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Coping Behavior Causes Asymmetric Changes in Neuronal Activation in the Prefrontal Cortex and Amygdala

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Abstract

When faced with an inescapable stressor, animals may engage in ‘coping’ behaviors, such as chewing inedible objects, that attenuate some physiological responses to the stressor. Previous evidence indicates that dopamine neurotransmission in the right prefrontal cortex is modulated by coping processes. Here we tested whether medial prefrontal cortical (mPFC) neuronal activation, as measured by Fos-immunoreactivity (Fos-ir), was altered in rats chewing inedible objects during exposure to novelty stress. We found that chewing caused an increase in Fos-ir that was selective for the right hemisphere of the mPFC along with a decrease in Fos-ir that was selective for the right central nucleus of the amygdala (CeA), a region that may regulate dopamine neurotransmission in mPFC. These observations suggest that coping during stress engages mPFC and CeA neuronal activity asymmetrically.

Keywords

stress; hemispheric asymmetry; central nucleus of the amygdala; chewing behavior; dopamine

Stressful or challenging conditions elicit a constellation of physiological and behavioral responses. The medial prefrontal cortex (mPFC) is critically involved in the regulation of many of these responses (Amat et al., 2005; Arnsten, 1997; Jackson and Moghaddam, 2006; Sullivan, 2004). Substantial evidence indicates that dopamine (DA) plays a critical role in the stress-related actions of the mPFC (Sullivan and Gratton, 1998; Sullivan and Szechtman, 1995). In addition, a functional asymmetry exists within the PFC, with the right hemisphere being particularly responsive to aversive events (Czeh et al., 2006; Davidson, 2000). Consistent with this, stressor-induced increases in DA utilization are larger in the right hemisphere of the mPFC than in the left hemisphere (Berridge et al., 1999; Sullivan, 2004; Sullivan and Szechtman, 1995).

When exposed to an inescapable stressor, animals may engage in a limited set of “coping” behaviors, often involving oral behavior such as chewing, which act to attenuate certain components of the stress response (Berridge et al., 2002). For example, mice and rats exposed to an inescapable, novel, and brightly lit environment (novelty-stress) chew inedible material (wood, foil, etc) preferentially over highly palatable food (Berridge et al., 1999; Hennessy and Foy, 1987). Under these conditions, chewing suppresses the glucocorticoid

stress response (Hennessy and Foy, 1987). Moreover, chewing also attenuates stress-related DA utilization preferentially within the mPFC, having no noticeable effect on stressor-induced increases in DA utilization outside this region (Berridge et al., 1999). Interestingly, chewing-induced suppression of mPFC DA utilization is largely confined to the right hemisphere (Berridge et al., 1999).

Combined, these observations suggest a particularly important role of the right hemisphere of the mPFC in behavioral responding in stress and coping. Nonetheless, it remains unclear whether coping in stress significantly influences mPFC neuronal activity and, if so, whether this is lateralized. The current experiments were designed to provide an initial exploration of this issue. These studies examined the effect of chewing during stress on neuronal activity, as measured by Fos-immunoreactivity (Fos-ir), within the mPFC. In addition, Fos-ir levels were measured in the central nucleus (CeA) and the basolateral nucleus (BLA) of the amygdala, two nuclei that have been implicated in the regulation of mPFC DA utilization, particularly in stress (Ahn and Phillips, 2003; Davis et al., 1994; Goldstein et al., 1996).

Male Sprague-Dawley rats were housed in pairs for at least 14 days before testing. On the day of testing, animals were placed individually into novel, brightly lit Plexiglas chambers (46 cm × 46 cm × 21 cm) with ambient white noise played at a level of 80 dB throughout the experiment. Chambers were empty for the “no-chew” group ($n = 8$) and contained six small pieces each of foil, wood, styrofoam, and tissue paper for the “chew” group ($n = 16$) (Berridge et al., 1999; Hennessy and Foy, 1987). After 100 min, rats were removed from the testing chambers, deeply anesthetized with sodium pentobarbital (100 mg/kg), and perfused transcardially. Subsequently, the brains were removed, sectioned, and processed for Fos-immunohistochemistry using standard methods (España et al., 2003). In mounted sections, the number of Fos-ir nuclei within regions of interest in the left and right hemispheres (mPFC, CeA, and BLA) was analyzed using NIH Scion IMAGE software by examiners blind to treatment conditions from digital images captured by an Olympus OLY-750 digital video camera connected to an Olympus BX51 microscope (see Fig. 1 for capture sites). Data were analyzed using a mixed within (hemisphere)/between (treatment)-subjects ANOVA. When statistical significance ($P < 0.05$) was indicated, t -tests were used for post hoc analyses.

Behavior was scored from the videotape of the first 40-min of novelty exposure by a trained observer using a computer-based event recorder (Noldus Information, Wageningen, The Netherlands). The duration of grooming and chewing behavior and the frequency of quadrant entries and rearing were scored. Animals from the chew group who chewed less than 10 s were removed from the analysis ($n = 3$). Behavioral data were analyzed using t -tests to compare the chew ($n = 8$) vs. no-chew groups ($n = 13$).

The availability of inedible objects had no significant effect on any behavior scored except for chewing (data not shown; $t_{19} = 4.4$, $P < 0.001$).

Within the mPFC, ANOVA indicated a significant effect of chewing on the number of Fos-ir-positive nuclei (hemisphere × treatment, $F_{1,18} = 5.8$, $P = 0.027$). Chewing significantly increased Fos-ir in the right hemisphere but not the left, as shown in Figure 2 (planned comparisons: right, $t_{18} = 2.3$, $P = 0.031$; left, $t_{18} = 0.2$, $P = 0.89$). When examined in a subset of animals ($n = 14$), chewing was observed to affect Fos-ir within dorsal and ventral subregions of mPFC similarly.

As shown in Figure 3, chewing had the opposite effect in the CeA as it did in the mPFC, resulting in a statistically significant 54% decrease in the number of Fos-ir-positive nuclei within the right hemisphere and a nonsignificant 74% increase in the left hemisphere (hemisphere × treatment, $F_{1,18} = 10.6$, $P < 0.01$; planned comparisons: right, $t_{19} = 2.2$, $P = 0.044$; left, $t_{19} = 1.1$, $P = 0.27$).

Within BLA there was no significant effect of chewing on Fos-ir (data not shown; $F_s < 1.4$ $P_s > 0.26$).

Within the chew group, the amount of time spent in chewing was not correlated with Fos-ir counts in either hemisphere of any of the regions analyzed ($P_s > 0.11$).

Our previous studies demonstrated that under these experimental conditions, a nonescape coping behavior (chewing) preferentially decreases DA utilization in the right hemisphere of the mPFC of animals exposed to novelty stress. The current study was designed to investigate whether this coping behavior affects PFC neuronal activity. The results indicate that stress-related coping increases neuronal activity within the right hemisphere of the mPFC. The lateralization of the effect of coping on stress-related Fos-ir in mPFC is similar to that observed for DA utilization within this region (Berridge et al., 1999). Thus, within the mPFC, coping-induced alterations in Fos-ir and rates of DA release in stress are inversely related and both localized to the right hemisphere. Combined, these observations suggest that coping-induced reductions in DA release may contribute to coping-induced increases in mPFC neuronal activity in stress (Ferron et al., 1984). Such an action of DA is consistent with reports indicating that PFC catecholamines attenuate stress-related physiological and behavioral responding (Sullivan, 2004).

Pharmacological activation of the CeA, but not the BLA, increases DA efflux within the mPFC (Berridge et al., 2002; Stalnaker and Berridge, 2003). Thus, the current studies also examined the effects of coping on stress-related neuronal activity within both the CeA and BLA. Chewing did not affect Fos-ir within either hemisphere of the BLA. In contrast, chewing was associated with a large decrease in Fos-ir within the right hemisphere of the CeA. Combined with observations reviewed above, these observations suggest the possibility that chewing-induced suppression of the right hemisphere CeA may result in reduced drive on DA utilization within the right mPFC, which in turn influences stress-related neuronal activity within this hemisphere of the mPFC. Additionally, the CeA projects to a variety of nondopaminergic systems that could influence the PFC, including noradrenergic and serotonergic systems (Price and Amaral, 1981; Van Bockstaele et al., 1998). Chewing-related alterations in the activity of CeA projections to these regions could also influence mPFC neuronal activity.

The significant effects of chewing during stress in this and prior studies were localized to the right hemisphere of the mPFC and CeA. This accords with previous findings that the right hemisphere is in general preferentially involved in stress-related responding (Adamec, 2000; Coleman-Mesches and McGaugh, 1995; Davidson, 2000; Sullivan, 2004). However, chewing also appeared to cause a large, though non-significant, increase in Fos-ir in the left hemisphere of the CeA. Such an action would be consistent with studies that have found a reciprocal or mutually inhibitory relationship between the left and right amygdala (Adamec and Morgan, 1994).

While the functional significance of chewing behavior during stress is unclear, the current studies demonstrate a significant impact of this behavior on neuronal physiology within the mPFC. Given the pivotal role of the PFC in behavioral and physiological responding in stress, it would appear that coping-related alterations in mPFC neuronal activity could impact a variety of behavioral/physiological processes. Future studies will need to explicitly examine the impact of nonescape coping behavior on cognitive and affective responding in stress.

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Fig. 1. Photomicrographs of PFC, CeA, and BIA capture regions shown in neutral red-stained coronal sections. Panel **A**: The two PFC capture regions ($\times 12.5$). Panel **B**: CeA and BIA capture regions ($\times 40$). PFC, prefrontal cortex; CC, corpus callosum; ML, midline; CeA, central nucleus of the amygdala; BIA, basolateral nucleus of the amygdala; CP, caudate putamen; LA, lateral nucleus of the amygdala.

Fig. 2.

Effects of chewing during stress on Fos-ir in the left and right prefrontal cortex (PFC). Panel **A**: The mean ($\pm$ SEM) number of Fos-ir nuclei counted in the dorsal and ventral PFC capture sites combined, in left and right PFC, across chew and no-chew conditions. Only in the right PFC, chewing significantly increased the number of Fos-ir nuclei. Panel **B**: Typical examples of Fos-immunoreacted tissue from left and right PFC in both conditions ($\times 400$). Note that for the purposes of these examples, regions smaller than those used for analysis are shown. * $P < 0.05$ vs. no-chew controls.

Fig. 3.

Effects of chewing during stress on Fos-ir in the left and right central nucleus of the amygdala (CeA). Panel **A**: The mean ($\pm$ SEM) number of Fos-ir nuclei counted in left and right CeA, across chew and no-chew conditions. Only in the right CeA, chewing significantly attenuated the number of Fos-ir nuclei. In addition, within the no-chew condition, there was a trend toward greater Fos-ir in the right CeA as compared to the left ($P = 0.076$). Panel **B**: Typical examples of Fos-immunoreacted tissue from left and right CeA in both conditions ($\times 400$). * $P < 0.05$ vs. no-chew controls.