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Behavioral effects of chronically elevated corticosterone in subregions of the medial prefrontal cortex

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Abstract

The medial prefrontal cortex is a key mediator of behavioral aspects of the defense response. Since chronic exposure to elevated glucocorticoids alters the dendritic structure of neurons in the medial prefrontal cortex, such exposure may alter behavioral responses to danger as well. We examined the effects of chronically elevated corticosterone in discrete regions of the medial prefrontal cortex on exploration of the elevated plus-maze. Chronically elevated corticosterone in the prelimbic or infralimbic cortices reduced open arm exploration. This effect was specific to the ventral regions of the medial prefrontal cortex as corticosterone had no effect on plus-maze exploration when administered into the anterior cingulate cortex. Taken together, these findings demonstrate clear regional differences for the effects of corticosterone in the medial prefrontal cortex.

Keywords

prefrontal cortex; cingulate; prelimbic; infralimbic; fear; anxiety; corticosterone; glucocorticoids

Introduction

The defense response is a highly conserved process that promotes survival of the organism by activating a complex neural circuit. At the most basic level, this response consists of threat detection along with adaptive physiological and behavioral responses to danger [1, 2]. The autonomic and endocrine responses to a diverse array of threats have been well characterized and the neural circuits mediating these effects are beginning to emerge. However, less is known about how glucocorticoids released during the defense response affect the neural circuit itself. Glucocorticoids secreted in response to danger cross the blood brain barrier and may alter activity of the defense circuitry to subsequent threats [3, 4].

The medial prefrontal cortex is a key mediator of behavioral aspects of the defense response and expresses both type I and type II glucocorticoid receptors [5, 6]. Glucocorticoids

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administered directly into the anterior cingulate cortex (ACC) identified this structure as a negative feedback site for hypothalamic pituitary adrenal axis regulation [5]. Knockdown studies of glucocorticoid receptors in the prelimbic (PLC) and infralimbic cortices (ILC) reveal clear regional differences in glucocorticoid receptor mediated feedback and behavior in the forced swim test [7]. Chronically elevated glucocorticoids reduce the volume of the medial prefrontal cortex and alter dendritic morphology in this region of the brain [8, 9]. Such structural changes likely modify function of the medial prefrontal cortex and possibly include altered behavioral responses to danger.

Glucocorticoid receptors in the medial prefrontal cortex are involved in regulating endocrine and behavioral components of the defense response. Further, glucocorticoids secreted during chronic exposure to threats may increase the sensitivity of the neural circuit underlying behavioral responses to danger. Our previous work suggests the amygdala and dorsolateral bed nuclei of stria terminalis represent components of the defense circuit affected in this way [10, 11]. However, the behavioral effects of chronically elevated glucocorticoids within the medial prefrontal cortex remain largely unexplored.

As part of our ongoing efforts to understand the potential role of glucocorticoids on the defense circuit, we implanted corticosterone micropellets into distinct subregions of the medial prefrontal cortex. In a series of three experiments we systematically examined the effects of glucocorticoids in the ACC, PLC, and ILC on exploration of the elevated plus-maze.

Methods

Animals

Sixty-seven Male Wistar rats (Charles River Laboratories, Wilmington, MA) weighing 325–350g were habituated to the animal facility for at least 1 week prior to surgery then acclimated to the testing room for one additional week. All rats were handled and weighed daily. Rats were then randomly assigned to receive stereotaxic implantation of cholesterol (control) or corticosterone micropellets into one of three regions of the medial prefrontal cortex: anterior cingulate (ACC), prelimbic (PLC) or the infralimbic (ILC) region. All procedures were approved by the Towson University IACUC.

Micropellets

Micropellets of crystalline corticosterone and cholesterol were constructed according to the procedure developed and characterized in our laboratory [10–13]. Briefly, the pellet is constructed by tamping cholesterol or corticosterone into a 25-gauge stainless steel cannula then the cannula is mounted in a standard electrode holder. Once the cannula is lowered into the target tissue the micropellet is extruded by inserting a stylet into the cannula. The cannula is removed from the brain leaving the pellet behind. The pellet is clearly visible in tissue sections at the end of the one week treatment period and micropellet placement was verified histologically for each animal at the end of the study (Figure 1). This procedure yields a micropellet containing 30µg of corticosterone with a diffusion radius of 0.75 mm measured ventral to the pellet in the amygdala. Tissue levels of corticosterone are in the high

physiologic range [14]. The cytoarchitecture of the amygdala and frontal cortex are different and may affect the diffusion characteristics of corticosterone. Diorio et al. [5] found corticosterone diffusion up to 0.9 mm caudal to the implantation site in the ACC. This raises the possibility that diffusion from our micropellet may be greater in the cortex than in the amygdala. Alternatively, diffusion of corticosterone may be ovoid rather than spherical with more diffusion in the rostral caudal plane.

Stereotaxic surgery

Rats received bilateral implants containing 30 µg of cholesterol or corticosterone in the ACC (2.9 mm rostral to bregma, + 1.5 mm lateral to midline, -2.6 mm ventral), PLC (2.9 mm rostral to bregma, + 1.7 mm lateral to midline, -3.9 mm ventral) or ILC (2.9 mm rostral to bregma, + 2.0 mm lateral to midline, -5.1 mm ventral). Rostral/Caudal coordinates are with respect to bregma and ventral coordinates were referenced to the skull surface [15]. All coordinates are 16% from vertical to avoid cannula tracks through structures under investigation. Stereotaxic surgery was performed under ketamine/xylazine anesthesia (100/10 mg/kg, i.p.).

Elevated plus-maze

The elevated plus-maze is a validated method for assessing fear/anxiety in rats used for over 30 years [16]. The current experiments were performed using a commercially available elevated plus-maze constructed to standard dimensions for rats (Med Associates, St Albans, VT). The maze consists of two runways 10 cm wide that intersect in the center at 90° angles, elevated 50 cm above the floor. One runway is enclosed by walls (closed arms) and 2 runways without sides (open arms) that intersect in the center. A digital video camera is mounted above the maze and attached to a computer in an adjacent room where behavior can be monitored remotely in real time and recorded for behavioral analysis. All plus maze tests were conducted between 07:00 and 10:00 to keep diurnal influences constant across tests.

Rats were placed in the center of the plus-maze facing an open arm. Placing the rat on the maze facing a closed produces a bias for an initial closed arm entry while facing an open arm results in an equal probability for an initial open or closed arm entry [16]. Following a 5 min period of maze exploration the animal was returned to his home cage and the maze was cleaned with 20% ethanol and dried prior to additional testing. Behavioral measures from the plus-maze fall into two main categories; locomotion and anxiety-related behaviors [17]. Most studies using the elevated plus-maze rely on the percentage of open arm entries and the percentage of time spent in the open arms as an index of fear/anxiety [18]. The greater the initial state of fear/anxiety, the less the rat will explore the relatively bright, open arms [19]. We analyzed the percentage of open arm entries (open arm entries/total arm entries × 100) and percentage of time spent exploring the open arms (time in the open arms/total time on arms × 100) as indices of anxiety-like behavior. A detailed factorial analysis of behaviors on the plus-maze revealed that closed arm entries load most heavily with variables associated with locomotor activity while the total number of arm entries is related to both locomotion and anxiety-like behavior [17]. Accordingly, we analyzed closed arm entries as an index of locomotion.

Results

Corticosterone implants in the ACC region had no effect on open arm exploration or general locomotion on the elevated plus maze. The percentage of entries into the open arms, $t(22) = 0.46$; $p = 0.64$ and percentage of time exploring the open arms, $t(22) = 0.96$; $p = 0.35$ were not significantly different from control animals implanted with cholesterol in ACC (Figure 2). Corticosterone also had no effect on closed arm entries in this brain region indicating stable locomotion across treatment groups, $t(22) = 1.37$; $p = 0.18$.

In contrast, corticosterone implantation in the PLC reduced open arm exploration. The percentage of entries into the open arms, $t(21) = 3.47$; $p = 0.002$ and percentage of time spent exploring the open arms, $t(21) = 3.83$; $p = 0.001$ were both reduced by chronically elevated corticosterone in the PLC (Figure 3). The effects of corticosterone were specific to open arm exploration and had no effect on general locomotion as measured by closed arm entries, $t(21) = 0.14$; $p = 0.89$.

Corticosterone implanted in the ILC region also reduced exploration of the open arms on the elevated plus maze. Percentage of open arm entries, $t(18) = 2.36$; $p = 0.03$ and percentage of time spent exploring the open arms were both reduced by corticosterone implants in the ILC, $t(18) = 2.69$; $p = 0.02$ (Figure 4). Similar to the ACC and PLC, corticosterone administration in the ILC had no effect on closed arm entries, $t(18) = 0.37$; $p = 0.72$. Overall, chronically elevated corticosterone in the more ventral regions of the medial prefrontal cortex (PLC and ILC) produced a specific reduction in open arm exploration without affecting overall locomotion on the plus maze (Table 1).

Discussion

Elevated corticosterone in the PLC or ILC reduced exploration of the open arms on the elevated plus-maze in the current study. Reduced exploration of the central region of an arena in the open field test or open arms of the plus-maze is usually interpreted as increased anxiety-like behavior [16, 20]. While this interpretation can be applied to the current findings, we are considering plus-maze exploration in a broader context as exploration of an aversive, potentially dangerous environment. The broader view is consistent with the openness and relative brightness of the open arms being the main factors influencing plus-maze exploration [21]. This is the first study to assess exploration of a potentially threatening environment (open arms of the maze) when corticosterone is elevated in discrete subregions of the prefrontal cortex.

We found that elevated corticosterone in the PLC produced specific avoidance of the open arms of the plus maze without affecting overall locomotion. Mcklveen et al. [7] examined the potential role of glucocorticoids in the PLC and ILC by knocking down glucocorticoid receptors in these regions. Glucocorticoid receptor knockdown in the PLC increased total locomotion in the open field test including the central region of the arena. This finding is consistent with glucocorticoids acting on the PLC to alter exploration of a novel environment but avoidance of the center of the arena may depend on the level of corticosterone and duration of glucocorticoid exposure. Our experiments maintained

corticosterone levels in the high physiological range for seven days while the receptor knockdown studies reduced the effects of endogenous corticosterone for 5–6 weeks. It is possible that chronic corticosterone elevation in the PLC is necessary to produce avoidance of the center of the open field or the open arms of the plus maze.

Like the PLC, chronically elevated corticosterone in the ILC reduced open arm exploration in the current study. This is in contrast to the lack of observable effect with chronically elevated corticosterone in the ACC. Glucocorticoid treatment had no effect on overall locomotion in any of the three regions examined but produced specific avoidance of the open arms when administered to the more ventral regions of the medial prefrontal cortex, the PLC and ILC respectively. With diffusion from the micropellets at approximately 0.75–0.90 mm, corticosterone in the ventral margin of the ACC may include the dorsal aspect of the PLC. In our study, this did not alter exploration of the plus maze. Likewise, corticosterone treatment near the margin between the PLC and ILC may have included part of both structures. With these considerations, our findings demonstrate regional differences for the behavioral effects of corticosterone in the medial prefrontal cortex.

Glucocorticoid receptor knockdown in the ILC increased immobility in the forced swim test but had no effect on exploration of the center of an open field [6]. We found that elevated corticosterone in the same region reduces open arm exploration on the plus maze. It is possible that reducing the effects of endogenous glucocorticoids has no observable effect on anxiety-like behavior and chronic elevation of corticosterone is necessary to produce behaviors consistent with increased anxiety. Open arm avoidance and immobility in the forced swim test represent different complex behaviors influenced by glucocorticoids. While glucocorticoid receptor knockdown in the ILC is sufficient to increase immobility in the forced swim test [6], the effects of elevated corticosterone within this structure are unknown. It is possible that the effects of glucocorticoids on behavior in the forced swim test are biphasic with both abnormally low levels or chronic excess resulting in immobility. We did not perform the forced swim test in our experiments so further work is necessary to determine the effects of chronically elevated corticosterone in the ILC on forced swimming behavior.

Our experiments focused on open arm avoidance behavior when corticosterone is elevated locally in subregions of the medial prefrontal cortex. Additional work is necessary to determine the mechanism underlying these effects. Systemic elevation of corticosterone reduces the volume of the medial prefrontal cortex without reducing the number of neurons [22]. Such volume changes are attributed to altered dendritic structure following chronic stress or corticosterone treatment [8, 9, 23]. Local administration of corticosterone may produce similar structural changes and alter neuronal function within the medial prefrontal cortex and downstream limbic targets mediating exploratory behavior on the plus maze.

Reduced open arm exploration observed in the current study may be partly mediated by altering activity of limbic structures shown to reduce open arm exploration when corticosterone is elevated. Efferent projections from the medial prefrontal cortex are extensive and include a number of structures that may contribute to increased avoidance of potential danger when glucocorticoids are elevated [24–26]. Here we focus on structures that

reduce open arm exploration when corticosterone is chronically elevated within the structure. The behavioral effects of corticosterone in the PLC or ILC are consistent with effects of corticosterone in the central nucleus of the amygdala and dorsolateral bed nuclei of stria terminalis [10, 11]. The PLC sends monosynaptic efferents to the central and basolateral nuclei of the amygdala [27]. Reduced open arm exploration when corticosterone is elevated in the PLC could at least in part be through the central nucleus of the amygdala. The ILC also projects to the central nucleus of the amygdala and the BNST raising the possibility that the behavioral effects of elevated corticosterone in the ILC may be mediated through these structures [25]. The central nucleus of the amygdala projects to the dorsolateral bed nuclei of stria terminalis and elevated corticosterone in either of these structures reduces open arm exploration on the elevated plus-maze. We found the same effect by elevating corticosterone in either the PLC or ILC in the current study. Considering the same behavioral profile is observed when corticosterone is chronically elevated within each of these anatomically connected structures, the PLC and ILC may be part of a complex glucocorticoid sensitive circuit that underlies avoidance of potential danger.

In summary, chronically elevated corticosterone in the ACC did not alter exploration of the elevated plus-maze. However, the current studies support a role for the PLC and ILC in mediating exploratory behavior when corticosterone is chronically elevated in these structures. The ventral medial prefrontal cortex may be part of a glucocorticoid sensitive neural circuit underlying behavioral changes observed during glucocorticoid excess.

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Highlights

- Corticosterone implants in prelimbic or infralimbic cortices reduce open arm exploration.
- Elevated corticosterone in the anterior cingulate cortex didn't affect plus-maze behavior.
- All behavioral effects of corticosterone were specific to open arm exploration.
- Corticosterone's effects in the medial prefrontal cortex are anatomically specific.

Figure 1.

Location of micropellet implants from all three experiments. Different symbols indicate the site targeted for each experiment. Implants for the cingulate cortex from experiment 1 are indicated with circles. The prelimbic (triangles), and infralimbic (squares) cortices are from experiments 2 and 3 respectively. Corticosterone or cholesterol (control) were implanted in all three experiments. Black markers represent corticosterone micropellets and grey indicates cholesterol.

Figure 2.

Chronically elevated corticosterone in the anterior cingulate cortex (ACC) had no effect on plus maze exploration. Corticosterone implanted rats ($N = 12$) exhibited a similar percentage of open arm entries ($p = 0.64$) and percentage of time spent exploring the open arms ($p = 0.35$) as cholesterol implanted animals (control; $N = 12$)

Figure 3.

Chronically elevated corticosterone in the prelimbic cortex (PLC) reduced open arm exploration of the elevated plus maze. Corticosterone implanted in the PLC (N = 11) significantly reduced both the percentage of open arm entries and percent time spent exploring the open arms of the elevated plus maze compared to cholesterol implanted controls (N = 12). ** = $p < 0.01$ vs. cholesterol.

Figure 4.

Chronically elevated corticosterone in the infralimbic cortex (ILC) reduced open arm exploration of the elevated plus maze. Corticosterone implants in the ILC (N = 10) significantly reduced both the percentage of open arm entries and percent time spent exploring the open arms of the elevated plus maze compared to cholesterol implanted controls (N = 10). * = $p < 0.05$ vs. cholesterol.

Table 1

Locomotor behavior on the elevated plus maze.

Implant Site	CHOL	CORT	t	df	p
ACC	9.3	7.8	1.4	22	0.18
PLC	9.3	9.4	0.14	21	0.89
ILC	7.4	7.4	0.37	18	0.72

Mean closed arm entries for cholesterol (CHOL) or corticosterone (CORT) implanted animals. Anterior cingulate cortex (ACC); prelimbic cortex (PLC); infralimbic cortex (ILC)