Amygdala and Periaqueductal Gray Lesions Only Partially Attenuate Unconditional Defensive Responses in Rats Exposed to a Cat

BEATRICE M. DE OCA AND MICHAEL S. FANSELOW

University of California, Los Angeles

Defensive responses to a cat were observed in rats given excitotoxic lesions of the central nucleus of the amygdala (ACe), dorsolateral periaqueductal gray (dlPAG), ventral periaqueductal gray (vPAG), or sham lesions. Rats were placed adjacent to a compartment containing a cat. Sham-lesioned rats avoided the area nearest the cat and preferred the area furthest away from the cat. They also exhibited numerous defensive responses including, climbing, escape from the apparatus, and freezing. Rats with lesions of the ACe reacted like the sham lesioned rats by preferring the area of the apparatus furthest from the cat, however they climbed and escaped significantly less than sham lesioned rats. Avoidance of the area adjacent to the cat was attenuated in rats with lesions of the vPAG. Climbing along the walls of the apparatus was also attenuated in rats with lesions of the vPAG. Escapes from the apparatus were not significantly reduced by lesions of the vPAG and dlPAG. Thus, ACe lesions attenuated climbing and eliminated escapes, but did not impair locomotion of the rat away from the cat.

WHEN THREATENED BY A PREDATOR, organisms react with defensive behaviors. The functional behavior systems approach considers these behaviors within the context of adaptive responses that increase the chances of surviving the encounter with the predator. Some defensive behaviors may be effective against various predators and circumstances. Rats and other rodents will often assume a completely immobile posture called freezing to a variety of predators. Other defensive responses may be specific to a particular circumstance. Snake mobbing by squirrels (Coss & Owings, 1978) is an example of a predator-specific defense. In either case, each species has behaviors that help it survive within its ecological niche. Bolles (1971) has described these as species-specific defensive responses (SSDRs). These responses have been observed in rats when presented with predators as well as stimuli that acquire fear-eliciting properties through Pavlovian conditioning.

One important determinant of the type of defensive response that is engaged in by rats is the spatial or temporal distance of the predator (Fanselow & Lester, 1988). The spatial and temporal distance that dictates the type of behavioral responses that rats engage in has been divided into three stages of predatory imminence.

Address for Correspondence: Beatrice M. de Oca, One University Drive, Camarillo, CA 93012. E-mail: beatrice.deoca@csuci.edu

Integrative Physiological & Behavioral Science, October–December 2004, Vol. 39, No. 4, 318–333.

When a rat enters a potentially dangerous area without an immediate predator, it enters the pre-encounter stage of defense. In this stage of defense, the spatial and temporal predator distance is greatest. Laboratory simulation of this stage of predatory imminence has produced changes in foraging patterns (Fanselow, Lester, & Helmstetter, 1988; Helmstetter & Fanselow, 1993). Rats will take fewer meals when travels away from their nesting area expose them to the risk of an occasional electric shock, but they increase their meal size. With this modification in their foraging pattern, they reduce their exposure to potential threat while preserving their caloric intake.

When threat is perceived to be either spatially or temporally immediate, the rat is in the post-encounter stage of defense. Freezing has repeatedly been observed as the dominant response in this stage of defense in studies using fear conditioned danger signals associated with electric shock to elicit post-encounter defense (Fanselow, Sigmundi, & Williams, 1987; Fanselow & Lester, 1988). Rodents show the same freezing response to a variety of natural predators (Griffith, 1920; Blanchard & Blanchard, 1971; Satinder, 1976).

If post-encounter defenses are not successful in avoiding detection or attack, then circa-strike defensive behavior is observed in response to contact or near contact with the predator. This stage of defense includes rapid jumping, biting, and audible vocalizations. In the laboratory, these same behaviors are seen in response to the delivery of shock on very dense schedules (Ulrich & Azrin, 1961).

During the post-encounter stage elicited by the presence of a cat, freezing is the most commonly observed behavior in rats, especially in the laboratory. Some other responses that have been observed include analgesia (Lester & Fanselow, 1985), flight (Blanchard, Flannelly, & Blanchard, 1986), movement to an enclosure (McGregor & Dielenberg, 1999), and movement to enclosed areas of a simulated burrow system (Blanchard & Blanchard, 1989).

The brain regions that mediate these defensive behaviors include the amygdala and the periaqueductal gray (PAG). The amygdala is involved in the acquisition and expression of fear-motivated behavior. The periaqueductal gray is an efferent of the amygdala's central nucleus (ACe) and mediates specific defensive responses (Fanselow, 1994).

Within the amygdala, different aspects of defensive behaviors are mediated by different regions. The basolateral complex, including the lateral nucleus, the basolateral nucleus and the basomedial nucleus of the amygdala, is necessary for the acquisition of a variety of defensive responses, including fear-potentiated startle (e.g., Campeau and Davis, 1995), a step down inhibitory avoidance task (Roesler et al., 2000), conditional freezing and an escape from fear task using the same fear-conditioned CS (Amorapanth, LeDoux, & Nader, 2000). Indeed, virtually every Pavlovian conditional fear response, when evaluated against appropriate control conditions, is virtually eliminated by basolateral amygdala lesions.

The central nucleus of the amygdala (ACe) is thought to play a critical role in the expression of both conditional and unconditional fear. The ACe is the major response output area of the amygdala, directing autonomic changes through the hypothalamus and behavioral changes through the periaqueductal gray (LeDoux, 1993). Lesions of the ACe block conditional freezing to a context (Blanchard & Blanchard, 1972), but not unconditional freezing nor ultrasonic vocalization in male rats after ejaculation (Choi & Brown, 2003). They also abolish fear-potentiated startle (Campeau & Davis, 1995).

Cats can unconditionally elicit defense in rats (Blanchard & Blanchard, 1989; Blanchard & Blanchard, 1971; Fanselow & Lester, 1985; Zangrossi & File, 1992), as can the odor of fox feces, trimethylthiazoline (Wallace & Rosen, 2000). Freezing to a cat can be attenuated by lesions of the amygdala (Blanchard & Blanchard, 1972) and the ventral PAG (de Oca et al., 1998).

Lesions of the ACe also attenuate unconditional hypoalgesia produced by cats or noise and conditional hypoalgesia produced by placement in a context previously paired with an aversive stimulus (Helmstetter, 1992; Bellgowan & Helmstetter, 1996; Fox & Sorensen, 1994).

The amygdala's involvement in emotional behaviors also links it to a variety of ailments produced by stress. Stress can lead to gastric erosion and other physical symptoms (Selye, 1976), and it can impair cognitive functioning and the brain mechanisms that mediate it, including hippocampal long-term potentiation (LTP; e.g., Diamond & Rose, 1994). Lesions that damage the ACe prevent these effects; they preserve hippocampal LTP in stressed rats and block the stress-induced memory impairments produced by one hour of stress prior to a water maze learning task (Kim et al., 2001). Thus, the amygdala mediates multiple consequences that arise from aversive situations and may be necessary for the expression of many fear-motivated behaviors.

However, there are some aversive situations that do not appear to require involvement of the amygdala. In one study, rats explored a novel alley containing pins designed to model prickly spines on plants. Rats avoided crossing the area where the pins were and spent little time on the pins; amygdala lesions attenuated avoidance of the pins (Hebert et al., 1999). However, amygdala lesions did not affect the rats' normal risk assessment measures, stretch attend and stretch approach (Hebert et al., 1999). Thus, some aspects of defense belonging to the pre-encounter stage of predatory imminence like the stretch attend posture rats make when entering an area of possible danger (Blanchard, Flannelly, & Blanchard, 1986) do not appear to require the amygdala. The ACe also seems to leave offensive reactions to a conspecific male rat intact while dramatically decreasing defensive freezing to a cat (Blanchard & Takahashi, 1988).

As an efferent of the amygdala, the periaqueductal gray coordinates a host of defenses. Different responses appear to be differentially mediated by sites varying along the dorsal-ventral axis. In rats with lesions of the ventral periaqueductal gray (vPAG), freezing to cues paired with shock is greatly attenuated, but the shock-elicited circa-strike behavior is normal (Fanselow, 1991). The vPAG also mediates freezing elicited by unconditioned danger stimuli, including cats (de Oca et al., 1998). This role of the vPAG appears to be functional early on in development. Lesions of the vPAG decreased freezing to an adult male rat in 14-day-old rat pups and decreased ultrasonic vocalizations induced by isolation in ten-day-old pups (Wiedenmayer, Goodwin, & Barr, 2000). In contrast, rats with lesions of the dorsolateral periaqueductal gray (dlPAG) do not show normal levels of shock-elicited circa-strike behavior (Fanselow, 1991). Damage to the dlPAG produced by bilateral electrode tracts attenuated active defense, including an offensive posture, biting, freezing, and escape elicited by the presence of a conspecific (Adamec, 2001). Thus, the dorsolateral and ventral regions of the PAG appear to mediate two mutually-exclusive defensive responses; circa-strike responding by the dlPAG and freezing by the vPAG.

One difference between many laboratory studies of defense and defense in the natural environment is the laboratory's extensive use of shock. Shock permits the study of aversively-motivated behavior, but it uses pain to simulate post-encounter defense. Pain is unlikely to occur prior to circa-strike defense involving physical contact between predator and prey (Blanchard, 1997). Therefore a cat was used as a danger stimulus in this study. To determine if the midbrain PAG is necessary for defensive flight and avoidance, rats with lesions of the dorsal and ventral PAG were placed adjacent to a cat. Furthermore, rats with lesions of the ACe were tested in order to determine if these behaviors require involvement of the ACe, much like other fear-motivated defensive responses. We expected that lesions of the ACe would attenuate avoidance of the cat.

Method

Subjects

Forty female Long Evans-derived rats born and maintained at the University of California, Los Angeles Psychology Department vivarium served as subjects. The rats were approximately 120 days

old at the start of the experiment. Rats were individually housed in hanging standard stainless steel cages with *ad lib* access to food and water and maintained on a 14:10 hr light/dark cycle (lights on at 07:00, lights off at 21:00). All procedures were conducted during the light portion of the cycle. These rats previously served in a different experiment involving fear conditioning and food deprivation. Rats were returned to *ad-lib* food for three days prior to the start of this experiment. Rats were handled daily for five days prior to the start of the first experiment.

Surgery and Histology

Rats were anesthetized with sodium pentobarbital (55 mg/kg, i.p.), treated with atropine sulfate (0.12 mg/kg), and placed in a stereotaxic device with the head in a level position. A single incision was made on the scalp, the skull was exposed, and a small hole was made in the skull with a dental drill. Bilateral electrolytic lesions were made in the dlPAG (stereotaxic coordinates: A-P −7.3, −7.8; Lat ± 0.7; D-V −5.6 mm from bregma), the vPAG (stereotaxic coordinates: A-P −7.5, −7.8; Lat ± 0.7; D-V −6.0 mm from bregma), and the ACe (stereotaxic coordinates: A-P −2.3, Lat: ± 4.2 DV: −7.6). The electrode consisted of a stainless steel insect pin insulated with Epoxylite except for 250 μM at the tip. Lesions were made by passing DC current (Grass, D.C. Constant Current Lesion Maker, model D.C. LM5A) for 10 s for dlPAG and vPAG lesions (0.7 and 0.6 mA for dlPAG and vPAG, respectively). Current was passed for 15 s (1.0 mA) for ACe lesions. The electrode was not lowered for sham lesions. Half of all shams had a hole drilled in the area of the skull above the PAG and half had a hole drilled in the area of the skull over the ACe. Eleven subjects received lesions of the dlPAG or amygdala, ten subjects received lesions of the vPAG and eight subjects received sham lesions. After surgery, the rats were allowed to recover on a heating pad before being returned to their home cages. All subjects received seven days of recovery from surgery during which time they were handled daily. At the conclusion of the experiments, rats received an overdose of sodium pentobarbital and were transcardially perfused with 0.9% saline followed by 10% formalin. Brains were removed and fixed in formalin before being sectioned (50 μm coronal sections) on a cryostat. Every third section was mounted on a glass slide and subsequently stained with thionin. Lesion locations were verified using a dissecting scope.

Materials and Apparatus

The apparatus was made of two 2″ × 4″ pine boards cut to a length of 183 cm. These two pieces formed the bottom edges of the apparatus and were placed parallel to each other, spaced 30.5 cm apart on the linoleum floor of the laboratory. Hardware cloth was nailed to the boards and formed the 60 cm high sides and ends of the apparatus. The length of the apparatus was divided into six segments, each 30.5 cm. The sixth segment contained the cat or the control stimulus. Hardware cloth was used to form a barrier between the sixth segment and the rest of the apparatus. The sixth segment's top was enclosed by the hardware cloth to create an enclosure for the cat. Segments 1–5 were marked by a 2 cm wide strip of colored tape applied to the floor. See Figure 1 for a diagram. The floor was wiped with a 1% sodium hydroxide solution prior to placement of each rat.

A brown and white male calico cat served as the cat danger stimulus and a brown stuffed teddy bear approximately the same height as the cat served as the control stimulus. The image processing and analysis program, NIH Image, was used to analyze the movement of the rats in the apparatus for any differences in distance traveled, velocity, and acceleration.

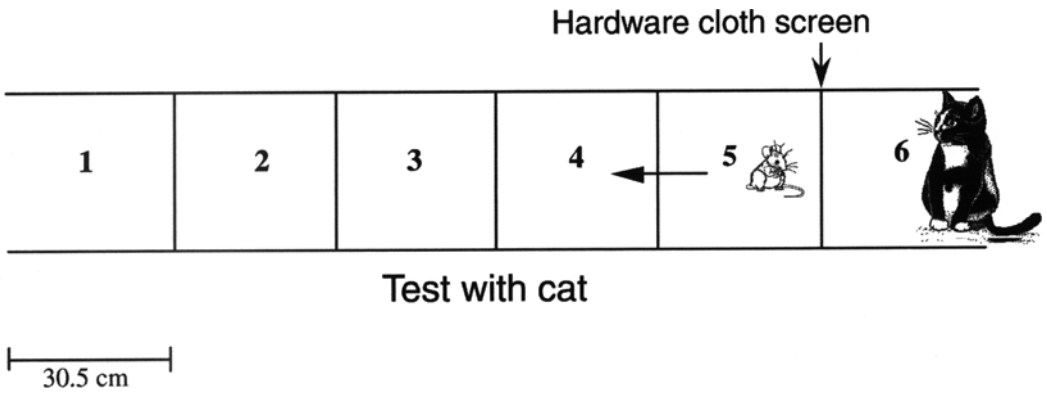

FIG. 1. A diagram of the apparatus used to compare responses of rats to a cat and a control stimulus.

Procedure

The experiment occurred over two trials, each occurring on a different day. Because of the rapid habituation rats demonstrated to cats in pilot work, the trials were limited to one minute. On the first day, the stuffed toy control stimulus was used in the sixth segment of the apparatus. Each rat was individually placed into the center of segment 5 for one minute by the experimenter. At the end of the one-minute trial, the rat was removed from the apparatus and returned to its home cage. On the next day, the control stimulus was replaced by the cat. The same procedure followed on the first day was repeated. On both days, the experimenter recorded the segment the rat was in, freezing, and whether or not the rat was climbing along the walls of the apparatus using a 2 sec time-sampling procedure. The number of escapes out of the apparatus by climbing or jumping over the walls of the apparatus was also recorded. When a rat escaped from the apparatus, the clock was stopped and the rat was replaced in the apparatus. A camcorder on a tripod also recorded the rat's behavior. The experimenter was blind to the lesion condition of the rats.

Results

Lesions

Figure 2 shows the location and extent of the bilateral lesions of the ACe. All lesions included the entire nucleus from −2.3 Bregma to beyond 3.14 Bregma. Figure 3 shows the location and extent of the PAG lesions. Lesions of the dlPAG typically extended into the deep layers of the superior colliculus and the lateral PAG. Lesions of the vPAG often included the lateral PAG as well. Lesion conditions were determined on the basis of histological analysis and resulted in five rats with amygdala lesions, five rats with vPAG lesions, eight subjects with dlPAG lesions, and eight rats with sham lesions.

Behavior

The time-sampled observations of the rat's location within the apparatus during the baseline and cat exposure days were converted to a percentage. During the control trial, all groups of rats spent

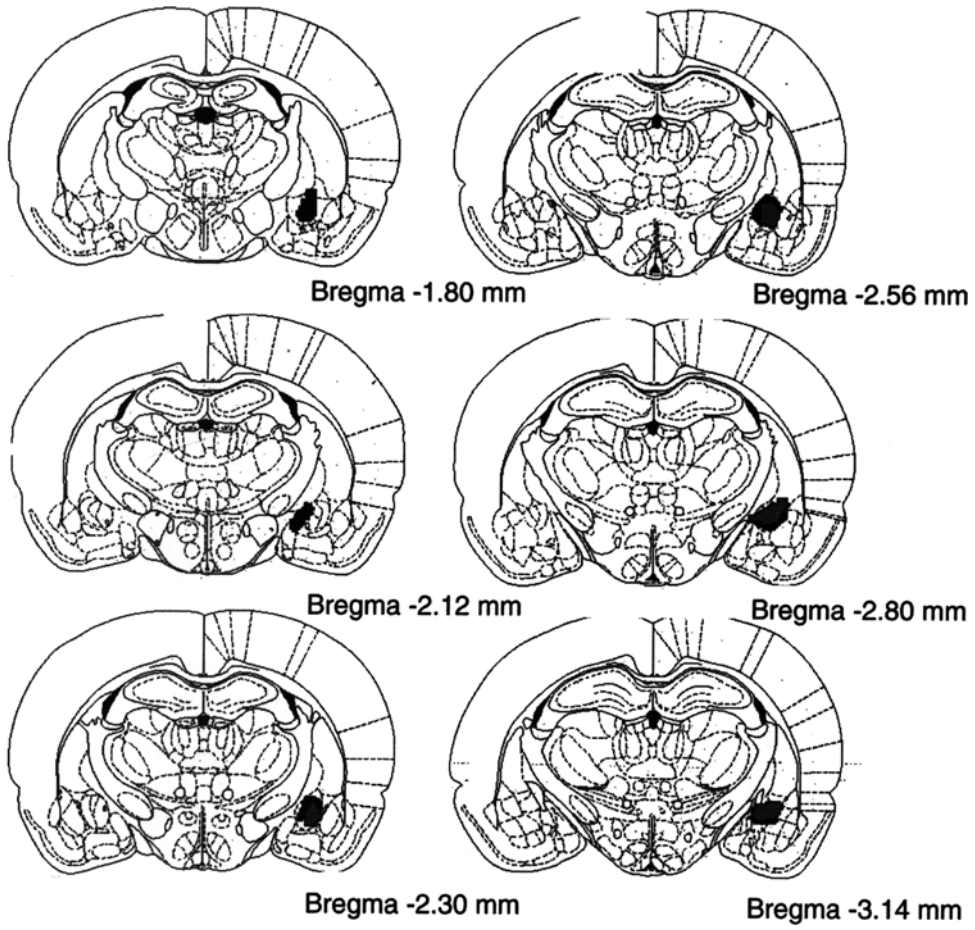

FIG. 2. Minimum (gray shading) and maximum (black shading) lesions of the ACe.

the majority of the time in segment five, nearest the stuffed toy control stimulus (see Figure 4). Figure 5 illustrates that on the test day, sham-lesioned rats spent less time in segment five near the cat and spent approximately half of the time in segment one furthest away from the cat. In order to compare the change from control to test trial, the percent time spent in each of the five segments on the control stimulus day and the cat danger stimulus day was converted to a difference score (cat-control). A one-way repeated measures Analysis of Variance (ANOVA) was conducted on these scores. As shown in Figure 6, rats spent less time in the segment of the apparatus closest to the cat on the test day. Avoidance of the cat was significantly attenuated in rats with lesions of the vPAG, but not in rats with ACe lesions or dlPAG lesions. The ANOVA conducted on the effect of lesion type on the difference scores between the percent time in each segment during the cat and control stimulus sessions revealed a main effect of lesion type [$F(3,88) = 3.133$, $p < .05$], a significant effect of repeated measures comparing the difference in time spent in each segment of the apparatus during the cat and control session [$F(4,88) = 19.708$, $p < .001$], and a marginal Lesion + Repeated Measures interaction [$F(12,88) = 1.718$, $p < .10$]. Post hoc tests (Dunnett t) indicate that rats with

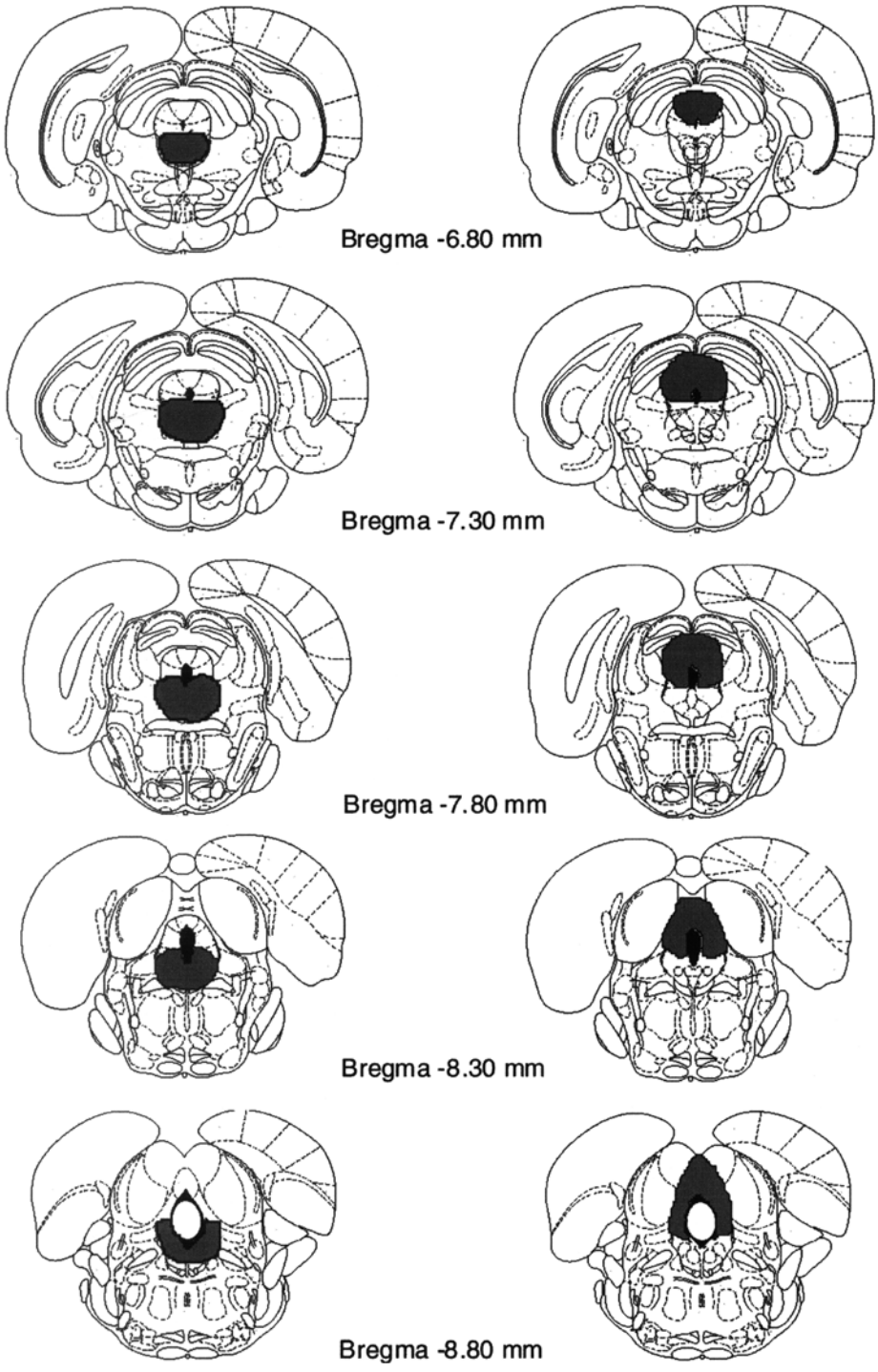

FIG. 3. Representative lesion of the vPAG (left column) and dlPAG (right column).

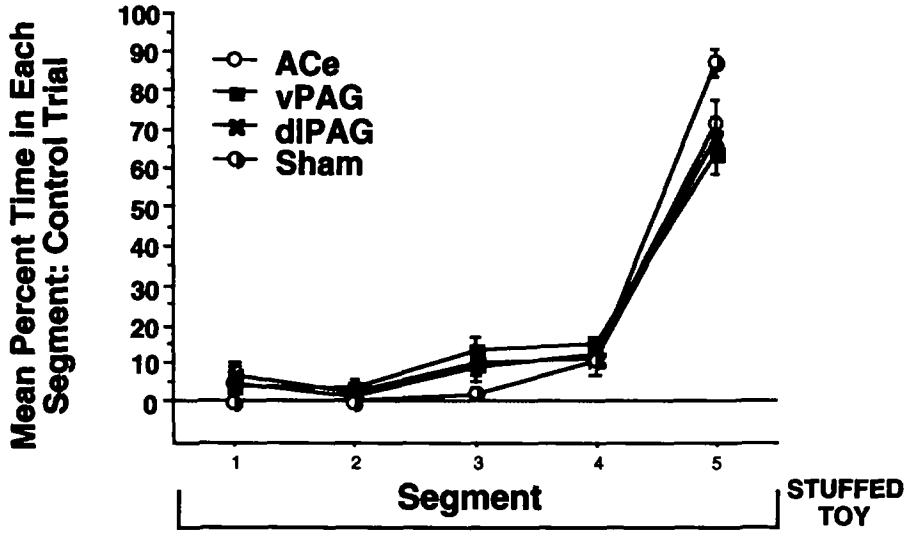

FIG. 4. Mean percent time rats spent in each segment of the apparatus when a control stimulus was placed inside.

vPAG lesions differed significantly from sham lesioned rats in the distribution of time spent in each segment of the apparatus ($p < .001$). Figure 6 shows that the Sham and Ace groups appear to be similar in the difference between the percent time spent in each segment of the apparatus during the control and cat trials. The dlPAG and vPAG groups differ from the Sham pattern and show less of a preference for the segment furthest from the cat. In order to compare the pattern of the time spent across all five segments of the apparatus, a trend analysis was done. This analysis revealed linear

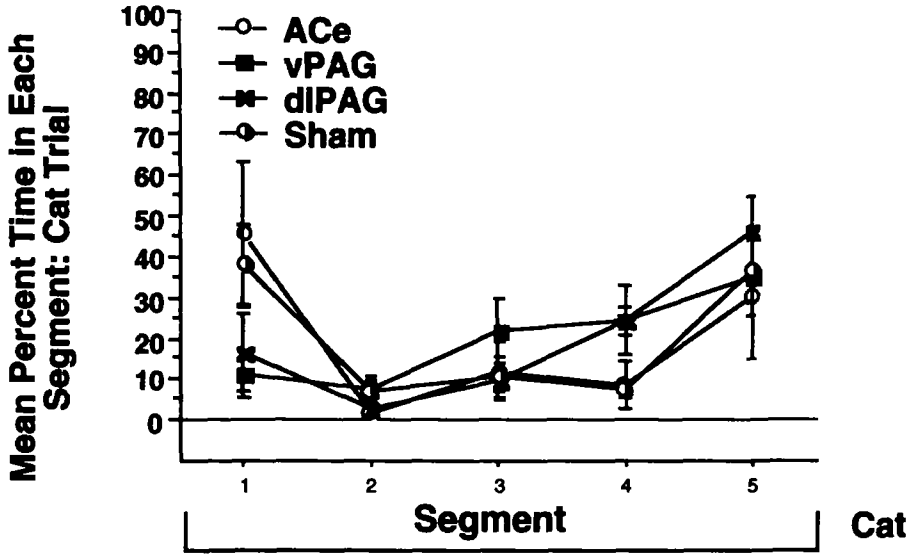

FIG. 4. Mean percent time rats spent in each segment of the apparatus when a control stimulus was placed inside.

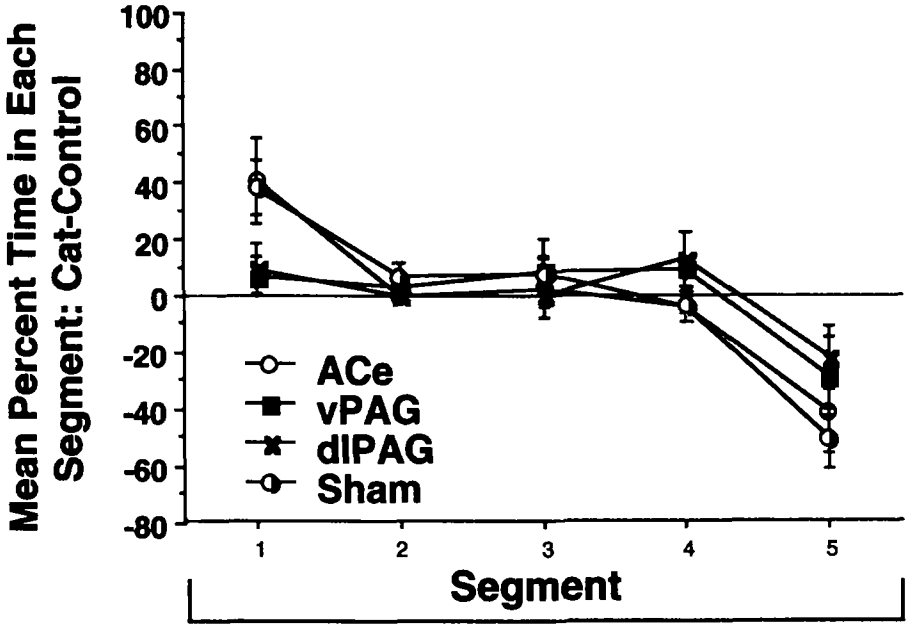

FIG. 6. Difference in the mean percent time spent in each segment of the apparatus between the cat test and the control stimulus test.

and cubic trends among both sham and ACe lesioned results, but not dlPAG and vPAG lesioned rats (see Table 1). Thus, rats with ACe lesions distributed their time in the apparatus in a way that was similar to rats with sham lesions.

Lesions of the vPAG and the ACe eliminated climbing along the walls of the apparatus when the cat was present (Figure 7). The ANOVA conducted on the effect of lesion type on the amount of time spent climbing along the walls of the apparatus during the control and cat tests revealed a significant effect of lesion [$F(3,22) = 3.721, p < .05$], a significant effect of repeated measures between the control and cat trials [$F(1,22) = 12.45, p < .01$], and a significant Lesion × Repeated Measure interaction [$F(3,22) = 6.371, p < .01$]. Post hoc tests (Dunnett t) indicate that rats with ACe lesions and vPAG lesions differed significantly from rats with sham lesions ($p < .05$).

TABLE 1

Linear trend analyses of the difference in the amount of time spent in each of the segments of the apparatus when the cat was present and when the cat was not present.

Trend	df	ACe	vPAG	dlPAG	Sham
Linear	1	10.98*	3.48	2.25	31.01*
Quadratic	1	0	2.26	1.66	1.57
Cubic	1	6.59**	5.67**	3.02	9.87*
Order 4	1	1.21	.20	2.04	1.33

*$p < .05$ **$p < .10$

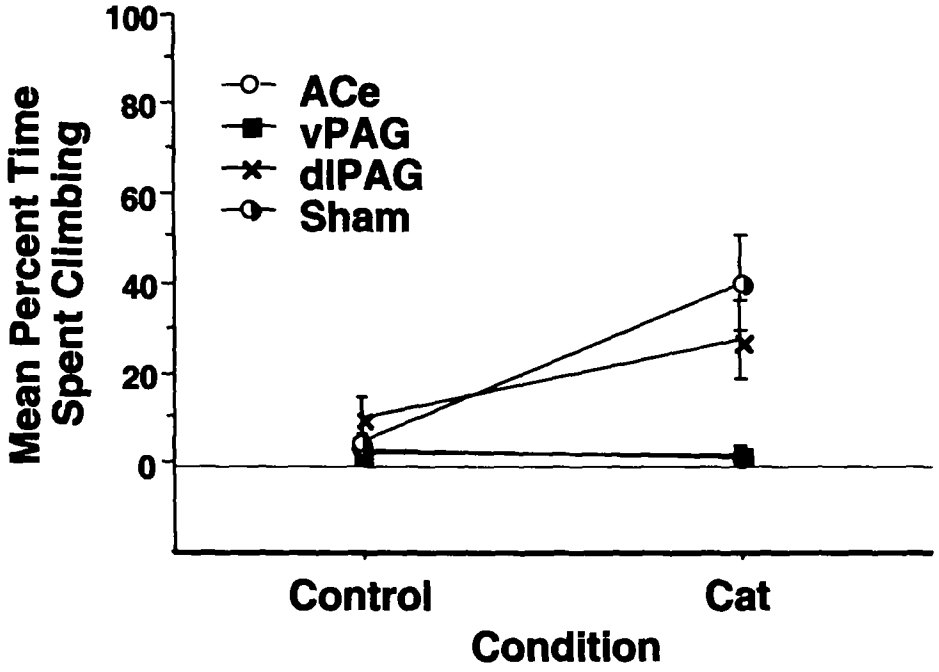

FIG. 7. Mean percent time spent climbing along the walls of the apparatus during the cat test.

Since the apparatus was not enclosed at the top, escape from the apparatus was possible. Any time a rat climbed or jumped over the 60 cm walls of the apparatus, it was counted as an escape. Although escapes appear to be attenuated in all three lesion groups compared to sham-lesioned controls when tested with the cat danger stimulus test, this effect was only significant in rats with ACe lesions (Figure 8). The ANOVA conducted on the effect of lesion type on the number of escapes out of the apparatus during the control and cat tests revealed a significant effect of lesion [$F(3,22) = 3.492, p < .05$] a significant effect of repeated measures [$F(1,22) = 9.42, p < .01$], and a significant Lesion × Repeated Measure interaction [$F(3,22) = 4.294, p < .05$]. Post hoc tests (Dunnett t) indicate that rats with ACe lesions differed significantly from rats with sham lesions ($p < .05$). No escapes were observed during the control stimulus test.

Freezing was quite sporadic on the cat danger stimulus test, and appeared only when rats reached the segment furthest away from the cat (Figure 9). The ANOVA conducted on the effect of lesion type on the percent freezing at each segment of the apparatus revealed no effect of lesion type [$F(3,22) < 1.0$], a significant effect of repeated measures [$F(4,88) = 2.524, p < .05$], and no Lesion × Repeated Measure interaction [$F(12,88) = 1.121, p > .2$]. No freezing was observed during the control stimulus test. Figure 9 seems to demonstrate a trend whereby ACe lesions enhanced freezing and vPAG lesions eliminated freezing. While no rats with vPAG lesions froze at all, only two sham, one ACe and one dlPAG rat froze at all during the test, limiting the conclusions that can be drawn regarding the effect of these lesions on the freezing in this test.

In order to determine whether any changes in behavior seen in the experiment can be attributed to motor impairments, further analysis of the movement of rats when tested with a cat was done using the video analysis program, NIH Image. Video analyses of the distance traveled [$F(3,21) <$

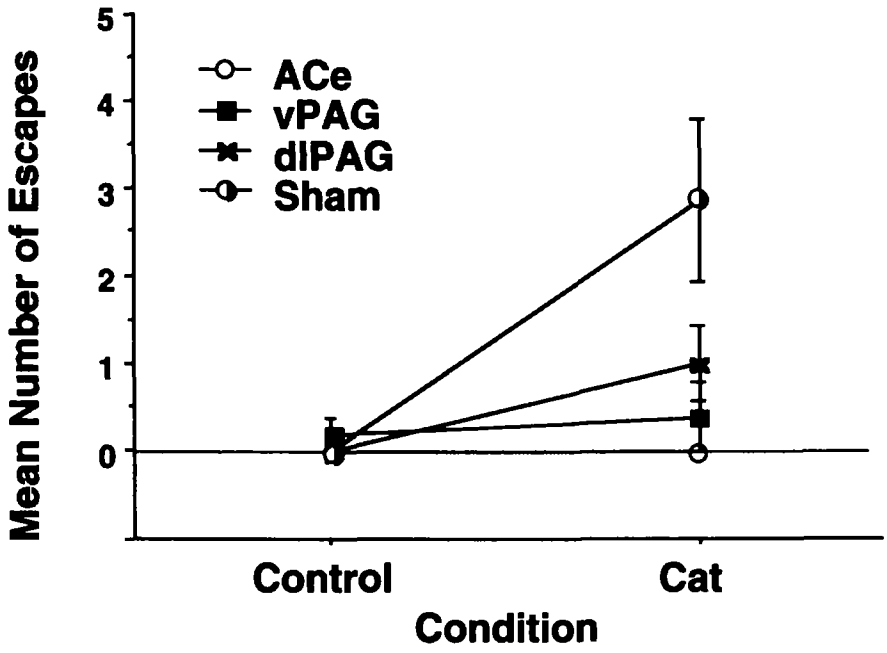

FIG. 8. Number of escapes from the apparatus made by jumping during the cat test.

1.0], velocity of locomotion [$F(3,21) < 1.0$] and acceleration of locomotion [$F(3,21) < 1.0$] during the cat trial were statistically analyzed using a MANOVA. One sham subject was excluded from the analysis due to technical failure of the recording. No differences were found between the four lesion conditions on any of these measures, suggesting that the lesions did not produce motor impairments.

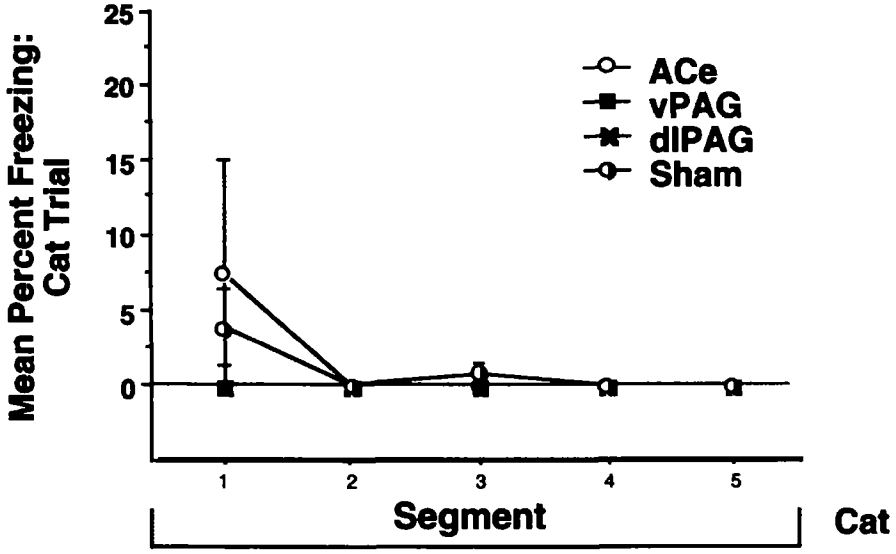

FIG. 9. Mean percent time spent freezing in each segment of the apparatus during the cat test.

Discussion

When placed in the apparatus with a cat, rats with sham lesions clearly preferred the segment furthest away from the cat, and decreased the time they spent in the area adjacent to the cat compared to the control trial. While vPAG lesions significantly reduced avoidance of the apparatus nearest the cat, ACe lesions had no such effect. ACe and Sham lesioned rats demonstrated almost identical changes in the time spent in each segment of the apparatus. Trend analyses looking at the time spent in each segment indicated that Sham and ACe lesioned rats distributed their behavior between sections similarly. Thus, ACe lesions did not affect avoidance of the cat.

However, ACe lesions did affect other reactions to the cat. Sham lesioned rats often climbed along the walls of the apparatus. Climbing along the walls of the apparatus during exposure to the cat was eliminated by lesions of the ACe and vPAG. Sham-lesioned rats also made numerous escapes from the apparatus when the cat was present. Escapes were eliminated by ACe lesions.

The ACe is generally considered to be the main output of the amygdala with efferent connections to the periaqueductal gray and the hypothalamus (Fanselow, 1994; LeDoux et al., 1988). Lesions of the amygdala, including the ACe, attenuate freezing to a cat (Blanchard & Blanchard, 1972) and a host of other defensive responses as described earlier. It is therefore initially surprising that these lesions did not block flight away from the cat.

However, other researchers that have examined the contributions made by specific amygdala nuclei have found evidence supporting unique contributions made by the different nuclei. For instance, excitotoxic lesions of the central amygdala attenuated a conditioned suppression response, which has been shown to be an indirect measure of freezing (Bouton & Bolles, 1980), but spared performance in a conditional stimulus avoidance task. In contrast, lesions of the basolateral amygdala attenuated the conditional stimulus avoidance task but spared conditioned suppression (Killcross, Robbins, & Everitt, 1997). This suggests that the ACe may not be a final common pathway for all conditional fear responses. Avoidance responses may be mediated by brain regions other than the central amygdala. However, this conclusion is complicated because the fear avoidance task may not depend on the reduction of conditioned fear to the CS during CS offset, but to feedback cues indicating the correct response has been made (Fanselow, 1997). Thus, it is possible that rats may continue responding in avoidance learning even when they have amygdala lesions because such responding may not depend on negative reinforcement from fear reduction due to CS offset, but on feedback cues acting as an informational cue that guides behavior (Fanselow, 1997).

A recent study of rhesus monkeys given selective fiber-sparing ibotenic acid lesions of the amygdala also found that freezing and hostile responses to a human intruder were preserved even in animals that had complete bilateral lesions of the ACe (Kalin et al., 2001). The authors of this study suggest that the orbitofrontal cortex may mediate anxious temperament along with other areas like the bed nucleus of the stria terminalis (BNST), allowing related behaviors to occur despite damage to the amygdala.

Walker and Davis (1997) found a double dissociation between the involvement of the BNST and ACe in potentiated startle. Potentiated startle elicited by a sudden, loud noise presented in the presence of fear-conditioned cues is blocked by inactivation of the ACe but not the BNST. In contrast, acoustic startle potentiated by exposure to a bright light instead of fear-conditioned cues is blocked by the BNST and not the ACe. Walker, Toufexis, and Davis (2003) suggest that conditioned fear reactions, especially those elicited by discrete stimuli, may require ACe involvement, but that unconditioned and long duration fear reactions may instead require involvement by the BNST. Both the BNST and ACe receive inputs from the basolateral amygdala (Alheid, Do Olmos, & Beltramino,

1995). Consistent with Walker, Toufexis, and Davis' (2003) hypothesis, lesions of the medial amygdala interfered with unconditional defensive reactions to cat odor in rats, but ACe lesions had no such effect (Li, Maglinao, & Takahashi, 2004).

If the central amygdala does not mediate all unconditional fear responses, then it may help explain why rats with ACe lesions avoided the cat as much as sham-lesioned rats in the present study. However, these same rats did not engage in climbing or escapes from the apparatus, two behaviors seen often in the sham-lesioned rats. One possibility is that there are differences in the specific behaviors these regions mediate. For instance, chemical stimulation of the ACe influences tonic immobility (Ramos, Leite-Panissi, & Menescal de Oliveira, 2002) and electrolytic lesions of the ACe blocked unconditional hypoalgesia produced by a brief, loud noise (Bellgowan & Helmstetter, 1996). One possibility is the ACe may be necessary for some reactions to unconditioned fear, while other brain regions like the BNST may be necessary for others, including potentiated startle and avoidance.

Further support for a lack of ACe involvement in unconditioned avoidance is found in studies using rats' unconditional fear of elevated areas. C-Fos labeling following exposure to an elevated plus maze found evidence of much activity in the BNST and several amygdala regions, but not the ACe (Silveira, Sandner, & Graeff, 1993). More studies of unconditioned fear with multiple behavior measures are necessary to test the hypothesis that some unconditioned fear responses are mediated by the BNST and other regions instead of the ACe.

One may attempt to explain this pattern of results by suggesting that the ACe lesions produced deficits in some aspects of movement, like the speed or pattern of flight responses, while leaving intact locomotion. Rats with amygdala central nucleus lesions neither escaped nor climbed along the walls of the apparatus during the test; two measures where sham-lesioned rats showed significantly elevated levels of responding during the test. However, these differences don't appear to be general deficits in motor behavior that can be noticed in regular handling and observation, as the lesioned animals were indistinguishable from sham-lesioned animals in these ways. Also, detailed analysis of the distance traveled, velocity and acceleration of the rats' movement did not reveal any group differences, suggesting that the lesions did not produce impairments in locomotion.

The results obtained with lesions of the PAG indicate that vPAG lesions attenuated flight to the segment furthest away from the cat. It is possible that these effects of vPAG lesions were mediated by the lateral PAG, which was damaged in both types of PAG lesions. The lateral regions of the PAG have been shown to mediate flight responses (Bandler & Shipley, 1994). Thus, while the dlPAG and vPAG appear to have mutually exclusive roles in freezing and circa-strike responding, the lateral zones may mediate flight.

In sum, the use of lesions and the task used here allowed multiple measures of unconditional defensive responses to be observed with a fairly high level of predatory imminence. This allowed for the unexpected result of normal levels of locomotive flight from the cat in rats with amygdala lesions, while other measures of flight like escape and climbing were eliminated. Along with other studies demonstrating preservation of some defensive responses after ACe lesions (Kalin et al., 2001; Hebert et al., 1999; Killcross, Robbins, & Everitt, 1997; Walker & Davis, 1997), these results suggest the ACe is not required for all types of post-encounter defense. The orbitofrontal cortex (Kalin et al., 2001) and/or the BNST (Walker & Davis, 1997; Walker, Toufexis, & Davis, 2003) may be able to mediate anxiety-related behaviors that the central nucleus is not necessary for. Since vPAG lesions eliminated or reduced the defensive responses observed here, forebrain structures seem to be able to signal midbrain structures that organize defense in a manner that can bypass the ACe.

Notes

Beatrice M. De Oca is now at California State University, Channel Islands. Research was funded by NIMH: RO1 MH 62122 awarded to MSF.

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