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Amygdaloid lesions and stimulus-reward associations in the rat

Robert W. Kentridge, Christine Shaw and John P. Aggleton

Department of Psychology, University of Durham, Durham (U.K.)

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Rats with amygdaloid lesions were trained on learning set tasks designed to tax stimulus–reward associations. Lesions centred in the medial and ventral half of the amygdala had no effect on the acquisition of two object discriminations but did impair successive reversals of the second discrimination. The same lesions had no effect, however, on the acquisition of a spatial win–stay lose–shift task which taxed one-trial place–reward associations. In a second experiment it was found that lesions in the central and basolateral regions of the amygdala disrupted performance of the same spatial win–stay lose–shift task although, as before, acquisition was unaffected. Taken together these findings support a role for the amygdala in stimulus–reward associations and indicate that it may be particularly important when differing values of reward must be distinguished.

INTRODUCTION

One influential notion concerning the function of the primate amygdala is that it is concerned with the rapid formation of stimulus–reward associations[2,17,36]. The first explicit evidence for this came from an examination of the effects of amygdaloid damage in monkeys on object and place discriminations and their successive reversals[18]. Further support for this proposal came from reports that amygdalectomy disrupts a range of other tasks which also tax rapid stimulus–reward associations, such as learning-set[33] and one trial win–stay lose–shift learning[12,31,35]. This proposed function is also consistent with the dramatic behavioural changes that follow amygdaloid damage in monkeys e.g. tameness and aberrant social behaviour[36]. The present study set out to determine whether the rat amygdala shares a similar function by examining the effects of amygdaloid damage on behavioural tasks taxing stimulus–reward associations. The tasks chosen were a simultaneous visual discrimination and its successive reversals, and a one-trial win–stay lose–shift task.

A number of studies have examined the effects of amygdaloid lesions in rats and mice on visual, spatial, auditory, and olfactory discriminations, although the conclusions are far from clear. While some studies have reported normal rates of acquisition[7,8,15,29], others have noted deficits[10,20,26,34]. A similar confused picture emerges over reversal learning, some studies finding no impairment[7,29], others finding deficits[8,20]. It should be noted that studies with rats have only involved up to three reversals[20] and hence have failed to examine the facilitation of learning that occurs with multiple reversals. We therefore trained rats with amygdaloid lesions on two object discriminations, the second of which was followed by a total of 7 reversals.

The reversals were followed by a one-trial win–stay lose–shift task. In this test of working memory the animal had to remember, for a given trial, whether the sample arm in a T-maze was baited or unbaited. If it was baited the animal had to return to that arm in order to receive food (win–stay), if it was unbaited the animal had to switch (lose–shift) to the other arm[11,14]. A spatial task was used as it has proved difficult for rats to learn an object win–stay lose–shift task[1]. In spite of this difference, the demands of this task appear in many respects similar to those of the one-trial object–reward tasks that have proved sensitive to amygdalectomy in monkeys[31,35].

The lesions in the present study were made with the neurotoxin ibotenic acid in order to minimise damage to fibres of passage. This follows from evidence that some

Correspondence: J.P. Aggleton, Department of Psychology, University of Durham, South Road, Durham, DH1 3LE, U.K.

well-established effects of amygdaloid lesions are in fact a result of damage to fibres passing through the structure[6].

EXPERIMENT 1

Methods

Subjects

The subjects were 14 naive, male rats of the DA strain (Bantin and Kingman, Hull, U.K.) which were caged individually. The animals were housed in a single room with a 14 : 10 h light/dark photoperiod, all testing taking place during the light period. The animals were fed approximately 15 g of laboratory diet ('Beekay rat and mouse', Bantin and Kingman) daily so that their body weights did not drop below 85% of normal body weight. At the start of the study the animals, which were aged about 4 months and weighed between 170 and 220 g, were allocated randomly to the two groups. One animal with an amygdaloid lesion fell ill during the reversal task and so was eliminated from this and the subsequent tasks.

Apparatus

Object discriminations and reversals. The apparatus, a Grice box, consisted of a small rectangular start box (13 × 18 cm) which was separated from a triangular test area by a guillotine door. The far wall of the test area was 43 cm long and 43 cm from the guillotine door. Two equal sides, 46 cm long, connected this far wall to the entrance of the start box. The walls of the apparatus, which were made of aluminium, were 24 cm high. The plastic floor contained two food wells 2.5 cm in diameter positioned 35 cm from the start box. An aluminium partition which protruded 16 cm from the far wall ensured that the rats could not run directly between the two food wells which were 21 cm apart. The luminant light level at the food wells was 250 lux.

Win–stay lose–shift. Training was conducted in a T-maze. The floor of the maze was 10 cm wide and made of aluminium. The stem was 80 cm long and had a guillotine door located 33 cm from the beginning. The cross-piece was 136 cm long and at each end there was a food-well 4 cm in diameter. The walls were 17 cm high and made of clear Plexiglas. The maze was supported on two stands 93 cm high.

For the final part of the win–stay lose–shift task an additional cross-arm was fitted onto the stem of the T-maze so forming a double T or H shape. Both cross-arms were 136 cm long and contained food-wells 4 cm from the ends, making a total of 4 wells. The central stem of the maze was now 97 cm long and two wooden blocks were placed 20 cm apart in the middle of the central stem to form a temporary start box.

Both the visual discrimination and the win–stay lose–shift tasks were conducted in the same large room which contained a variety of large distinctive objects. Illumination was provided by fluorescent lights suspended approximately 95 cm above the mazes. The luminant light level in the T-maze was 320 lux at the choice point and 280 lux at the two food wells.

Surgery and histology

All the rats were anaesthetized by intraperitoneal injection of 3 ml/kg of chloral hydrate-pentobarbitol mixture (containing 42 mg/ml chloral hydrate and 9.7 mg/ml Nembutal). They were then placed in a stereotaxic headholder (David Kopf Instruments) and the scalp was retracted to expose the skull. A dental drill was used to make an opening exposing the cortex above the amygdala.

Two injections of ibotenic acid (1 mg/100 μl, Sigma) dissolved in phosphate buffer (pH 7.2) were made in each hemisphere through a 1-μl Hamilton syringe. Each injection of 0.3 μl took 5 min and the needle was left in position for a further 5 min after each injection. The injection coordinates relative to ear-bar 0 with the incisor bar set at +5.0 were: rostral AP + 5.1, DV + 1.6, LAT ± 3.6; Caudal AP + 4.1, DV + 1.6, LAT ± 3.7. An identical procedure was used for the sham-operated controls except that the needle was only lowered to a height of +4.0 and then withdrawn immediately. Sulphanilamide powder was applied in all the rats before the skin was sutured. Seven animals received amygdaloid lesions (AMG) while seven served as sham controls (SHAM).

At the end of the study the AMG and SHAM rats were perfused intracardially with 5% formol saline. The brains were subsequently blocked, embedded in wax (Paraplast), and cut in 10-μm coronal sections. Every tenth section was mounted and stained with Cresyl violet, a Nissl stain. Every adjacent section was reacted with Luxol fast blue, a fibre stain. Each lesion was examined under light microscopy and their extent was estimated by plotting the regions of cell loss onto 5 standard coronal sections (AP 6.2, 5.6, 5.2, 4.6, 4.2) from a stereotaxic atlas[30].

Procedure

Object discriminations and reversals. Pretraining began 14 days after surgery. During pretraining the rats were trained to run from the start box to find food pellets (45 mg, Campden Instruments Ltd.) in either food-well by pushing aside a circular wooden disc (4.5 cm diame-

ter, 1.5 cm high). Ten trials were given daily for an average of 5 days.

This pretraining procedure was followed by two object discriminations, the second of which was followed by 7 successive reversals. The stimuli for the discriminations were made of plain wood and were small enough so that they could be easily displaced but large enough so that the base covered the food-well. Multiple copies were made in order to eliminate the use of incidental cues that might aid the discrimination.

The test stimuli for the first discrimination were a cube (2.5 cm sides) and a cone (3.5 cm base diameter, 4 cm high). Three animals in each group were assigned a particular correct object (S +), the remaining animals received the other object as S + . To start each trial the guillotine door was raised and the rat allowed to select one of the objects, a choice occurring when the rat had pushed the object sufficiently to reveal the edge of the food-well. Following a choice the rat was allowed to eat any pellets it found and was then returned to the start box. The left–right positions of the test objects were then varied according to a pseudorandom schedule, the appropriate food-well baited with three 45-mg reward pellets (Campden Instruments) and the next trial begun. Each session consisted of 10 trials. Testing continued until each rat reached a criterion score of 26 correct responses over 3 consecutive days (87%).

Following an interval of two weeks the animals were tested on a second object discrimination followed immediately by 7 successive reversals. The test stimuli were an arch (3.5 cm high, set on a round base with a diameter of 3.5 cm) and a square (3.5 cm wide, 1.5 cm high) from which a single rod (2 cm long, 0.8 cm diameter) protruded. On the first test trial of the first session both objects were baited and the rat allowed to choose one. Whichever object had been selected became S + for the initial discrimination. The rest of the testing procedure was the same as for the first discrimination except that the animals now received 20 trials a day and only two reward pellets were used per trial. Testing continued in this manner until a criterion score of 18/20 followed by at least 16/20 correct responses on the next session[18]. The first reversal began on the following session. That is, the object previously designated as S + became S – and vice versa. Testing continued until the criterion level was once again reached. A total of 7 reversals were carried out.

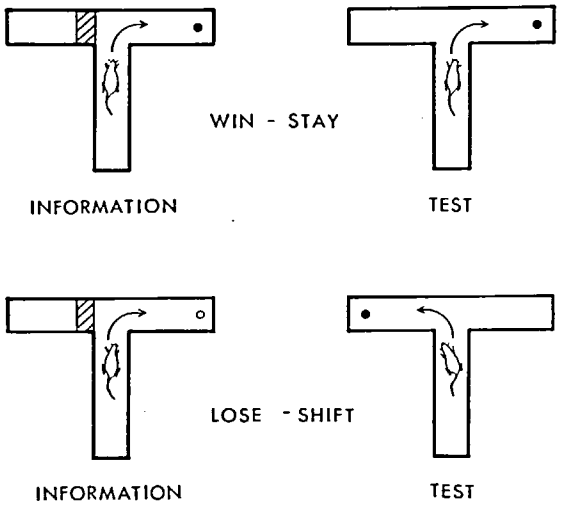

Fig. 1. Diagrammatic representation of the win–stay (upper) and lose–shift (lower) task. The filled and empty circles represent respectively the presence or absence of food in the food-well.

In order to confirm that the rats were not using olfactory cues to solve the discrimination, each animal was tested for a further 4 sessions shortly after the final discrimination. For these additional sessions the test stimuli and the reward contingencies were unchanged except that the stimuli were sealed with several coats of a strong-smelling, clear varnish.

Win–stay lose–shift

Between 2 and 7 days after completion of the reversal task the animals received 4 days pretraining in the T-maze in which they were familiarized with the apparatus. In the last of these pretraining sessions each rat received 6 forced runs, 3 to each arm, in which 3 food pellets (45 mg Campden Instruments) had been placed in the food-well.

For the experiment proper each test session contained 8 trials, a trial consisting of a forced 'sample' run followed by a 'choice' run. On each day there were 4 win–stay and 4 lose–shift trials and an equal number of correct right and left responses. There were never more than 3 consecutive trials with the same rule or to the same arm. On win–stay trials a wooden block was placed at the choice point so forcing the rat to enter the arm containing 3 food pellets (Fig. 1). After the rat had eaten the pellets it was returned to the start box and the same arm, i.e. the 'sample' arm, rebaited with 6 pellets (win–stay). At the same time the experimenter pretended to bait the incorrect arm. On the 'choice' run the animal was allowed free access to both arms, a choice being defined by all 4 feet entering one arm. On lose–shift trials the sample arm was left unbaited and the opposite arm was baited with 6 pellets for the 'choice' run (condition '3 vs 0'). There was an intertrial interval of approximately 5 min, while the delay between the 'sample' and 'choice' trials was approximately 20 s.

After the first 15 sessions the testing procedure was changed slightly in order to aid acquisition of the

win–stay rule[11,14]. For all subsequent sessions a partial depletion procedure was used for win–stay sample trials. That is, 6 pellets were now used to bait the food-well but the rat was removed and returned to the start box after it had eaten only 3 of the pellets (condition '6/3 vs 0'). Three more pellets were then added to the 3 remaining pellets prior to the choice run. The lose–shift trials were unchanged.

After a total of 39 sessions, 24 with partial depletion, the maze was altered so that the animals now had to remember a list of two sample runs. For this a double T-maze was used (Fig. 1). Each session now consisted of 6 pairs of trials i.e. 12 trials, and the rats received a total of 6 sessions. For these sessions the rat was confined in the centre of the stem by two wooden blocks. One of these blocks was then removed and a sample run was made to one end of the maze as described previously. The rat was then returned to the start area and allowed to make a second sample run to the other end of the maze. Two choice runs were then made to each end of the maze in the same order as the sample runs. Each block of two trials was separated by an interval of approximately 10 min. Six right and left sample runs and 6 win–stay and lose–shift trials were given randomly in the same manner as before. The partial depletion procedure was used for the win–stay sample runs and 6 reward pellets were provided on all correct choice runs.

RESULTS

Histological findings

The amygdaloid lesions, which were intended to damage all nuclei, were consistently located in the medial and ventral half of the amygdala (Fig. 2). The lesions resulted in considerable damage to the medial, anterior cortical and posterior cortical nuclei, with variable involvement of the basal and central nuclei. In contrast, the lateral nucleus was spared. The lesions did not extend beyond the anterior or posterior limits of the structure, while there was very little damage to regions dorsal to the amygdala. The placement of the lesions was very consistent although the total extent of amygdaloid damage ranged from approximately 25%–57% (median 32%). Within this region the loss of cells appeared complete. The Luxol fast blue stain confirmed that the neurotoxin had spared fibres.

Object discriminations and reversals

There were no differences between the number of trials required by the SHAM (mean = 74.3) and AMG (mean = 74.0) groups to reach the learning criterion on the first discrimination ($t < 1$). Similarly, there were no differences in the number of errors made by the SHAM (mean = 20.4) and AMG (mean = 18.1) groups.

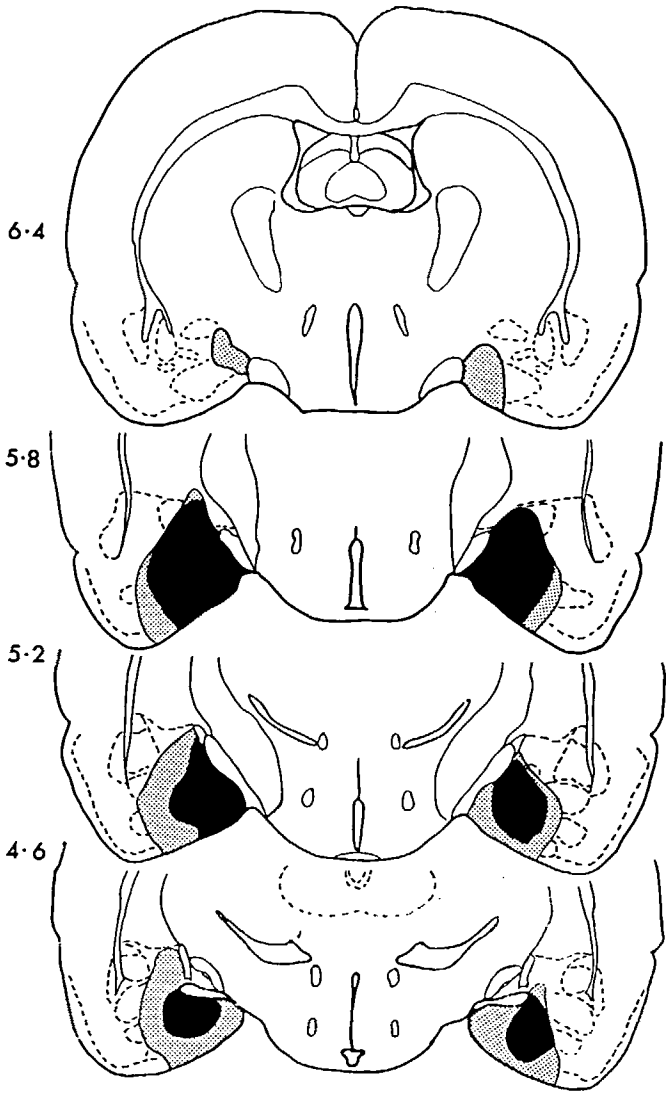

Fig. 2. The extent of cell loss in the largest (stippled) and the smallest (black) of the AMG lesions in Expt. 1. The lesions are shown on coronal sections and the numbers indicate AP level[30].

The acquisition performance of the SHAM and AMG groups on the second discrimination and its 7 subsequent reversals are shown in Fig. 3. As before, there were no group differences on the initial discrimination, as measured by total errors ($t < 1$) or total trials ($t < 1$) to complete the acquisition criterion, indeed the AMG groups made on average slightly fewer errors and required fewer trials than the SHAM animals (Fig. 3).

The error scores for reversals 1–7 were logarithmically transformed prior to analysis in order to conform with the homogeneity of variance assumption of analysis of variance. Evidence of a group difference emerged over the successive reversals as the AMG animals made

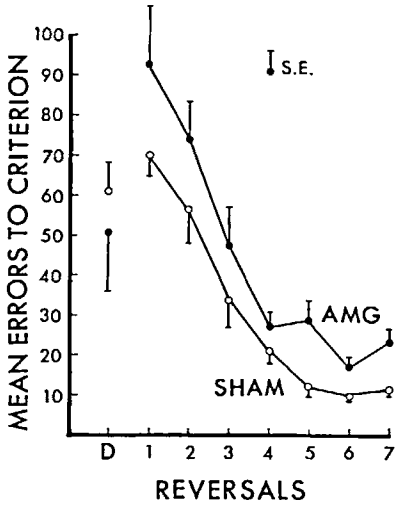

Fig. 3. Expt. 1. Mean errors to criterion on object discrimination (D) and subsequent reversals (1–7).

more errors than the control SHAM group (Fig. 3). This difference was supported by a two-way analysis of variance with a between–subjects factor 'lesion' and a within–subjects factor 'reversals'. This analysis revealed a lesion effect ($F_{1,11} = 9.34$, $P = 0.011$) and an effect of successive reversals ($F_{6,66} = 53.49$, $P < 0.001$), although there was no clear evidence of a significant interaction ($F_{6,66} = 1.87$). The total numbers of errors made by the AMG animals over the 7 reversals were compared with the extent of the individual lesions, but no clear correlation emerged ($r = 0.26$).

As previous studies of reversal learning and amygdaloid damage in rats have only used up to 3 reversals, we also examined the transformed total errors scores for reversals 1–3 and 4–7 separately. An analysis of reversals 1–3 failed to provide evidence of a lesion effect ($F_{1,11} = 2.10$), while a clear lesion effect was found for reversals 4–7 ($F_{1,11} = 12.29$, $P = 0.005$).

The error scores from the 7 reversals were classified into 3 stages as this has proved sensitive to the effects of amygdala removal in monkeys[18]: Stage I, sessions with below chance scores of less than 6 correct responses; Stage 2, sessions with between 7 and 13 correct trials; Stage 3, sessions with between 14 and 18 correct trials. The total number of such errors made over reversals 1–7 were compared and although there was evidence of a lesion effect ($F_{1,11} = 4.84$, $P = 0.05$), there was no evidence of an interaction ($F_{1,11} = 1.34$), i.e. by this classification the distribution of errors was similar in the two groups with the majority for both groups falling in Stage II.

After completion of the reversals the animals were tested for a further 4 sessions using stimuli that had been freshly coated with varnish. On the fourth and final session with these stimuli the mean score of the SHAM groups was 17.6, while that of the AMG group was 17.7. These scores correspond to 88%, the very rapid transfer showing that the rats were not using olfactory cues to solve the object discrimination.

Win–stay lose–shift

Fig. 4 shows the mean acquisition scores of the AMG and SHAM groups. It is evident that throughout the acquisition period there were no differences between the two groups although there was clear evidence of learning the win–stay lose–shift rule. These conclusions were supported by an analysis of variance which compared the scores from Blocks 6–13, i.e. those blocks of sessions in the single T-maze during which the rat was removed before all of the food pellets had been eaten ('partial depletion'). There was no evidence of a lesion effect ($F < 1$), although there was a clear improvement over the blocks of sessions ($F_{7,77} = 9.03$, $P < 0.001$), reflecting the animals' acquisition of the task (Fig. 4).

After completion of 39 sessions the rats received a further 6 sessions, 12 trials per session, on the double T-maze in which the animals had to remember two sample trials at a time. It can be seen that the animals transferred readily to this harder task and by the last 3 sessions were correct on approximately 75% of the trials (Fig. 4).

DISCUSSION

Although amygdaloid damage did not affect the acquisition of two simultaneous object discriminations a deficit did emerge when the animals were trained on

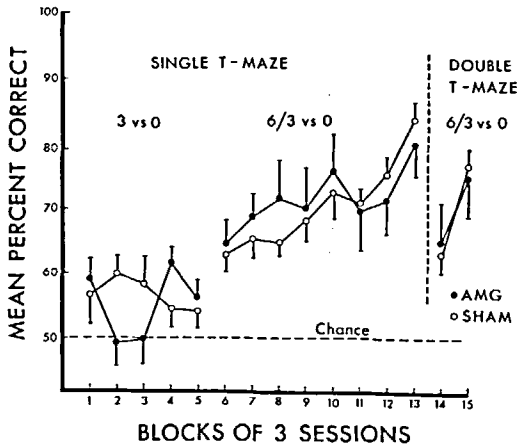

Fig. 4. Expt. 1. Acquisition of the win–stay lose–shift task. The results are divided between the 3 test conditions; '3 vs 0', '6/3 vs 0' and '6/3 vs 0' in double T-maze. The vertical bars show the standard errors of the means.

successive reversals of the second discrimination. No lesion effect was found, however, on the spatial win–stay lose–shift task.

The normal discrimination performance of the AMG rats was consistent with some[7,15,29] but not all[10,20,26,34] previous studies of discrimination learning by rats with amygdaloid damage. Attempts to isolate the critical factor responsible for the observed deficits have unfortunately proved frustrating. A review of earlier findings shows that studies using simultaneous discriminations have reported both normal[15] and impaired[20] rates of acquisition. Similarly, those studies using go/no-go procedures in an operant chamber have found deficits in some[10,26,34] but not all[29] cases. Likewise, there does not appear to be a particular amygdaloid region that is necessary for discrimination learning. Although it has been suggested that the lateral nucleus is especially important for discrimination learning[26,27], there are studies with lesions involving nearly all of this region which have failed to find deficits[28,29]. Similarly, there is no clear evidence that a factor such as the number of trials per session relates in any simple manner to the presence or absence of a learning deficit. This distinction is of interest as there is growing evidence for a neurological dissociation involving the amygdala between the slow, gradual learning typically observed in many discrimination tasks and the very rapid learning observed in learning set or reversal set[24]. Recent evidence does, however, indicate that more clear-cut deficits may be found when rats have to discriminate reward from non-reward on the basis of a secondary cue[5,9].

In spite of normal scores on the original learning, the AMG rats were impaired over a series of 7 reversals. This impairment was clearest over the later reversals when both the SHAM and AMG groups showed formation of a discrimination-reversal set (Fig. 3) and if, as in previous studies of rats, testing had stopped after just 3 reversals, no statistically significant lesion effect would have been apparent. It seems unlikely that the AMG impairment reflected an inhibitory deficit as these animals showed a normal proportion of Stage I errors and, indeed, there was no lesion effect when the total numbers of errors made on the first session of each reversal was compared ($F_{1,11} = 2.87$). An alternative possibility is that the AMG deficit is related to the emergence of the reversal discrimination set.

If effective use of a reversal set underlies the lesion effect it might be expected that the AMG animals would also be impaired on the acquisition and performance of the spatial win–stay lose–shift task. This was not found as the AMG rats were indistinguishable from the SHAM controls. One possible explanation is that the animals had made the task easier by using the presence or absence of food in the mouth to guide choice trials. This is, however, very unlikely as both groups showed rapid transfer to the double T-maze where such cues would only be of help if both trials in a pair were the same, i.e. only win–stay or lose–shift. It is also the case that a previous study using a very similar design found that an interpolated feeding experience did not stop acquisition of the task[14]. The transfer of the animals onto the double T-maze condition also helps rule out the possibility that the animals were relying on body turns rather than place cues as the former strategy should result in better performance when both body turns in a double trial are in the same rather than the opposite direction. Such a result was not found. These findings all suggest that the rats were relying on place–reward associations to solve the task.

An alternative explanation for the lack of any lesion effect is that the partial depletion procedure, which is known to aid acquisition[11], eases the demand on place–reward associations. It is also the case that the lesions spared much of the lateral and central amygdaloid nuclei, regions that others have tried to implicate in win–stay lose–shift behaviour[22,27]. With these considerations in mind, a second experiment examined performance on a harder version the win–stay lose–shift task, while the lesion coordinates were altered to incorporate more of the lateral and dorsal amygdala.

EXPERIMENT 2

Rats with amygdaloid lesions were tested on a more difficult version of the spatial win–stay lose–shift task in which the partial depletion procedure was stopped after acquisition and the amount of reward used on the sample runs was varied. In addition, the effects of the dopaminergic blocker α-flupenthixol were assessed. This follows from a wealth of evidence indicating that neuroleptic drugs, such as flupenthixol, disrupt the rewarding properties of a range of natural reinforcers[3,23,37]. The doses of α-flupenthixol were determined from previous studies on the same strain of rat which showed that these levels of the drug appeared to attenuate the perceived reward value of food pellets[21].

Subjects and apparatus

The subjects were 21 naive, male rats of the DA strain (Bantin and Kingman, Hull, U.K.) which weighed between 203 and 260 g at the start of the experiment. The housing conditions and the apparatus were the same as in Expt. 1.

Surgery and histology

All procedures matched those in the previous experiment except that new stereotaxic coordinates were used (rostral AP + 5.0, DV + 1.6, LAT ± 3.8; caudal AP + 4.1, DV + 1.6, LAT ± 3.9) and 0.35 μl of ibotenic acid (1 mg/100 μl) was injected in each site. Eleven rats received amygdaloid lesions (AMG) while 10 served as surgical controls (SHAM).

Procedure

The initial acquisition stage closely matched the partial depletion condition used in Expt. 1. On win–stay trials the animal received 3 of the 6 reward pellets (45 mg Campden Instruments) in the sample arm before being removed. On the choice run the animal received the remaining 3 pellets if it returned to the same arm. On lose–shift trials the sample arm was not baited and the opposite arm was baited with 3 pellets for the choice run (condition '6/3 vs 0'). After 10 such sessions the rats received a further 6 sessions in which the rewards were increased so that on win–stay trials the animal ate 6 of 12 pellets in the food-well and received the remaining 6 if it returned to the same arm (condition '12/6 vs 0'). On lose–shift trials the sample arm was unbaited and the animals were rewarded with 6 pellets if they alternated on the choice trial. This condition was added in order to increase the saliency of the discrimination.

The partial depletion procedure was then stopped and for the next 12 sessions (condition '6 vs 0') the rats were allowed to eat all 6 pellets in the sample arm on win–stay trials. The animals were rewarded with a further 6 pellets for returning to the same arm on the choice run. The sample and reward on the lose–shift trials was the same as in the previous condition, i.e. 0 and 6 pellets, respectively. The task was made more difficult during the next 12 sessions (condition '5 vs 1') as the sample reward on win–stay trials was reduced from 6 to 5 pellets while the sample reward on lose–shift trials was increased from zero to one pellet. For both win–stay and lose–shift trials a correct choice was rewarded with 6 pellets. The rats were then returned to the '6 vs 0' condition for a further 6 sessions before receiving a final 9 sessions of the '6 vs 0' condition during which all the animals received i.p. injections of either 0.03 or 0.09 mg/kg of α-flupenthixol (Lundbeck, Copenhagen) in a 1 ml/kg 0.9% saline vehicle or vehicle alone. The injections, which were given 150 min before testing, were arranged in a counterbalanced sequence.

RESULTS

Histological findings

Two cases with asymmetric lesions were deleted from the study leaving 9 AMG cases and 10 SHAM controls. The lesions in the 9 remaining AMG animals were consistently placed in the central regions of the structure (Fig. 5) but there was often much damage to the lateral and basolateral nuclei. The total extent of amygdaloid damage ranged from 22% to 68% (median 46%). In the case with the largest lesion there was some unilateral damage to the piriform cortex although the entorhinal cortex was spared. In several animals there was slight involvement of the most ventral portions of the putamen. In those animals with the larger lesions a vacuole often formed in the amygdala, indicating that

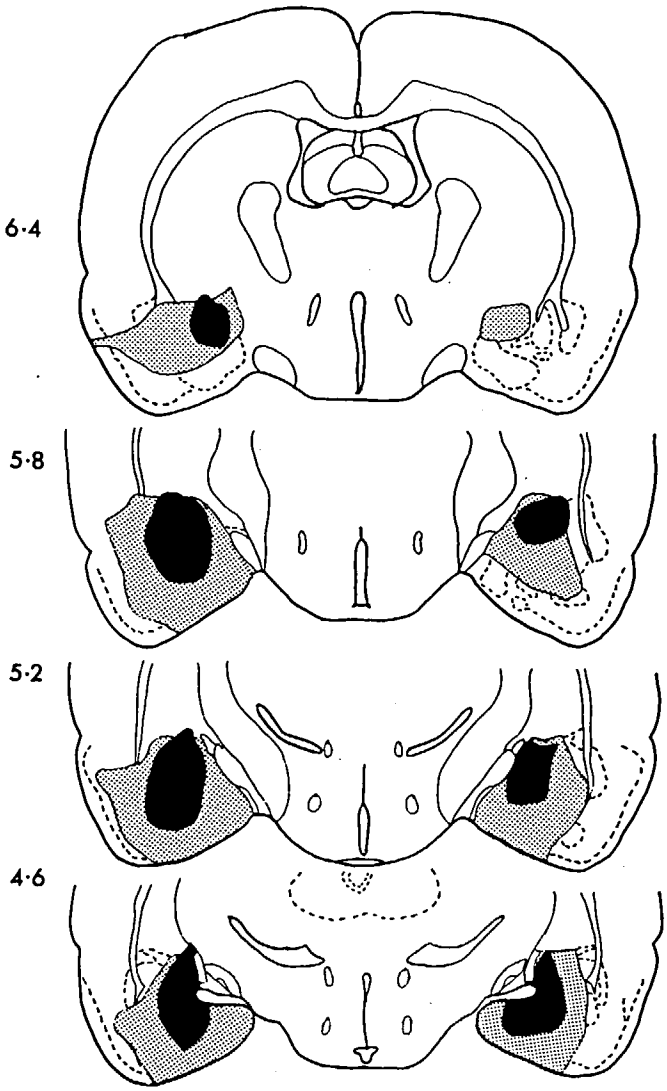

Fig. 5. The extent of cell loss in the largest (stippled) and the smallest (black) of the AMG lesions in Expt. 2. The lesions are shown on coronal sections and the numbers indicate AP level[30].

although the neurotoxin reduced fibre damage it was not eliminated.

Win–stay lose–shift

Fig. 6 shows the mean performance of the two groups of animals over successive blocks of 3 sessions (24 trials). Comparisons using the scores from these blocks of trials confirmed the lack of any lesion effect ($F < 1$) on the initial 10 blocks of partial depletion trials ('6/3 vs 0'). As expected, increasing the size of the sample reward on win–stay trials improved performance ('12/6 vs 0') and this condition provided the first indications of a lesion effect ($F_{1,17} = 3.73$, $0.1 > P > 0.05$).

A much clearer difference between the AMG and SHAM animals emerged over the remaining blocks of sessions, the amygdaloid surgeries consistently impairing overall performance (Fig. 6). Statistical comparisons between the performances of the two groups on the '6 vs 0' condition revealed both a highly significant lesion effect ($F_{1,17} = 19.47$, $P < 0.001$) and a practice effect ($F_{3,51} = 3.85$, $P < 0.025$), although there was no interaction between these factors ($F < 1$). Once again, there was a highly significant group effect on the '5 vs 1' condition ($F_{1,17} = 17.92$, $P < 0.001$), although there was also a lesion × practice effect ($F_{3,51} = 4.54$, $P < 0.01$) reflecting the divergent pattern of scores over the first two blocks of this condition (Fig. 6). That is, the SHAM rats were transiently disrupted by the new condition in which, for the first time, a sample reward was present on lose–shift trials. As expected the lesion effect was still present when the animals were returned to the '6 vs 0' condition for two blocks of sessions ($F_{1,17} = 9.18$, $P < 0.01$).

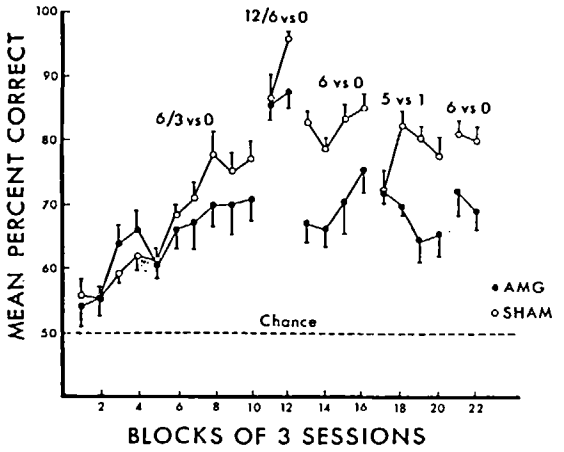

Fig. 6. Expt. 2. Acquisition and performance on the win–stay lose–shift task over the various test conditions. The vertical bars show the standard errors of the means.

In an additional series of analyses the performance test scores on the win–stay and lose–shift trials were considered separately. Given the innate preference of rats to alternate, it was no surprise to discover that the animals made more mistakes on the win–stay trials (condition '6 vs 0', $F_{1,17} = 219.7$, $P < 0.001$; condition '5 vs 1', $F_{1,17} = 12.49$, $P < 0.005$). For both the '6 vs 0' and '5 vs 1' conditions the SHAM animals were significantly better than the AMG animals on both win–stay ('6 vs 0', $F_{1,17} = 18.45$, $P < 0.001$; '5 vs 1', $F_{1,17} = 4.90$, $P < 0.05$) and lose–shift ('6 vs 0', $F_{1,17} = 4.65$, $P < 0.05$; '5 vs 1' $F_{1,17} = 10.79$, $P < 0.005$) trials.

The injection of two different doses of α-flupenthixol failed to have any effect on the performance of the animals on the '6 vs 0' condition ($F_{2,18} = 1.51$), nor was there any evidence of an interaction between the two experimental groups ($F_{2,18} = 1.39$). There was, however, a lesion effect ($F_{1,9} = 9.26$, $P < 0.05$) consistent with all of the previous performance tests.

GENERAL DISCUSSION

In the second experiment the amygdaloid lesions produced a pronounced performance deficit on a working memory test of win–stay lose–shift behaviour. The deficit during conditions '6 vs 0' and '5 vs 1' contrasted, however, with the lack of a clear lesion effect during acquisition (Fig. 6). This difference between acquisition and performance appears to reflect the use of the partial depletion procedure which makes the task appreciably easier[11], i.e. the deficit only emerged when the distinction between the win–stay and lose–shift trials became either harder to identify or harder to remember. The lack of a clear deficit during acquisition is also consistent with the findings from Expt. 1 which only looked at acquisition. It should be added that although the task clearly taxed spatial working memory there was no evidence from Expt. 1, or from previous studies[4,25], that this feature alone was responsible for the deficit in the animals with amygdaloid lesions. Taken together, the findings from the two experiments indicate that amygdaloid lesions in rats can impair win–stay lose–shift behaviour, both in a test of reference memory (serial reversals) and in a test of working memory (win–stay lose–shift).

The present findings support the recent report that lesions of the central amygdala impair the formation of place–reward associations[22]. In that experiment rats were trained in an 8-arm radial maze using a working memory procedure to choose between an arm which had contained 7 pieces of food and an arm which had contained one piece of food. It was found that electro-

lytic lesions of the central but not the basolateral amygdala impaired the more difficult versions of this task. The present task differs from this earlier study[22] in that win–stay and lose–shift trials were kept separate and that eight, rather than one, trials were given per session. Nevertheless, both studies point to an involvement of the amygdala in learning set behaviour, but it remains to be determined whether there is any difference in the involvement of the lateral and central amygdala in the present task.

It has already been noted that the performance deficit on the win–stay lose–shift task could stem from a number of different causes. Possible candidates include a failure to form or remember place–reward associations, a failure to discriminate between different magnitudes of reward, or an attenuation of the affective responses to different rewards. Evidence that amygdaloid damage disrupts the learning or retention of place–reward associations comes from the finding that amygdaloid lesions can impair performance on a win–stay lose–shift task when there are retention intervals of 5 min or more, but that there is no deficit with 5 s retention intervals[22]. Further support comes from the deficit on the '6 vs 0' condition in which there was no demand for rats to discriminate between different magnitudes of reward. This is consistent both with the poor performance of the SHAM animals on the first block of the '5 vs 1' condition (Fig. 6), a variant which places more emphasis on the ability to differentiate between magnitudes of reward, and with the lack of effect of dopaminergic blockade on this task, a manipulation that should attenuate the magnitude of the perceived reward[3,23,37]. The lack of an impairment on the partial depletion procedure, a procedure likely to increase the affective state on win–stay trials and hence their salience, also appears to run counter to the notion that the primary deficit is a change in affect. Nevertheless, there is behavioural evidence that amygdalectomy can attenuate the responsiveness to reward and non-reward[13,16,19] and these various explanations still have to be clearly dissociated.

The main rationale behind the present study was to determine whether amygdaloid damage in the rat has similar consequences to those observed in monkeys. In particular, the study focussed on those tasks that have most strongly implicated the primate amygdala in the rapid formation of stimulus–reward associations. The present finding provides at least superficial support for the notion that the monkey and rat amygdala, which share many anatomical features[32], also share this important function.

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