Contribution of Ventrolateral Prefrontal Cortex to the Acquisition and Extinction of Conditioned Fear in Rats

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The ventrolateral, agranular insular portion of prefrontal cortex (PFC) in rats is involved in visceral functions and has been shown to be involved in emotional processes. However, its contribution to aversive learning has not been well defined. Classical fear conditioning has been a powerful tool for illuminating some of the primary neural structures involved in aversive emotional learning. We measured both the acquisition and the extinction of conditioned fear following lesions of the ventrolateral PFC of rats. Lesions reduced fear reactivity to contextual stimuli associated with conditioning without affecting CS acquisition, and had no effect on response extinction. Ventrolateral PFC may normally be involved in the processing of contextual information while not being directly involved in extinction processes within the aversive domain.

Key Words: ventrolateral prefrontal cortex; fear conditioning; acquisition; extinction; context.

The ventrolateral, agranular insular cortex (AI) lining the dorsal bank of the rhinal sulcus has been described as the visceral-sensory region of prefrontal cortex (PFC; Cechetto & Saper, 1987), in part due to inputs from parabrachial nucleus and outputs to the nucleus of the solitary tract (Groenewegen, Berendse, Wolters, & Lohman, 1990). It has a number of other projections to brain stem and spinal cord autonomic regions (Groenewegen, 1988; Kapp, Schwaber, & Driscoll, 1985; Neafsey, Hurley-Gius, & Arvanitis, 1986) and stimulation here leads to changes in blood pressure and heart rate (Hardy & Holmes, 1988; Powell, Buchanan, & Hernandez, 1985). Lesions of AI have resulted in deficits in aversive classical conditioning (Hankins, Garcia, & Rusiniak, 1974), inhibitory avoidance, and conditioned taste aversion (CTA; Bermudez-Rattoni & McGaugh, 1991). It has been likened to primate orbital cortex (Leonard, 1969), a conclusion that has gone largely unchallenged (e.g., Groenewegen, 1988; Preuss, 1995). This is due in part to similarities in its connectivity, e.g., with the medial portion of MD thalamus (Groenewegen, 1988; Groenewegen et al., 1990) and with the amygdala (Sarter & Marko-
witsch, 1984) and its implication in emotional processes (Nonneman, Voigt, & Kolb, 1974). Both primate orbital cortex and rat sulcal, ventrolateral prefrontal cortex have also been implicated in the extinction of appetitively motivated bar-press responses (Butter, 1969; Kolb, Nonneman, & Singh, 1974), suggesting that damage in this region may contribute to the decreased ability to adopt new appropriate response strategies to changing stimulus values, or the perseveration so typical of frontal damage (Kolb et al., 1974; Nonneman et al., 1974). As part of a series of experiments examining the role of PFC subregions in fear (Morgan & LeDoux, 1995), in the present study we looked at the effect of agranular insular/lateral orbital (AI/LO) lesions on both the acquisition and the extinction of classical fear conditioning.

Male Sprague-Dawley rats were randomly assigned to two groups: AI/LO lesion and control. Lesions were bilateral, with two lesions per side. Coordinates for AI/LO (measured in mm relative to the interaural line and with the surface of the skull level) were 13.0–13.2 anterior, 2.8–3.2 lateral, and 5.1–5.6 dorsal; 11.5–11.8 anterior, 3.8–4.2 lateral, and 3.7–4.8 dorsal. An epoxy-coated, stainless-steel insect pin (500-μm exposed tip) was lowered into the brain and anodal direct current (1 mA) was passed for 8–12 s at each site. Control rats were treated in the same way as lesion rats except that no electrode was lowered into the brain. After 14 days of recovery, the animals were trained in a classical fear conditioning paradigm involving the presentation of a tone conditioned stimulus (CS; 10 kHz tone, 80 dB, 20 s) coterminal with a foot shock unconditioned stimulus (US; 0.5 mA, 500 ms delivery of direct current produced by a grid floor shocker). We measured freezing behavior as the conditioned emotional response (Bouton & Bolles, 1980). Animals received two CS-US pairings a day for 2 days, after which the CS was presented alone. Freezing was measured during the 20 s CS and during the 20 s prior to the CS (pre-CS, or context), allowing assessment of the extent of conditioning to the CS and to the context in which CS-US pairing took place, respectively (Phillips & LeDoux, 1992). Testing continued daily until extinction criterion was met.

Following completion of behavioral testing, rats were given an overdose of chloral hydrate (4%, 1 cc/100 g) and were perfused with 100 ml of saline followed by 500 ml of 10% buffered formalin. Brains were frozen and cut into 40-μm sections, with every third section mounted on acid-cleaned gelatin-coated slides and stained with thionin. Lesion placement was verified by microscopic examination, and all lesion boundaries were traced. Lesion site volumes were estimated, using a 0.5 × 0.5-cm grid, in comparison to a nonlesioned control brain analyzed at equivalent levels rostral-to-caudal to typical lesioned brains.

Eleven animals received bilateral lesions that included extensive, if not complete, damage to AI cortex rostral to the genu of the corpus callosum, with extensive spread of the lesion into the medially adjacent LO cortex; there was some spread of the lesion into dorsally adjacent cortex. A typical AI/LO lesion, depicted in Fig. 1A, included 86–95% destruction of the AI region rostral to the genu of the corpus callosum, and closer to 50% destruction at the level of the genu. Damage to LO started slightly more caudally and ranged rostrally to caudally from 50–83% destruction. Nine animals received bilateral lesions in tissue dorsal to AI that largely spared AI/LO cortex, though some cases had damage to most rostral AI. A typical dorsal lateral prefrontal cortex (IPFCd)
FIG. 1. Lateral prefrontal cortex lesions. (A) A typical agranular insular/lateral orbital cortex (AI/LO) lesion is shown here. (B) A typical dorsal lateral prefrontal cortex control lesion is shown here. The areas depicted include the zone of gliosis as well as the lesion cavity. See text for a more detailed description of lesion sites. AI, agranular insular cortex; Cg, cingulate cortex; LO, lateral orbital cortex; MO/VO, medial orbital/ventral orbital cortex; Fr, frontal cortex; IL, infralimbic cortex; Par 1, parietal cortex, area 1; VO, ventral orbital cortex.
FIG. 1—Continued
lesion, depicted in Fig. 1B, included destruction to 31–68% of more rostral AI, and spared it more caudally. LO received less than 20% damage most rostrally and was otherwise spared. These animals were used as a separate control-lesion group, called lPFCd control. All lesion-group assignments were made without knowledge of individual rats’ performances. Thus three groups were included in statistical analyses: AI/LO lesions (n = 11), IPFCd control lesions (n = 9), and controls (n = 26).

Days 1 and 2 of the experiment were conditioning days. The effects of conditioning on a given day were defined by the amount of freezing during the first trial of the following day (Phillips & LeDoux, 1992). Changes in freezing over Days 1–3 thus reflect fear acquisition. A repeated measures 3 x 2 x 3 ANOVA of lesion group (AI/LO, IPFCd, control) by stimulus type (pre-CS, CS) by acquisition day (Days 1–3) resulted in main effects of stimulus type (F(1, 43) = 40.496, p < .001), and day (F(2, 86) = 330.404, p < .001), and interactions of stimulus type by lesion (F(2, 43) = 6.282, p < .01), stimulus type by day (F(2, 86) = 35.522, p < .001), and stimulus type by day by lesion (F(4, 86) = 2.496, p < .05). This indicates that animals acquired the freezing response more rapidly to the CS than context, but that lesion groups differed in their rates of acquisition depending on the stimulus type. We conducted three follow-up 2 x 2 ANOVAs of lesion group by day (Days 2 and 3 only, since the effects of conditioning cannot be measured until Day 2) only for the context and found that the AI/LO group differed from the control group (F(1, 35) = 7.464, p < .01), and from the IPFCd group (F(1, 18) = 7.056, p < .02), but the IPFCd group did not differ from the controls (p = .687). In none of the analyses was there a significant interaction of day by lesion. Thus, AI/LO-lesioned rats froze less than control and IPFCd groups to the context over Acquisition Days 2 and 3, and this reduced freezing on Day 2 was not significantly greater than the reduced freezing on Day 3. In sum, animals in the AI/LO lesion group exhibited a smaller increase in the amount of freezing expressed during the pre-CS context period over Days 1–3 than did the IPFCd or control groups (see Fig. 2A); animals in all three groups conditioned to the CS at the same rate as each other (see Fig. 2B).

Days taken to reach extinction criterion (2 consecutive days of 5 s or fewer spent freezing during CS and pre-CS) were examined between groups and across stimulus type (pre-CS, CS) using a 3 x 2 ANOVA. There was only a main effect of stimulus type (F(1, 43) = 29.379, p < .001), indicating that all animals took longer to give up the freezing response to the CS than to the context, but that no group differed significantly from the others (see Fig. 3). Thus all groups extinguished the freezing response to both the context and CS at approximately the same time.

The present results show that lateral PFC may be involved in the acquisition or expression of conditioned responding to contextual stimuli. While significant, the decreased freezing by AI/LO-lesioned rats is not a complete elimination of contextual conditioning as has been demonstrated with hippocampal-lesioned rats in the same behavioral paradigm (Phillips & LeDoux, 1992). Figure 2A shows that AI/LO-lesioned rats froze substantially to the context by Day 3, though less than IPFCd and control groups. The extinction results reflect this. While AI/LO-lesioned rats appear to take slightly less time to extinguish the conditioned response overall than the control and IPFCd
groups, the effect is not significant. Thus, the extinction results seem simply to reflect the somewhat reduced freezing during acquisition.

A possible explanation for this decreased freezing to context is that AI/LO is strongly associated with olfactory structures, and damage here has been shown to interfere with the acquisition of odor discrimination (Eichenbaum, Shedlack, & Eckmann, 1980). Schoenbaum and Eichenbaum (1995) suggested that this region of PFC may provide odor-based contextual information during learning. Reduced contextual salience is consistent with a reduced rate of
contextual conditioning. It may be that our AI/LO-lesioned animals are less efficient at identifying contextual cues, which may be primarily olfactory for rats. Alternatively, contextual conditioning may be more generally susceptible to the disruptive effects of these PFC lesions than CS conditioning, or the task may be more sensitive to changes in contextual fear levels.

The sulcal region of PFC has a history of being associated with primate orbital cortex (Groenewegen, 1988; Leonard, 1969; Preuss, 1995), based in part on its implication in emotional processes (Bermudez-Rattoni & McGaugh, 1991; Nonneman et al., 1974) and in bar-press extinction (Butter, 1969; Kolb et al., 1974). Nonneman et al. (1974) found that lesions of lateral PFC resulted in increased emotionality relative to controls. In contrast, Powell et al. (1985) found that AI lesions attenuated the magnitude of classically conditioned bradycardia, Bermudez-Rattoni and McGaugh (1991) found that such lesions disrupted inhibitory avoidance learning (essentially a version of contextual fear conditioning), and studies have found that sulcal PFC lesions disrupt CTA (Bermudez-Rattoni & McGaugh, 1991). These results are in line with our findings of reduced fear, here specific to the context.

Our results did not support previous findings showing resistance to extinction of a bar-press response following large lateral lesions (Kolb et al., 1974). In fact, there was a mild trend toward facilitation of extinction, though this was presumably due to the decreased rate of contextual conditioning. It may be that appetitive and aversive extinction are governed by differing neural substrates. The lack of any measurable effects following the more dorsally placed cortical lesions (group IPFCd) emphasizes that the effects seen with AI/LO lesions are due to the damage in the rhinal sulcal area. They further show that a sizable amount of frontal cortex can be removed without noticeable effects. In sum, damage to lateral PFC disrupts the acquisition of conditioned fear to contextual stimuli, perhaps due to its role in olfactory functions.

**FIG. 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 and during the context test period on 2 consecutive days (see text for more details). AI/LO, rats with lesions focused in the agranular insular/lateral orbital cortex. IPFCd, cortical control rats with lesions focused in cortex dorsal to AI.
REFERENCES


