Behavioral Neuroscience
1999, Vol. 113, No. 2, 283–290

0735-7044/99/$3.00

Neurotoxic or Electrolytic Lesions of the Ventral Subiculum Produce Deficits in the Acquisition and Expression of Pavlovian Fear Conditioning in Rats

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The effects of neurotoxic or electrolytic ventral subicular (vSUB) lesions on the acquisition and expression of Pavlovian fear conditioning in rats were examined. Conditioning consisted of the delivery of tone–footshock trials in a novel observation chamber, and freezing served as the measure of conditional fear. Pretraining vSUB lesions produced a severe tone freezing deficit and a modest context freezing deficit, whereas posttraining lesions produced severe deficits in freezing to both a tone and a context conditional stimulus (CS). Similar impairments were produced by neurotoxic and electrolytic lesions. Increases in motor activity associated with the lesions could not account for freezing deficits. These results reveal that neurons in the vSUB have an important role in both the acquisition and expression of Pavlovian fear conditioning to contextual and acoustic CSs.

The study of the neurobiology of learning and memory has profited greatly from the analysis of simple behavioral systems (for a review, see Milner, Squire, & Kandel, 1998). In recent years considerable progress has been made in understanding the neurobiology of simple forms of emotional learning and memory in mammals, such as Pavlovian fear conditioning in rats (Davis, Rainnie, & Cassell, 1994; Fanselow, 1994; Maren, 1996; Rogan & LeDoux, 1996). For Pavlovian fear conditioning tasks, a neutral conditional stimulus (CS), such as a tone or a context (the training environment), is paired with an aversive unconditional stimulus (US), such as an electric footshock. After a few conditioning trials, the CS comes to elicit robust conditional fear responses (CRs), including freezing, potentiated acoustic startle, hypoalgesia, and increased blood pressure. This form of learning is rapidly acquired, stable over long periods of time, and easily quantified.

Because of the potential relevance of fear conditioning to disorders of fear and anxiety in humans, a major goal has been to identify the critical brain substrates underlying Pavlovian fear conditioning in rats. For conditioning to acoustic CSs, there is consensus that projections from the auditory thalamus to the amygdala are essential (Campeau & Davis, 1995a; Maren & Fanselow, 1996; Rogan & LeDoux, 1996). For conditioning to contextual CSs, the amygdala is also essential (Helmstetter, 1992; Maren, 1998; Maren, Aharonov, & Fanselow, 1996; McNish, Gewiritz, & Davis, 1997; Phillips & LeDoux, 1992), although the afferent neural structures and pathways underlying conditioning to more complex contextual CSs are less clear. Several reports have suggested a role for the dorsal hippocampus (DH), a structure known to be important for spatial learning and memory (e.g., Morris, Garrud, Rawlins, & O'Keefe, 1982). For example, DH lesions attenuate both the acquisition and expression of contextual fear conditioning in rats (Kim & Fanselow, 1992; Maren, Aharonov, & Fanselow, 1997; Maren & Fanselow, 1997; Phillips & LeDoux, 1992; Selden, Everitt, Jarrard, & Robbins, 1991), and lesions that interrupt projections from the DH to the amygdala interfere with contextual fear conditioning (Maren & Fanselow, 1995).

Although several studies have implicated the DH in contextual fear conditioning, recent reports indicate that neural pathways that traverse the DH, rather than DH neurons per se, may have a role in contextual fear conditioning. For example, we have found that the acquisition of contextual fear conditioning is not affected by neurotoxic (axon-sparing) lesions in the DH but is impaired by electrolytic (axon-damaging) lesions in the DH (Maren et al., 1997; Maren & Fanselow, 1997). This observation suggests that either damage to axons that traverse the DH ("fibers of passage") or combined damage to fibers of passage and DH neurons is responsible for contextual fear conditioning deficits in rats with electrolytic DH lesions. In either case, fibers of passage in the DH would appear to have an important role in contextual fear conditioning.

One structure that may be the source of these critical fibers of passage within the DH is the ventral subiculum (vSUB). Like the DH, the vSUB has been implicated in a variety of functions including exploratory behavior (Burns, Annett, Kelley, Everitt, & Robbins, 1996) and spatial learning (Floresco, Seamans, & Phillips, 1997). The vSUB is located at the temporal pole of the hippocampal formation and sends axonal projections to wide areas of the brain, including the nucleus accumbens (NAcc) and amygdala (Canteras & Swanson, 1992). It is important to note that the

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This work was supported by grants from the National Institute of Mental Health (R03MH57360 and R29MH57865) and the University of Michigan. I thank William Holt for technical assistance.

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projections from the vSUB to the NAcc are located in the fimbria, which traverses the DH, and recent reports indicate that fimbria–fornix lesions disrupt contextual fear conditioning (Maren & Fanselow, 1997; Phillips & LeDoux, 1995). Moreover, either NAcc lesions (Kiernan, Bailey, Sims, Lukes, & Cranney, 1996; Riedel, Harrington, Hall, & Macphail, 1997) or reversible inactivation of the NAcc (Westbrook, Good, & Kiernan, 1997) impairs the acquisition of contextual fear. Together with the different effects of neurotoxic and electrolytic DH lesions on the acquisition of contextual fear conditioning, these reports suggest that neural projections from the vSUB to the NAcc are involved in contextual fear conditioning (Maren et al., 1997; Riedel et al., 1997; Westbrook et al., 1997).

In light of the potential importance of the vSUB–NAcc system in contextual fear conditioning, we investigated the role of the vSUB in the acquisition and expression of Pavlovian fear conditioning in rats. To examine whether neurons in the vSUB are required for contextual fear conditioning, we compared the effects of electrolytic or neurotoxic lesions of the vSUB on the acquisition and expression of conditional fear. We hypothesized that deficits in contextual fear conditioning would be associated with both types of lesions. In addition, lesions were made either before or after training to assess the role of the vSUB in the acquisition as compared with the expression of Pavlovian fear conditioning.

Method

Subjects

The subjects were 80 adult male Long-Evans rats (200–224 g) obtained from a commercial supplier (Harlan Sprague Dawley, Indianapolis, IN). After arrival, the rats were individually housed in standard stainless steel hanging cages on a 14:10-hr light–dark cycle (lights on at 7 a.m.) and were provided free access to food and tap water. After housing, the rats were handled daily (10–20 s per rat) for 5 days to acclimate them to the experimenter.

Behavioral Apparatus

Eight identical observation chambers (30 × 24 × 21 cm; MED Associates, Burlington, VT) were used for both conditioning and contextual fear testing. The chambers were constructed from aluminum (side walls) and Plexiglas (rear wall, ceiling, and hinged front door) and were situated in sound-attenuating cabinets located in a brightly lit and isolated room. The floor of each chamber consisted of 19 stainless steel rods (4 mm in diameter) spaced 1.5 cm apart (center-to-center). The rods were wired to a shock source and solid-state grid scrambler (MED Associates, Burlington, VT) for the delivery of footshock USs. A speaker mounted outside a grating in one wall of the chamber was used for the delivery of acoustic CSs. The chambers were cleaned with a 5% ammonium hydroxide solution, and stainless steel pans containing a thin film of the same solution were placed underneath the grid floors before the rats were placed inside. Background noise (65 dB, A scale) was supplied by ventilation fans in each cabinet.

Each conditioning chamber rested on a load-cell platform that was used to record chamber displacement in response to each rat's motor activity. To ensure interchamber reliability, each load-cell amplifier was calibrated to a fixed chamber displacement. The output of each chamber's load cell was set to a gain (vernier dial = 8) that was optimized for detecting freezing behavior. Load-cell amplifier output (−10 to +10 V) from each chamber was digitized and acquired on-line using Threshold Activity software (Version 1.06, MED Associates). The absolute values of the load-cell voltages were computed and these absolute values were multiplied by 10 to yield a load-cell activity scale that ranged from 0 to 100.

Design and Procedure

The rats were randomly assigned to groups that received sham surgery or vSUB lesions either 1 week before or 1 day after training. In each lesion group, rats received either a neurotoxic or electrolytic lesion in the vSUB; sham groups were established for each of these lesion groups. This yielded four groups of rats (SH-Electro, SUB-Electro, SH-Excito, SUB-Excito; $n = 10$/group) for both the pre- and posttraining lesion groups. On the conditioning day, the rats were transported to the laboratory in squads of 8 and placed in the conditioning chambers; the chamber position was counterbalanced for each squad and group. The rats received 15 tone (10-s, 90-dB, 2-kHz)–footshock (2-s, 1.0-mA; 70-s intertrial interval) trials 3 min after being placed in the chambers. Sixty seconds after the final shock, the rats were returned to their home cages. One week after training (to allow for recovery from surgery in the posttraining groups), fear conditioning to the context was assessed by returning the rats to the conditioning chambers and measuring freezing behavior (somatomotor immobility except that necessitated by breathing) during an 8-min extinction test. Twenty-four hours after the context extinction test, fear to the tone CS was measured by placing the rats in a novel context and presenting a 6-min tone 2 min after placement in the context. The novel context consisted of the same chambers described above, but the room housing the chambers was darkened (illumination in the room was provided by a 40-W red light), the doors on the sound-attenuating cabinets were closed, the ventilation fans were turned off, and the chambers were cleaned with a 1% acetic acid solution.

During both the conditioning and extinction sessions, each rat's activity was monitored continuously using the data acquisition system described above. For each chamber, load-cell activity was digitized at 5 Hz yielding one observation per rat every 200 ms (300 observations/rat/min). In all experiments, freezing was quantified by computing the number of observations for each rat that had a value less than the freezing threshold (load-cell activity = 5; animals exhibit freezing when load-cell activity is at or below this value; see Maren, 1998). To avoid counting momentary inactivity as freezing, an observation was only scored as freezing if it fell within a contiguous group of at least five observations that were all less than the freezing threshold. Thus, freezing was only scored if the rat was immobile for at least 1 s. For each session, the freezing observations were transformed to a percentage of total observations. In addition to freezing, motor activity was quantified during the preshock period on the conditioning day using the raw load-cell output.

Surgery

One week before or 1 day after fear conditioning, the rats were treated with atropine methyl nitrate (0.4 mg/kg body weight), anesthetized with an intraperitoneal injection of Nembutal (sodium pentobarbital, 65 mg/kg body weight), and mounted in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). The scalp was incised and retracted, and head position was adjusted to place

bregma and lambda in the same horizontal plane. Small burr holes (2 mm in diameter) were drilled bilaterally in the skull for the placement of 28-gauge cannulas or Epoxylite-insulated electrodes in the vSUB. Half of the rats in each lesion group received either neurotoxic or electrolytic lesions of the vSUB. For neurotoxic lesions, a 10-μl Hamilton syringe was mounted in an infusion pump (Harvard Apparatus, South Natick, MA) and connected to the injection cannula with polyethylene tubing. *N*-methyl-D-aspartate (20 μg/μl; Sigma Chemical, St. Louis, MO) in 100 mM phosphate-buffered saline (pH = 7.4) was infused (0.1 μl/min for 2 min, 0.2 μl per site) at three sites in the vSUB (AP: −5.8, −6.3, and −6.8 mm from bregma; ML: ±4.0, ±5.0, and ±5.0 mm from bregma; DV: −7.5, −6.0, and −4.0 mm from dura, respectively). Five minutes were allowed after each infusion for diffusion of the drug. For electrolytic lesions, an insulated electrode (except for 500 μm at the tip) was lowered into the vSUB (same coordinates as above) and anodal current (1.0 mA, 10 s) was injected. Following surgery, the incision was closed with stainless steel wound clips, and the rats were allowed to recover on a heating pad before returning to their home cage. Rats in the pretraining groups recovered for 1 week before training, and rats in the posttraining groups recovered for 1 week before extinction testing (rats with pretraining lesions experienced the same training-to-testing interval). Thus, the training-to-testing interval was equated for all groups, insofar as all of the rats were tested 1 week following training. In the pretraining groups, the data from 1 sham rat were lost because of an equipment failure. In the posttraining groups, 4 sham rats and 1 SUB-Excito rat died during surgery. This yielded the following number of subjects per group: Pretraining, SH-Electro (n = 9), SUB-Electro (n = 10), SH-Excito (n = 10), SUB-Excito (n = 10); Posttraining, SH-Electro (n = 8), SUB-Electro (n = 10), SH-Excito (n = 8), SUB-Excito (n = 9).

Histology

Histological verification of lesion location was performed after behavioral testing. Rats were perfused across the heart with 0.9% saline followed by 10% formalin. After extraction from the skull, the brains were postfixed in 10% formalin for 2 days and 10% formalin (vol/vol)/30% sucrose (wt/vol) until sectioning. Coronal sections (50 μm thick, taken every 200 μm) were cut on a cryostat (−16 °C) and wet mounted on glass microscope slides with 70% ethanol. After drying, the sections were stained with 0.25% thionin to visualize neuronal cell bodies. Lesions were verified by reconstructing the damage on stereotaxic atlas templates. The extent of damage to the vSUB, medial entorhinal cortex (mEC), ventral CA1, and caudal dentate gyrus–CA3 (DG/CA3) was rated by inspection of the lesion reconstructions and assigned a numeric score. For each brain region, scores of 4, 2, or 1 were assigned for severe, moderate, or minimal damage, respectively, and a score of 0 was assigned if there was no damage present. Severe damage involved complete or near complete lesions (approximately 100%), moderate damage involved substantial, but not complete, lesions (approximately 50%), and minimal damage involved limited lesions (25% or less).

Data Analysis

For each session, the freezing data were transformed to a percentage of total observations, a probability estimate that is amenable to analysis with parametric statistics. These probability estimates of freezing were analyzed using analysis of variance (ANOVA). Post hoc comparisons in the form of Fisher protected least significant difference tests were performed following a significant omnibus F ratio. Lesion severity ratings were analyzed with nonparametric Kruskal-Wallis tests. All data are represented as means (±*SEM*s).

Results

Histology

An illustration of the extent of maximal and minimal brain damage in representative rats with vSUB lesions is shown in Figure 1. The extent of brain damage was comparable with electrolytic and neurotoxic lesions, and Figure 1 illustrates the typical lesion extent for both types of lesion. In all subjects, the vSUB was destroyed completely in both hemispheres. In addition, the lesions typically encroached upon the the ventral hippocampal formation, including hippocampal area CA1, the dentate gyrus, and hippocampal area CA3. Many rats exhibited minor damage to the medial entorhinal cortex; however, damage to perirhinal cortex was not observed in any of the animals. Electrolytic lesions damaged white matter tracts in the vicinity of the lesion, such as the ventral angular bundle, whereas these tracts were spared in rats with neurotoxic vSUB lesions.

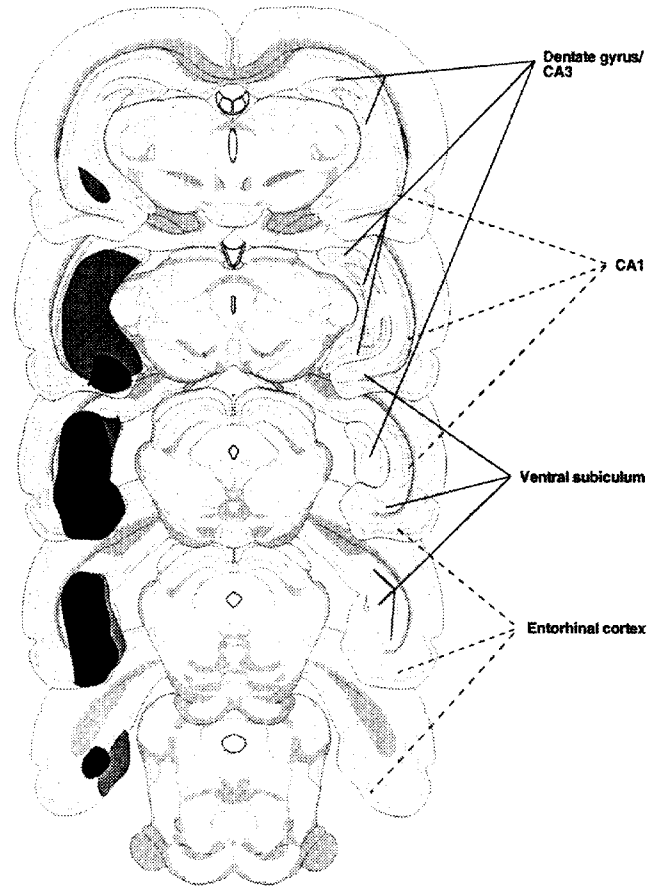

Figure 1. Schematic representation of the extent of lesions (maximum, light shading; minimum, dark shading) of the ventral subiculum. Unilateral representations of bilateral lesions are shown so that the damaged areas can be visualized with respect to the labeled areas. Coronal brain section images adapted from Swanson (1992).

Table 1
Mean Ratings of Lesion Severity in Rats With Ventral Subicular Lesions

Group	*n*	Brain region			
		vSUB	mEC	vCA1	DG/CA3
Electrolytic					
Pretraining	10	4.0	1.2	1.8	1.7
Posttraining	10	3.8	1.5	1.4	1.7
Neurotoxic					
Pretraining	10	3.8	1.0	3.1	3.1
Posttraining	9	3.6	0.7	2.7	2.7

Note. Maximum score = 4; severe damage = 4, moderate damage = 2, minimal damage = 1, no damage = 0. vSUB = ventral subiculum; mEC = medial entorhinal cortex; vCA1 = ventral hippocampal area CA1; DG/CA3 = caudal dentate gyrus and hippocampal area CA3.

Mean ratings of lesion severity for the rats in each experimental group are shown in Table 1. There were no significant differences in the extent of lesions between rats in the pre- and posttraining groups for any of the brain regions examined (Kruskal-Wallis test, $ps > .05$), and the extent of vSUB lesions was similar in all of the rats, $H(3) = 2.48, p = .48$. However, rats receiving neurotoxic lesions exhibited significantly more damage in the ventral CA1, $H(3) = 10.2$, $p < .05$, and DG/CA3, $H(3) = 9.3, p < .05$, and significantly less damage in the mEC, $H(3) = 9.2$, $p < .05$, than rats in the electrolytic lesion groups. None of the rats were excluded from the statistical analysis of freezing behavior.

Conditional Freezing

For the sake of clarity, data from the SH-Excito and SH-Electro groups were collapsed because the groups did not differ statistically from one another. Freezing during the 8-min context and tone extinction tests in rats receiving pretraining surgery is shown in Figure 2, A and B, respectively. As illustrated, vSUB lesions attenuated conditional freezing to both the contextual and acoustic CSs. An ANOVA on the context extinction data revealed significant main effects of group, $F(2, 36) = 5.0$, $p < .05$, and test minute, $F(7, 252) = 10.2$, $p < .0001$, and a significant Group × Minute interaction, $F(14, 252) = 3.0$, $p < .0005$. Post hoc comparisons ($p < .05$) on the group means revealed that rats receiving electrolytic or neurotoxic lesions differed from sham controls but did not differ from one another. This pattern of results varied across the test minutes insofar as the two lesion groups did not differ from sham controls early in the test (Minutes 1 and 2), but did differ from controls in the remainder of the test (Minutes 3–8). A similar pattern of results was obtained from the analysis of the tone extinction test. Freezing during the 2-min pretone baseline was low and did not differ between the groups. An ANOVA on the tone extinction data (Minutes 3–6) revealed significant main effects of group, $F(2, 36) = 18.5$, $p < .0001$, and test minute, $F(5, 180) = 9.3$, $p < .0001$. Again, post hoc comparisons ($p < .05$) on the group means revealed that rats receiving electrolytic or neurotoxic lesions differed from sham controls but did not differ from one another. Thus, the method by which vSUB lesions were made did not interact with the deficits these lesions imparted on conditional freezing.

A within-subjects comparison of the deficits produced by vSUB lesions on context and tone fear is shown in Figure 2C. These data are collapsed across the first 6 min of the context test and the 6-min tone test for clarity; the tone data were normalized by subtracting the 2-min baseline. It is apparent that deficits in freezing were more pronounced to the tone CS than to the context CS. An ANOVA on these data confirmed this impression and revealed significant main effects of group, $F(2, 36) = 20.5$, $p < .0001$, and CS, $F(1, 36) = 13.4$, $p < .001$, and a significant Group × CS interaction, $F(2, 36) = 5.1$, $p < .02$. Post hoc comparisons ($p < .05$) confirmed that freezing in the vSUB groups differed from their respective sham controls and was greater to the context CS than to the tone CS. Thus, under the training conditions used in the present experiment, pretraining vSUB lesions produce more severe deficits in freezing to a tone as compared with a context CS.

Freezing during the 8-min context and tone extinction tests in rats receiving posttraining surgery is shown in Figures 2D and 2E, respectively. As illustrated, vSUB lesions attenuated conditional freezing to both the contextual and acoustic CSs. An ANOVA on the context extinction data revealed a significant main effect of group, $F(2, 32) = 16.1$, $p < .0001$, and a significant Group × Minute interaction, $F(14, 224) = 2.9$, $p < .0006$. Post hoc comparisons ($p < .05$) on the group means revealed that rats receiving electrolytic or neurotoxic lesions differed from sham controls but did not differ from one another. This pattern of results varied across the test minutes insofar as the SUB-Electro group did not differ from sham controls in the final test (Minute 8), but did differ from controls in the remainder of the test (Minutes 1–7). A similar pattern of results was obtained from the analysis of the tone extinction test. Freezing during the 2-min pretone baseline was low and did not differ between the groups. An ANOVA on the tone extinction data (Minutes 3–6) revealed significant main effects of group, $F(2, 32) = 29.3$, $p < .0001$, and test minute, $F(5, 160) = 4.6$, $p < .001$, and a significant Group × Minute interaction, $F(10, 160) = 3.3$, $p < .001$. Again, post hoc comparisons ($p < .05$) on the group means revealed that rats receiving electrolytic or neurotoxic lesions differed from sham controls but did not differ from one another. As with the pretraining data, the method by which vSUB lesions were made did not interact with the deficits these lesions imparted on conditional freezing.

A within-subjects comparison of the deficits produced by vSUB lesions on context and tone fear in rats receiving posttraining lesions is shown in Figure 2F. These data are collapsed across the first 6 min of the context test and the 6-min tone test for clarity; the tone data were normalized by subtracting the 2-min baseline. Unlike the pretraining data, it is apparent that deficits in freezing to the tone CS were comparable to deficits in freezing to the context CS. An ANOVA on these data confirmed this impression and revealed a significant main effect of group, $F(2, 32) = 36.9$,

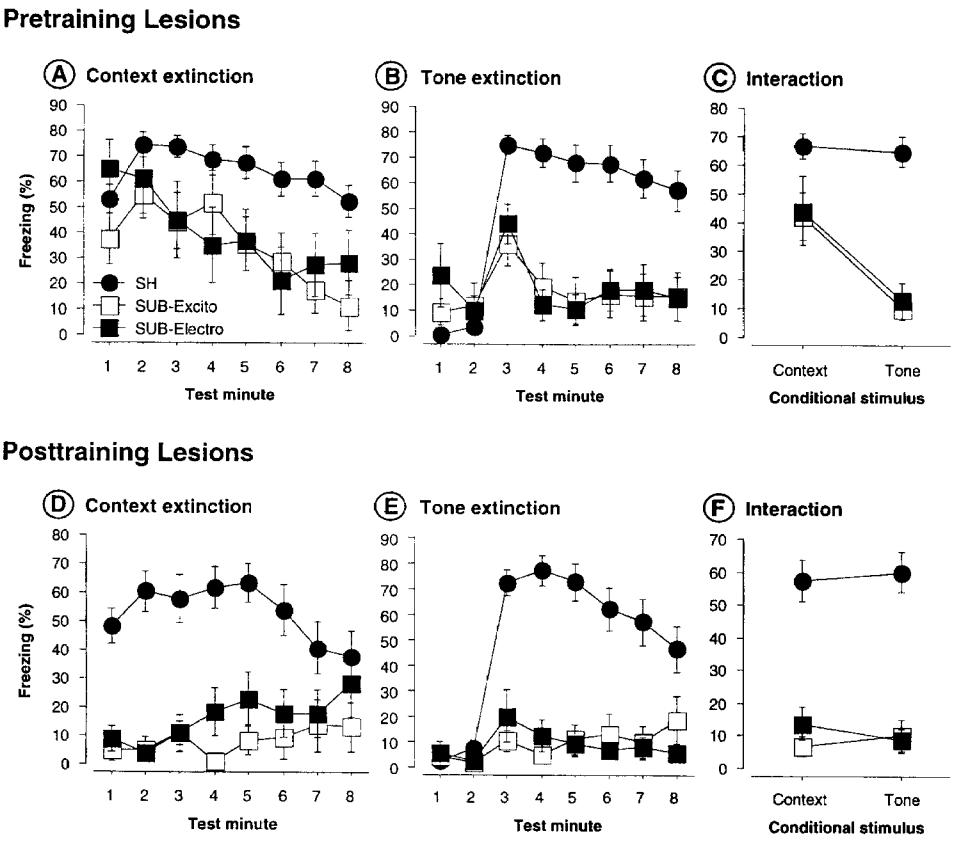

Figure 2. Pre- (A, B, C) and posttraining (D, E, F) neurotoxic lesions of the ventral subiculum (vSUB) and conditional freezing. A and D: Mean ($\pm SEM$) percentage of freezing during the 8-min context extinction tests conducted 1 day after conditioning in rats that received sham surgery (SH), electrolytic vSUB lesions (SUB-Electro) or excitotoxic vSUB lesions (SUB-Excito). B and E: Mean ($\pm SEM$) percentage of freezing during the 8-min tone extinction tests conducted 1 day after conditioning in rats that received sham surgery, electrolytic vSUB lesions, or excitotoxic vSUB lesions. Tone onset occurred at the start of the 3rd min of the test. C and F: Interaction plots illustrating the mean ($\pm SEM$) percentage of freezing collapsed across the first 6 min of the context and tone extinction tests, respectively, in rats that received sham surgery, electrolytic vSUB lesions, or excitotoxic vSUB lesions.

$p < .0001$, and a nonsignificant Group × CS interaction, $F(2, 32) = 0.5$. Post hoc comparisons ($p < .05$) confirmed that freezing in the vSUB groups differed from their respective sham controls, but was not different from the context and tone CSs. Thus, posttraining vSUB lesions produced severe deficits in freezing to both a tone and a context CS.

Motor Activity

We have previously reported that DH lesions increase preshock locomotor activity (Maren & Fanselow, 1997; Maren et al., 1997), a change that is frequently observed in rats with DH lesions (Blanchard, Blanchard, Lee, & Fukunaga, 1977; Douglas & Isaacson, 1964). This increase in activity was only observed in rats receiving electrolytic DH lesions (Maren et al., 1997). We speculated that the increase in activity was due to a disruption of vSUB projections that passed through the DH. Thus, we predicted in the present experiment that either electrolytic or neurotoxic vSUB lesions would increase preshock motor activity. The activity data from rats receiving pretraining surgery are shown in Figure 3. As shown, both neurotoxic and electrolytic vSUB lesions produced modest increases in motor activity. An ANOVA on these data confirmed this observation and revealed a significant main effect of lesion, $F(1, 36) = 7.5$, $p < .01$, and a nonsignificant Lesion × Procedure interaction, $F(1, 36) = 0.5$. Thus, vSUB lesions increased motor activity, and this increase did not interact with the techniques by which the lesions were made. Interestingly, another study reported decreases in motor activity after vSUB lesions (Burns et al., 1996). However, the task these investigators used to assess activity was quite different from that in the present study. Activity was assessed in a large open field in food-deprived rats, two factors that greatly increase activity in intact rats.

The increase in motor activity produced by pretraining vSUB lesions raises the possibility that freezing deficits are

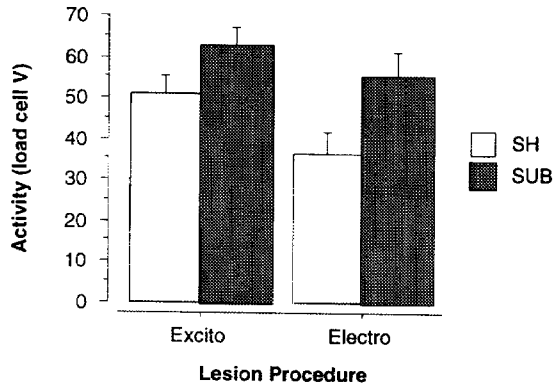

Figure 3. Motor activity in rats with ventral subiculum (vSUB) lesions. Mean (± *SEM*) activity (load cell voltage) in rats that received sham surgery (SH) or vSUB lesions (SUB) using either excitotoxic (Excito) or electrolytic (Electro) lesion procedures.

related to motor hyperactivity rather than to learning and memory deficits per se. Although we have argued that increased motor activity cannot account for freezing deficits in rats with DH lesions (Maren, Anagnostaras, & Fanselow, 1998), it is possible that the situation is different in rats with vSUB lesions. This issue was addressed in two ways. First, we examined whether vSUB lesions produced reliable deficits in freezing when preshock motor activity was used as a covariate. Freezing data from rats with pretraining vSUB lesions were submitted to an analysis of covariance (ANCOVA) using preshock motor activity as the covariate. For the purposes of this analysis, rats with electrolytic or neurotoxic lesions were collapsed, as were their respective sham control groups. For both context and tone freezing, the ANCOVAs revealed significant main effects of lesion: context, $F(1, 36) = 4.8, p < .05$; tone, $F(1, 36) = 27.7, p < .0001$. That is, although preshock activity accounted for 23% of the variance in context freezing and 31% of the variance in tone freezing in rats with vSUB lesions, there was significant residual variance in freezing behavior that was accounted for by the vSUB lesion. Hence, vSUB lesions produced reliable impairments in context and tone freezing even after the variance associated with preshock activity was taken into account.

Second, we examined whether rats with pretraining vSUB lesions (collapsed across lesion type) differed from controls in the degree to which they suppressed their motor activity after fear conditioning. If rats with vSUB lesions condition fear normally, then they should show normal activity suppression despite their relatively high baseline (preshock) motor activity. Mean motor activity from the 6-min tone and 8-min context extinction tests was measured in the same manner as described for preshock activity. An activity suppression ratio (SR) was generated by computing the proportion of activity during each extinction test compared to the sum of activity during the preshock period and the extinction test: SR = (extinction activity)/[(preshock activity) + (extinction activity)]. With this measure, an SR equal to 0.5 indicates an absence of suppression (preshock and extinction activity are equal), and an SR equal to 0 indicates a complete suppression of activity. The SR normalizes each subject to its own preshock activity levels. As shown in Figure 4, rats with vSUB lesions were severely deficient in the magnitude of their activity suppression during both context and tone extinction testing, whereas sham rats showed substantial suppression. This was confirmed by a significant main effect of lesion, $F(1, 37) = 43.5, p < .0001$, in a repeated measures ANOVA with factors of lesion (SH, vSUB) and CS (context, tone). Thus, vSUB lesions not only attenuate conditional freezing, but they also impair conditional activity suppression. Together with the ANCOVA, these data suggest that rats with vSUB lesions have an associative deficit in acquiring conditional fear, rather than a performance deficit in expressing conditional fear.

Discussion

The present results indicate that either electrolytic or neurotoxic vSUB lesions produce impairments in conditional freezing in rats. These results extend and complement earlier reports on the effects of DH lesions on Pavlovian fear conditioning. Together, these data suggest that the vSUB is an important component of the neural circuitry mediating Pavlovian fear conditioning. Moreover, both neurotoxic and electrolytic lesions of the vSUB reproduced the effects of electrolytic DH lesions on contextual freezing. These results implicate damage to vSUB fibers within the DH for the pattern of deficits obtained by electrolytic DH lesions and suggest that the vSUB-NAcc pathway is important for contextual fear conditioning.

In addition to producing context freezing deficits, vSUB lesions produced a nearly complete abolition of freezing to the tone CS. This effect was independent of whether the brain lesions were made before or after training. The tone freezing impairments in this study were much larger than those observed following neurotoxic DH damage (e.g., Maren et al., 1997), but in both cases tone freezing deficits were more severe than context freezing deficits when the lesions were made before training. This pattern of results is

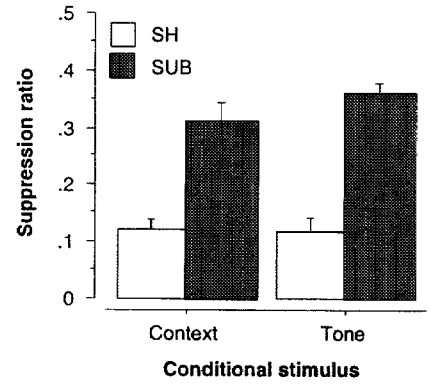

Figure 4. Activity suppression in rats with ventral subiculum (vSUB) lesions. Mean (± *SEM*) activity suppression ratios in rats that received either sham surgery (SH) or vSUB lesions (SUB) during the context and tone extinction tests.

different from that observed in rats with electrolytic DH lesions. Typically, electrolytic lesions of the DH produce context freezing deficits but spare conditional freezing to a tone CS (e.g., Kim & Fanselow, 1992; Phillips & LeDoux, 1992; but see Maren et al., 1997). It is not clear why tone freezing in rats with hippocampal formation damage is impaired in some cases but not others, but it would appear that damage to the vSUB has consequences for tone freezing that are different from those resulting from damage to the DH—tone freezing in rats with vSUB lesions is substantially lower than that observed in rats with DH lesions.

Why do rats with vSUB lesions exhibit such pronounced conditional freezing deficits? One possibility that has been raised in a recent study is that freezing deficits following hippocampal damage are the result of motor hyperactivity that competes with the freezing response (McNish et al., 1997). From this viewpoint, rats with vSUB lesions exhibit attenuated freezing (to both tones and contexts) because they cannot stand still. We have argued that this response competition hypothesis cannot account for the pattern of results obtained with DH lesions (see Maren et al., 1998). Indeed, applied to the present data, it is difficult to imagine how simple response competition could produce a near-complete freezing deficit in rats with posttraining lesions, yet a rather modest deficit (at least to context) in rats with pretraining lesions. If the motor hyperactivity induced by vSUB lesions were simply to interfere with performance of freezing, one would not expect this to vary as a function of when the brain lesions were made with respect to training. Moreover, the existence of a significant effect of vSUB lesions after the variance associated with preshock motor activity was taken into account and the impaired activity suppression in rats with vSUB lesions suggests that they have an associative deficit in fear conditioning. The motor hyperactivity observed in rats with vSUB lesions may be due to a failure of these rats to habituate exploration in a novel environment, which has been observed in rats with DH lesions (e.g., Blanchard et al., 1977; Mitchell, Maren, & Hwang, 1993). As in rats with DH lesions, this habituation deficit may be due to an inability of rats with vSUB lesions to form contextual representations during exploration.

We have suggested that the deficits obtained following vSUB lesions arise from a disconnection of the vSUB and NAcc. It is also possible that the effects of vSUB lesions on fear conditioning are due to a disruption of vSUB input to the amygdala. Ventral subicular projections to the basolateral amygdala may be the pathway by which information about contextual CSs is transmitted to the amygdala (Maren & Fanselow, 1995). However, to account for both the tone and context conditioning deficits in rats with vSUB lesions one must assume that the vSUB also has a more general role in modulating memory functions in the amygdala. There is some precedence for this view insofar as lesions placed in other components of the hippocampal system affect the expression of fear to both contexts and tones. For example, posttraining lesions of the perirhinal cortex produce robust deficits in conditional fear to both acoustic and contextual CSs (Campeau & Davis, 1995b; Corodimas & LeDoux, 1995; Rosen et al., 1992). In this case, it has been suggested that the perirhinal cortex is not an obligatory CS pathway for tone or context information but may play a more general role in regulating memory retrieval in the amygdala. Likewise, the vSUB may also regulate the associative functions of the amygdala independent of CS modality.

Finally, an interesting outcome of this study was that pretraining vSUB lesions produced weaker impairments in contextual freezing than did posttraining lesions. A similar pattern has been observed in rats with neurotoxic DH lesions (Maren et al., 1997) and, more recently, in mice with electrolytic DH lesions (Frankland, Cestari, Filipkowski, McDonald, & Silva, 1998). The relatively weak impairment in contextual freezing following pretraining lesions might be due to rats with vSUB lesions adopting a different strategy to acquire contextual fear. We have argued that contextual representations (and associations based on those representations) can be acquired using elemental or configural strategies, with only the latter requiring the hippocampal system (Maren et al., 1997). Thus, rats with vSUB lesions may use the hippocampus-independent, elemental strategy to acquire contextual fear. Similarly, posttraining vSUB lesions should be devastating to contextual fear, assuming that configural strategies are used by intact rats. Based on this logic, however, it is not clear why rats with vSUB lesions would still be deficient in acquiring fear to a tone CS. Indeed, a tone is presumably composed of fewer elements than a context. One possibility is that rats with vSUB lesions, unlike intact rats, require contextual cues to retrieve fear memories for tone CSs, regardless of how contextual representations are acquired (e.g., Corodimas & LeDoux, 1995). Additional studies are required to determine if the vSUB has such a role in retrieving fear memories.

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Received August 13, 1998
Revision received September 29, 1998
Accepted October 26, 1998 ■