Groenewegen et al. 1991).

Animals that have acquired the self-administration of psychostimulants normally *increase* responding (perhaps to maintain an optimal plasma level of the drug) as the dose is systematically reduced (Yokel and Wise 1976). Therefore the elevated rate of IV cocaine self-administration in basolateral amygdala-lesioned animals may have resulted from a decrease in the reinforcing efficacy of the drug that would be characterised by a shift to the right in the typical inverted U-shaped cocaine dose-response function (Yokel and Wise 1975). Were this the case, then the threshold dose of IV cocaine for maintaining responding should also be higher in lesioned animals when compared to sham-operated controls. However, in the present within-session cocaine dose-response study, lesioned animals did not show any tendency to extinguish responding before sham-operated controls as the dose of cocaine reached threshold. Instead, basolateral amygdala-lesioned animals maintained a slightly elevated rate of self-administration over the entire range of cocaine doses. Although increases in responding on the inactive control lever occurred at the same dose for both groups, the lesioned group displayed an elevated rate of inactive lever pressing with no complementary decrease in drug lever responding until the threshold drug doses had been reduced to approximately four-fold less than that in the sham-operated control group (0.012 mg/kg as opposed to 0.047 mg/kg). This result suggests that lesions of the basolateral amygdala do not obviously alter the reinforcing efficacy of IV cocaine, but may either increase spontaneous locomotor activity, thereby generally increasing the propensity to lever press, or increase the tendency to perseverate in one pattern of behaviour, regardless of the outcome, which may be heightened by the psychomotor stimulant properties of cocaine. On the basis of previous experiments (Cador et al. 1989; Burns et al. 1993) and the photocell activity test of experiment 4, the latter seems to be the more likely alternative.

An effect of response perseveration following amygdala lesions has previously been reported (Kemble and Beckman 1970), as well as a deficit in appropriate behavioural modification in response to alterations in reward magnitude. Lesions of the amygdala were therefore suggested to result in a lack of frustrative emotionality (Henke and Maxwell 1973). However, McDonough and Manning (1979) demonstrated that neither postulated mechanism was sufficient to explain the patterns of behaviour seen following lesions of the amygdala and concluded that the effects were a direct result of the lesioned animals being less under the control of conditioned reinforcers, an explanation that is consistent with our earlier work (Cador et al. 1989; Everitt et al. 1989; Robbins et al. 1989; Everitt and Robbins 1992).

## Implications for the amygdala in the reinforcing effects of cocaine

One of the advantages of studying IV drug self-administration is that it enables the investigation of mechanisms underlying contingent drug reinforcement. The neural mechanisms underlying response-contingent behaviour may depend more upon the amygdala (Wilson et al. 1994), than those underlying locomotor responses to an acute systemic injection of cocaine, over which the animal has no control. The present locomotor activity study indicated that there were no overall differences in the spontaneous, or cocaine-driven locomotor activity of the lesioned and sham operated groups, but it is possible that the non-contingent manner in which the drug was administered concealed any possible effects of the basolateral amygdala lesion. However, our earlier studies showing the lack of effect of basolateral amygdala lesions on the locomotor response to intra-accumbens infusions of *d*-amphetamine suggest this is unlikely (Cador et al. 1989; Burns et al. 1993). Moreover, informal observation of the lesioned animals did not indicate qualitative differences from sham-operated controls in their exploratory or locomotor behaviour during the cocaine self-administration sessions. It would nevertheless be of interest to assess the locomotor stimulant effects of cocaine during contingent self-administration in drug-naive and experienced animals.

McGregor and Roberts (1993) have also studied the effects of manipulations of the amygdala on cocaine self-administration by investigating the impact of intra-amygdala (and intra-accumbens) infusions of the $D_1$ dopamine receptor antagonist SCH23390, using both continuous reinforcement and progressive ratio schedules. The results showed that infusing SCH23390 into the amygdala increased cocaine self-administration (an observation confirmed by Caine et al. 1995), but to a greater extent than that seen to follow infusions of the drug into the nucleus accumbens. By contrast, the break-point for IV cocaine self-administration measured under a progressive ratio schedule was decreased more by infusions of SCH23390 into the nucleus accumbens than into the amygdala. However, responding maintained by cocaine under a progressive ratio differs from responding under a second-order schedule. Thus, as responding may be modified by the rate-altering effects of the cocaine in the former case, whereas under the second-order schedule employed in the present experiments, responding is maintained solely by the contingent presentation of conditioned stimuli previously associated with cocaine.

Superficially, lesions of the basolateral amygdala reported here and infusions of a $D_1$ dopamine receptor antagonist infused into the amygdala appear to affect IV cocaine self-administration similarly. But the effects of basolateral amygdala lesions were seen during the acquisition of cocaine self-administration, whereas the effects of SCH23390 were seen post-acquisition. It will be important in future studies to investigate whether basolateral amygdala lesions made *after* acquisition also result in

elevated IV cocaine self-administration, although preliminary data indicate that this is not the case (Whitelaw and Everitt, unpublished observations). Moreover, the distribution of $D_1$ dopamine receptors in the amygdala (Wamsley et al. 1989) suggests that the central nucleus may be the important region mediating the drug effects. The central nucleus was spared by the basolateral amygdala lesions in the present study, thus enabling the effects to be specifically localised to the basolateral amygdala. The increases in cocaine self-administration seen following either basolateral amygdala lesions or intra-amygdala infusions of SCH23390 may, therefore, result from alterations in quite distinct neural and psychological mechanisms.

Rats infused intra-amygdala with $D_1$ dopamine receptor antagonists have also been shown to be impaired in the discrimination of a cocaine cue (McGregor and Roberts 1993). This interesting observation of altered subjective effects of cocaine following manipulation of the amygdala might usefully be investigated in animals with basolateral amygdala lesions, since this may help to clarify the mechanism underlying the altered propensity to self-administer cocaine. Indeed, one possible explanation for the apparently increased reinforcing efficacy of cocaine is that this was the indirect consequence of a decrease in its aversive (anxiogenic) effects (see Ettenberg and Geist 1991) following basolateral amygdala lesions. The amygdala has long been accepted to be a critical element in the neural circuitry underlying fear motivated behaviour and anxiety (Selden et al. 1991; Davis 1992; Le Doux 1992; Davis et al. 1994). Moreover, anxiogenic drug cue discrimination as well as its generalisation to states induced by aggressive defeat, are impaired following lesions of the basolateral amygdala (Vellucci et al. 1988; Vivian et al. 1994). Thus, in the present experiment, lesions of the basolateral amygdala may in some way have mitigated the initial aversive effects of IV cocaine (Glick et al. 1994) thus resulting in the accelerated acquisition of its self-administration. It will be important to study this directly in future experiments.

## Second-order schedule of IV cocaine self-administration

Second-order schedules of cocaine and heroin self-administration have been established in monkeys and studied parametrically in terms of alterations of schedule requirements (for example, fixed interval or fixed ratios of CS+ or drug presentation), the monotonic increase in responding that is seen when doses of IV drug are increased and also the importance of drug-associated conditioned stimuli in maintaining responding (Goldberg 1973; Goldberg et al. 1976; Goldberg and Tang 1977; Katz 1979). There are few examples where responding for cocaine or heroin under second-order schedules has been established in rats. Corrigall and Coen (1989) demonstrated that rats would respond for heroin and cocaine under brief fixed interval second-order schedules and that responding varied with dose. However, such a procedure has not been used to study the neural basis of drug-seeking behaviour to date. The importance of this form of schedule of reinforcement is that it allows the study of relatively prolonged periods of instrumental responding for cocaine that are not affected by any rate-altering or other property of the drug itself. In the experiments reported here, cocaine was available contingent upon a fixed ratio of responses that were themselves reinforced under a unit fixed ratio of responding for a cocaine-associated CS+. This may not be the optimal form of second-order schedule (see Goldberg 1973) and we have now established a fixed interval 15-min second-order schedule of IV cocaine self-administration that generates high rates of responding maintained by presentations of a CS+, each contingent upon completion of small fixed ratios of responding (Weissenborn et al. 1995; Arroyo et al. 1996; Markou et al. 1996). However, in the present experiments the failure of rats with basolateral amygdala lesions to acquire the earliest stages of responding under a second-order schedule obviated the need to progress to more demanding fixed interval-fixed ratio schedules of reinforcement.

Despite more rapid acquisition and increased rates of IV cocaine self-administration (and, therefore, increased numbers of cocaine-CS+ pairings), rats with basolateral amygdala lesions were severely impaired in the acquisition of a second-order schedule of cocaine self-administration. Such a schedule maximises the importance of drug-associated conditioned reinforcers in maintaining cocaine-seeking behaviour and progressively fewer basolateral amygdala lesioned rats were able to maintain their responding as the unit schedule response requirement (i.e. the fixed ratio of responses for the CS) was progressively increased. This result is consistent with our earlier work demonstrating the importance of the integrity of the basolateral amygdala in mediating associations between previously neutral stimuli and primary reinforcers, including water (Cador et al. 1989) sucrose (Burns et al. 1993) and sexual interaction (Everitt et al. 1989) such that these stimuli do not acquire conditioned reinforcing properties. More recently, it has also been demonstrated that cues associated with cocaine fail to reinstate cocaine self-administration following extinction in animals with lesions of the basolateral amygdala (Meil and See 1995). Thus a body of data now strongly suggests that the basolateral amygdala is part of a neural system through which stimuli acquire motivational salience and thereby control over instrumental behaviour, including cocaine self-administration. The behavioural effects of such stimuli are greatly potentiated by stimulant drugs acting within the nucleus accumbens (Taylor and Robbins 1984 1986). This interaction between the basolateral amygdala and the dopaminergic innervation of the nucleus accumbens may represent an important element of the neural system involved in mediating the reinforcing effects of psychomotor stimulants, as well as the impact of drug-associated stimuli on drug-seeking behaviour (see also Robbins et al. 1989; Everitt and Robbins 1992; Markou et al. 1993; Robinson and Berridge 1993).

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**Note added in proof** It has come to our attention in a paper by Steven Grant and his colleagues to be published in October 1996 in the Proceedings of the National Academy of Sciences, USA that increased glucose metabolism, measured using positron emission tomography, is seen in the amygdala, as well as in other medial temporal and prefrontal cortical structures, in human cocaine abusers exposed to environmental cues associated with cocaine self-administration and craving. These results in human subjects are clearly consistent with the results of our study showing that the basolateral amygdala is an important structure mediating the control by cocaine-associated cues over the acquisition of cocaine-seeking behaviour in rats.

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