Behavioural Brain Research 236 (2013) 48–55

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Within-event learning in rats with lesions of the basolateral amygdala

Pam Blundell[a,*], Michelle Symonds[b], Geoffrey Hall[b,c], Simon Killcross[c], Glynis. K. Bailey[b,c]

[a] University of Leeds, Leeds, LS2 9JT, UK
[b] University of York, York, YO10 5DD, UK
[c] University of New South Wales, Sydney, Australia

HIGHLIGHTS

- We examine BLA- and sham-lesioned rats' ability to form sensory–sensory associations.
- BLA-lesioned rats can acquire sensory preconditioning.
- BLA-lesioned rats are impaired at taste-potentiated odor aversion.
- BLA needed for learning sensory properties of motivationally significant stimuli.

ARTICLE INFO

Article history:
Received 11 October 2011
Received in revised form 17 August 2012
Accepted 18 August 2012
Available online 25 August 2012

Keywords:
Basolateral amygdala
Taste
Odor
Aversion learning

ABSTRACT

Rats with neurotoxic lesions of the basolateral amygdala were trained in procedures designed to assess the formation of within-event, taste–odor associations. In Experiments 1 and 2 the animals were given initial exposure to a taste–odor compound; the value of the taste was then modified, and the consequent change in responding to the odor was taken to indicate that an odor–taste association had been formed. In Experiment 1 the value of the taste (saline) was enhanced by means of salt-depletion procedure; in Experiment 2 the taste was devalued by aversive conditioning. In neither procedure did lesioned animals differ from sham-operated controls. Experiment 3 confirmed, however, that taste-potentiation of odor aversion learning (an effect thought to depend on the formation of a taste–odor association) is abolished by the lesion. Implications for the view that the amygdala is necessary for sensory–sensory associations between events in different modalities are considered.

1. Introduction

It is widely accepted that the amygdala complex is involved in learning about events of emotional or motivational significance. Much of the evidence comes from work with aversive events (e.g., [1–3]) but effects have also been obtained with appetitive reinforcers (e.g., [4,5]). In their analysis of these latter effects, Blundell et al. [6] demonstrated that lesions of the basolateral amygdala (BLA) had no effect on a rat's ability to acquire a Pavlovian conditioned response but that in instrumental learning the normal sensitivity to devaluation of the reinforcer was not obtained. Specifically, BLA-lesioned rats trained to make different responses for different reinforcers did not a show a selective reduction in responding when one of the reinforcers was devalued (e.g., by feeding the animal to satiety with that reinforcer). It was suggested in explanation, that the BLA is necessary for animals to form a representation of the reinforcer that integrates its sensory and motivational properties (see also Ref. [7]).

According to this interpretation, the deficit shown by rats with BLA lesions lies in their inability to learn normally about the sensory aspects of motivational events. The effect appears to be specific to the combination of motivational and sensory properties as other research has shown that their ability to form associations between two sensory aspects of a compound stimulus is unaffected. Thus, Blundell et al. [6] gave lesioned rats exposure to a compound of two tastes (e.g., salt and sucrose). One of these tastes was then devalued by pairing it with a nausea-inducing injection of lithium chloride (LiCl). In a subsequent test, the rats showed an aversion to the other taste; that is, a standard sensory preconditioning effect was obtained. Learning about the properties of the taste compound in the first stage, when the motivationally significance of the stimuli was not relevant, proceeded normally. (It is true that the rats were water-deprived during this experiment and that the flavors were presented as solutions, but the motivational significance of the stimuli was not relevant to the test which assessed only the association between the two tastes.)

* Corresponding author at: Institute of Psychological Sciences, University of Leeds, Leeds, LS2 9JT, UK. Tel.: +44 0113 343 5735; fax: +44 0113 343 5749.
E-mail address: p.blundell@leeds.ac.uk (P. Blundell).

0166-4328/$ – see front matter
http://dx.doi.org/10.1016/j.bbr.2012.08.030

Dwyer and Killcross [8] confirmed this result, and then went on to look at a parallel procedure with hungry subjects. They gave rats exposure to a motivationally significant taste (e.g., sucrose) in a particular place (one arm of a Y-maze). They then devalued that arm by associating it with the effects of an injection of LiCl. When given a place-preference test, rats with lesions of the BLA showed a normal tendency to avoid that arm, demonstrating that they had learned about the motivational significance of that place. But, in contrast to control subjects, they showed no evidence of having acquired an aversion to the sucrose that had experienced there. Dwyer and Killcross concluded that, for rats with BLA lesions, the place cues were able to retrieve only a general motivational representation, so that experience of an aversion in that place would be unable to generate an aversion to the specific taste of sucrose [8].

This account of the effects of BLA lesions – that they impair the ability to learn about the sensory aspects of a motivationally significant event, but that sensory–sensory associations between neutral stimuli are not affected – is challenged by the results of studies of the phenomenon of taste-potentiated odor-aversion learning (TPOA). Rats given exposure to a taste shortly before a state of nausea is induced will readily develop an aversion to the taste; it is assumed that an association is formed between the taste as the conditioned stimulus (CS) and nausea as the unconditioned stimulus (US). Aversion learning with an odor as the CS, by contrast, proceeds only slowly. If, however, a taste is presented in compound with the odor, the aversion acquired by the odor is found to be stronger (e.g., [9]; see Ref. [10], for a review); that is, the presence of the taste potentiates conditioning to the odor. One interpretation of this effect is that it depends on a process of within-event learning [11]. The suggestion is that experience of the compound CS during conditioning allows the rat to form an odor–taste association. The taste also forms a strong association with the US, whereas the odor does not. The odor will be able to elicit a conditioned response (CR), however, not because of its own association with the US, but by way of the associative chain: odor–taste-US. Evidence in favor of this interpretation comes from the observation that extinction of the taste prior to the test with the odor (a procedure that will break the last link in the chain), reduces the ability of the odor to evoke the CR [12]. Thus, TPOA appears to rely on sensory–sensory learning, between neutral events.

If the BLA is specifically involved in learning about the sensory properties of motivationally significant events, there is no reason to expect an effect of BLA lesions on the sensory–sensory association taken to be responsible for TPOA. It has been reliably demonstrated, however, that procedures that disrupt the normal functioning of the BLA attenuate or eliminate TPOA. This result was first demonstrated in studies using electrolytic lesions [13] but has since been obtained with neurotoxic lesioning techniques [14,15], and with infusions into the BLA of the GABA receptor agonist muscimol [16] of the NMDA antagonist APV [17,18], of lidocaine [19] and of the noradrenalin antagonist propanolol [20]. Hatfield and Gallagher [17] further reported that the TPOA shown by normal animals in their training preparation is sensitive to the effects of extinguishing the taste, confirming the interpretation that the effect depends on the formation of the within-event, odor–taste association. In contrast to the results obtained with the sensory-preconditioning procedure then, these experiments appear to demonstrate a role for the BLA in the formation of sensory–sensory associations between motivationally neutral cues.

The TPOA procedure involves a compound cue with elements drawn from different modalities (i.e., a taste and an odor), and Hatfield and Gallagher [17] offered the interpretation that a normally functioning BLA is needed for the formation of associations between such cues. This interpretation can accommodate the results described so far. According to this line of reasoning, the failure of lesioned animals to a show a reinforcer devaluation effect is taken to be a specific instance of their inability to form a cross-modal association, in this case between the sensory and motivational properties of the reinforcer. Sensory preconditioning proceeded normally in the experiments cited earlier [6] because the critical stimuli were drawn from the same modality (both were tastes). The obvious implication of this analysis is that sensory preconditioning would be disrupted by BLA lesions if the relevant stimuli were taken from different modalities (e.g., if a taste and an odor were used, as in the TPOA procedure). The first two experiments to be reported here test this prediction; the final experiment looks again at TPOA using our stimuli and procedures.

Experiment 1 employed a procedure [21] that makes use of an experimentally induced salt need to demonstrate the formation of within-event taste–odor associations. In the version of this procedure developed in our laboratory, rats are allowed to consume a saline solution to which an odorant (iso-amyl acetate, AA) had been added. At the concentration used in this procedure, AA has been demonstrated to function strictly as an odor without taste properties [22]. The rats are then injected with an agent that produces sodium depletion and thus renders saline particularly valued. When given a choice between plain water and water to which AA had been added, normal rats show a marked preference for the latter. This outcome depends on the rats having experienced saline and AA together in the first phase; control subjects given exposure to saline and AA on separate trials during this phase show no preference for the odor of AA on test.

We investigated the effects of neurotoxic lesions of the BLA on performance on this task. There were four groups of subjects – BLA-lesioned and sham-lesioned subjects given preexposure to the taste–odor compound (Group Lesion-Comp and Sham-Comp), and lesioned and sham-lesioned subjects given separate exposure to the elements of the compound (Groups Lesion-Ele and Sham-Ele). We anticipated that animals in Group Sham-Comp would show a preference for AA on the test whereas those in Group Sham-Ele would not. If an intact BLA is necessary for the formation of a within-event, odor–taste, association, then neither of the lesioned groups would be expected to show such a preference.

Experiment 2 utilized an aversive conditioning paradigm to examine the effect of BLA lesions on the formation of taste–odor associations, using a procedure that has been widely applied in demonstrations of sensory preconditioning in flavor aversion learning [11]. This experiment employed a within-subject design. All rats were initially exposed to two compound stimuli, AX and BY, where A and B were different tastes and X and Y different odors. Again, there is good evidence that these stimuli function as odors at the concentrations used here [23]. They then received aversion conditioning with LiCl as the US and taste A as the CS; taste B was presented nonreinforced in this stage. The test assessed the extent to which odors X and Y controlled an aversion. If preexposure establishes within-event associations then it can be expected that establishing an aversion to X's associate would result in the animals showing an aversion to this odor on the test. Since Y's associate B does not undergo conditioning, no aversion to Y is to be expected. The question of central interest was whether lesions of the BLA would eliminate the X/Y difference.

Experiment 3 was a replication of the basic taste-potentiation effect, using a within-subject design. This experiment employed two odors, almond and vanilla. All animals receive two conditioning trials, one with each odor. On one of these trials an odor was presented alone; on the other trial, the other odor was presented in compound with a taste (sucrose). Separate tests assessed the aversion controlled by each odor. A stronger aversion to that trained in compound with sucrose than to that trained alone would indicate potentiation by taste. The question of interest was whether this effect would be found in rats with BLA lesions.

2. Materials and methods

2.1. Subjects

The subjects for Experiment 1 were 32, male, hooded Lister rats, 16 of which had undergone surgery to destroy the cell bodies of the BLA, and the remainder had undergone a sham procedure. They had a mean weight of 305 g (range: 290–315 g) at the time of surgery. Shortly after surgery they had been used in a study of Pavlovian-instrumental interaction in an operant chamber [4]. After completion of this study (which involved food deprivation) they were returned to ad libitum food and water for a period, before being used in the present series of experiments. All rats were naive with respect to the stimuli used in this study. At the start of this experiment they had a mean weight of 403 g (range 325–460 g). The rats were housed singly in a colony room that was lit from 8:00 a.m. to 8:00 p.m. Behavioral testing was carried out during the light portion of the cycle.

Experiment 2 used 16 of the rats that were used in Experiment 1, eight with BLA lesions and eight sham-lesioned animals. The animals were selected at random apart from the constraint that subjects from each of the experimental conditions of the previous experiment were equally represented in the Sham and BLA groups of Experiment 2.

Experiment 3 used the 16 rats from Experiment 1 that were not used in Experiment 2. One of the animals in the sham-lesioned group became ill during the course of the experiment and an error on the part of the experimenter meant that data were lost for a second, reducing the group size to six.

2.2. Surgery

Anesthesia was induced with 4% halothane delivered in O_2 and N_2O gas in an induction chamber. The rat was then transferred to a stereotaxic frame (Stoelting Inc., USA) and its nose placed in a facemask; anesthesia was maintained by the delivery of 1.5% halothane in O_2 and N_2O (approximately 0.7 l/min of each). The depth of anesthesia was monitored by assessing the pedal withdrawal reflex and responsivity to a mild tail pinch. An incision was made along the skull and the skin and fascia were cleared to reveal bregma. A drill mounted on the stereotaxic frame was used to make burr holes above the injection sites. Two injections were made on each side through a 30-gauge needle attached by polythene tubing to a 1-ml SGE syringe controlled by a Harvard infusion pump. Each injection was of 0.25 ml of .09 M quinolinic acid. Injections were made at the following coordinates: Lateral + and −4.6; anterior–posterior −2.3; ventral (from dura) −7.3. Each injection was made over 2.5 min, and the injection needle was left in place for a further 2.5 min to allow the neurotoxin to diffuse. The procedure for sham-operated animals was identical except that no neurotoxin was injected. The wound was then closed with a suture. Animals were observed during recovery from the operation and were given an injection of saline if they showed signs of dehydration. Once they had been observed eating and drinking, they were returned to their home cages and allowed to recover, with free access to food and water, before the start of behavioral training.

2.3. Apparatus

Inverted 50-ml centrifuge tubes, equipped with stainless steel, ball-bearing-tipped spouts, were used to present measured amounts of the various solutions. Fluid consumption was measured, by weighing, to the nearest 0.1 g.

In Experiment 1, training and testing occurred in cages different from the home cages and housed in a separate, dark room. These cages measured 35 cm × 22 cm × 19 cm, had walls and floor made of transparent plastic and a roof of wire mesh through which drinking bottles could be inserted. The solutions presented were 0.16 M NaCl (N), 0.05% iso-amyl acetate (AA), and the compound of N plus AA (made up so as to maintain these concentrations for the individual elements). Salt need was induced by subcutaneous injection of 0.5 ml of FuroDoca – a mixture of 10.0 mg of furosemide (Furo; supplied by Sigma) and 5.0 mg deoxycorticosterone acetate (Doca; Sigma) dispersed in deionized water by the addition of Tween 80 (Sigma; 1 drop per 20.0 ml).

Procedures for Experiment 2 and 3 were carried out in a context different from that used for Experiment 1. This consisted of a small, well lit, room containing cages measuring 36 cm × 23 cm × 18 cm, which had walls made of white plastic and both a roof and floor made of wire mesh. The taste stimuli (sour and bitter) consisted of a 0.01 M solution of HCl and a 0.0006 M solution of quinine sulphate. The odor stimuli consisted of a 2% (v/v) solution of almond essence and a 1% solution of vanilla essence (Supercook, Leeds, UK). The unconditioned stimulus for aversion conditioning was an intraperitoneal injection of 0.30 M LiCl at 10 ml/kg of body weight.

2.4. Behavioral testing

2.4.1. Experiment 1

The rats were deprived of fluid for 24 h prior to the first training day and maintained on a water deprivation schedule for the next four days, receiving 20-min access to water in their home cages half an hour after training finished each day. On each of the four training days, the rats were exposed to a solution in the test environment for two 10-min sessions, 5 h apart, each day. The animals were assigned to four equal-sized groups. Those in groups Sham-Comp and Lesion-Comp received four exposures to a compound composed of iso-amyl acetate (AA) and saline (N) once per day over four days, and four exposures to water on the alternate sessions. The order was pseudo-random in that they received two exposures to the compound in the morning sessions, and two exposures to the compound in the afternoon sessions. Rats in groups Sham-Ele and Lesion-Ele received four exposures to AA alone and four exposures to N alone. The order of presentation was matched to that given to the compound groups in that these subjects received exposure to AA alone when the compound groups were exposed to the compound. Three hours after the final session on Day 4, all rats were injected with FuroDoca. The rats were given access to distilled water overnight, but not to food. Three hours prior to test on Day 5, the water was removed from the home cages.

The first test took place 20 h following injection with FuroDoca. The test consisted of a choice between a drinking tube containing the AA solution and a drinking tube containing water. The duration of the test was 10 min, and the position of the drinking tubes was counterbalanced across animals. A second test session, identical to the first, was given 5 h later.

2.4.2. Experiment 2

Three days before the start of training the standard water bottles were removed and for the next two days access to water was given during two 30-min drinking sessions, initiated at 11:00 a.m. and 4:00 p.m. Fluids continued to be given at these times throughout the experiment. On each of the next four days the animals were exposed, in the experimental cages, to the flavor compounds AX and BY, where A and B represent the tastes, and X and Y the odors. Each exposure session lasted for 30 min; one compound was consistently presented in the morning session and the other in the afternoon. Half the animals received AX in the morning and half received AX in the afternoon. The identity of the tastes and odors used as AX and BY was fully counterbalanced within each group. Over the next eight days, all animals received two reinforced trials with A and two nonreinforced presentations of B; half of the animals received the sequence A, B, A, B, and half the sequence B, A, B, A. The flavored solutions were presented for 30 min in the morning drinking sessions with water being made available for 30 min in the home cages in the afternoon sessions. Consumption of A was followed immediately by an injection of LiCl. Each training day was followed by a recovery day in which water was presented in the home cage during both drinking sessions.

The test was conducted over the next two days. On the first, half of the rats in each group were given odor X (the odor associated with the reinforced taste), and half were given odor Y. On the second test the other odor was presented. On each test the animals were given access to two bottles for 30 min during the morning session, one bottle containing the odor, the other water. Water was given in the home cage in the afternoon drinking sessions.

2.4.3. Experiment 3

After the schedule of water deprivation had been established as in Experiment 2, all rats underwent two conditioning trials, separated by a recovery day. On one trial the rats were given access for 30 min, in the morning drinking session, to 13 ml of a compound consisting of an odor in a 10% (w/v) sucrose solution. This was followed by an injection of LiCl. On the other trial the rats received access for 30 min to 13 ml of a solution that contained the other odor, consumption of which was followed by an injection of LiCl. The order of presentation of the trials, and the identity of the odor (almond or vanilla) that was presented in compound, was counterbalanced within each group. Following a further recovery day, the consumption of vanilla and almond was assessed, on consecutive days, in two test sessions. As in Experiment 2, on each session the rats were given access to two drinking tubes, one containing water and the other a solution containing the odor. Half the animals in each group were tested first with the odor that had been conditioned in compound and half were tested first with the odor that had been conditioned alone.

2.5. Histology

After completing the series of experiment reported here, the rats were given a lethal dose of sodium pentobarbitol (Sagital, 2 ml, ip) and perfused via the ascending aorta with cold 0.1 M phosphate buffered saline, pH 7.4, followed by 4% paraformaldehyde. The brains were then removed and postfixed with 4% paraformaldehyde before being transferred to 20% sucrose. The brains were frozen in solid CO_2 and coronal sections (40 mm) were cut throughout the extent of the lesioned area. Every fourth section was mounted on a gelatine-coated slide, and stained using cresyl violet. Slides were examined under a microscope to assess the extent of the excitotoxin-induced neuronal damage. Areas of neuronal loss were mapped onto standardized rostrocaudal section drawings of the rat brain [24], using ImageJ [25]. The total area of the lateral amydala (LA) and BLA at each of the five rostrocaudal levels of the atlas was measured, and then the area of damage was measured, such that the percentage damage for each rat at each level of the BLA could be calculated. This allowed calculation of

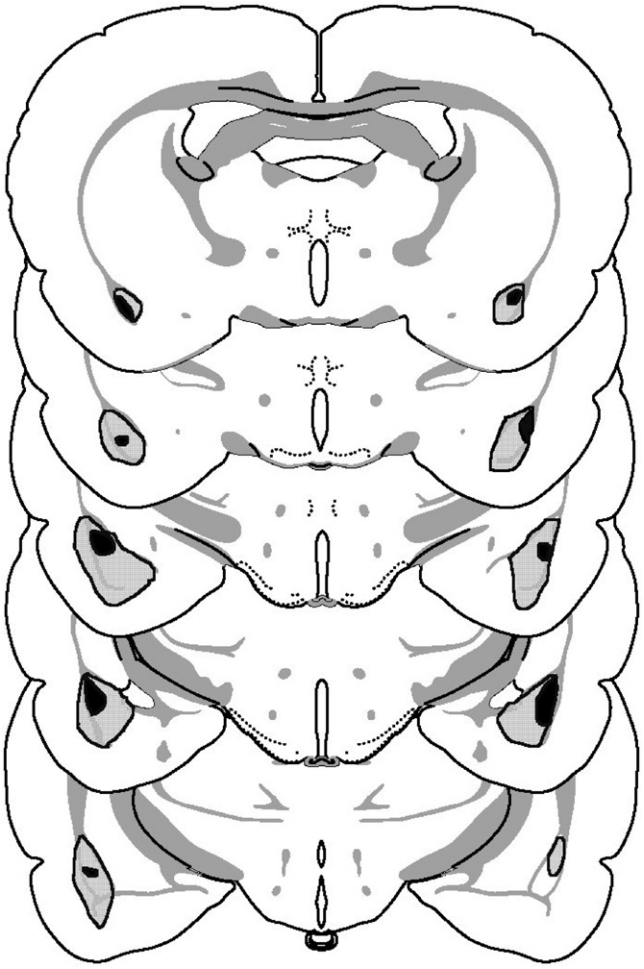

Fig. 1. Schematic representation of excitotoxic lesions to the basolateral amygdala. Shaded areas represent the smallest (black) and largest (gray) extent of neuronal damage in a single animal. Coronal sections are −1.3 mm to −3.9 mm relative to bregma (Swanson, 1998).

the mean (±SEM) percentage area of the BLA damaged at each level for each experiment.

3. Results

3.1. Histology

Fig. 1 shows the extent of the smallest and largest lesions. On the whole the lesions proved to be rather small, and in two cases there was no damage to the BLA. These animals (which were both assigned to Lesion-Ele, in Experiment 1, and were also used in Experiment 3) were excluded from further analysis. Shrinkage had occurred in all of the accepted cases, as did gliosis (although often this was not complete). In some animals the damage extended to the lateral nucleus, and in one case there was limited unilateral damage to the cortical nucleus of the amygdala. All animals had an intact central nucleus. Lesions were drawn using ImageJ [25], and the mean (±SEM) percentage area of the BLA and the LA destroyed at each level for each experiment was calculated (Table 1). The brains with acceptable histologies ($n = 14$) averaged over 50% damage to LA and BLA (levels 24–32). Sparing of the neurons was mostly in the lateral nucleus, and in the posterior region. The lesions are comparable across all three experiments, with slightly less damage in the animals in Experiment 3.

Table 1
Mean (±SEM) percentage area of BLA and LA destroyed by lesion. Levels refer to plates in Swanson [24], with distance from bregma in brackets.

	Experiment 1 ($n = 14$)	Experiment 2 ($n = 8$)	Experiment 3 ($n = 6$)
Level 24 (−1.33)	64.6 (5.3)	64.1 (8.1)	65.2 (8.0)
Level 26 (−1.78)	59.5 (5.5)	66.3 (8.3)	50.4 (5.7)
Level 28 (−2.45)	53.1 (5.7)	59.4 (7.5)	44.6 (8.9)
Level 30 (−3.25)	56.6 (3.2)	58.2 (4.2)	54.6 (5.8)
Level 32 (−3.90)	31.5 (7.3)	37.1 (12.3)	23.9 (7.9)

3.2. Behavior

3.2.1. Experiment 1

Fig. 2 shows, separately for the BLA-lesioned and the sham-lesioned groups, the mean volume of each fluid consumed during the preexposure phase of the experiment. The data are pooled over all four trials with a given fluid. Rats in the Ele groups consumed more saline than amyl acetate, and rats in group Comp consumed more of the AA + N compound than water; but there was no clear difference between BLA-lesioned and sham-lesioned subjects. Statistical analysis confirmed this description of the data. An analysis of variance, conducted on the data from the animals in the Ele groups, with the variables of lesion (BLA, Sham) and flavor (AA, N), revealed a significant main effect of flavor, $F(1, 12) = 164.55$. (In this and subsequent analyses a significance level of $p < .05$ was adopted.) No other effects were significant (all $F_s < 1$). An equivalent analysis, conducted on the data from the animals in the Comp groups again revealed a significant main effect of flavor, $F(1, 14) = 59.56$. No other effects were significant; for the main effect of lesion, $F < 1$, and for the interaction, Flavor x Lesion, $F(1, 14) = 4.45$.

The test performance was expressed as a preference ratio: volume of AA consumed/total intake. A higher ratio score in the Comp condition than in the Ele condition would indicate the occurrence of a sensory-preconditioning effect. Fig. 3 shows group mean preference ratios for the four groups on each of the test trials. In all groups the ratio score was somewhat lower on test 2 than on test

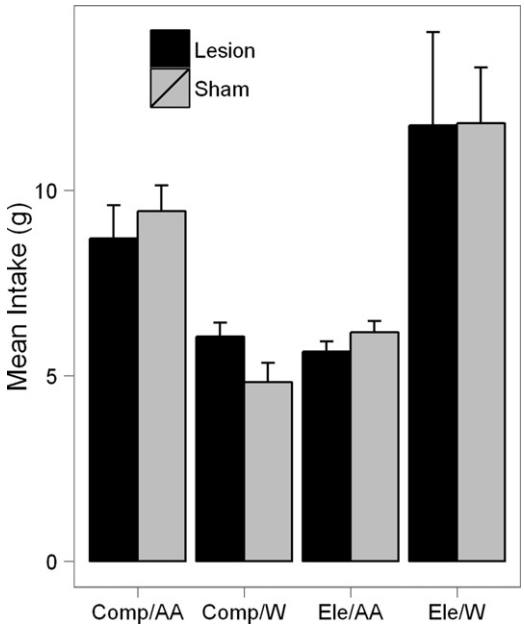

Fig. 2. Experiment 1: Group mean volumes consumed per trial during the preexposure phase. Comp groups were exposed to a compound of amyl acetate and saline (AAN); on other trials they received water (W). Ele groups received separate trials with the elements of the compound, amyl acetate (AA) and saline (N). BLA refers to animals with lesions of the basolateral amygdala.

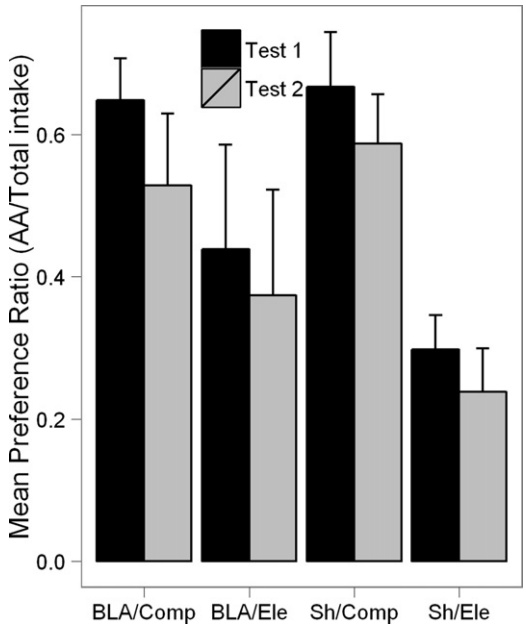

Fig. 3. Experiment 1: Group mean preference ratio scores for the two test sessions after induction of a salt need. Comp groups had received preexposure to a compound of amyl acetate (AA) and saline; Ele groups had received separate preexposure to AA and saline. On each test the animals had access to two bottles one containing a solution of AA, the other water.

1, but the same basic pattern was evident on both trials. Subjects in the Ele groups had low ratio scores (showing a preference for water over AA); subjects in the Comp groups showed a preference for AA. Critically, this preference was seen both in sham-lesioned and in BLA-lesioned animals and there was no obvious difference between the lesioned and sham animals in the size of this effect. Interpretation of this finding would be complicated if absolute levels of consumption differed between groups. Thus, the total amount of fluid consumed by each group on test is presented in Table 2. This shows that there was slightly more consumption in test 2 than in test 1, but there was no other systematic variation. A mixed three-way analysis of variance was conducted on the preference data summarized in the Fig. 2, with the variables of lesion (BLA, sham), group (Comp, Ele) and test (1, 2). This revealed a significant main effect of group, $F(1, 26) = 18.57$. No other effects were significant: for all other main effects and interactions, $F_s < 1$, apart from the interaction of Lesion × Group, where $F(1, 26) = 2.00$. A similar analysis of variance was conducted on the baseline data. This revealed no statistically significant effects – largest F for the factor of test, $F(1, 26) = 3.16$.

Table 2
Group mean total intake (g) on test sessions.

	Sham-Comp	Sham-Ele	Lesion-Comp	Lesion-Ele
Experiment 1				
Test 1	3.5	3.5	3.9	3.1
Test 2	4.9	3.7	3.7	3.9
		Sham		Lesion
Experiment 2				
X test		7.3		7.9
Y test		7.0		8.9
		Sham		Lesion
Experiment 3				
Odor 1		7.0		8.1
Odor 2		7.3		8.3

A preference for an odorant previously associated with saline in animals in a state of salt need may be taken to indicate that a within-event (odor-saline) association has been formed. The present results demonstrate that lesions of the BLA are without influence on this form of learning. It should be noted that, although the lesions suffered by the animals used in this study were rather small, there is no doubt that they are capable of exerting an effect on behavior in other situations. Prior to the experiment reported here, the same rats were used in a study of the effects of superimposing a Pavlovian CS on instrumental responding and the BLA-lesioned subjects were found to show pattern of behavior distinctively different from that shown by the sham-lesioned subjects [4].

3.2.2. Experiment 2

An error on the part of the experimenter meant that data were lost for one of the animals in the sham-lesioned group, reducing the group size to seven. Although levels of consumption were not high (acid and quinine solutions are not usually drunk in large amounts) all animals consumed reasonable amounts of AX and BY during the preexposure phase, and there were no obvious differences between the groups. On the last trial with AX, the group mean score for the BLA rats was 7.2 g and that for the sham rats was 7.8 g; the equivalent scores for the BY solution were 8.4 g and 7.8 g. An analysis of variance with group and solution as the variables revealed no significant effects (all $F_s < 1$).

Conditioning with A established an aversion to this taste. On the first trial of the conditioning phase, the group mean consumption scores were 9.2 g for the sham group and 9.3 g for the BLA group; on the second trial the equivalent scores were 3.6 g and 3.9 g. Scores for the consumption of the nonreinforced taste B on the first presentation in this phase were 8.4 g for the sham group and 8.6 g for the BLA group. Consumption was reduced somewhat on the second presentation of B (perhaps as a result of generalization from the reinforcement of A), but levels still remained high at 7.4 g for the sham group and 7.8 g for the BLA group. An analysis of variance with group, stimulus (A or B), and trial (first or second) as the variables revealed no significant effect of group and no significant interaction involving this variable (all $F_s < 1$). There was, however, a significant interaction between stimulus and trial number, $F(1, 13) = 27.36$. A further analysis conducted on the data for the first trial revealed no significant effects: For the main effect of stimulus, $F(1, 13) = 4.14$; for the main effect of group and the interaction, $F_s < 1$. An equivalent analysis for the second trial showed there to be a significant difference between the reinforced and nonreinforced tastes, $F(1, 13) = 14.63$; no other effects were significant ($F_s < 1$). Thus an aversion was acquired to A but not B in both groups, and there was no difference between the groups in the magnitude of this aversion.

The results of the test phase are presented in Fig. 4, which shows, for each group, the mean preference ratio (consumption of the odor-containing fluid/total consumption) for the test trials with odors X and Y. These preference scores were based on comparable levels of overall consumption. As Table 2 shows, total intake in the test phase did not differ between groups or according to trial type; an analysis of variance conducted on the scores shown in the table, with group and trial type as the variables revealed no significant effects (largest $F < 2$).

It is clear from the figure that both groups consumed odor Y (that associated with the nonreinforced taste) as readily as they consumed water, having ratio scores of about .5. Both groups showed an aversion to odor X (that associated with the reinforced taste), suggesting that the within-event, A–X, association had been formed in both groups. There is an indication that the magnitude of the aversion to X was slightly less profound in the BLA-lesioned rats than in the shams, but the difference was small and proved not to be statistically reliable. An analysis of variance conducted on the data summarized in the figure, with group (BLA and sham) and test

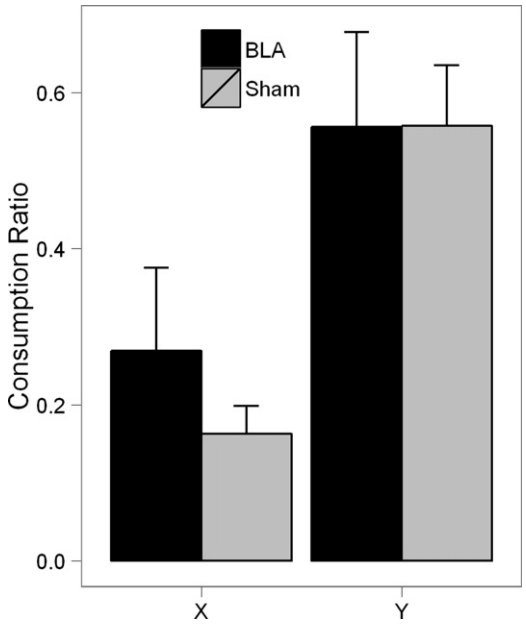

Fig. 4. Experiment 2. Group mean preference ratio scores (volume consumed of odor-containing fluid/total consumption) on the test sessions. All animals had been preexposed to the taste–odor compounds AX and BY (where A and B represent tastes and X and Y odors). Prior to the test A was subjected to aversion conditioning but B was not. BLA refers to animals with lesions of the basolateral amygdala. Vertical bars indicate SEMs.

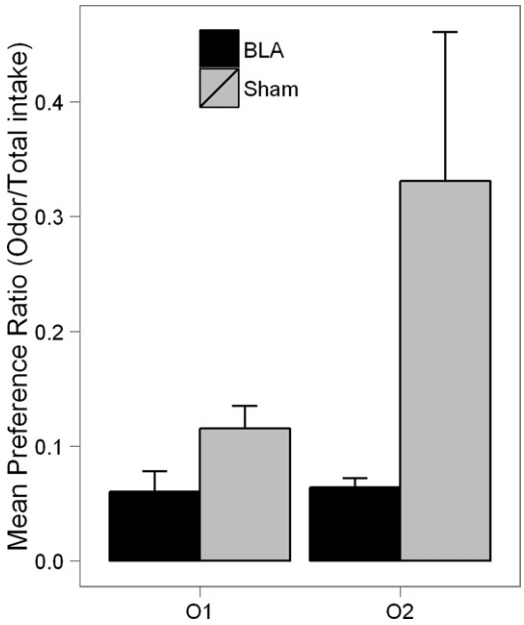

Fig. 5. Experiment 3. Group mean preference ratio scores (volume consumed of odor-containing fluid/total consumption) for the test sessions. O1 refers to the odor conditioned alone; O2 refers to the odor conditioned in compound with a taste. BLA refers to animals with lesions of the basolateral amygdala. Vertical bars indicate SEMs.

odor (X and Y) as the variables revealed a significant effect only of odor, $F(1, 13) = 10.00$; all other $F_s < 1$. We conclude that rats with BLA lesions show no deficit in sensory preconditioning with this training procedure.

3.2.3. Experiment 3

Both groups readily drank the solutions presented on the conditioning trials. Group means for the odor presented alone were 10.7 g for the sham-lesioned animals and 9.8 g for the BLA-lesioned animals; the equivalent scores for the compound solution were somewhat higher (12.0 g and 12.3 g), presumably reflecting the palatability of the sucrose solution. An analysis of variance confirmed the reliability of the difference between consumption of the odor alone and of the odor when presented in compound with sucrose, $F(1, 10) = 7.93$, but there was no significant difference between the groups and no significant interaction ($F_s < 1$)

Group mean ratio scores (consumption of the odor/total consumption) for the tests with the odor conditioned alone (O1) and that conditioned in compound (O2) are presented in Fig. 5. As Table 2 shows, there were no differences in total consumption; an analysis of variance conducted on the data shown in the table, with group and odor type as the variables, revealed no significant effects (all $F_s < 1$). Both groups showed evidence of having acquired an aversion to both odors, in that all ratio scores were less than .5. The sham-lesioned subjects, however, showed the potentiation effect, having a stronger aversion to the odor conditioned in compound with the taste than to the odor conditioned alone. BLA-lesioned animals had equally strong aversions to both odors. An analysis of variance was conducted on the data summarized in the figure with group (lesioned and sham) and odor (conditioned in compound or alone) as the variables. For the main effect of group, $F(1, 10) = 6.24$; for the effect of odor, $F(1, 10) = 4.31$, $p = .065$; and for the interaction of these variables, $F(1,10) = 3.98$, $p = .07$. Analysis of simple main effect showed that the scores for the two odors for the sham-lesioned group differed significantly, $F(1, 10) = 8.28$, whereas those for the BLA-lesioned group did not ($F < 1$).

Although the results just presented confirm that the standard taste-potentiation effect is not found in rats with BLA lesions, it should be noted that the pattern of results obtained here differs from that previously reported. The experiments by Hatfield et al. [15] and by Hatfield and Gallagher [17] found that rats with BLA lesions learned poorly about an odor both when it was presented alone and when it was presented in compound with a taste. In the present experiment the BLA group showed a substantial aversion to the odor under both conditions of training. The reason for this difference is unclear. One procedural difference between this and the earlier experiments was that in the latter the odor was presented separately (on a filter paper disk adjacent to the drinking tube containing water) whereas in our experiment the odorant was dissolved in the water. It seems unlikely, however, that this is a critical difference. Our procedure was based on that used by Rescorla and Durlach [11] who have successfully demonstrated the standard features of odor conditioning with this procedure; and indeed, the results for the sham-lesioned animals in the present experiment provide a clear example of the taste-potentiation effect. A second difference is that the earlier experiments made a between-group comparison, one group being conditioned with the odor alone and the other with the odor–taste compound. Our within-subject design, in which each subject is trained with both odors, requires that the animal be able to discriminate between the two odors if a difference is to be seen on test. It is in principle possible, then, that our results might simply reflect an inability of the BLA-lesioned rats to make this discrimination – that taste-potentiation occurred and that the aversion acquired to the odor trained in compound generalized fully to the odor trained alone. But again, there are good reasons to reject this interpretation. In particular, the within-subject procedure used in Experiment 2 required rats to discriminate between the same odors as were used in the present experiment, and in this, the BLA-lesioned rats proved to be just as capable as the shams.

Whatever its source, the fact the BLA-lesioned animals consumed rather little of both X and Y on the test sessions leaves open the possibility that the failure to find a difference between the odors

in this group might be the consequence of a "floor effect", and that a difference might have been obtained had levels of consumption been higher. In order to address this possibility, the animals were given a second pair of test trials identical to the first. We expected that the opportunity for extinction of the aversion allowed by the first set of test trials would be likely to produce a raised level of consumption on the second set. This proved to be the case, but there was still no evidence of taste-potentiation of conditioning in the BLA-lesioned animals; in fact the mean ratio for the odor trained in compound with the taste (0.39) was higher than that for the odor trained alone (0.26). The sham-lesioned animals also showed extinction of aversion, but the TPOA pattern remained - the mean ratio for the odor trained in compound with the taste (0.30) was still less than that of the odor trained alone (0.39). These scores did not differ significantly (all $F_s < 1$).

4. Discussion

It has been reported [14,15,17,20] that lesions of the BLA abolish the TPOA effect (the enhancement of aversion conditioning to an odor when it is trained in compound with a taste). Although differing in some details, the results reported in the present Experiment 3 confirmed that rats with BLA lesions do not show the same potentiation effect as normal rats. The potentiation effect has been interpreted in terms of within-event learning; specifically, it has been argued that the ability of the odor to control a strong aversion after this sort of training is mediated by way of an odor–taste association formed during the compound conditioning trial. Its abolition by lesions of the BLA has thus been taken as support for the proposal that the BLA is necessary for the formation of sensory–sensory associations between stimuli from different modalities. This interpretation can accommodate the finding that BLA lesions disrupt the normal sensitivity of rats to the devaluation of an instrumental reinforcer (e.g., [6]) without the need to suppose that the BLA is specifically involved in the formation of representations of motivational significant events. The failure to form an association between the sensory and motivational properties of a reinforcer, taken to underlie the absence of the reinforcer-devaulation effect, could be interpreted as being just a further example of the inability to form a cross-modal association.

Experiments 1 and 2 provided a direct test of this proposal. They used versions of the sensory preconditioning design in which the animals were initially given preexposure to a taste–odor compound (i.e., to a compound with elements from different modalities, as in the TPOA procedure). The value of the taste was then modified (enhanced in Experiment 1 and reduced in Experiment 2) and the effect on the conditioned response evoked by the odor was tested. In both cases the response elicited by the odor was appropriate to the value acquired by its taste associate (i.e., consumption of the odor was enhanced in Experiment 1 and suppressed in Experiment 2). This outcome, which is usually interpreted as indicating the operation of an odor–taste association, was found both in normal animals and in rats with lesions of the BLA. According to these results, the ability to form the odor–taste association was not impaired by the BLA lesion. The notion that animals with such lesions have a general inability to form cross-modal within-event associations appears to be disconfirmed.

It remains to explain why the BLA lesion should disrupt TPOA but not sensory preconditioning when both phenomena are assumed to depend on the formation of a taste–odor association. One possibility is that we have been mistaken in assuming that the same basic psychological process is responsible for both sensory preconditioning and the taste-potentiation effect. What we have assumed so far is that test performance in both procedures is determined by the operation of an associative chain, linking odor to taste and (in Experiments 2 and 3) taste to the US. But alternative interpretations are available for both. According to some authors, (e.g., [9]) taste potentiation of odor aversion learning may occur because the presence of the taste allows the odor to form a direct association with the US – the presence of the taste is thought to open a "gate", allowing the odor entry into an aversion learning mechanism that would otherwise not be accessible to such stimuli. To adopt this account allows the possibility that BLA lesions disrupt taste potentiation of odor aversion learning not because they prevent the formation of taste–odor associations (the results of the sensory preconditioning experiments would then be taken as demonstrations of that), but because they disrupt the operation of the gating mechanism. The problem for this interpretation is that there is good evidence that the potentiation effect obtained with the procedures used here does in fact operate by way of the odor–taste-US associative chain. As we have already noted, Hatfield and Gallagher [17] report that extinction of the aversion to the taste will attenuate that to the odor – not what would be expected if the aversion to the odor was the result of a direct odor-US association.

An alternative possibility is that the taste-potentiation effect depends on the formation of a taste–odor association (formation of which is disrupted by BLA lesions) but that the sensory preconditioning effect obtained in these experiments is the product of some other mechanism that is independent of the BLA. Although it has usually been assumed that the test stimulus in sensory preconditioning gains access to the US representation by way of an association with the CS, an alternative possibility is that this procedure allows the formation of a direct association between the test stimulus and the US. For animals preexposed to the taste–odor compound, presentation of the taste on the conditioning trial might be expected to activate the representation of its associate, the odor. Some authors (see Ref. [26], for a review; see also Ref. [8]) have supposed that the associative activation of a stimulus representation along with the presentation of a US will result in excitatory conditioning establishing that stimulus as a CS. It will be noted that this analysis of sensory preconditioning still requires that the animals form a taste–odor association in the first stage of training (without this, the taste would not be able to activate the odor representation during the conditioning phase). In consequence, even if this account were to be adopted, the view that the BLA lesion disrupts the formation of sensory–sensory associations would still be untenable. What remains possible is that the BLA is necessary for test performance when, as in the taste-potentiation procedure, this depends on the associative chain taste–odor-US, but is not necessary when test performance depends on a direct odor-US association. Unfortunately for this interpretation, there is evidence that the effect of BLA lesions on the taste-potentiation effect does not depend on processes that operate during the test stage of the experiment. Ferry et al. [16] investigated the effects of injections of the GABA agonist muscimol into the BLA at various stages in the taste-potentiation procedure. Injections given at the time of conditioning with the taste–odor compound impaired the taste-potentiation effect, whereas an injection given just before the test was without effect.

It will be evident from these observations, that we cannot yet present a theoretical account that can accommodate the entire pattern of results. At the empirical level, however, we can conclude that rats with BLA lesions are quite capable of forming cross-modal, sensory–sensory associations (between taste and odor in our sensory preconditioning procedure) but that they fail to do so when (as in the taste-potentiation procedure) the taste–odor compound is presented along with a motivationally significant US. It is possible that the effect of BLA lesions on standard reinforce-devalution tasks (e.g., [6]), should be interpreted in this way, as being not so much a failure specifically to integrate the sensory and motivational

properties of the reinforcer as a more general inability to learn about or process a sensory cue when a significant motivational event is also present.

Acknowledgements

This work was conducted at the University of York during a study visit by G. Bailey supported by a Young Australian Researchers Award from the Australian Academy of Science and an award from the Experimental Psychology Society. P. Blundell was supported by a studentship from the UK Medical Research Council. Correspondence concerning this article should be addressed to P. Blundell, Institute of Psychological Sciences, University of Leeds, Leeds, LS2 9JT, UK.

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