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Amygdaloid And Non-amygdaloid Fear Both Influence Avoidance Of Risky Foraging In Hungry Rats

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Considerable evidence seems to show that emotional and reflex reactions to feared situations are mediated by the amygdala. It might therefore seem plausible to expect that amygdala-coded fear should also influence decisions when animals make choices about instrumental actions. However, there is not good evidence of this. In particular, it appears, though the literature is conflicted, that once learning is complete, the amygdala may often not be involved in instrumental avoidance behaviours. It is therefore of interest that we have found in rats living for extended periods in a semi-naturalistic 'closed economy', where they were given random shocks in regions that had to be entered to obtain food, choices about feeding behaviour were in fact influenced by amygdala-coded fear, in spite of the null effect of amygdalar lesions on fear of dangerous location *per se*. We suggest that avoidance of highly motivated voluntary behaviour does depend in part on fear signals originating in the amygdala. Such signalling may be one role of well-known projections from amygdala to cortico-striate circuitry.

1. Introduction

Contemporary research on fear provides evidence that both amygdala and amygdala-independent circuitry are involved in storing and using memories of painful or frightening events. Reflex and emotional reactions to environmental stimuli that have previously been associated with danger are thought to depend upon learned synaptic alterations within the amygdala [1–5]. However, voluntary avoidance of such stimuli, once learned, often seems to depend upon extra-amygdalar memories, presumably coded within cortico-striate circuitry [6–11], though the amygdala does appear to be needed for the initial learning of voluntary avoidance [12–16].

This picture rests almost entirely on experiments in which animals are removed from their home cages and taken for short periods to experimental chambers for training and testing. It seemed possible to us that a quite different picture might emerge under more naturalistic conditions arranged so that fear and avoidance, as well as appetitive behaviours were all a meaningful, integrated part of animal's lives. For example, perhaps the amygdala *would* then contribute to expression of avoidance, or cortico-striate circuitry *would* learn avoidance responses without the aid of the amygdala.

We decided to look into this by setting up a semi-naturalistic 'closed economy' in which rats lived for extended periods [17,18]. It consisted of a foraging zone that had to be entered to press levers to 'forage' for food and that could be rendered dangerous by the administration of footshocks and a nesting region that was always safe but where no food was ever available (figure 1). Our initial observations showed that animals in this environment commonly spent a great deal of time in the foraging zone, even when they were not working for food, and, as might be expected, when the region was made dangerous (via unsignalled footshocks), the rats greatly reduced, though not totally, the

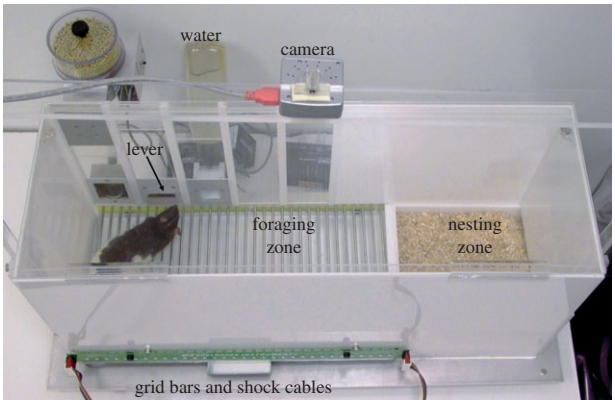

Figure 1. The closed economy apparatus. The live-in apparatus consisted of a safe nest and a risky foraging zone where the animal had to press a lever to obtain food pellets and had access to water. A camera mounted above the chamber tracked the animal's movement constantly. Another lever and pellet receptacle were added in the inserts closer to the nest in experiment 2.

amount of time they spent there, and they greatly diminished their active foraging (i.e. lever pressing) for food. Moreover, in accord with previous findings [19,20], the amygdala did not appear to be needed for avoidance of the foraging zone, especially if it was lesioned once the avoidance response was well learned. However, the amygdala *did* appear to be needed in order for shock to substantially reduce *lever pressing* for food. These observations suggested to us the possibility that the amygdala might be important for expressing (not just learning) avoidance responses when strongly motivated appetitive behaviours and avoidance compete.

Given the essentially observational character of our initial closed economy experiment, where we were not testing a specific hypothesis but were just asking whether under more realistic conditions there might be differences from the standard picture, we felt the need to run an experiment in which we explicitly tested the hypothesis that extra-amygdala mechanisms, while sufficient to mediate avoidance of dangerous places, are not sufficient for avoidance of strongly desired places or activities. Additionally, in this second experiment, rats could obtain food from either of two different levers, one closer to the safe nesting chamber and the other farther into the dangerous region. We anticipated that once shock was introduced, animals would tend to choose the closer, safer lever and avoid the distant, more dangerous one. If so, this would give us the opportunity to test the hypothesis that the preference for the safer lever would depend at least in part on the amygdala.

2. Material and methods

(a) Subjects

Male Charles River Long Evan rats (initially weighing 275–300 g) were individually housed in eight 'closed economy' chambers (figure 1) in a climate-controlled vivarium (accredited by the Association for Assessment and Accreditation of Laboratory Animal Care) on a reverse 12 L : 12 D cycle (lights on at 19.00).

(b) Surgery

Under anaesthesia (94 mg kg^{-1} ketamine and 6.2 mg kg^{-1} xylazine, i.p.), rats were implanted with lesion electrodes (epoxy-coated insect pins no. 00, approx. 0.75 mm tip exposed) bilaterally to their amygdala (from bregma: anterior posterior −2.5; medial lateral ±4.2/5.0; dorsal ventral −8.4/8.6 mm). Lesions were made by passing 1 mA constant current for 10 s (preshock lesions animals). Intact animals underwent the same surgery without lesions. Postshock lesion animals (see below) were re-anaesthetized with light halothane anaesthesia prior to making their lesions [21,22].

(c) Apparatus

Closed economy chambers were custom-built from Plexiglas with the following dimensions: 74.3 cm × 25.4 cm × 33 cm (length × width × height). Each chamber consisted of a 'foraging' arena (54 × 25.4 cm) and a 'nest' (20.3 × 25.4 cm). The nest floor was covered with sawdust, while the floor of the foraging zone was composed of 32 stainless steel rods (4.5 mm diameter) wired to a precision animal shocker (Coulbourn Instruments, Allentown, PA, USA). As can be seen in figure 1 (video: http://faculty.washington.edu/jeansokk/Closed_economy.html), a pellet receptacle-dispenser, a lever and a water bottle (Med Associates, Fairfax, VT, USA) were accessible 47, 39 and 30 cm, respectively, from the nest. In the two lever experiment, another lever and pellet receptacle-dispenser were affixed 13 and 22 cm, respectively, from the nest. The ANY-maze video tracking system (Stoelting Co., Woodale, IL, USA) was used to track the animal's movement, via a Fire-I B/W Board Camera (Unibrain Inc., San Ramon, CA, USA) placed above each closed economy apparatus and to control all input/output devices connected to an AMi interface (Stoelting Co.).

(d) Experimental procedure

In all experiments, the animals' behaviours were continuously recorded except for a 1 h break (every 1–2 days) during which the chamber and bedding pan (underneath the shock floor) were cleaned and food and water reservoirs were refilled.

(i) Experiment 1: unpredictable shocks and foraging behaviour

Animals were run in three groups named 'intact', 'preshock lesion' and 'postshock lesion', as defined in table 1. All animals underwent three successive conditions referred to as phase I, II and III as specified in the table, but the lesion treatment was different in each group. For convenience, we sometimes refer to phases I–III as 'baseline', 'shock' and 'extinction' phases, respectively, though in the postshock lesion group, where during phase III we wanted to assess the post-lesion avoidance that had been learned during phase II, shock was continued during phase III (table 1).

During the baseline phase, rats were shaped to press the lever to attain pellets (45 mg dustless precision pellet; Bio-Serv, Frenchtown, NJ, USA) at a 'fixed ratio 50-continuous reinforcement' (FR50-CRF) schedule (50 lever presses required for the first pellet and then subsequent lever presses delivered a pellet/press) (cf. [17]) by gradually increasing the lever pressing schedule (i.e. FR1-CRF, FR5-CRF, FR10-CRF, FR20-CRF, FR30-CRF, FR40-CRF and FR50-CRF). During each FR-CRF schedule, if the animal did not make sequential lever pressings within 1 min, then the FR-CRF requirement was reset.

After 7 days of stable baseline meal patterns were recorded at the FR50-CRF schedule, all animals entered phase II (the 'shock' phase), which lasted 7 days. During this period, unsignalled footshocks (0.8 mA) were presented randomly every hour regardless of the animal's location (nest or foraging zone). If the animal was in the nest, the shock immediately turned off; if the animal was in the foraging zone, the shock stayed on until the rat escaped to the nest (or a maximum of 10 s).

Following 7 days of shock, animals entered phase III, which lasted 7 days. Shock was discontinued during phase III for intact

Table 1. Group designations for experiments 1 and 2.

		treatments				
exp	group name		phase I	phase II		phase III
exp 1	intact		no shock	shock		no shock
	preshock lesion	lesion	no shock	shock		no shock
	postshock lesion		no shock	shock	lesion	shock
exp 2	intact		no shock	shock		—
	preshock lesion	lesion	no shock	shock		—

and preshock lesion animals. Postshock lesion animals were lesioned (as above) during the cleaning break and then underwent further testing with random shocks continued.

(ii) Experiment 2: unpredictable shocks and two lever preference

Separate groups of preshock lesion and intact rats (defined as above) underwent 10 daily sessions of baseline, shock and extinction, except two levers (one closer to the nest than the other) were available for procuring food pellets, both on a CRF schedule. All animals displayed a stable bias to one of the levers during the baseline days; hence, their lever selections were normalized by dividing the preferred lever presses with the total lever presses for each day.

(e) Histology

At the completion of testing, animals were overdosed with Buthanesia and perfused intracardially with 0.9% saline followed by 10% buffered formalin. The brains were removed and stored in 10% formalin overnight and then kept in 30% sucrose solution until they sank. Coronal 50 µm sections were taken through the extent of the lesion, mounted on gelatin-coated slides and stained with cresyl violet and Prussian blue dyes (electronic supplementary material, figure S6) [23].

(f) Statistical analyses

The daily meal frequency, pellet consumption and time spent in the foraging zone were normalized to the mean baseline values of each animal. The normalized values were analysed by paired or independent *t*-tests, and one-way repeated measures ANOVA where appropriate.

3. Results

(a) Experiment 1. Amygdaloid fear contributes to suppression of lever pressing when the foraging region becomes dangerous

Experiment 1 was carried out in three phases. During all phases, animals moved at will between a nesting chamber and foraging region that contained a lever that could be pressed to obtain meals on a FR50-CRF schedule (figure 1; see Material and methods). Phase I was a baseline period used to determine each animal's undisturbed behaviour. During phase II, animals were shocked at random times (average about twice per hour) when in the foraging region; shock was continued until they returned to the nesting region but for not more than 10 s. During phase III, shock was either discontinued, allowing fear to extinguish, or continued, depending on an animal's experimental group.

Three groups of rats were run. In an intact group, electrodes were implanted in the amygdala to provide a sham control, but no lesions were ever made; members of this group were shocked during phase II but not during phase III. In a second preshock lesion group, amygdala lesions were made at the start of the experiment (and before any shocks were experienced); in these animals, phase III of the experiment was free of shocks, as in the intact group. In a third, postshock lesion group, lesions were made at the end of phase II; in these animals, shock was continued during phase III.

(i) Intact animals

Prior to the introduction of shock, animals spent an average of about 7.90 h (s.d. = 2.97) per day in the foraging zone, about 16% (1.3 h) of which were devoted to lever pressing for food. When shock was introduced during phase II of the experiment (figure 2*a*), these animals fairly rapidly reduced the amount of time they spent in the foraging zone. On the last day of phase II, time in the foraging zone averaged about 3 h, or some 40% of its average baseline (phase I) value ($t_8 = 13.72$, $p < 0.001$). When shock was discontinued for these animals in phase III, time in the foraging zone gradually increased again. Extinction of foraging zone avoidance occurred much less rapidly than acquisition. It should be noted that since shocks occurred at random times at an average rate of $2\,h^{-1}$, it would take some time before an observer could be sure that shock had really stopped and the foraging zone was now safe.

In intact animals, the development of shock-induced suppression of lever pressing followed a similar time course to that of zone avoidance; however, acquisition of lever suppression occurred more slowly than that of foraging zone avoidance. On the last day of phase II, the number of meals earned was about 45% of average meals earned per day during the baseline days of phase I ($t_8 = 4.31$, $p = 0.003$).

During extinction, time in the foraging zone recovered gradually towards baseline values, whereas the shock-caused depression of meals earned nearly recovered to baseline within the first day of extinction (figure 2; paired *t*-test of % recovery of time in foraging zone versus meal frequency on day 2 of phase III: $t_8 = 5.69$, $p < 0.001$). This suggests that working for meals was suppressed only when fear of the foraging zone, as indexed by time spent there, was fairly extreme, a point to which we will return below.

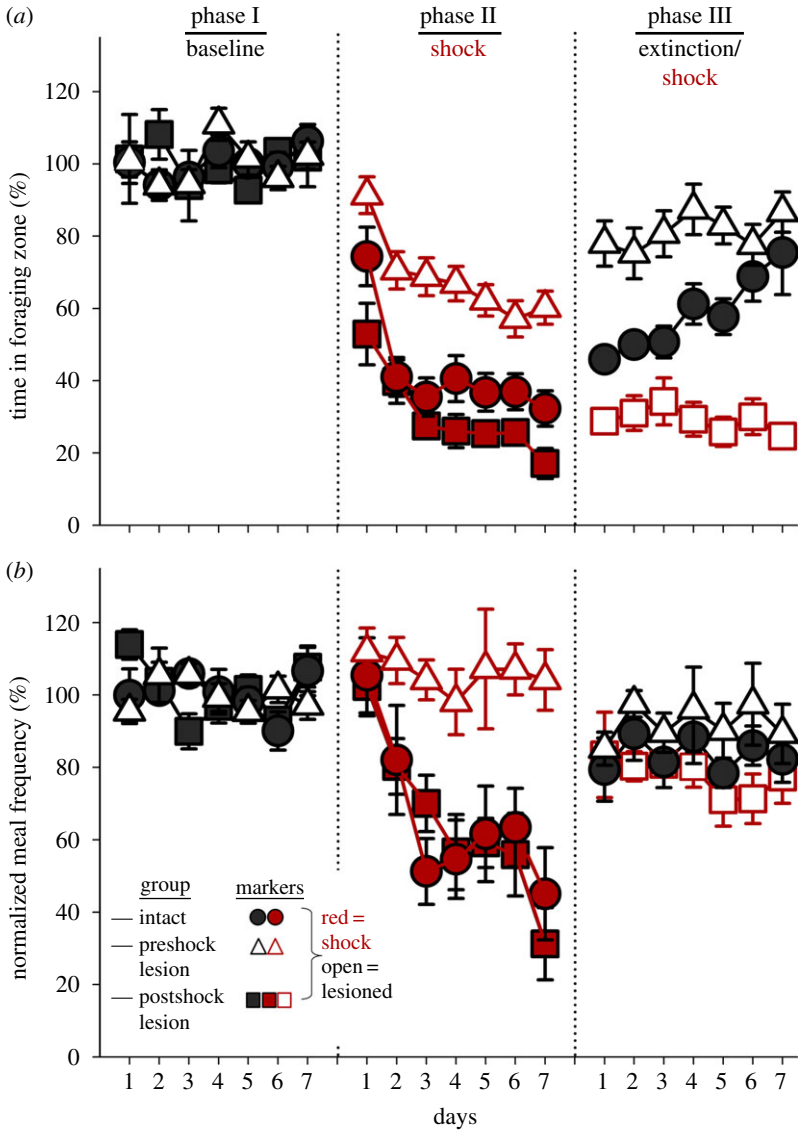

Figure 2. Time in foraging zone and meal frequency in experiment 1. (*a*) Per cent time spent in the foraging zone. Each animal's daily score was normalized to the mean across the baseline sessions (phase I). Animals in the intact group went through phase I (baseline), phase II (shock) and phase III (extinction); the postshock lesion group underwent phase I (baseline), phase II (shock), taken out briefly and given amygdalar lesions, and then phase III (continued shock as phase II); the preshock lesion group received amygdalar lesions prior to going through phase I (baseline), phase II (shock) and phase III (extinction). Open markers indicate amygdala lesions; red markers and lettering indicate random shocks. (*b*) Daily meal frequency. The number of meals earned each day was normalized to the mean across the baseline sessions.

(ii) Animals given amygdala lesions prior to the introduction of shock (preshock lesion group)

During the shock phase of the experiment, animals that had previously been given amygdala lesions reduced their time in the foraging zone (% time in foraging zone last day of phase II as a % of baseline period less than 100%, $t_8 = 8.70$, $p < 0.001$) but to a lesser extent than intact animals (% time in foraging zone for intact versus preshock lesion groups, last day phase II: $t_{16} = 4.15$, $p = 0.001$). During extinction, this avoidance of the foraging zone abated. Shock caused relatively little reduction in lever pressing at any time during the experiment in these animals that were lesioned prior to the start of testing (figure 2*b*).

(iii) Animals given amygdala lesions after experience with shock (postshock lesion group)

Diminished avoidance of the foraging region and/or diminished suppression of lever pressing (within the shocked region) in amygdala-lesioned animals could be taken to suggest a contribution of amygdala-mediated fear to avoidance behaviour. However, effects of the amygdala lesions could also be owing to slower or less complete consolidation of fear, which has often been reported in amygdalectomized rats [24,25]. In order to try to discriminate these possibilities, we ran a group of rats that were not lesioned until *after* a number of days of receiving shocks in the foraging zone and in which shock was continued after lesioning their amygdalae. By the time of lesion, these postshock lesion animals had experienced considerable shock in the foraging zone; and their avoidance behaviour had become almost asymptotic (one-way repeated measures ANOVA 4th–7th day: $F_{3,21} = 2.35$, $p = 0.10$), and their lever pressing strongly depressed (% meal frequency last day of phase II as a % of baseline period less than 100%, $t_7 = 6.88$, $p < 0.001$) (figure 2). If the amygdala's contribution to such depression were only a consequence of reduced consolidation of fear that was itself of extra-amygdala origin, then this already established depression would be expected to continue after amygdala removal. However, if the fear that was driving foraging zone avoidance and/or diminished lever pressing

Table 2. Time spent in foraging zone and pellets earned in experiment 2. (Values are mean (±s.e.m.) per cent decrease scores for the last 5 days of the shock phase relative to the mean baseline scores for each animal.)

	time in foraging zone		feeding		foraging time—feeding
	% differences	t(d.f.), p	% differences	t(d.f.), p	t(d.f.), p
intact	66.7 ± 3.8	17.8(8) < 0.001	21.7 ± 6.8	3.2(8) = 0.01	
pre	51.4 ± 4.9	10.5(8) < 0.001	5.9 ± 1.3	0.2(8) < 0.82	(49.9)
$\frac{\text{pre}}{\text{intact}} \times 100$	77.1		27.2		5.8(16) < 0.001

stemmed in part from the amygdala itself, these effects should have been reduced when the amygdala was removed. As seen in figure 2, loss of the amygdala in these animals that had considerable experience with foraging region shock before the lesion was made caused a minimal (and non-significant) increase in time spent in the foraging zone (normalized % time in foraging zone last day of phase II versus first day of phase III: $t_{13} = 2.06$, $p = 0.06$; note that shock continued in these animals, so extinction would not have been expected during phase III in these animals). However, *lever pressing* suddenly returned when the amygdala was lesioned (% meals earned last day of phase II versus first day of phase III: $t_{13} = 4.27$, $p = 0.001$). This suggests that non-amygdalar fear is sufficient to mediate avoidance of the shocked region, but amygdalar fear is needed to keep the animals from lever pressing for food.

(iv) Water-tube licking

The same pattern of behaviour seen in lever pressing for food was seen in water-tube licking. Licks were substantially depressed by shock in intact animals but very little in lesioned ones (electronic supplementary material, figure S1).

(v) Time budgets

During phase I of the experiment, average time in the foraging zone, across all animals, was 7.2 h (s.d. = 2.72) per day; the mean for the unlesioned animals of the intact and postshock lesion groups (the latter not yet lesioned) was higher than that for the lesioned preshock lesion animals, but this difference was not statistically significant (see the electronic supplementary material, figures S2–S3, for details of time budgets). When shock was introduced, time in the foraging zone decreased in all groups. In the intact group, mean time in the foraging zone fell to 2.0 h (s.d. = 0.86), and in the postshock lesion animals, measured at the start of phase III just after they had been lesioned, it fell to 2.67 h (s.d. = 1.11). In the preshock lesion animals, measured at the end of phase II, it fell to only 3.4 h (s.d. = 1.01) (this difference from the other two groups significant at $p < 0.001$, as is consistent with avoidance learning being dependent on the amygdala). Time spent lever pressing, fell with a pattern similar that of overall time in the foraging zone: in the intact group, it fell to 0.84 h (a 34.4% decrease), in the postshock lesion group (measured at the start of phase III) to 0.87 h (a 33.1% decrease) and in the preshock lesion group to 0.95 h (a 11% decrease). Whereas time lever pressing fell in all groups, rate of lever pressing fell in the intact animals relative to baseline ($t_{16} = 3.95$, $p < 0.001$; this was a 65% decrease compared with a 34% decrease in time pressing) but *rose* relative to baseline (phase I) levels in the lesioned animals ($t_{16} = 2.90$, $p < 0.01$). The net result of these changes, as seen in figure 2 was that less food than normal was earned by the shocked intact group animals but approximately a normal amount was earned by the shocked lesioned animals, because they compensated for their reduced time pressing by pressing more efficiently. We note that the behaviour of the lesioned animals seems adaptive in that it minimizes time at the potentially dangerous location of the lever. However, it seems less than optimal, because the lesioned animals are spending considerably more time in the dangerous foraging zone than is needed for acquiring food and water.

(b) Experiment 2. Amygdaloid fear biases near lever pressing over distant lever pressing as well as suppressing lever pressing when the foraging region becomes dangerous

A second experiment was run that was very similar to that just described except for a change in the schedule of food reinforcement, the availability of two rather than one lever (see Material and methods), and the lack of a postshock lesion group. The day by day behaviour of these animals (not shown) was similar to that of the intact and preshock lesion groups of figure 2. Values at the end of phase II relative to average baseline values from phase I and statistical test values are shown in table 2. As in experiment 1, introduction of shock during phase II depressed both time in the foraging region and lever pressing for food in intact animals, whereas in animals lesioned prior to the start of testing, shock depressed time in the foraging zone but had little effect on lever pressing for food. The lever-choice behaviours of intact versus preshock lesioned groups in this experiment were very different; this will be described below.

(i) Comparison of avoidance in intact versus amygdala-lesioned animals

A selective role for the amygdala in shock-induced suppression of lever pressing for food versus suppression of foraging zone occupancy is made particularly clear if we look at the effect of amygdala lesions on both time in the foraging zone and food earned specifically at those times when fear is likely to be maximal. This is done for both experiments 1 and 2 in figure 3, with measurements normalized so that intact animal values are 100%. This figure shows clearly that amygdala removal causes avoidance of lever pressing to be greatly reduced, whereas time in the foraging zone is reduced relatively little regardless of different schedules of food reinforcement

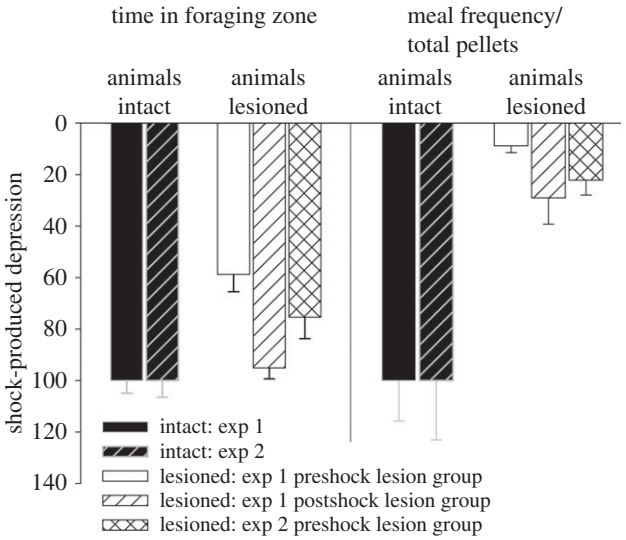

Figure 3. Amygdala lesion effects on shock-produced depression of time spent in the foraging zone and feeding in experiments 1 and 2. Per cent time in foraging zone and meal frequency (exp. 1, figure 2) or total pellets (exp. 2, table 2) scores were normalized so that the intact condition value would be 100%. For experiment 1, intact scores are last phase II day scores for both intact and postshock lesion groups (which had not yet been lesioned), and lesion scores are last phase II day scores for the preshock lesion group and first phase III day scores for the postshock lesion group (first lesioned day). For experiment 2, both intact scores and lesioned scores are mean values over the last 8 days of phase II. Statistics were carried out on each group separately (see text) from those of the preshock lesion group. It should be noted that amygdalectomy did not reduce foraging zone avoidance at all in postshock lesion animals, in which avoidance was already well learned by the time they were lesioned.

employed in experiments 1 (FR50-CRF) and 2 (CRF) (tests of normalized % shock-induced depression of time in foraging zone versus depression of meal frequency: exp. 1: preshock lesion group, $t_{16} = 4.6$, $p < 0.001$; postshock lesion group, $t_{14} = 5.98$, $p < 0.001$; exp. 2: $t_{16} = 5.23$, $p < 0.001$). In fact, consistent with previous reports [13,15,24], the postshock lesion animals, in which amygdala lesions were made *after* avoidance of the foraging region had been well learned, avoided the foraging region just as much as did intact animals.

(ii) Role of amygdala in altering choice behaviour

The threat of shocks in the foraging region not only suppresses lever pressing by intact animals, it also affects which lever they choose in the two lever situation of experiment 2. One lever was closer to the boundary between the foraging region and the always safe nesting region, while the other was more distal (see Material and methods). Prior to the onset of shocks all animals, both intact and lesioned, had initial strong, though not absolute preferences for one of the two levers, and about equal numbers of animals preferred each lever (figure 4). However, after random shocks began in the foraging region, intact animals, switched their initial preferences ($\chi^2_1 = 14.4$, $p < 0.001$).

The choice behaviour of lesioned animals was entirely different (figure 4). They invariably continued to greatly prefer the same lever that they had preferred prior to the introduction of shock. To compare the degree to which animals originally preferring the far lever switched to the near one in intact versus lesioned groups, we calculated the increase in percentage of near-lever presses for the five animals of each group that initially preferred the far lever. The mean increase for the intact animals was 77.6% (s.d.: 25.8), whereas for the lesioned animals it was 8.3% (s.d.: 10.8) (intacts: near versus far lever, $t_8 = 3.39$, $p = 0.01$; lesions: near versus far lever, $t_8 = 0.25$, $p = 0.81$, intact versus lesions initially preferring far lever, $t_8 = 5.49$, $p = 0.001$). This shows that intact animals developed a preference for the lever nearer the safe region, whereas lesioned animals continued to choose the lever they had initially preferred.

(iii) Relationship between avoidance of foraging region and avoidance of lever pressing

It was noted in discussing experiment 1 that working for meals seemed to be suppressed only when fear of the foraging zone, as indexed by time spent there, was fairly extreme. Figure 5 shows the relationship between these two variables for all the data of both experiments 1 and 2. The percentage of time (relative to baseline values) spent in the foraging chamber is plotted on the *x*-axis. Variation in this variable, for both intact and lesioned animals, was the result of acquisition and extinction of foraging zone avoidance, as shock was introduced or removed. Values at the far right come from the baseline period before shock was introduced as well as from the end of the extinction period. Values at the far left come from the end of the acquisition period in intact animals. Intermediate values come from animals midway in the acquisition or extinction process or from the end of acquisition in preshock lesion animals, who acquire avoidance responses slowly. The *y*-axis is a measure of amount of lever pressing by these hungry animals, again as a percentage of its baseline value. Consider first the lesioned condition, in which amygdala-coded fear is minimal or absent. These animals tend to press the lever almost the same amount no matter how afraid of the foraging region they appear to be. There is only a small, but significant, decrease in lever pressing as the animals reduce their time in the foraging zone because of the shocks they are receiving there (regression coefficient of lesioned animals, $\beta_{\text{lesions}} = 0.503$, $p < 0.01$). The situation for the animals with intact amygdalae, who presumably can express emotional fear, is different. When foraging zone avoidance ranges from low to anything short of fairly strong, lever pressing is only slightly depressed and is almost identical in amount to that shown by the animals with lesioned amygdalae. However, when foraging zone avoidance is in the top third of its range, lever pressing in the intact animals becomes substantially depressed, but this only happens if the amygdala is intact (regression coefficients for intacts versus lesions; $\beta_{\text{lesions}}/\beta_{\text{intacts}} = 2.16$; intacts versus lesioned last two bins food depression, $t_{123} = 5.32$, $p < 0.001$). Thus, modest levels of fear suppressed lever pressing for food only slightly and did so similarly in intact and amygdala-lesioned animals. It was only the highest levels of fear that suppressed lever pressing substantially, and this happened only in animals with intact amygdalae.

4. Discussion

The animals in the closed economy of this experiment are living in a situation where they have available a safe nesting region and an adjacent foraging region in the interior of which are levers that must be pressed in order to earn the only food they get. During the acquisition phases of our

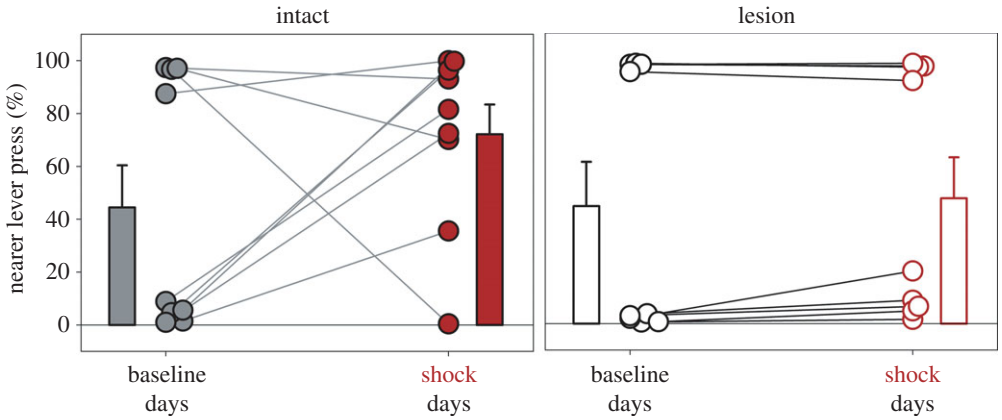

Figure 4. Amygdala lesion effects on near versus far lever preference in experiment 2. Initially, animals had strong lever preferences that were approximately random. On baseline days, animals almost always pressed their preferred lever. When random shock was introduced (red markers), intact animals modified their behaviour in the direction of pressing the lever nearer the safe region. However, lever choice was unaffected by shock in the amygdala-lesioned animals (open markers).

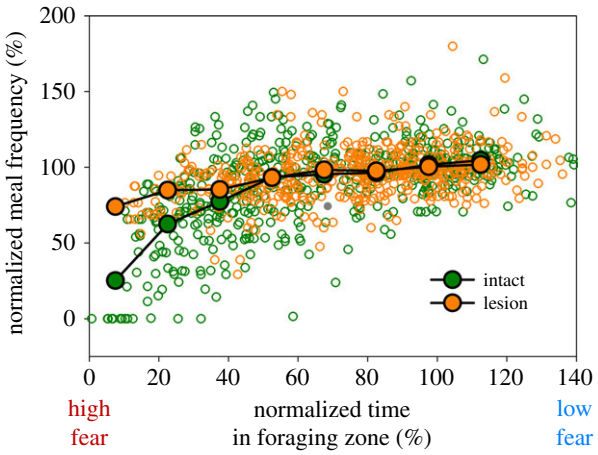

Figure 5. Relationship between per cent time in foraging zone and meals/pellets earned. Daily scores for each animal in experiments 1 and 2 are represented in a scatter plot. Intact and lesioned animals are represented in green and orange, respectively. Large markers show averages for 15% wide bins on the *x*-axis.

experiments, the grid floor of the foraging region occasionally becomes electrified. If an animal is there, it gets shocked until it can make its way back to the nesting region. Thus, the animals need to enter the foraging region and go far enough into it to press the lever in order to get food, but the farther in they go, the longer it will take to escape the shock should it turn on.

When, after a period of baseline testing (phase I), random grid electrification is introduced, animals with intact amygdalae substantially diminish the amount of time they spend in the foraging region and diminish their lever pressing, though on average they continue to spend enough time in the foraging region to earn their normal amounts of food if they were to lever-press efficiently while there. We think of these animals, as being in an approach-avoidance conflict situation during the shock phase of the experiment. They are drawn to the foraging region and its levers by a need for food, but they presumably fear the region, and this fear presumably becomes greater, the farther into the region they go. Therefore, at each moment they must weigh positive and negative factors and decide to approach or retreat from the region of the lever. The central issue of this report is the question of whether fear coded in the amygdala influences the outcome of these decisions.

Based on both the animal and human literature, it is widely believed that fear, as well as probably both negative and positive valence are coded within the amygdala [26–29]. There is good evidence from the animal literature that the amygdala is a major origin of the emotional expressions of fear [4,30]. It would therefore be natural to suppose that when some possible choices or activities are feared, this amygdala-coded fear should enter into decisions about what instrumental (voluntary) actions should be made. However, available animal literature puts this supposition into considerable doubt. It is generally agreed that the amygdala plays important roles in the learning and consolidation of both active and passive instrumental/voluntary avoidance responses [14,16,31], but there are many experiments which seem to find that once instrumental avoidance responses are well learned, inactivation of the amygdala does not affect their performance ([12,13,32], but see [14]) and thus would probably not be expected to affect decisions involving feared alternatives.

It has been reported that the amygdala contributes importantly to avoidance of food approach when a predator-like robot is located near food [21]. While this does suggest that amygdala-coded fear contributes to decisions about voluntary behaviour, it would be interesting to know whether a similar result holds for cases where the fear is learned (using footshock pain) rather than innate (without pain). One also wonders whether fear of the artificial predator actually entered into a decision to not approach or simply interfered with approach owing to some innate reaction to the feared object, and a similar concern applies to some of the experiments reported here.

In so far as the amygdala is not involved in the performance of avoidance responses, it is presumed that fearful memories are coded elsewhere, presumably somewhere in the cortico-striate circuitry that participates in voluntary choice behaviour [23–25]. We thus distinguish between amygdala-coded 'emotional' fear, which determines reflex and emotional responses, and whatever extra-amygdala-coded factor causes avoidance of places associated with danger, which we will refer to as 'extra-amygdaloid fear'.

In the human literature, it seems often to be supposed that amygdala-based fear affects voluntary choice behaviour [33,34], but this is difficult to establish without the possibility of experimental inactivation of the amygdala. There are a few

'experiments of nature' that seem to provide evidence for a role of the amygdala in cases where some choices are feared [35], and behavioral tests show clear signs of decreased risk aversion in the absence of amygdala function [36]. But it is difficult to know in all these cases whether it is learning of fear or post-learning expression of fear that required the amygdala, though there is some evidence which suggests that, as in much avoidance learning in animals, it might be primarily the former [36].

The present observations based on rats living for extended periods in a semi-naturalistic setting, however, do seem to provide good evidence for a post-learning contribution of amygdala-based 'emotional fear' in decisions about voluntary actions in animals. They clearly show that suppression of instrumental lever pressing in animals shocked for being in the general region of the lever is amygdala-dependent. It is true that this could possibly be an indirect consequence of amygdala-dependent emotional reactions to the foraging region. Thus, the hunger that is driving lever pressing might be suppressed by fear, though it is difficult to see why the animals would enter the region in the first place if they were not hungry. It is also possible that the animals might become hyper-vigilant when they go far enough into the forging region to press the lever, and they then spend their time assessing threats rather than pressing the lever or they might even show some degree of freezing when they get well into the foraging zone. However, the fact that the choice of the safer of two levers in our two lever experiment depends on the amygdala seems to provide fairly compelling evidence for amygdala involvement in voluntary choice behaviour.

Our experiments were planned and have been discussed from the perspective of the well-established role of the amygdala in fear conditioning. However, it must be acknowledged that there is considerable evidence that the amygdala codes learned positive and negative values and/or affects that have become associated with neutral cues, over and beyond its perhaps special role in fear conditioning [28,29,37]. Thus, it may well be that loss of the amygdala would be expected to have direct effects on tendency to approach and press the levers in our experiments, as well as effects on tendencies to avoid the levers. Moreover, there is some reason to believe that, in addition to providing signals coding for valence, value or emotional significance for use by other brain regions where decisions are actually made, the amygdala itself may participate in the decision-making process [38]. In so far as either of the above aspects of amygdala function were affecting the outcome of our experiments, some reinterpretation of their meaning would be necessary. However, we feel that the directions such reinterpretation should take are not clear at our current state of knowledge about decision-making mechanisms and the role of the amygdala therein.

In addition to providing evidence that amygdala-based fear can in fact promote avoidance behaviour and affect voluntary choices, our observations also suggest an interesting possibility concerning the circumstances under which this does and does not happen. The avoidance of lever pressing that occurred in our intact animals was almost entirely owing to reduced lever pressing at the highest levels of fear, and there were differences in lever pressing between intact and lesioned animals *only* at these high fear levels. Our observations thus suggest that only the highest levels of fear are able to compete effectively with the sort of strong motivation to feed that was present in our closed economy animals and, moreover, that even these highest levels of fear could not effectively suppress feeding without the aid of the amygdala.

All experiments were performed in strict compliance with the University of Washington Institutional Animal Care and Use Committee guidelines.

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