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THE NON-HUMAN PRIMATE AMYGDALA IS NECESSARY FOR THE ACQUISITION BUT NOT THE RETENTION OF FEAR-POTENTIATED STARTLE

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Abstract

Background—In a previous study (1), we found that rhesus monkeys prepared with bilateral lesions of the amygdala failed to acquire fear-potentiated startle to a visual cue. However, a second group of monkeys, that received the lesion after training, successfully demonstrated fear-potentiated startle learned prior to the lesion.

Methods—In the current experiment, the eight monkeys used in the second part of the original study (1), four of whom had bilateral amygdala lesions and their four controls, were trained using an auditory cue and tested in the fear-potentiated startle paradigm. This test was performed to determine whether they could acquire fear-potentiated startle to a new cue.

Results—Monkeys with essentially complete damage to the amygdala (based on histological analysis), who had retained and expressed fear-potentiated startle to a visual cue learned before the lesion (1), failed to acquire fear-potentiated startle to an auditory cue, when training occurred after the lesion.

Conclusions—The results suggest that while the non-human primate amygdala is essential for the initial acquisition of fear conditioning, it does not appear to be necessary for the memory and expression of conditioned fear. These findings are discussed in relation to a network of connections

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between the amygdala and the orbitofrontal cortex that may subserve different component processes of fear conditioning.

Keywords

fear-conditioning; fear; amygdaloid complex; emotional learning; memory

Introduction

We previously used a non-human primate version of the fear-potentiated startle paradigm to investigate the role of the macaque monkey amygdala in the development and expression of learned fear (1). In this emotional learning task, the presentation of a fear conditioned stimulus creates a state of anticipatory fear that potentiates the acoustic startle reflex (2,3). We have found that monkeys with bilateral lesions of the amygdala were unable to acquire fear-potentiated startle (1). However, a different group of monkeys, who underwent fear conditioning prior to receiving bilateral lesions of the amygdala, were able to demonstrate fear-potentiated startle after the amygdala was lesioned. This suggests that, in contrast to rodents, in the non-human primate the neural circuits responsible for retention and the expression of fear-potentiated startle are operative in the absence of an intact amygdala.

To strengthen the case that the amygdala is essential for the acquisition of fear-potentiated startle and that the preserved expression of fear-potentiated startle was not due to incomplete lesions of the amygdala, in the current study, we trained the same lesioned animals used in experiment 2 of our previous study (1) and their controls using an auditory cue paired with an aversive airblast. If the amygdala is required for new learning, then the amygdala lesioned animals should fail to demonstrate the fear-potentiated startle.

Methods and Materials

The institutional Animal Care and Use Committee of the University of California, Davis approved the protocol for the experimental procedures used in these studies. The protocol adheres to National Institutes of Health guidelines.

Subjects and Living Arrangements

The eight adult male rhesus monkeys (*Macaca mulatta*) used in this study were born and mother-reared in outdoor half-acre enclosures at the California National Primate Research Center (CNPRC). Prior to participating in these studies, they had all been relocated from outdoor cages to indoor CNPRC housing facilities for at least 2 months and were habituated to indoor living conditions. All animals were pair-housed within two joined individual cages (28 X 22 X 46 inches). The rooms were automatically regulated on a 12-h light/dark cycle, with lights on at 6:00 A.M. and off at 6:00 P.M., and room temperature set to 75° – 85° F. Subjects' diet consisted of monkey chow (Ralston Purina, St. Louis, MO) supplemented with fruit and vegetables and *ad-libitum* water.

Surgical procedures

The surgical and imaging procedures for these animals were performed 14–45 days after acquisition of fear-potentiated startle to a visual conditioned stimulus (1) and were thoroughly presented in the previous publication; methods will only be briefly described here.

Magnetic resonance imaging

Animals were anesthetized and were then placed in a magnetic resonance imaging (MRI)-compatible stereotaxic apparatus (Crist Instruments, Hagerstown, MD). MRI scans served as brain atlases and were used to generate individualized injection coordinate matrices.

Lesion surgery: ibotenic acid injections

Anesthesia was induced and, using sterile procedures, the skull was exposed and craniotomies were made dorsal to the amygdala. The dorsoventral location of the amygdala was verified electrophysiologically and injection coordinates altered accordingly. Two identical 10 μ l Hamilton syringes were used to simultaneously infuse ibotenic acid (10 mg/ml in 0.1 M PBS; Biosearch Technologies, Novato, CA) into each amygdala. A unilateral amygdala lesion required three to four rostrocaudal injection planes, each with two to three mediolateral levels and one to three dorsoventral infusion sites. The ibotenic acid injections were followed by (a) suturing of the dura (b) filling the craniotomy with GelFoam (Amersham Biosciences, Peapack, NJ) and (c) suturing of the fascia and skin in three layers.

Apparatus

A detailed description of the apparatus is provided in Antoniadis et al (2007) (1). Briefly, a custom-built primate restraint chair that allowed the monkey to comfortably stand on a floor was enclosed within a ventilated, light and sound-attenuated chamber.

Stimuli

The startle stimulus was a 40-millisecond (ms) burst of white noise (5–20 kHz). The conditioned stimulus (CS) consisted of a 500 Hz, low intensity (40-dB) tone presented for 2.1 seconds. The aversive unconditioned stimulus (US) was a 1.2-sec, 100-pound per square inch burst of compressed air delivered through 4 nozzles located about 26 cm from the animal's face and neck (see Antoniadis et al (2007) for details).

Specific behavioral procedures

Phase 1: Baseline startle amplitude assessment—The animal was transferred to the experimental chair and placed in the test chamber. For the first 10 min there were no startle stimuli and the animal was acclimated to the environment. During the next 40-min, blocks of startle stimuli consisting of white noise bursts were presented at each of the following intensities: 80, 90, 100, 110 and 115 dB. There were four blocks of the five startle stimuli, so the animal was exposed to 20 randomly presented noise bursts at a 120-sec inter-trial interval (ITI). Animals underwent four baseline days.

Phase 2: Tone-test to measure unconditioned effects of the tone on startle amplitude—This phase was carried out to evaluate whether the tone would unconditionally enhance or inhibit startle amplitude. The animal was placed in the test chamber and acclimated for 10 min. During the next 40-min there were 20 testing trials presented at a 120-second ITI: ten 100-dB white noise bursts delivered alone (noise- alone) and intermixed with ten 100-dB noise bursts delivered 1 sec after the presentation of the 40-dB (500 Hz) tone (tone-noise). The 1-sec delay was introduced to reduce the probability that the tone would have an unconditioned facilitatory effect on startle, given that an elevation in background noise has been reported to increase acoustic startle amplitude in rodents (4), perhaps via summation of loudness effects (5). Animals were exposed to daily tone-test sessions until tone-induced startle enhancement was less than 10% on two successive days.

Phase 3: Fear-potentiated startle training and testing—The animal was placed in the test chamber and acclimated for 10 min. In the next 50-min, five training trials were randomly

intermixed with 20 testing trials and separated by a 120-sec ITI. The first trial was always a training trial. It was initiated by the 2.1-sec tone, and the air-puff was delivered at the end of the tone. Testing trials were of two types. Either a startle stimulus was presented alone or it was delivered 1 sec after the 2.1-sec tone. In this mixed design, there is no cue to signal whether the tone will be followed by a startle stimulus, to measure conditioned fear, or by an aversive air-blast, to condition fear to the tone (6). This design produces stable levels of fear-potentiated startle (6).

Statistical Analysis

Statistical analyses were performed on the raw startle scores using Analysis of variance (ANOVA) procedures and Fisher's least significant difference (LSD) post hoc comparisons. The fear-potentiated startle scores (percentage) were included for descriptive purposes. Percent fear-potentiated startle is calculated as: $[(\text{tone-noise}) - (\text{noise alone}) / \text{noise alone}] \times 100$.

Histology

Approximately 5 months after the experiment, amygdala-lesioned animals were individually immobilized with ketamine hydrochloride (8mg/kg), deeply anesthetized with Nembutal (50 – 100 mg/kg, i.v.), and prepared for intracardiac perfusion. Briefly, 1% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.2 at 4 °C) was infused at a rate of 250 ml/min for 2 min, followed by 4% paraformaldehyde infused at 250 ml/min for 10 min and at 100 ml/min for 50 min. The brain was removed from the skull, postfixed for 6 hours in 4% paraformaldehyde in 0.1 M phosphate buffer solution (PBS), cryoprotected overnight in 10% glycerol in PBS and 2% dimethylsulfoxide, and finally cryoprotected for 3 days using 20% glycerol in PBS and 2% dimethylsulfoxide. Brains were frozen using the isopentane procedure (7), and stored at –70° C. A sliding microtome was used to cut coronal brain sections that were either 30 or 60µm thick. Tissue was preserved in cryoprotectant tissue-collecting solution (30% ethylene glycol and 20% glycerin in 0.005 M sodium phosphate buffer). A series of the 30-µm-thick sections was postfixed in 10% formaldehyde solution in phosphate buffer at 4°C for two weeks, rinsed, mounted onto gelatin-coated slides, and processed for Nissl staining. Individual amygdala volumes were estimated with the Cavalieri method (8). The control brains used for comparison came from a library of unlesioned rhesus monkeys.

Results

Histological evaluation of brain lesions

Table 1 lists the volumes of the amygdala in the four experimental subjects and in six unlesioned monkeys.

Table 2 lists the percentage loss of the amygdala in the experimental monkeys relative to the mean value obtained from the unlesioned monkeys.

We include photomicrographs (Figure 1) of three representative levels through the full rostrocaudal extent of the amygdala in control case #4, the matching levels in the case with the most complete lesion to the amygdala (A-Ibo #1) and the case with the smallest lesion in the experimental group (A-Ibo #4). Figure 1 about here Higher magnification photomicrographs of sections through the amygdala at four rostrocaudal levels in A-Ibo #1 with the most complete lesion to the amygdala are shown in Figure 2. Figure 2 about here

Amygdala lesioned brains—The ibotenic acid injections produced substantial cell loss in the amygdala in each of the experimental monkeys. The neurosurgery was designed to produce discrete damage to the entire amygdala. In general, the lateral, basal, accessory basal, and central nuclei were damaged. During the planning of these surgeries, every effort was made to

include the central nucleus in the lesion. In a few cases, this resulted in some extraneous damage to the rostral hippocampus. Given that there was some variability in the amount of the amygdala damage, we include a qualitative description of the lesion in each case.

To summarize the extent of the lesion in A-Ibo cases 1, and 2, the deep nuclei (lateral, basal and accessory basal) were completely eliminated with the exception of slight sparing in A-Ibo #2 (right side). Slight sparing was found in the posterior cortical nucleus of A-Ibo #1 (right side). On both sides of each case, sparing was detected in the medial nucleus, the periamygdaloid cortex, the anterior cortical nucleus and the amygdalo-hippocampal area. Interestingly, the volume of ibotenic acid used (39.4 μ ls) in the case with the largest lesion (A-Ibo #1) was smaller than the largest volume used on an individual subject (43 μ ls). Thus, the completeness of the lesion was due to both an adequate volume of the neurotoxin and the precise placement of the injections. In A-Ibo #3, there was only sparing of the lateral nucleus, the central nucleus (left side) and of the paralaminar nucleus (right side). In A-Ibo #4 there was slight sparing of the central nucleus on the right side. The relatively smaller lesion in this animal may be accounted for, in part, by the fact that the brain in this animal is notably larger than that in the other experimental cases (Fig. 1).

Postlesion retention of fear-potentiated startle to the light—The results from the presurgical acquisition of fear-potentiated startle to the light are presented in Antoniadis et al., (1). Following a post-surgical recovery period ranging from 26 to 36 days, retention testing was initiated. These results are reviewed here in order to provide a context for results from the auditory fear conditioning.

As reported previously (1), there was no significant group effect indicating that the control and the amygdala lesion group expressed fear-potentiated startle. The light CS potentiated the startle response by 79% in the control group and by 121% in the amygdala lesion group (Figure 3). The analysis on the light-induced startle change (percentage) indicated that the light did not produce a significantly higher fear-potentiated startle effect in the amygdala lesion than in the control group ($F_{(1,6)} = 1.33, p > .05$). Thus, despite receiving a near complete bilateral lesion of the amygdala 4–5 weeks before testing, the amygdala lesioned animals were able to express fear conditioning that had been learned prior to the amygdalectomy.

Acquisition of auditory fear-potentiated startle after amygdala lesions

Phase I: Baseline startle amplitude assessment—A statistical analysis was performed to assess whether startle response amplitude to different noise intensities varied from the presurgical (Figure 4, left) to the postsurgical (Figure 4, right) periods. The pre- and post-lesion baseline startle tests occurred 21 months apart. The amygdala lesions did not alter baseline startle to different noise intensities. This conclusion is supported by a mixed factors ANOVA with period (presurgical versus postsurgical) and lesion (control versus amygdala) as between-subjects factors, startle probe intensity (80–115 dB), day (1–4) and block (1–4) as within-subjects factors. Startle amplitude increased as a function of noise intensity ($F_{(4,52)} = 24.85, p < .0001$) during both periods and in both groups but there was no significant effect of period (presurgical vs postsurgical) ($p > 0.05$) and no significant interactions. Figure 4 about here

Phase II: Response to tone—In 4 out of 8 animals, the preconditioned tone initially led to a greater than 10% increase in startle amplitude. After seven habituation sessions, seven monkeys had reached a criterion of less than 10% tone-induced startle increase on two successive days. These seven monkeys proceeded to fear-potentiated startle training. The last monkey required 2 additional sessions to reach criterion. Mean tone-induced startle change was –12 % in the control group and –9 % in the amygdala lesion group, indicating that the unconditioned tone no longer enhanced startle amplitude (Figure 5).

Phase III: Fear-potentiated startle testing—Only the control group acquired fear-potentiated startle to the new auditory cue; the amygdala lesion group failed to demonstrate fear-potentiated startle (Figure 6). This conclusion was supported by a mixed factors ANOVA with lesion condition (control versus amygdala) as a between-subjects factor, trial type (noise-alone versus tone-noise) and trial number (1–10) as the within-subjects factors. The analysis revealed a significant fear-potentiated startle effect ($F_{(1,30)} = 4.61$, $p < 0.05$) indicating that startle was enhanced when the tone was turned on. The significant fear-potentiated startle by lesion condition interaction ($F_{(1,30)} = 5.33$, $p < 0.03$) reflected the fact that the control group ($p < 0.01$) acquired fear-potentiated startle whereas the amygdala lesion group did not. The tone increased the startle response by 45% in the control group and by 2% in the amygdala lesion group (Figure 6). It is noteworthy that some animals showed some signs of agitation or restlessness during this training/test session. This may have been an indication of anxiety produced by enclosure within the testing chamber. However, this behavior was seen in animals in both groups but was not seen in all animals. Given that the control monkeys showed significantly higher startle with the tone on (tone-noise trials) than with the tone off (noise-alone trials) seems to suggest that anxiety to the chamber was not a determining factor.

Discussion

In a previous study (1) we found that rhesus monkeys prepared with bilateral lesions of the amygdala failed to acquire fear-potentiated startle to a visual cue. This finding is in line with a large body of research carried out in a variety of species (c.f. 10, 11, 12). We also found, however, that if monkeys received a bilateral amygdala lesion after they successfully acquired conditioned fear, they retained the memory of the conditioning and demonstrated levels of fear-potentiated startle similar to a control group.

The animals that constituted the cohort that received fear-potentiated startle training prior to amygdala lesion had not been subjected to histological analysis at the time of that last report. While MRI analysis documented substantial bilateral shrinkage of the amygdala, it was nonetheless possible that sufficient amygdala tissue remained intact to mediate the conditioned fear memory and fear-potentiated startle performance. This possibility has now been refuted in two ways. First, histological analysis has demonstrated that the lesions of the monkeys that demonstrated preserved startle performance removed as much as 97% of the amygdala bilaterally. In 3 of the 4 cases, the deep nuclei were entirely eliminated and in the fourth case the deep nuclei were almost entirely eliminated. Thus, the amygdala was not available to sustain the memory of the fear conditioning that is necessary for normal fear-potentiated startle behavior. Second, these same animals failed to acquire new fear conditioning to a tone while controls demonstrated robust fear conditioning.

These results, in conjunction with our earlier findings in the rhesus monkey (1), provide strong evidence for the conclusion that the amygdala is essential for the fear conditioning that is a prerequisite for normal fear-potentiated startle. However, the memory of the conditioning and the expression of anticipatory fear leading to enhanced startle in the fear-potentiated startle paradigm can be mediated by other brain regions in the nonhuman primate. Support for this view comes from functional magnetic resonance imaging (fMRI) studies in human subjects demonstrating that the human amygdala is active during the acquisition of fear conditioning but not during the retrieval and expression of the conditioned fear (13,14).

There is a long history of studies conducted in the nonhuman primate indicating that the amygdala is an important component of the circuit involved in perceiving fear stimuli and affecting a fear-related response. The classical studies of Kluver and Bucy (15) which were refined and extended by Weiskrantz (16) and more recently by Aggleton and Passingham (17), have clearly demonstrated that bilateral lesions of the amygdala dramatically decrease

the emotional response to a normally fear-eliciting stimulus. We have also shown that ibotenic acid lesions of the amygdala blunts the emotional responses to novel objects (18). A general view is that the substantial sensory input that the lateral nucleus receives from all sensory modalities (19) is used to evaluate environmental stimuli for the presence of danger (20). Neurons in the primate amygdala are certainly tuned to detect emotionally salient stimuli. For example, Gothard et al (21) demonstrated that viewing images of monkey fear expressions induces increased firing of amygdaloid neurons.

Germane to the findings of the current study, LeDoux and colleagues have demonstrated in the rat that associative learning of a classically conditioned fear response to an auditory cue is dependent on neuronal activity and plasticity in the lateral nucleus of the amygdala (22). Consistent with this, Salzman and colleagues (23,24) demonstrated that neurons in the amygdala of macaque monkeys can code the valence of initially neutral stimuli that are paired with reinforcing or aversive stimuli in a classical conditioning paradigm. Interestingly, different sets of neurons appear to code for positive and negative associations. There appears to be little doubt, therefore, that the amygdala is involved in evaluating and responding to environmental danger signals and can learn that initially neutral or ambiguous stimuli are also potential dangers through classical conditioning.

To this point, we have been discussing the response of an animal to an innate fear-provoking stimulus that the animal detects in the environment. In the fear-potentiated startle situation, a neutral stimulus signals to the animal that a noxious event will take place in the future. Presumably, this induces a state of anticipatory fear in the animal that has the consequence of facilitating the startle motor reflex which we showed can take place independently of a functional amygdala. We have consistently found that post-training lesions of the amygdala block the expression of fear-potentiated startle (c.f. 10). Although it is possible for rats to show fear conditioning with *pre-training* lesions of the basolateral complex of the amygdala (BLA) when extensive overtraining is used (25,26), *post-training* lesions of the BLA still block the expression of conditioned fear in over-trained (27) or extensively over-trained (26,28) rats, with no evidence of savings (29). This was seen with several training-to-lesion intervals, with no difference in the magnitude of the impairment as a function of the training-to-lesion interval (30). Blockade of fear expression occurred when lesions were made 16 months after original training, nearly the entire life of the rat (31).

On the other hand, normal fear expression in amygdalotomized monkeys appears to be in line with findings that damage to the human amygdala does not interfere with the generation of fear responses. Indeed, patients with amygdala damage (unilateral) report negative affective experiences of normal magnitude and frequency (32), demonstrate normal affective evaluation of negative emotional scenes (33) and words (34), and normally generate fearful facial expression (35).

Our results in the monkey are consistent with the view that the amygdala has a time-limited role in fear learning (36). This study reports normal avoidance of a shock-associated compartment in amygdala-lesioned rats. These results suggest that the amygdala is not necessary to retain and express the memory of fear conditioning to context. However, more relevant to fear-potentiated startle, the metric that we have used in the monkey, eleven papers from the Davis laboratory show that post-training lesions (37–43) or transient inactivation (44–47) of the amygdala totally block the expression of fear-potentiated startle in the rat. These latter findings are in direct contrast to the results of our prior work in the rhesus monkey (1).

This species difference has also been supported by the work of Kalin and colleagues (48) who have shown that monkeys with bilateral lesions of the amygdala have a generally blunted emotional response to innate elicitors of fear such as snakes. But, they report that lesions of

the amygdala do not markedly alter the fearful response of monkeys in the human intruder paradigm that is an assay of dispositional behavioral and physiological characteristics of anxious temperament. This is consistent with the report of Walker et al (49) based on work in the rat that demonstrated that the amygdala is important in mediating conditioned fear, whereas other structures such as the bed nucleus of the stria terminalis, may be more involved in mediating anxiety. Recently, studies in monkeys and in humans have implicated the orbitofrontal cortex in fear and anxiety. The orbitofrontal cortex receives multimodal sensory information about the environment, and as such is ideally positioned to process stimuli that signal oncoming danger to the animal. Lesions to the monkey orbitofrontal cortex significantly reduced freezing in the human intruder task, where the presence of a human signals potential threat. The lesions also resulted in a leftward shift in frontal brain electrical activity, an effect seen in humans in conjunction with a reduction in anxiety. However, the same lesions did not markedly alter the responses to innate fear elicitors such as snakes (50). This finding is consistent with the idea that the orbitofrontal cortex generates a state of anticipatory anxiety to cues that signal oncoming danger. And, with multiple projections to the hypothalamus and the brainstem (51), the orbitofrontal cortex has the necessary connections for the expression of anticipatory anxiety. Interestingly, in humans, neuropsychiatric disorders associated with the orbitofrontal cortex include anxiety disorders (52).

Since fear-conditioned stimuli also signal oncoming danger, they induce a similar state of anticipatory fear that is presumably generated through the same orbitofrontal cortex areas. For this to occur, the fear associations that are established in the amygdala during fear conditioning, would have to be transferred to the orbitofrontal cortex. In support of this idea, the portions of the rat medial prefrontal cortex that are innervated by the amygdala also encode and retain fear associations (53). Another potential brain area might be the bed nucleus of the stria terminalis which has been shown in an abstract to block amygdala independent fear memories (54).

To conclude, data presented in this paper are consistent with the idea that the amygdala is essential for establishing the association of a neutral stimulus with an aversive event. In the fear-potentiated startle paradigm, the neutral stimulus, once it has been paired with the aversive event, generates a state of anticipatory fear that facilitates reflexes. If the amygdala is not available, the neutral stimulus cannot be associated with the aversive event and can not induce the state of anticipatory fear that facilitates startle. However, if the amygdala is available during conditioning, the memory of the association may be distributed to one or more brain regions. Strong candidates are the orbitofrontal cortex and/or bed nucleus of the stria terminalis. Because anticipatory fear and normal fear-potentiated startle can be elicited without a functioning amygdala, we propose that the anticipatory fear is generated by one or both of these structures in the primate. If true, this predicts that damage to these areas should markedly decrease the memory of fear-potentiated startle even in the presence of a functional amygdala. We will test this hypothesis in future studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Photomicrographs of three levels (top to bottom) through the full rostrocaudal extent of the amygdala in control case #4 (middle), the matching levels in the case with the most complete and discrete lesion to the amygdala (A-Ibo #1; left) and in the case with the smallest lesion in the experimental group (A-Ibo#4; right).

Figure 2.

Higher magnification photomicrographs at four rostrocaudal levels (AD) in case A-Ibo #1 with the most complete and discrete lesion to the amygdala. Left and right hemisphere sections are displayed in a rostral (top) to caudal (bottom) direction with a 0.48 mm inter-section distance. The lesion substantially eliminated the amygdala so other neuroanatomical landmarks were used to select these sections. CL, claustrum; SI, substantia innominata; PAC, periamygdaloid cortex; EC, entorhinal cortex; V, ventricle; H, hippocampus; rs, rhinal sulcus; OC, optic chiasm. The open arrows indicate injection sites.

Figure 3.

Mean startle amplitude (in V) to 100-dB startle stimuli in the absence (noise alone) or presence (light-noise) of a 4.2 sec light during the post-lesion fear-potentiated startle testing phase. The striped and dotted bars illustrate startle response magnitude (left axis), and the gray bars represent the difference in startle amplitude (light-noise minus noise-alone). Error bars denote standard error of the mean (SEM). The black bars represent the percentage change in startle in the presence of the light (right-axis). The following symbols denote the mean startle amplitudes (V) of individual animals in each group; square: A-Ibo #1 and Control #1; triangle: A-Ibo #2 and Control #2; circle: A-Ibo #3 and Control #3; diamond: A-Ibo #4 and Control #4. Asterisks

(*) (n=2) denote a significant fear-potentiated startle effect in both groups indicating that startle was enhanced when the lights were turned on.

Figure 4.

Mean startle amplitude (in V) as a function of stimulus intensity during the presurgery (left column) and the postsurgery (right column) baseline startle amplitude assessment. Acoustic stimuli vary from 80 dB – 115 dB. Data are averaged across four daily sessions in both graphs. Error bars at each data point denote SEM.

Figure 5.

Mean startle amplitude (in V) to 100-dB startle stimuli presented alone (noise alone) or 1 sec after the presentation of the 2.1-sec 40-dB/500-Hz tone (tone-noise) prior to conditioning. The striped and dotted bars illustrate startle response magnitude (left axis), and the gray bars represent the difference in startle amplitude (tone-noise minus noise-alone). Error bars denote SEM. The black bars represent the percentage change in startle in the presence of the tone (right axis).

Figure 6.

Mean startle amplitude (in V) to 100 dB startle stimuli presented alone (noise alone) or 1 sec after the presentation of the 2.1 - sec 40 dB/500 Hz tone (tone-noise) during the fear-potentiated startle testing phase. The striped and dotted bars illustrate startle response magnitude (left axis), and the gray bars represent the difference in startle amplitude (tone-noise minus noise-alone). Error bars denote SEM. The black bars represent the percentage change in startle produced by tone presentation (right axis). The following symbols denote the mean startle amplitudes (V) of individual animals in each group; square: A-Ibo #1 and Control #1; triangle: A-Ibo #2 and Control #2; circle: A-Ibo #3 and Control #3; diamond: A-Ibo #4 and Control #4. Asterisks (*) (n=2) denote a significant fear-potentiated startle effect indicating that startle was enhanced when the tone was turned on and a significant group effect showing that only the control group acquired fear-potentiated startle.

Table 1

Volume (in mm³) of left and right amygdala in unlesioned monkeys and in the experimental subjects with bilateral ibotenic acid injections of the amygdala

Group	Subject Identification	Amygdala	
		Left	Right
Unlesioned monkeys	Control case #1	169.44	171.60
	Control case #2	161.76	159.36
	Control case #3	167.28	166.08
	Control case #4	178.56	174.96
	Control case #5	170.88	168.48
	Control case #6	215.76	218.16
	Mean +/- standard error	177.28 +/- 8.02	176.44 +/- 8.62
Amygdala lesion	A-Ibo #1	5.18	3.59
	A-Ibo #2	6.07	39.59
	A-Ibo #3	34.26	19.63
	A-Ibo #4	73.85	65.68
	Mean +/- standard error	29.84 +/- 16.15	32.12 +/- 13.40

Table 2

Percentage loss of the amygdala in the experimental monkeys relative to the mean value obtained from the unlesioned monkeys

Subject Identification	Amygdala	
	Left	Right
A-Ibo #1	97%	98%
A-Ibo #2	97%	78%
A-Ibo #3	81%	89%
A-Ibo #4	58%	63%
Mean	83%	82%