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Differential Contribution of Dorsal and Ventral Medial Prefrontal Cortex to the Acquisition and Extinction of Conditioned Fear in Rats

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The emotional reactivity of rats with lesions of the dorsal portion of medial prefrontal cortex (mPFC) was examined using a classical fear conditioning paradigm. Conditioned fear behavior (freezing responses) was measured during both the acquisition and extinction phases of the task. Lesions enhanced fear reactivity to both the conditioned stimlulus (CS) and contextual stimuli during both phases, suggesting that dorsal mPFC lesions produce a general increase in fear reactivity in response to fear conditioning. M. A. Morgan, L. M. Romanski, and J. E. LeDoux (1993) found that lesions just ventral to the present lesions had no effect during acquisition of the same task and prolonged the fear response to the CS (but not the context) during extinction. Thus, both dorsal and ventral regions of mPFC are involved in the fear system, but each modulates different aspects of fear responsivity.

The prefrontal cortex contains a number of anatomically and functionally distinct subregions, one of which is the midline area, the medial prefrontal cortex (mPFC; see Kolb, 1990, for review). There is now strong evidence that mPFC is involved in emotional processes (Divac, Mogenson, Blanchard, & Blanchard, 1984; Frysztak & Neafsey, 1991; Holson, 1986; Kolb, 1974; Markowska & Lukaszewska, 1980), particularly in the aversive domain (Al Maskati & Zbrozyna, 1989). It is part of the circuitry involved in modulating cardiovascular (Powell, Watson, & Maxwell, 1994), dopamine (Thierry et al., 1994; Thierry, Tassin, Blanc, & Glowinski, 1976), and ACTH/corticosterone (Diorio, Viau, & Meaney, 1993) responsivity to aversive stimuli. Moreover, mPFC projects to nuclei of the amygdala and anatomically related brainstem areas (Berendse, Galis-de Graff, & Groenewegen, 1992; Hurley, Herbert, Moga, & Saper, 1991; Ottersen, 1982; Terreberry & Neafsey, 1983, 1987; van der Kooy, Koda, McGinty, Gerfen, & Bloom, 1984). These regions are known to be involved in the acquisition and/or expression of fear conditioning (Davis, 1992; Kapp, Wilson, Pascoe, Supple, & Whalen, 1990; LeDoux, 1987, 1992), one of the most widely used techniques for studying aversive emotional reactions.

Behavioral studies have produced conflicting results concerning the function of mPFC in fear conditioning, with lesions giving rise to increases (Frysztak & Neafsey, 1994; Holson, 1986; Morgan, Coons, & LeDoux, 1993; Morgan, Romanski, & LeDoux, 1993), decreases (Frysztak & Neafsey, 1991), or no change (Divac et al., 1984) in fear reactivity. It is possible that the contradictory findings are related to the placement of lesions in different aspects of mPFC. Anatomical studies have shown that the connections of mPFC progressively change over its dorsal–ventral extent (Sesack, Deutch, Roth, & Bunney, 1989), and it has been divided into dorsal and ventral regions on the basis of connectivity and functional observations (Neafsey, Terreberry, Hurley, Ruit, & Frysztak, 1993). Damage to different functional subregions may contribute to the discrepant behavioral findings.

In a previous study (Morgan, Romanski, & LeDoux, 1993), we examined the contribution of the ventral portion of mPFC to both the acquisition and extinction of emotional reactions during classical fear conditioning. We found that lesions of ventral mPFC had an effect on the extinction phase but not the acquisition phase of a fear conditioning task. In the present study, using the same behavioral task, we examined the effects of lesions of the dorsal portion of mPFC to determine whether it is also involved in overt reactivity to fear conditioning and, if so, to determine whether its contributions to fear conditioning are distinct from those of ventral mPFC.

Method

Anatomical Terminology

In the rat, the frontal midline area is divided into a number of distinct cortical regions. On the basis of Paxinos and Watson's (1986) terminology, from dorsal to ventral, these regions include cingulate area 1 (Cg1), cingulate area 3 (Cg3), infralimbic (IL), and medial orbital (MO) cortices. Cg1 is also referred to as area 24b or dorsal anterior cingulate cortex (ACd), Cg3 is variously called area 32 or prelimbic cortex (PL), and IL is also called area 25. A number of authors have further divided PL into dorsal and ventral subregions based on efferent connections (Sesack et al., 1989), afferent inputs from the hippocampal formation (Jay & Witter, 1991), and thalamic inputs (Berendse & Groenewegen, 1991). In the present lesion studies we have divided the mPFC only into dorsal and ventral regions. The dorsal mPFC (mPFCd) includes rostral Cg1 and the dorsal half of PL, and the ventral mPFC (mPFCv) includes the ventral half of PL, IL, and MO. A similar partition was made by Neafsey et al. (1993). Morgan, Romanski, and LeDoux (1993) made lesions of mPFCv, and in the present study we lesioned mPFCd.

Animals and Surgical Procedure

Male Sprague-Dawley rats, weighing 250-275 g on arrival, were housed in pairs for 5 to 7 days in a colony room where they had

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unlimited access to rat chow and water and were exposed to a 12-hr light–dark cycle. Five to 7 days after arrival, rats were randomly assigned to two groups: mPFCd-lesioned (n = 22), and control (n = 17). Lesioned animals were anesthetized with ketamine (100 mg/kg) and Rompun (5 mg/kg) and placed in a stereotaxic frame. The skull was exposed, and a hole over the mPFC was made using a dental drill. Coordinates (in millimeters relative to the interaural line) were AP = 12, ML = 0.6, and DV = 6.8 (Paxinos & Watson, 1986). An epoxy-coated, stainless steel insect pin (500-μm exposed tip) was lowered into the brain, and anodal constant current (1 mA) was applied for 10 s. All lesions were bilateral. The electrode was removed, the wound was sutured, and each rat was put in its own cage and returned to the colony room to recover. Control rats were treated in the same way except that no electrode was used. All rats were housed individually for the remainder of the experiment, which commenced 2 weeks after surgery.

Apparatus and Behavioral Procedures

Conditioning and extinction. All stages of experimentation took place in a single behavioral room. The apparatus and procedures have been described elsewhere (Morgan, Romanski, & LeDoux, 1993; Phillips & LeDoux, 1992) and are only summarized here. Rats were randomly assigned to one of two identical conditioning boxes (Coulbourn Instruments, Lehigh Valley, PA, Model E10-10) contained within sound-attenuating chambers (Model E10-20). A houselight (Model E11-01, 14v) was continuously on within the box, and a speaker (Model E12-01) through which the conditioned stimulus (CS) was delivered was mounted on the front wall. The unconditioned stimulus (US) was delivered through a grid floor (Model E10-10SF) attached to a grid floor shocker (Model E13-08). CS and US delivery were controlled by a personal computer (IBM 8086). The sound-attenuating chamber contained a peephole in its door through which the experimenter observed the rat's activity.

Prior to conditioning–testing on each day, rats were carried in their home cages, to a room adjacent to the behavioral room, where they remained in their cages for 20 to 55 min (depending on running order, which was randomly assigned each day) before the experiment began. This outer room was separated from the testing room by a sound-attenuating wall and a door sealed with a sound barrier to prevent any testing noises from reaching the waiting rats.

The experiment began on Day 0 with a 20-min period of exposure to the conditioning box, during which the computer and all other equipment were turned on but no explicit CS or US was presented. Rats were observed during this period, and general activity level and number of fecal boluses produced were recorded. Days 1 and 2 of the experiment were conditioning days, and consisted of two CS–US pairings on each day. The rat was placed in the chamber, and 90 to 210 s later a visual cue on the computer monitor signaled to the experimenter that the pre-CS (context test) time was beginning. After 20 s, the CS (a 20-s, 10-kHz, 80-dB tone) was presented and coterminated with the US (a 0.5-s, 0.5-mA shock delivered through the grid floor). Trial 2 was the same. Thirty seconds after the second CS–US offset, the rat was removed from the conditioning chamber, placed in its home cage, and transferred to the outer room and remained there until testing was completed on all rats. The chamber was wiped out with soap and water and thoroughly dried between rats. From Day 3 onward, the procedures for testing days were exactly the same as for conditioning days except that the US was never presented. Testing continued, with two CS presentations a day, until rats reached the criterion for extinction.

Freezing behavior was used as the measure of conditioned emotional responding (Blanchard & Blanchard, 1969; Bouton & Bolles, 1980; Fanselow, 1980; LeDoux, Sakaguchi, & Reis, 1984), and was assessed by observing the rat's behavior in the conditioning box. Stopwatches were used to time the total amount of freezing. Freezing was measured during the 20 s prior to the CS and during the 20-s CS to obtain a measure of conditioned fear both to the context in which conditioning took place and to the explicit CS. Only data from the first trial of each day were used, to avoid the possibly confounding effects of shock and CS delivery on responding during the second trial. Daily trials terminated only after the rat reached the extinction criterion of two consecutive days of 5 s or fewer spent freezing during the pre-CS and CS periods.

US test. For the final replication of the experiment, we also tested sensitivity to the US. Three weeks after the last rat had reached extinction criterion on the above task, lesioned (n = 5) and control (n = 4) rats were tested for their responsivity to a US alone. To reduce any effect of contextual conditioning, testing took place in a novel conditioning chamber that was not enclosed in a sound-attenuating chamber. All animals actively explored the box during the period before shock delivery, suggesting that they were not conditioned to contextual stimuli present in this test situation. Ten 1-s shocks of intensities ranging from 0.1 mA to 1 mA (in 0.1-mA increments) were delivered in ascending succession 45 s apart. The intensities at which the rat first appeared to notice, first flinched, and first jumped in response to the shock were recorded. The rat was removed from the box immediately after receiving the last shock.

Histology

Following completion of behavioral testing, rats were given an overdose of chloralhydrate (4%, 1 cc/100 g) and were perfused with 100 ml of saline followed by 500 ml of 10% buffered formalin. Brains were removed from the skull and postfixed in buffered formalin with 15% sucrose. Brains were then frozen and cut into 30-μm sections with a cryostat, with every fourth section before and after the lesion site and every section through the lesion site mounted on acid-cleaned gelatin-coated slides. All mounted tissue was then stained with thionin (0.5%). Lesion placement was verified by microscopic examination, and all lesion boundaries were traced.

Results

Histology

Figure 1 depicts a typical mPFCd lesion, with damage centered on rostral Cg1 and dorsal PL. All bilateral lesions that were centered in the rostral Cg1/PL region and that did not encroach noticeably on other areas (e.g., IL and MO) were included in statistical analyses. Two rats that sustained only unilateral damage to mPFCd were excluded from the study.

Acquisition

The amount of freezing elicited by exposure to the context and to the CS over Days 1–4 was used to measure fear acquisition. A 2 × 2 × 4 analysis of variance (ANOVA) of Lesion Group (mPFCd n = 18, control n = 16)[1] × Stimulus Type (pre-CS and CS) × Acquisition Day (Days 1–4) was performed, with stimulus type and day as repeated measures. The analysis resulted in a main effect of day, $F(3, 96) = 145.08$,

[1] One lesioned and 1 control rat failed to learn the task, and 1 lesioned rat froze substantially to the CS on Day 1 before the US was ever presented; thus, these 3 rats were not included in statistical analyses.

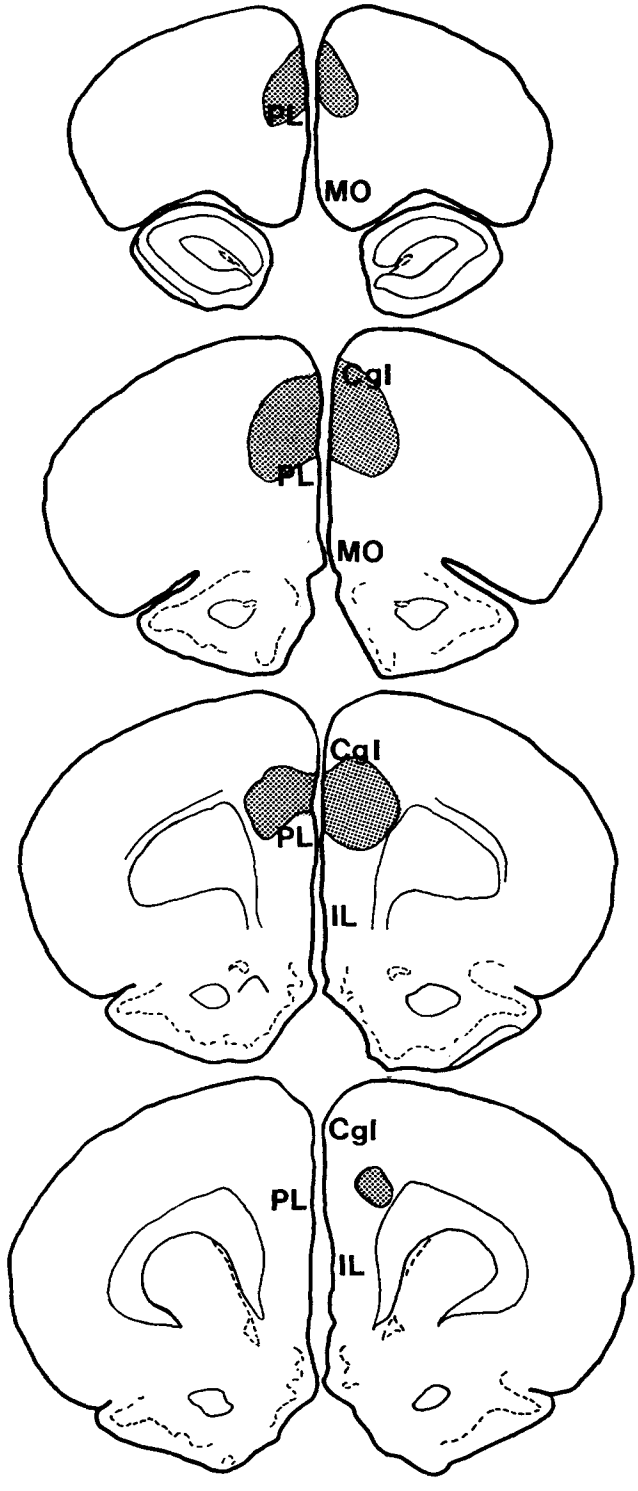

Figure 1. Medial prefrontal cortex lesions. The dorsal portion of medial prefrontal cortex (mPFCd) as defined here includes the dorsal cortical areas lying along the medial wall of the anterior frontal lobe, specifically dorsal prelimbic cortex (PL; cingulate area 3 [Cg3]) and rostral cingulate area 1 (Cg1). A typical dorsal mPFC lesion is shown here (stippling). The area depicted includes the zone of gliosis as well as the lesion cavity. PL = prelimbic cortex; MO = medial orbital cortex; and IL = infralimbic cortex.

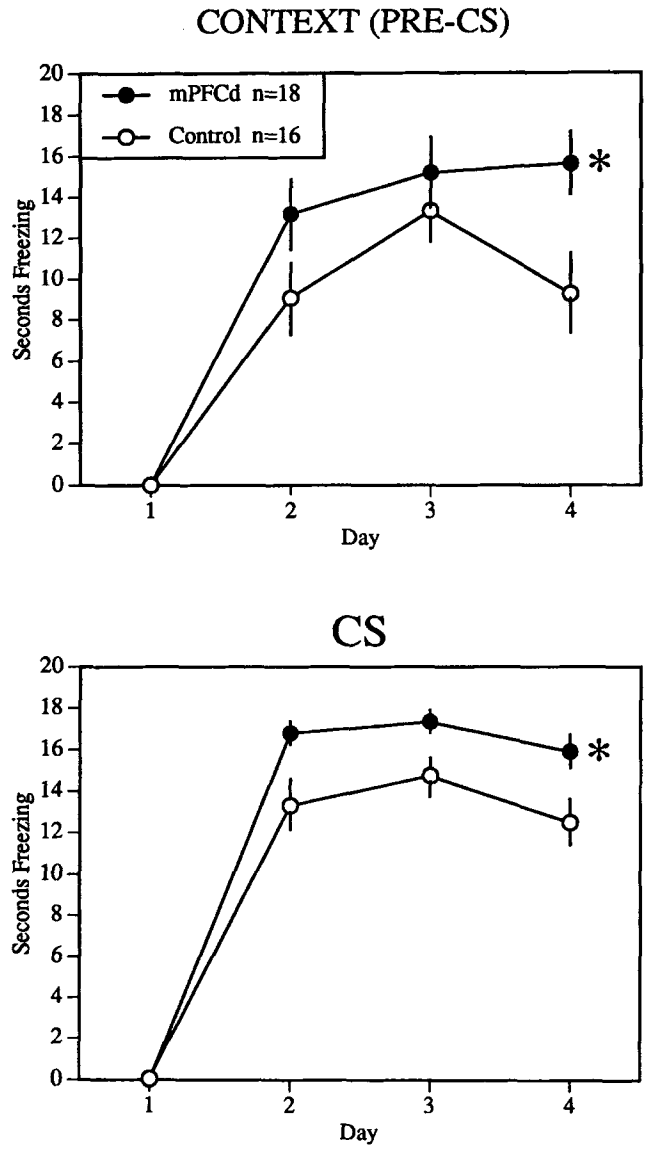

Figure 2. Acquisition of fear. Mean number of seconds spent freezing during an exposure to the conditioning context (pre-CS) and to the conditioned stimulus (CS) is shown. Freezing was measured during the 20 s prior to the onset of the conditioned stimulus (context test) and during the 20-s CS (CS test) on each day. Freezing responses during the first trial of Days 2 and 3 reflect the effects of conditioning trials on Days 1 and 2 (US presented). mPFCd = rats with lesions to the dorsal portion of the medial prefrontal cortex. *$p < .01$.

$p < .001$, indicating that acquisition took place. This can be seen most prominently in the change in the amount of freezing from Day 1 to Day 2 (see Figure 2). There was a main effect of lesion, $F(1, 32) = 8.59, p < .01$, and a main effect of stimulus type, $F(1, 32) = 6.66, p < .05$, but no Lesion × Stimulus Type interaction, $F(1, 32) = .26$, $p = .62$. Thus, lesioned rats exhibited more freezing than did controls, and both groups generally froze more to the CS than to the context. There was also an interaction of Day × Lesion, $F(3, 96) = 76.79, p < .05$, which was presumably due to the greater increase in freezing

from Day 1 to Day 2 by lesioned rats than by controls, and to the decrease on Day 4 by controls to context. There was not a significant interaction of Stimulus × Day, indicating that the actual rate of acquisition did not differ for the two stimulus conditions, though the main effect of stimulus mentioned above indicates that the amount of freezing to the CS was generally greater than freezing to context. The three-way interaction of Stimulus × Day × Lesion was not significant. To summarize, all rats tended to freeze more to the CS than to context, and lesioned rats exhibited more freezing overall to both stimulus types than did controls.

It became clear during a pilot experiment that mPFCd-lesioned rats were frequently at ceiling during training and testing. As another means of evaluating freezing during acquisition, we also calculated the number of times each rat responded at ceiling, that is, responded maximally during the 20-s intervals in which we measured behavior. This calculation was made to see the extent to which some rats' freezing behavior was not allowed to be expressed due to the 20-s time limit imposed by the experimental design. We set a criterion of 18 to 20 s spent freezing during a stimulus-measurement period as being "at ceiling," allowing time for the animal to initially orient to the stimuli and for experimenter error. We looked only at the first trial of Days 2 through 4 to make these analyses comparable with the previous acquisition analyses (with the exclusion of Day 1, because animals were not exhibiting conditioned freezing yet) and again to avoid the possibly confounding effects of shock and CS delivery on responding during the second trial. Because the number of times at ceiling was similar for the pre-CS and CS, we combined these data. Thus, the maximum possible number of times at ceiling was 6 (3 days × 2 stimulus types per day in Trial 1). Lesioned rats were at ceiling a mean of 3.44 out of six possible measurement periods, or 57% of the time, whereas controls were at ceiling a mean of 1.38 times, or 23% of the time. A t test showed that this difference was significant, $t(32) = 3.71, p < .01$.

Extinction

As another measure of contextual and CS learning, we looked at the number of days taken to extinguish the freezing response when the stimuli no longer signaled danger. The extinction criterion was set at 2 consecutive days of 5 s or fewer spent freezing during both the pre-CS and the CS (Figure 3). Days to extinction were examined between lesion groups and across stimulus type using a 2 × 2 ANOVA. There was a main effect of lesion, $F(1, 32) = 21.37, p < .001$, and a main effect of stimulus type, $F(1, 32) = 52.58$, $p < .001$, indicating that lesioned rats took longer than did controls to extinguish the freezing response, and that all rats generally took longer to extinguish the freezing response to the CS than to the context. There was not a significant interaction of Stimulus Type × Lesion, suggesting that the effect of increased resistance to extinction for lesioned rats was similar to both the context and the CS. In sum, lesioned rats took longer to extinguish the freezing response than did controls. In addition, by the end of Day 7, when controls typically began to reach extinction criterion, lesioned rats were at ceiling 49% of the time (a mean of 5.89 out of 12 measurement periods), whereas control rats were at ceiling only 14% of the time (a mean of 1.63 out of 12 measurement periods), $t(32) = 4.43; p < .001$.

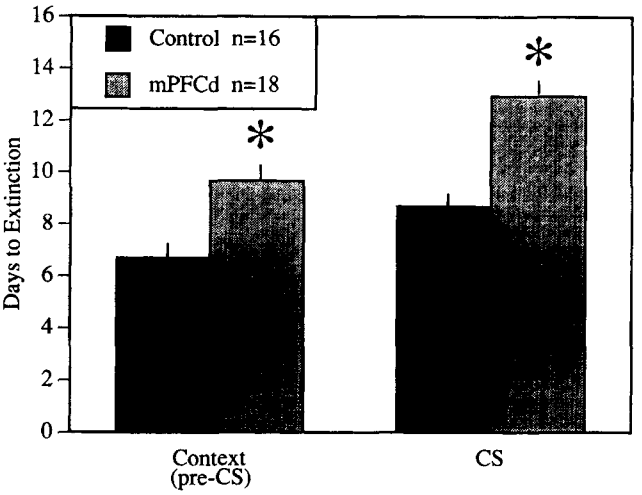

Figure 3. Extinction of fear. Mean number of days to reach criterion is shown. Extinction criterion was defined as 5 s or fewer of freezing during the conditioned stimulus (CS) and during the context test period on 2 consecutive days. mPFCd = rats with lesions to the dorsal portion of the medial prefrontal cortex. $^*p < .001$.

US Test

Our examination of nonassociative reactivity to the US involved 1-s footshocks of intensities ranging from 0.1 mA to 1 mA delivered in ascending succession 45 s apart. The amplitudes at which the rat first noticed, startled, and jumped in response to the shock were recorded. Separate t tests on the three measures (notice, startle, and jump) revealed that lesioned and control rats did not differ significantly on the notice or jump measures. They did differ significantly on the startle measure, $t(7) = 2.61, p < .05$, with controls tending to startle at lower shock intensities than lesioned rats. Thus, control and lesioned rats appear to have similar pain-response thresholds, though, if anything, controls have a lower threshold to startle.

mPFCd Versus mPFCv

Previously we examined the effect of lesions immediately ventral to the present lesions (Morgan, Romanski, & LeDoux, 1993). These mPFCv lesions, which included major portions of IL, ventral PL, and MO cortices, produced results quite different from the present dorsal lesions: Dorsal-lesioned rats showed an increase in responding to all phases of the task, whereas ventral-lesioned rats differed significantly from their controls only during the extinction phase of the task, showing an increase in resistance to extinction of responding to the CS but not to the context. A direct comparison of the two sets of findings seems appropriate here.

Of the two measures used to examine acquisition performance, amount of freezing over Days 1–4 and times at ceiling,

the latter measure is most effective in displaying the differences between the two lesion groups. Although in the present study dorsal-lesioned rats were at ceiling far more than controls, ventral-lesioned rats were not (see Figure 4A).

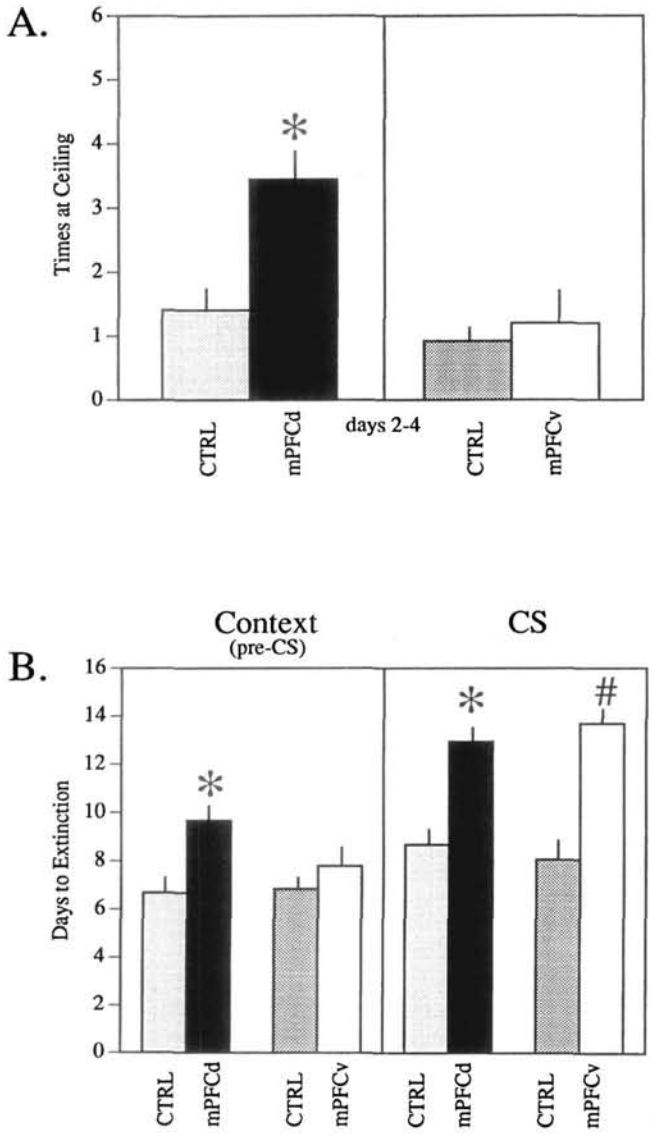

Figure 4. A: Times at ceiling: rats with lesions to the dorsal portion of the medial prefrontal cortex (mPFCd) versus to the ventral portion (mPFCv). Mean number of times at ceiling out of six possible measurement periods (see text) is shown. * mPFCd lesioned rats were at ceiling significantly more than their controls (CTRL; $p < .01$) and significantly more than mPFCv lesioned rats relative to their respective control groups ($p < .02$). B: Extinction of fear: mPFCd versus mPFCv. Mean number of days to reach extinction criterion for both lesion groups is shown. * mPFCd rats took significantly longer than did their controls to extinguish the freezing response overall (see Figure 3). # mPFCv rats took significantly longer than did their controls to extinguish the freezing response only to the CS ($p < .001$). The two lesion groups also responded significantly differently from each other relative to their controls as a function of the stimulus type ($p < .01$).

Ventral-lesioned rats were at ceiling a mean of 1.2 times ut of six possible measurement periods, or 20% of the time, whereas their controls were at ceiling a mean of 0.92 times, or 15% of the time. A t test showed that this difference was not significant, $t(33) = .59; p > .5$. A direct comparison of the two lesion types using a 2 × 2 ANOVA of Lesion Group (lesion vs. control) × Experiment (mPFCv-lesioned and their controls vs. mPFCd-lesioned and their controls) showed that dorsal-lesioned rats were freezing at ceiling significantly more often than ventral-lesioned rats, $F(1, 65) = 5.86, p < .02$.

Looking at the other measure of acquisition, amount of freezing elicited by exposure to the context and CS over Days 1–4, one can see that dorsal-lesioned rats froze significantly more than their controls ($p < .01$); ventral-lesioned rats did not ($p > .4$). A direct comparison of the two lesion areas using a 2 × 2 × 2 × 4 ANOVA of Lesion Group (lesion vs. control) × Experiment (mPFCv-lesioned and their controls vs. mPFCd-lesioned and their controls) × Stimulus Type (pre-CS vs. CS) × Day (1–4) showed that the increased freezing seen in dorsal-lesioned rats was not quite enough to produce a significant difference from ventral-lesioned rats (each relative to its own controls) when examined across all 4 days, $F(1, 65) = 2.6$, $p = .112$. However, two factors need to be considered. The times at ceiling data indicate that, in contrast to the ventral lesions, the full effect of dorsal lesions on increased freezing was not captured by this measure. In addition, rats with dorsal lesions were already freezing significantly more than their controls on Day 2, $F(1, 32) = 6.05, p < .02$, the first opportunity to see the effects of conditioning (see Figure 2). Ventral rats were not significantly elevated relative to their controls until Day 4, and then only to the CS, $F(1, 33) = 4.26, p = .047$. Because Day 4 was technically the second day of extinction testing, the increase at this point most likely reflects the beginning of their CS-specific increase in freezing during extinction rather than acquisition. Thus, dorsal-lesioned rats showed increased fear during acquisition whereas ventral-lesioned rats did not.

These two lesion groups also differ during extinction of fear responding. Morgan, Romanski, and LeDoux's (1993) study showed that ventral lesions produce an increased resistance to extinction that was present only in responding to the CS, whereas our present work indicates that dorsal lesions produce this increased resistance to both the context and the CS (see Figure 4B). An ANOVA combining both sets of data (2 × 2 × 2 of Lesion Group [lesion vs. control] × Experiment [mPFCv-lesioned and their controls vs. mPFCd-lesioned and their controls] × Stimulus Type [pre-CS vs. CS]) produced a significant three-way interaction, $F(1, 65) = 10.95, p < .01$, showing that the two lesion groups respond differently relative to their controls, depending on the stimulus type. In Figure 4B one can see that whereas mPFCv-lesioned rats were elevated only to the CS, mPFCd-lesioned rats were elevated to both the CS and context.

Discussion

In the present study, we found that lesions of dorsal mPFC increase freezing to all four aspects of the conditioning task that were measured: contextual (pre-CS) acquisition, CS

acquisition, contextual extinction, and CS extinction. Because of the lack of a particularly striking increase in any one of these components, it appears that lesions to the dorsal portion of mPFC produce a general increase in fear in response to fear conditioning.

That this increased fear is limited to the associative components of the conditioning task is suggested by several observations. It does not appear that such lesions simply produce a general change in activity level, because lesioned rats displayed a comparable amount of activity in the novel conditioning chamber on Day 0 when no explicit stimuli had yet been presented. Nor do such lesions appear to produce a nonspecific, chronic increase in fear, given that lesioned rats (a) did not produce any more fecal boluses than did controls during Day 0, (b) typically did not display any more signs of fear to the CS when it was initially presented on Day 1 prior to its pairing with the US, and (c) were not noticeably more fearful during the handling required during the experiment before testing. Similar conclusions were reached by Holson (1986) and Jaskiw and Weinberger (1992) in their studies of the effects of mPFC lesions. The US test also suggests that the lesioned rats were no more reactive to a nonassociative US than were controls, ruling out an increased sensitivity to pain as an explanation for increased freezing.

Other studies, using various manipulations and response measurement techniques, also suggest that mPFC is involved in emotion, particularly fear reactivity. Frysztak and Neafsey (1994) found that lesions of mPFC produce an increased tachycardia response to a CS previously paired with footshock. Al Maskati and Zbrozyna (1989) found that stimulation of rostral mPFC inhibits the defensive response elicited by stimulation of the amygdala or hypothalamus, while stimulation of mPFC alone produced no cardiovascular changes. Holson (1986) reported that mPFC lesions increase timidity in a situation-specific manner; lesioned rats behaved like controls under moderately stressful conditions, but they displayed increased fear under highly aversive conditions.[2] Additionally, there is a selective and high dopaminergic reactivity in this area in response to aversive situations such as mild footshock (Thierry et al., 1994, 1976). Lesions of mPFC result in significantly increased plasma levels of ACTH and corticosteroids after restraint stress (Diorio et al., 1993). Also rats with mPFC lesions are deficient in coping with aversive conditions involving more than a single exposure, as determined by gastric pathology, indicating that this area is an essential part of a coping system (Henke, 1990; Sullivan & Henke, 1986).

On the other hand, some studies have found no effect of mPFC lesions on fear reactivity. Powell et al. (1994) found that lesions of mPFCd (area 24) in rabbits did not effect the magnitude of conditioned fear, as measured by cardiovascular conditioned responses. Divac et al. (1984), using wild rats, found that lesions of mPFC had no effect on fear, and Holson (1986) found that such lesions did not produce increased freezing to a box (context) associated with footshock. However, these last two studies indicate that both control and lesioned rats were near ceiling in their respective tasks and any signs of increased fear may have been difficult to detect.

Some of the inconsistencies in behavioral and cardiovascular changes following lesions or stimulation of medial prefrontal cortex may well be due not only to the use of differing tasks and response measures but also to the area of cortex examined. On the basis of anatomical criteria, mPFC has been divided into several distinct subdivisions (see Sesack et al., 1989). However, these subdivisions have often not been explicitly acknowledged in behavioral studies, which tend to refer to the region as an undifferentiated whole. In notable exception, Frysztak and Neafsey (1994) and Powell et al. (1994) have examined the functions of discrete subdivisions of mPFC, primarily in terms of cardiovascular responding. Frysztak and Neafsey found that lesions of dorsal mPFC produced an increase in sympathetically mediated tachycardia in response to an excitatory conditioned stimulus (CS+), whereas ventral lesions decreased sympathetic activation. Powell's group looked at three subdivisions of rabbit mPFC, making lesions centered on areas 24 (ACd), 32 (PL), and 25 (IL), with some spread of the lesion sites into the other areas. They found that lesions centered in dorsal area 24 had no effect on the magnitude of the conditioned cardiovascular response but decreased discrimination between a CS+ and inhibitory conditioned stimulus (CS−; primarily by increasing responding to the CS−). Lesions centered in area 32 decreased the magnitude of the conditioned response and decreased discrimination, whereas lesions centered in ventral area 25 produced no significant changes in response magnitude or in discrimination. Although these two groups of findings are apparently somewhat incomparable due to the use of freely moving animals in Frysztak and Neafsey's work and restrained animals in Powell et al.'s work, they support the validity of viewing medial prefrontal cortex as a functionally heterogeneous area involved in various aspects of fear conditioning.

The above findings are relevant to understanding our findings about lesions of dorsal and ventral mPFC. We found that dorsal lesions produce an increased fear response to all four aspects of the task measured: CS and contextual stimuli during acquisition and extinction. Although an inability to suppress fear responses (e.g., Diorio et al., 1993; Neafsey et al., 1993) may be sufficient to explain all four changes, it may be that an inability to block out irrelevant stimuli (see Crino, Morrison, & Hof, 1993; Neafsey et al., 1993) or a decreased ability to discriminate between a CS+ and CS− (primarily displayed in increased responding to the CS−; Powell et al., 1994) contributes to the increased freezing to contextual stimuli in both the acquisition and extinction phases of the task. Furthermore, this inability to accurately identify the signal for an aversive event (i.e., the CS+, rather than CS− or irrelevant stimuli) might make the whole episode more fear provoking.

Regarding lesions of the ventral portion of mPFC, our finding of no behavioral changes during acquisition is more difficult to explain in light of Frysztak and Neafsey's (1994)

[2] The dependence of the effects of the lesion on the level of aversiveness of the stimuli may be related to our finding that when the intensity of the US is 0.3 mA instead of 0.5 mA, half of the lesioned rats behaved like controls and half showed the full-blown increased freezing response (Morgan, Coons, & LeDoux, 1993), suggesting that mPFCd-lesioned rats become increasingly more fearful than controls as the aversiveness of the situation increases beyond a certain threshold.

findings of decreased sympathetic activation and mPFCv's extensive connectivity with visceral control areas. However, Powell et al. (1994) also reported no effects on fear conditioning after lesions of ventral mPFC (area 25). Whereas neither Frysztak and Neafsey nor Powell looked at extinction, this is where we found our effects. Findings that cells in the amygdala change their response in a reversal learning task more slowly than cells in ventral PFC suggest that this area of PFC allows for the updating of behavioral responses to stimuli with changing reinforcement value (Rolls, 1992; Thorpe, Rolls, & Maddison, 1983). It has also been reported that rats with ventral frontal lesions will continue making previously reinforced responses (e.g., bar presses for food) long after the response is no longer reinforced (see Kolb, 1984). It may be that ventral lesions allow for normal acquisition of the task but prevent readjustment in responding to the CS when it no longer signals danger, thus explaining the increased resistance to extinction. This is reminiscent of the response perseveration found in humans after damage to frontal cortex (Fuster, 1989; Goldman-Rakic, 1987) and may be an example of extending response perseveration into the emotional domain (Morgan, Romanski, & LeDoux, 1993).

In the cognitive domain, rats with mPFC lesions are frequently impaired on working/representational memory type tasks (see Brito & Brito, 1990; Kolb, 1990), particularly if they contain a spatial or delay component. However, despite the substantial connectivity of the mPFC with the hippocampus (Jay & Witter, 1991), it is believed that it is not the spatial component of the task that disrupts performance (Kolb, 1990; de Bruin, Sanchez-Santed, Heinsbroek, Donker, & Postmes, 1994) but rather an inability to shift cognitive strategies (Brito & Brito, 1990) or a reduction in behavioral flexibility (de Bruin et al., 1994; Kolb, 1990). These conclusions seem compatible with the aforementioned idea that a decreased ability to appropriately adjust responding to changing stimulus values and decreased response inhibition are consequences of mPFC lesions.

What are the underlying mechanisms by which mPFC might be exerting its influence? It is by now well-known that the amygdala is a crucial component in the neural system involved in the acquisition and expression of fear, presumably by determining the emotional significance of threatening stimuli (Davis, 1992; Kapp et al., 1990; LeDoux, 1987, 1992). The mPFC has extensive reciprocal connections with the amygdala, as well as projections to several areas in the brainstem (to which the amygdala also projects) that are involved in the expression of conditioned fear (Berendse et al., 1992; Hurley et al., 1991; Terreberry & Neafsey, 1983, 1987; van der Kooy et al., 1984). It has been suggested that the mPFC monitors the internal state of the organism (see Damasio, 1994; Frysztak & Neafsey, 1994; Neafsey et al., 1993; Vogt, Sikes, & Vogt, 1993) and that one of its functions is to initiate motor output accordingly (Musil & Olson, 1993; Vives & Mogenson, 1985; Vogt et al., 1993). One possible scenario is that while the amygdala determines the emotional significance of threatening stimuli, mPFC uses this information to monitor and give feedback about the internal state of the animal and to update appropriate response outputs dependent on this internal state. Without the internal feedback as to the level of threat posed by the stimulus at any given time, the animal might, for adaptive purposes, remain in the defensive response state longer than necessary. This inability to coordinate behavior with the actual threat value of the stimulus could exhibit itself in two ways: by modulating the magnitude of the response in a particular threat session or across several sessions. One can see the response pattern corresponding to the former case with lesions of dorsal mPFC, exhibited through increased freezing during each session (e.g., see times at ceiling data). The response pattern corresponding to the latter case is exhibited in animals with lesions of ventral mPFC, where the animals continue to freeze at moderate levels across a number of test days. Our findings add strength to the view that subdivisions of mPFC are involved in different aspects of fear conditioning and further suggest various aspects of fear learning and extinction that need to be examined.

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