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Ventral and Dorsolateral Regions of the Midbrain Periaqueductal Gray (PAG) Control Different Stages of Defensive Behavior: Dorsolateral PAG Lesions Enhance the Defensive Freezing Produced by Massed and Immediate Shock

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Rats that receive nociceptive electric shock in an environment normally show the conditional fear-induced defensive response of freezing when returned to that environment. If several electric shocks are given in a massed manner they will condition less freezing than the same shocks given in a distributed manner. If a single shock is given immediately after placement in the chamber it does not support any conditioning, although the same shock given after a brief delay does. Electrolytic lesions of the dorsolateral periaqueductal gray (PAG), which damaged dorsomedial, dorsolateral, and lateral PAG, enhanced freezing under these conditions. Lesions of the ventral PAG, which caused extensive damage to the central gray below the aqueduct, reduced conditioning under the more optimal parameters (distributed or delayed shock). This was taken to indicate that both of these regions support different modes of defensive behavior and that when activated, the dorsolateral PAG inhibits conditional fear-induced defensive behavior.

Key words: periaqueductal gray, defensive behavior, freezing, immediate shock

INTRODUCTION

Many animal species have evolved elaborate means to defend themselves against environmental threats such as predation [Edmunds, 1974]. Often, such defense is accomplished behaviorally. Since the consequences of predation are particularly grave,

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there is little opportunity for the acquistion of new responses through trial and error [Bolles, 1970]. Therefore, even in animals that have the ability to learn elaborate response patterns such as the rat, defensive behavior is limited to innately preprogrammed species-specific defensive responses (SSDRs) [Bolles, 1975: pp 357–366]. Any learning in severely threatening situations appears limited to identifying signals for danger and safety as opposed to response patterns per se. Despite such constraints on defensive responding, rats have evolved a defensive behavior system that affords them some adaptive flexibility. This defensive behavior system is comprised of a number of SSDRs and an ability to match a particular SSDR to the demands of the immediate threatening stimulus configuration that confronts the organism [e.g., Bolles and Fanselow, 1980; Fanselow and Lester, 1988].

One step in analyzing the defensive behavior system of a particular species is to specify the environmental stimuli that select a particular SSDR from the animal's repertoire of defensive responses. One attractive notion has been the concept of behavioral support stimuli [Blanchard et al., 1976; Bolles and Collier, 1976; Bolles and Fanselow, 1980; Tolman, 1932]. According to this view, given a constant level of threat the neutral features of the environment will dictate the SSDR provoked. For example, the presence or absence of an exit might determine whether or not a rat engages in escape or freezing [Blanchard et al., 1976]. However, despite its intuitive appeal, the evidence taken as affirmation of the behavioral support stimulus hypothesis has been severely criticized [Fanselow, 1986; Fanselow and Lester, 1988]. Experiments that purported to find that alterations in neutral environmental features changed the type of SSDR confounded the level of threat or fear with the change in neutral features. A large series of experiments that held the level of threat constant found that manipulation of neutral environmental features such as escape exits made no difference in the type or amount of defensive behavior observed [Fanselow and Lester, 1988].

An alternative conceptualization is that the level of threat or fear determines the form of the SSDR observed in a given situation. The distance of a predator, the speed and direction of its movements, or the temporal imminence of a painful electric shock influence both the qualitative and quantitative nature of the SSDRs observed [see Fanselow, 1989; Fanselow and Lester, 1988]. A clear illustration of this pattern of findings can be obtained, in rats, by manipulating the temporal delivery patterns of a brief electric shock. During and immediately after an electric shock, rats show active defensive behaviors that resemble what would be seen at the time of the strike of a predator. Such *circa-strike defensive behaviors* consist of audible vocalizations and vigorous locomotor behavior [Fanselow, 1982]. If the shock is sufficiently intense, attack behaviors such as biting conspecifics or shock delivery grids may be seen [e.g., Fanselow et al., 1980]. These circa-strike reactions are unconditionally elicited by the shock and rapidly fade. They are replaced by *postencounter defensive* behaviors that are conditional responses to the contextual cues that were present at the time of shock. Freezing is the most obvious of these responses and its conditional control by contextual cues can be demonstrated by the reduction in freezing that ensues upon removal of the contextual cues and the reinstatment of freezing upon presentation of such cues [Blanchard and Blanchard, 1969; Fanselow, 1980]. Rats will show similar freezing responses if a predator is in its vicinity as long as the predator does not move so close as to provoke circa-strike behaviors [e.g., Blanchard et al., 1986]. If shock is delivered in a context in too infrequent a manner to support freezing, postencounter defenses are replaced by *preencounter de-*

fensive behaviors such as changes in meal patterns that reduce the time the subject is at risk while foraging in the potentially threatening environment [Fanselow et al., 1988; Helmstetter and Fanselow, 1993]. Often entrance into such mildly threatening environments is characterized by stretched approach movements [Pinel and Mana, 1989].

Elsewhere, we have argued that the midbrain periaqueductal gray (PAG) acts to select and coordinate these stages of defensive behavior [Fanselow, 1991]. This structure has long been implicated in fear- and defensive-related responding [e.g., Bandler, 1988; Lyon, 1964]. It seems that particular regions of this structure control different stages of defense. Regions lateral and dorsal to the aqueduct correspond to circa-strike behaviors. For convenience, in this paper we will refer to these areas as the dorsolateral PAG (dlPAG). Such behaviors can be elicited by direct electrical and chemical stimulation of this region [e.g., Beckett et al., 1992; Depaulis et al., 1992; Fanselow, 1991]. Lesions limited to the dlPAG block the circa-strike behaviors elicited by electric shock but do not reduce postencounter responses such as freezing to contextual cues paired with shock [Fanselow, 1991]. The dlPAG also appears to mediate autonomic changes that support the execution of more overt circa-strike defenses [Carrive, 1991]. We [Fanselow, 1991; Fanselow et al., 1993] recently proposed a model that suggests that these reactions are mediated by projections carrying primary nociceptive information to the dlPAG.

Quite the opposite pattern is found when lesions include the area of the PAG ventral to the aqueduct (vPAG). While circa-strike responses are preserved, postencounter freezing is severely impaired [e.g., Fanselow, 1991; Kim et al., 1993; LeDoux et al., 1988; Liebman et al., 1970]. Stimulation of this area immediately results in freezing [Fanselow, 1991]. Since freezing is a response to cues associated with shock, the Fanselow et al. [1993] model proposes that the information that activates these behaviors arrives at vPAG from the forebrain structures that mediate Pavlovian fear conditioning such as the amygdala. Lesions of the amygdala block a rat's freezing to cats and shock-associated cues in a manner that is similar to lesions of the vPAG [Blanchard and Blanchard, 1972; Kiernan and Cranney, 1992; Kim et al., 1993; LeDoux et al., 1988]. While postencounter defenses are accompanied by a distinct pattern of autonomic activity, these autonomic changes do not involve the PAG. Rather, amygdalar projections to the hypothalamus appear critical to the autonomic component of postencounter defenses [LeDoux et al., 1988].

The circa-strike defenses mediated by dlPAG and the postencounter defenses mediated by vPAG are physically incompatible. A rat engaging in freezing must be able to immediately switch to circa-strike defenses if the threatening situation changes. The abruptness of the change in behavior can be easily observed by administering a brief electric shock to a freezing rat; at the onset of shock, freezing is immediately replaced by an activity burst [Fanselow, 1982]. Since both forms of defense are not simply motor patterns but consist of several behavioral dimensions, including an appropriately supportive pattern of autonomic activity, it is probably not sufficient to merely drive a motor pattern. One coordinated set of activities must replace another. A rapid change in the total pattern would be best accomplished if activation of circa-strike behaviors was accompanied by an active inhibition of postencounter defensive responding. Our theoretical model [Fanselow et al., 1993] suggests that this is accomplished by an inhibitory influence of the dlPAG on the neuroanatomical loci supporting postencounter defenses (vPAG and amygdala).

While at present there is little evidence for such an inhibitory influence of dlPAG on

vPAG, the proposal is testable. In a situation where dlPAG inhibitory influences served to attenuate vPAG-mediated postencounter SSDRs, lesions of the dlPAG that spare vPAG should actually enhance postencounter defenses. There are two effects in the behavioral literature where this inhibitory influence has been suggested to interfere with freezing [Fanselow et al., 1993]. These are two situations that produce surprisingly little freezing given the amount of aversive stimulation. One is when rats are given several electric shocks that are spaced closely together; this procedure produces about half the level of freezing that more distributed shocks produce [Fanselow et al., 1993; Fanselow and Tighe, 1988]. The second is when shock is given immediately upon placement in an observation chamber. Rats do not freeze under such conditions; yet considerable freezing is observed if the same shock is presented a minute or more after placement in the chamber [Blanchard et al., 1976; Fanselow, 1986, 1990]. If these deficits arise because the dlPAG inhibits postencounter defense, then lesions confined to the dlPAG should serve to reverse them. Alternatively, lesions of the vPAG should serve to reduce freezing even under the more optimal conditioning parameters. Therefore, we conducted two experiments to test these predictions. Experiment 1 made lesions of either the dlPAG or the vPAG and examined freezing in rats given shock under both massed and spaced conditions. Experiment 2 made similar lesions and examined the immediate shock freezing deficit.

EXPERIMENT 1

Fanselow and Tighe [1988] reported that rats given massed unsignaled shocks (3 sec apart) freeze less in the shock-associated context than do rats given the same shocks in a more distributed manner (e.g., 60 sec apart). This finding has theoretical importance because it is contrary to many influential theories of Pavlovian conditioning. Recently, Fanselow et al. [1993] suggested that this deficit arises partly because shock has a disruptive influence on conditioning. This disruptive influence could be mediated by shock's activation of the dlPAG and its presumptive inhibitory effects on postencounter defense.

Subjects and Surgery

The subjects were 33 male Long-Evans derived rats born and maintained at the University of California, Los Angeles, psychology department colony. The animals were approximately 120 days old at the start of the experiment when they were individually housed with continuous access to food and water in a colony room maintained on a 14:10 hr day:night cycle. All experimental procedures were conducted during the light portion of the cycle. For 2 days before surgery the animals were adapted to handling. They were removed from their home cage and held for approximately 30 sec.

Animals were anesthetized with 55 mg/kg sodium pentobarbital, treated with atropine (0.12 mg/kg, ip) and placed in a stereotaxic instrument. With the head leveled between lambda and bregma, the skull was exposed and a small hole was made with a dental drill. A monopolar, stainless-steel electrode (00 insect pin, insulated to the exposed tip) was lowered to the lesion site. The lesion was produced by passing anodal current (1.0 mA, 10 sec) through the electrode (Grass, D.C. Constant Current Lesion Maker, Model D.C. LM5A, Quincy, MA). Animals were assigned to one of three groups. There was a sham lesion group that received identical surgery procedures except that

the electrode was not lowered into the brain. The other two groups received lesions of either the dlPAG or the vPAG. Four lesions were made for both of these groups. Stereotaxic coordinates for the dlPAG were 7.2 and 8.0 mm posterior to bregma, –0.6 and +0.6 mm lateral to the midline, and 5.8 mm ventral to the skull. The coordinates for the vPAG lesions were the same except that the dorsoventral coordinate was changed to 6.4 mm ventral from the skull. All animals were given 7–10 days to recover from the surgery. During this period they were handled daily and transported to the laboratory on the 2 days prior to the conditioning phase.

Apparatus

All phases of the experiment were conducted in identical observation chambers (28 × 21 × 10.5 cm; Lafayette Instruments Co., Lafayette, IN) that were contained within sound-attenuating chests with the front panel removed for observation of the animal. These chests were equipped with ventilation fans that supplied background masking noise (68 db A scale). The floor of each chamber consisted of 4 mm diameter stainless-steel rods spaced 1.5 cm apart center-to-center. Scrambled electric shock originated from a custom 450 V ac shock source wired through a commercial mechanical scrambler (Lafayette Instruments Co.). These units were adjusted to deliver a 0.5 mA shock to the grid floor of each chamber. The ceiling, front, and back walls of the chambers were constructed of clear Plexiglas and the two side walls of stainless steel. The chambers were located in a room isolated from the control equipment and the observer who scored behavior from a video monitor. The chambers were cleaned with a 5% ammonium hydroxide solution between sessions.

Procedure

Animals from each surgery group were assigned to one of two conditioning treatments. The treatments differed only in the interval between shocks. One group received massed shocks spaced 3 sec apart and the other received spaced trials where the interval between shock presentations was 60 sec. Each animal in both conditions received the first of three 1 sec, 0.5 mA footshocks 130 sec after placement into the chamber and was removed 30 sec after the last shock and returned to its home cage. An 8 min fear test commenced 24 hr later. Each animal was returned to its respective conditioning chamber and was observed for the level of fear conditioned to the context. The measure used to assess fear was the percent of time spent freezing during the test period. This behavior was defined as the total absence of movement of the body and vibrissa except that required for respiration [e.g., Fanselow, 1980]. A time sampling procedure was used to score this behavior. Every 2 sec a single animal was observed and its behavior was scored as freezing or not freezing. These data were used to create a percent score of the total time an animal spent freezing while in that context. In addition to this postconditioning fear test, freezing was measured during the 130 sec baseline period prior to the first shock on the conditioning day. There was no freezing behavior observed during this baseline period.

Histology

The day following the fear test, animals in the lesion groups received a lethal dose of sodium pentobarbital and were perfused intracardially with 0.9% saline followed by 10% formalin. The brains were removed and sectioned at 50 μm; every third section

through the lesion site was saved and stained with thionin. The location of the lesion was inspected under a dissection scope and transcribed onto diagrams of the rat brain copied from a stereotaxic atlas [Paxinos and Watson, 1986]. These transcriptions were conducted by a trained rater who was blind to the experimental treatment and results. Animals with misplaced lesions were excluded from analysis. Representative lesions are diagrammed in Figure 1.

Results and Discussion

Following recovery from anesthesia, rats in the dlPAG were somewhat more reactive to handling. Sham and vPAG rats were not distinguishable. This increased reactivity normalized during the recovery period prior to the experiment. The behavior of all three groups was indistinguishable during the 2 min preshock period on the test day. None of the groups showed substantial freezing during this period (<2%).

The results, in terms of the percentage of time the rats froze during the test session, are presented in Figure 2. Inspection of just the sham lesioned rats reveals the massed shock deficit in freezing. Nonlesioned rats that received shocks 3 sec apart froze less than half as much as the rats given the same shocks spaced 60 sec apart. Rats given lesions of the vPAG showed reduced freezing compared to the sham controls. Despite this overall reduction in freezing in vPAG lesioned rats, distributed shock produced more than twice as much freezing as massed shock. Lesions of the dlPAG had a markedly different effect. These lesions enhanced defensive freezing in the massed shock rats such that they were more like the distributed sham rats. Freezing was uniformly high in all dlPAG lesioned rats.

These conclusions were supported statistically by an overall analysis of variance (ANOVA) that indicated the presence of reliable group differences, $F(5,27) = 24.35$, $P < 0.001$. The data were further analyzed by a set of planned pairwise comparisons made at the 0.05 level. These indicated that the sham 3 and 60 sec groups differed reliably from each other. The two dlPAG groups did not differ from each other. Both froze reliably more than the sham 3 sec group but did not differ from the sham 60 sec group. The two vPAG groups showed reliably less freezing than their corresponding sham controls.

These data are consistent with previous reports that the vPAG is essential for freezing behavior [e.g., Borszcz et al., 1989; Fanselow, 1991; Kim et al., in press; LeDoux et al., 1988; Liebman et al., 1970]. They also indicate that the more dorsal portions of this structure are not necessary for the execution of this SSDR. Indeed, under massed shock conditions, lesions of the dlPAG significantly increased freezing. This finding is consistent with the hypothesis that shock activates dlPAG and produces, along with circa-strike defensive responses, an inhibition of postencounter defenses. Lesions of the dlPAG eliminate this inhibition. This finding supports Fanselow et al.'s [1993] contention that a major source of the massed shock deficit is the inhibitory effects of the dlPAG on postencounter defensive behavior. More generally, the data support the view that the ventral and dorsolateral portions of the PAG organize separate and distinct defensive behavior modules.

Very dense shock schedules produce little freezing [Bolles and Riley, 1973; Fanselow, 1989; Fanselow and Tighe, 1988]. This occurs, in part, because each successive shock replaces freezing with the activity burst characteristic of circa-strike defenses [Fanselow, 1982]. Since lesions of the dlPAG reduce these activity bursts [Fanselow, 1991], greater levels of freezing are promoted with massed shock in lesioned animals.

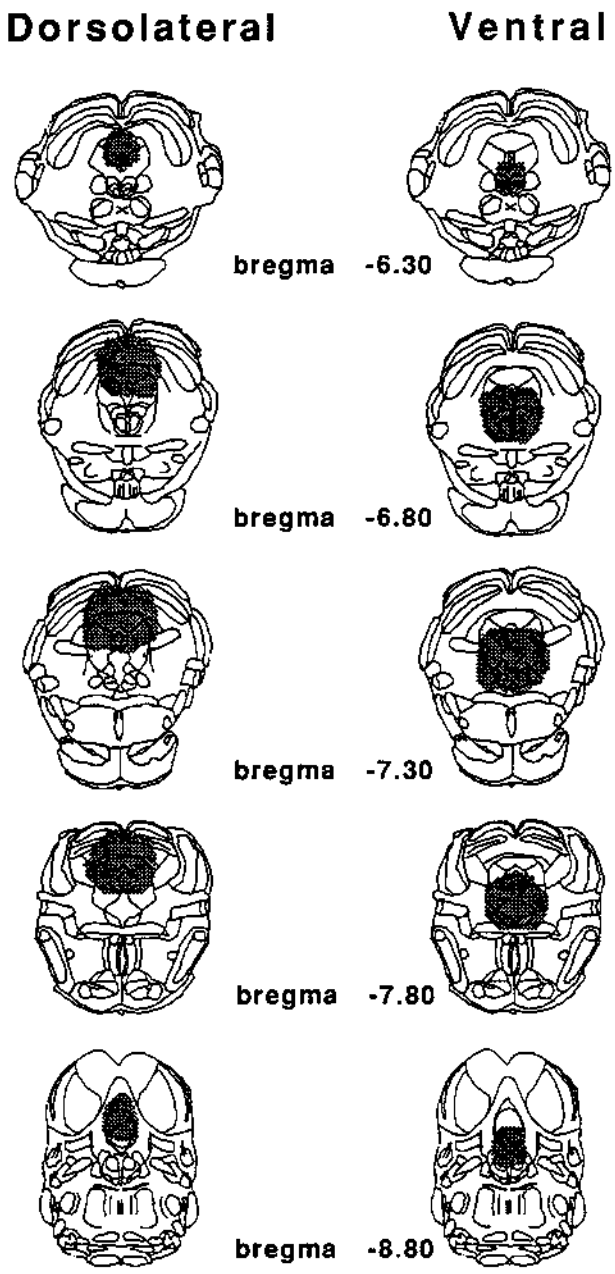

Fig. 1. The shading shows representative examples of the dlPAG and vPAG lesions made in experiment 1. Dorsolateral lesions included extensive damage to the lateral, dorsolateral, dorsomedial PAG, and deep layers of the superior colliculus. Ventral lesions included extensive damage to the ventromedial, ventrolateral, and lateral PAG as well as the dorsal raphe.

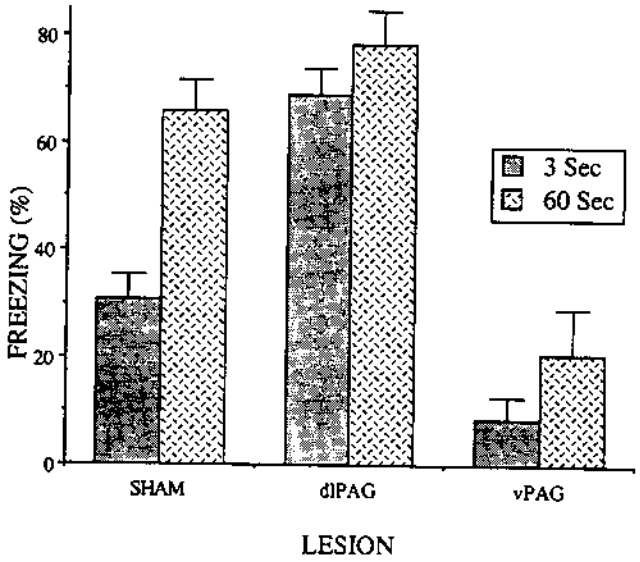

Fig. 2. The percentage of time spent freezing is a function of lesion type and the interval between shocks. These data from experiment 1 were taken during an 8 min shock-free test conducted 24 hr after the rats received three shocks. Both vPAG groups and the 3 sec dlPAG group contained five rats each. Each of the other groups contained six subjects.

EXPERIMENT 2

Blanchard et al. [1976] reported that rats given a single shock immediately after placement in an observation chamber behaved much like unshocked controls. This differed markedly from rats given the same shock a few minutes after placement in the chamber. Those rats showed a pronounced suppression in activity. These researchers suggested that rats given a few minutes to explore the chamber learned that there were no escape exits and therefore froze following shock. They suggested that rats not afforded such an opportunity to learn the environment were inescapably engaged in what they presumed to be a more dominant SSDR—flight.

Subsequently, Fanselow [1986] demonstrated that this immediate shock deficit was indeed a deficit in freezing. However, it was also manifest in defecation. More recently, we [Fanselow et al., 1994] found that the deficit is found in another defensive response—fear-induced analgesia. The fact that the immediate shock deficit is found with multiple response measures suggests that it is actually a failure to learn fear of the context paired with shock and not a change in response selection from freezing to flight. Several additional experiments found that manipulations of the presence or absence of escape exits had little effect on this deficit, while manipulations designed to influence association formation had a large impact on the deficit [Fanselow, 1986, 1990; Fanselow et al., 1993]. In total, the results indicate that rats do not freeze in response to immediate shock because it does not condition fear of the environment.

Fanselow et al. [1993] suggested that one source of the immediate shock deficit is that shock sets up an inhibitory process that interferes with conditioning. This suggestion was motivated by the finding that habituation to the shock in a different environ-

ment, before immediate shock in the test environment, resulted in an attenuation of the immediate shock deficit. Such an inhibitory influence may be mediated by the dlPAG. If this were the case, dlPAG lesions would attenuate the immediate shock deficit; this was tested in experiment 2. Rats were given several exposures to immediate shock. This was done to maximize our ability to detect an enhancement of the conditioning process. Following these multiple shocks, rats were given a savings test by exposing them to a delayed shock that normally supports conditioning and a series of shock-free extinction tests. This allowed us to further tease apart the differential effects of dlPAG and vPAG lesions.

Subjects, Surgery, Histology, and Apparatus

This experiment used 24 rats, similar to those of the first experiment. The details of the apparatus, surgery, and histology were the same as the previous experiment. However, there were three lesion groups (dlPAG, vPAG, superior colliculus) and a sham surgery control. All the lesion groups had four lesions (7.4 and 8.0 mm posterior to bregma and 0.5 mm lateral to each side of midline) that varied with respect to their dorsal/ventral coordinates. The dlPAG, vPAG, and superior colliculus lesions were 5.5, 6.3, and 4.0 mm ventral to the skull, respectively. The results of these lesions are diagrammed in Figure 3. Three rats with misplaced lesions were eliminated from the analysis.

Procedure

The experiment began 1 week after surgery. There were three immediate shock sessions, each on successive days. Immediate shock consisted of placing a rat in the observation chamber and giving it a 2 mA, 3 sec shock as soon as the door could be closed. The rat remained in the chamber for 3 min following shock during which time it was observed for freezing. Then it was returned to the home cage.

On the 4th day all the rats received a delayed shock. Each rat was placed in the chamber and nothing happened for 3 min. The rats were observed during this time, so this served as a *test* of the amount of conditioning supported by the three previous immediate shocks. Then they received a 2 mA, 3 sec shock and were returned to their home cages 30 sec later.

Over the next 4 days the rats were given, once daily, an 8 min extinction test session. No shocks were administered during this time; the rats were merely observed for freezing.

Results

The percentage of the 3 min period following each of the three immediate shocks that the rats spent freezing is shown in the leftmost portion of Figure 4. There was little freezing during this time and there were no statistically reliable differences between groups at any of these points.

The data from the 3 min test are shown in the center of Figure 4. This test occurred 24 hr after the last of the three immediate shocks. Therefore, this period gives an indication of the amount of freezing conditioned by repeated experience with immediate shock. Note that in the sham controls there was no freezing. These rats received considerable exposure to the context (9 min) and several immediate shocks but still did not freeze. The same was true of the rats with lesions of the vPAG or superior colliculus. However, there was quite a different picture for the rats with dlPAG lesions. Those rats froze considerably. A one-way ANOVA on those data indicated a reliable difference between

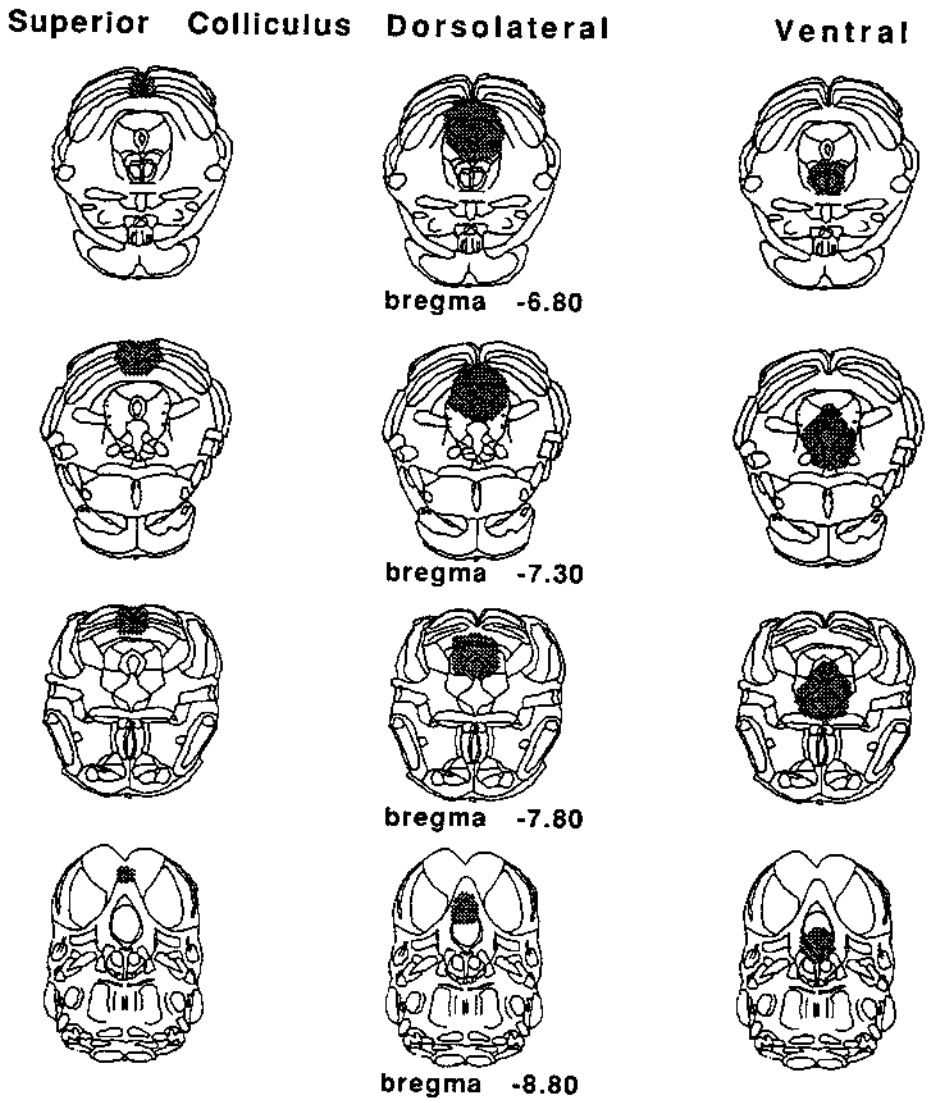

Fig. 3. The lesions of the superior colliculus, dlPAG, and vPAG in experiment 2 are indicated by the shading on coronal planes from Paxinos and Watson [1986]. Superior colliculus lesions included extensive damage to the medial portions of the superficial and deep layers of the superior colliculus but no damage to PAG. Dorsolateral lesions caused extensive damage to the lateral, dorsolateral, and dorsomedial PAG as well as the medial portions of the deep layers of the superior colliculus. Ventral lesions caused extensive damage to the ventromedial and ventrolateral PAG and the dorsal raphe nucleus. There was some minimal damage to the lateral PAG as well.

groups, $F(3,17) = 5.09$, $P < 0.05$. Newman-Keuls comparisons made at the $P < 0.05$ level confirmed that the dlPAG rats froze more than all other groups, which did not differ from each other.

The rats received a shock after the 3 min test. This is essentially a 3 min delayed

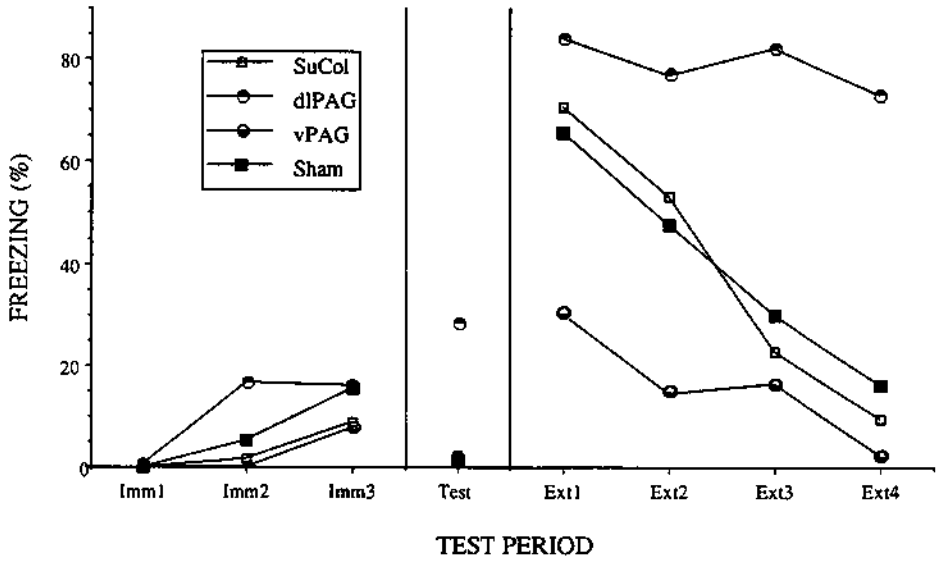

Fig. 4. These are the data of experiment 2. **Left:** The percentage of time spent freezing during 3 min periods that followed each of three immediate shocks (Imm1–3). **Center:** A 3 min observation period (Test) that occurred 24 hr after the last immediate shock. **Right:** All the rats received a shock after that 3 min period (delayed shock). Starting the day after the test the rats received four shock-free extinction tests that were 8 min long and 24 hr apart (Ext1–4). The sham and superior colliculus (SuCol) each had six rats. The dlPAG and vPAG groups contained five and four rats, respectively.

shock, which should be sufficient to support conditional freezing. As can be seen in the rightmost portion of Figure 4, this was the case. A large amount of freezing was seen over the four shock-free extinction tests. What is clear is that over the course of these tests the dlPAG animals froze the most and maintained a high level of freezing. That is, they showed little extinction. All the other groups tended to show a pattern of reduced freezing over trials; this represents extinction of fear. The vPAG animals showed the least freezing.

These conclusions were supported by a 4 (lesion type) × 4 (extinction trial) repeated measures ANOVA. There was a reliable lesion × extinction interaction, $F(9,51) = 4.37$, $P < 0.01$. Because of the large number of comparisons possible with a repeated measures analysis, subsequent comparisons were made with Scheffe's test, protected at the $P < 0.05$ level. These contrasts indicated that the dlPAG rats froze more than the sham controls on the 2nd, 3rd, and 4th extinction tests. The vPAG rats froze less than the controls on the first two tests. The superior colliculus lesioned rats never differed from the controls. The sham, superior colliculus, and vPAG groups showed statistically reliable decreasing linear trends in freezing over extinction trials. The dlPAG rats did not show this trend.

Discussion

Examination of the sham rats tells us something about the nature of the immediate shock deficit. These rats received three immediate shocks and stayed in the chambers for 3 min after each. On the 4th day, when the rats were placed in the chamber for the test, they did not freeze. These data strongly contradict the escape exit search explana-

tion of the immediate shock deficit provided by Blanchard et al. [1976]. According to that view, after receiving an immediate shock these rats should have been attempting to escape and searching for escape exits. Escape and search attempts should only persist until the rat is convinced that no escape exits are available. Note that in order to explain the freezing that follows a 3 min delayed shock (e.g., that seen in experiment 1) the escape exit search view suggests that just 3 min *before* shock is enough to normally convince rats to abandon attempts at escape and to freeze. The rats in this experiment had an extensive period of time to look for escape exits (9 min) and since that time occurred after shock they should have been highly motivated to look for these exits. Any such efforts would have obviously ended in failure. Therefore, the Blanchard et al. [1976] view predicts that these rats should have evidenced high levels of freezing during the test, but no freezing was observed. The data are much more in accord with the view that immediate shock simply does not support contextual fear conditioning and thus are consistent with associative analyses of this phenomenon [Fanselow, 1986, 1990; Fanselow et al., 1993].

It is important to note that the results of the test session before delayed shock indicate that the rats with dlPAG lesions were successfully conditioned to associate a context with immediate shock. The data are consistent with the hypothesis that the immediate shock deficit arises partly because of an inhibitory influence of the dlPAG on the conditional fear system. That dlPAG lesions reduced the deficits that occur with both massed and immediate shock supports the contention that both effects arise from similar processes [Fanselow et al., 1993].

Once these rats received a delayed shock they frozen considerably during the extinction tests. At this point, rats with vPAG lesions showed a reduced level of freezing. Again we see that the vPAG is important for this defensive response. On the other hand, lesions of the dlPAG had the opposite effect: they served to enhance this defensive response. The rats with dlPAG lesions did not extinguish over the shock-free extinction tests. This does not necessarily mean that the dlPAG is involved in the extinction of fear. The dlPAG rats started extinction with a higher level of fear because the immediate shock successfully conditioned fear in them but not the other animals. This initially higher level of fear may be the reason for the persistent freezing that those rats showed as opposed to a deficit in extinction of fear.

GENERAL DISCUSSION

In two experiments we found that vPAG and dlPAG lesions produced strikingly opposite alterations in a conditional fear-induced defensive response—freezing. In situations where freezing was normally strong, vPAG lesions attenuated the response. This is consistent with several other studies using electrolytic [e.g., Borszcz et al., 1989; Fanselow, 1991; Kim et al., 1993; LeDoux et al., 1988; Liebman et al., 1970] and cytotoxic [Kiernan and Cranney, 1992; LeDoux et al., 1988] lesions of the vPAG. In two situations that produced weak levels of freezing, given the amount of shock experienced (massed or immediate shock), dlPAG lesions increased the level of freezing. To account for this pattern of results we suggest that the vPAG is critical for the expression of conditional fear-induced defensive responses. Alternatively, the dlPAG acts to inhibit the conditional fear system.

It is clear that both of these portions of the PAG play a role in promoting defense. However, they promote different forms of defense. The dlPAG organizes circa-strike

defenses. These are elicited unconditionally by a nociceptive stimulus such as shock and thus are temporally bound to the time of shock delivery [Fanselow, 1991]. They are also elicited by other contact stimuli, such as the strike of a predator [e.g., Blanchard et al., 1981]. On the other hand, the vPAG coordinates certain components of postencounter defenses. These are provoked by stimuli that predict a nociceptive event but not the nociceptive event itself [Bolles and Fanselow, 1980]. They also occur when a predator is in the vicinity but contact is not immediately imminent [Lester and Fanselow, 1985]. Since these behaviors can be produced by learned cues, the vPAG is dependent on the forebrain structures involved in the learning of fear such as the amygdala [e.g., Davis, 1992]. These forebrain structures are also critical in producing the same defensive responses when they are elicited by the presence of a predator [Blanchard and Blanchard, 1972]. The amygdala has been shown to be critical to every component of postencounter defense tested. This includes freezing [e.g., LeDoux et al., 1988], potentiated startle [Hitchcock and Davis, 1986], hypertension [LeDoux et al., 1988], and analgesia [Helmstetter, 1992]. Not all components of postencounter defense depend on the vPAG. Responses such as freezing and opioid analgesia do [Fanselow, 1991; Helmstetter and Landeira-Fernandez, 1991]. However, the concurrent autonomic changes and potentiated startle appear to be mediated by projections from the amygdala to other structures [Kiernan and Cranney, 1992; LeDoux et al., 1988]. If the function of the inhibition from dlPAG is to turn off the integrated postencounter defensive pattern to replace it with its own, integrated, circa-strike pattern, this would be most efficiently done by inhibiting the amygdala as it appears to be the last common point for all the postencounter responses. There are monosynaptic projections from dlPAG to amygdala [Rizvi et al., 1991]. The finding of experiment 2, that dlPAG lesions enhanced conditioning, also suggests an influence of dlPAG on forebrain structures.

There was some overlap in the dlPAG and vPAG lesions (see Figs. 1, 3). Inspection of the lesions from this experiment, those in other work from this laboratory [e.g., Fanselow, 1991; Kim et al., 1993; unpublished results], and the lesion literature as a whole [e.g., Borszcz et al., 1989; Kiernan and Cranney, 1992; LeDoux et al., 1988; Liebman et al., 1970; Lyon, 1964] suggests that the critical region is the portion of the PAG ventral and ventrolateral to the most ventral portion of the aqueduct. If this region is damaged there is a reduction in freezing regardless as to whether or not there is more dorsal damage. For example, Liebman et al. [1970] found reduced freezing with lesions that involved the entire PAG. On the other hand, lesions confined to areas below the aqueduct reduce freezing [Borszcz et al., 1989; Fanselow, 1991; Kim et al., in press]. Such ventral lesions usually contain damage to the dorsal raphe nucleus. However, recently Maier et al. [1993] reported that lesions confined to the dorsal raphe nucleus did not reduce freezing, while lesions that transgressed into the vPAG did. Additionally, Kim et al. [in press] reported that rats with ventrolateral PAG lesions that spared dorsal raphe showed reduced freezing. Thus it appears that if there is any damage to the ventral PAG then the dominant effect is a deficit in freezing. Enhancement of freezing depends on leaving this ventral area intact while there is damage to the more dorsal regions of the PAG. Electrolytic lesions not only damage cells intrinsic to the lesioned structure; axons passing through the lesioned area are also interrupted. Therefore, it is possible that the present results are partly mediated by damage to fibers of passage. However, chemical lesions of the ventral PAG, which destroy neurons but leave fibers of passage intact, reduce freezing as well [LeDoux et al., 1988; Kiernan and Cranney,

1992]. The locations of these chemical lesions are similar to the electrolytic lesions investigated here.

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