Stress and the female brain. The effects of estradiol on the neurobiological reactions to chronic stress.
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Increased stress vulnerability after a prefrontal cortex lesion in female rats

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Abstract

Neuroimaging studies in patients suffering from affective disorders have shown decreased volume and reduced regional cerebral blood flow in multiple areas of the prefrontal cortex, including the medial prefrontal cortex and the orbitofrontal cortex. This aberrant brain activity is among other things attributed to chronic stress. Affective disorders occur more often in women than in men. In the current experiment, female mPFC lesioned and non-lesioned rats were subjected to 3 weeks of chronic unpredictable stress in order to determine the role of the mPFC in dealing with chronic stress, and the consequences of mPFC damage for coping with consecutive stressful events. mPFC damage in female rats intensified the stress-induced activation of the dorsomedial nucleus of the hypothalamus and the paraventricular nucleus of the hypothalamus as measured with c-Fos expression changes and markedly increased plasma catecholamine levels after 3 weeks of unpredictable stress. Additionally, an mPFC lesion significantly reduced the time of appearance of stress-induced behavioral changes in the open field. Altogether, mPFC dysfunction affects the way female rats react to chronic stress, it not only increased the activation of brain regions involved in neuroendocrine and autonomic responses to stress but it also significantly reduced the time of onset of behavioral changes.
The role of the mPFC in stress responses

Introduction

Major depression and anxiety disorders, such as posttraumatic stress disorder are associated with morphological and functional changes in the limbic system and the prefrontal cortex (PFC). Clinical studies have shown that stressful experiences, like severe and sequential life events, are important etiological factors in the development of affective disorders (Post, 1992; Kendler et al., 1995). Moreover, it has been reported in humans and experimental animals that stress, particularly chronic stress, can affect brain activity and induce localized structural changes and neuronal damage (Post, 1992; Kaufman et al., 2000; McEwen, 2000).

Dysfunction of the medial and orbital PFC can disarrange the adequate processing of emotional stimuli (i.e. severe stressors) (Drevets, 1998; Rajkowska, 2000a). Postmortem studies of patients suffering of major depression have shown decreased number and size of neurons and glia in the medial prefrontal cortex (mPFC) and a related decreased volume of the mPFC (Ongur et al., 1998; Rajkowska, 2000b). In addition, neuroimaging studies in depressed subjects and subjects suffering from post traumatic stress disorder showed disturbed cerebral blood flow and metabolic activity of the mPFC (hypofrontality) (Drevets et al., 1997; Galynker et al., 1998; Drevets, 2000; Lanius et al., 2001), which could be improved by antidepressant treatment with selective serotonin re-uptake inhibitors (SSRIs) (Kennedy et al., 2001).

Also in rats, the mPFC in is important for neuroendocrine and autonomic responses to stressful situations. Particularly, the prelimbic and infralimbic regions have been shown to modulate hypothalamic-pituitary-adrenal (HPA)-axis activity and are involved in glucocorticoid-mediated negative feedback mechanisms (Diorio et al., 1993). Several tracing studies have shown that the infralimbic and prelimbic cortices project to a number of diencephalic, brainstem and spinal control centers and to limbic areas including the dorsomedial nucleus of the hypothalamus (DMH) and the paraventricular nucleus of the hypothalamus (PVN), which may enable direct and indirect modulation of autonomic functions (Terreberry et al., 1987; Bacon et al., 1993; Ter Horst et al., 1996; McEwen, 2000; Buijs et al., 2000; Hata et al., 2000).

Affective disorders occur approximately twice as often in women as in men (Kessler et al., 1993; Weissman et al., 1995; Kessler et al., 1995) which may be partly explained by neurobiological effects of circulating ovarian hormones. Estrogen can modulate the function of the serotonergic system (Joffe et al., 1998; Rubinow et al., 1998; Osterlund et al., 2000; Raap et al., 2000; Birzniece et al., 2002), the limbic activity (George et al., 1996) and the HPA-axis (Handa et al., 1994; Carey et al., 1995; DeLeo et al., 1998; Shupnik, 2002). Biological differences in stress processing between men and women could underlie the higher liability for stress-related diseases in females.

The aim of this study was to investigate whether experimentally induced hypofrontality in rats will increase their stress vulnerability. We used female rats because of their suspected increased sensitivity to chronic stress and because of the considerable relevance for the human affective disorder research and the lack of information concerning the development of
stress-related pathology in female rats. These data may provide new insight into the role of the mPFC in dealing with chronic stress, and the consequences of mPFC damage for coping with consecutive stressful events.

**Materials and methods**

**Animals**

Thirty-four cyclic female Wistar rats (Harlan, The Netherlands) weighing 208 ± 1.3 g at the start of the experiment were individually housed with a 12 hr light-dark cycle (lights on at 7:00 PM). Each rat had a piece of PVC tube in its homecage (d=8 cm, l=17 cm). Food and water was available *ad libitum* (standard rat chow, Hopefarms). Protocols were approved by the animals ethics committee of the University of Groningen (FDC 2509). Twenty-two animals were bilaterally mPFC lesioned and subdivided in a control group (n=10) and a stress group (n=12). The twelve non-lesioned animals were also subdivided in a control and a stress group (n=6/group). All animals were handled and weighed daily to minimize handling stress during the experiment.

**PFC lesion**

To induce electrolytic lesions in the mPFC the rats were anaesthetized with halothane. A stereotaxic apparatus was used to temporarily implant an electrode. Bilateral coordinates for electrode placements were AP 10.6 mm from intraoral, L ±0.5 mm from the midline and V -3.6 mm from the dura. During 8-10 sec a current of 1.2 mA was delivered. After the surgery the rats received 0.1 ml Temgesic (buprenorfinehydrochloride i.p. 6.5 μg/ml) and were allowed to recover for 11 days before the start of the chronic footshock stress procedure.

**Experimental paradigm**

The rats were subjected to a daily footshock protocol for 22 days as described earlier (Trentani et al., 2002; Westenbroek et al., 2003). In brief, the rats allocated to the stress group were placed in a footshock box with a gridfloor daily during the dark period and received 5 inescapable footshocks with an intensity of 0.8 mA and a duration of 8 sec (unconditioned stimulus). Each footshock was preceded by 5 sec of light in order to condition the rats. This was to add a ‘psychological’ component to the footshocks, which is useful on the last day of the experiment when only the conditioned stimulus is presented to induce stress-related responses but to avoid pain-induced changes in the central nervous system.
The time between the shocks and the duration of the session varied in each session, in order to make the stressor as unpredictable as possible. The non-stressed rats were kept in their homecages to avoid any extra stress in this group.

**Open field**

The behavioral changes during the chronic stress period were recorded in an open field (OF). The test was performed under red-light conditions, during the early phase of the active period of the animals, and before the footshocks of that day. The open field consisted of a circular black arena with a diameter of 1 meter and contained a familiar object next to the wall, i.e. the homecage tube of the tested animal. The test was repeated 3 times; after 1 day (OF1), after 13 days (OF2), and after 20 days (OF3) of stress. Rats were gently placed in the tube at the start of the trial; the duration of each trial was 8 minutes. Locomotor behavior was recorded with a video tracking system (EthoVision, Noldus Information Technology, Wageningen, The Netherlands). The distance moved in inner zone and in the whole arena, and the time spent in the tube area was analyzed (see Figure 2A for zone definition).

On day 22 of the chronic stress procedure the rats allocated to the stress group were placed in the shock box for 30 minutes and received only stimulation with light (the conditioned stimulus). Two hours later the rats were anaesthetized with halothane and 3 ml blood was collected by cardiac puncture to determine plasma corticosterone, noradrenaline and adrenaline concentrations. Subsequently, the rats were perfused with 0.9 % saline, followed by 300 ml 4 % paraformaldehyde (in 0.1 M sodium phosphate buffer (PB), pH 7.4). Adrenal glands were weighed. The brains were carefully removed and postfixed overnight in 4 % paraformaldehyde and stored in 0.1 M phosphate buffered saline (PBS) with 1 % sodium-azide (pH 7.4) at 4 °C.

**Hormone assays**

Plasma corticosterone and noradrenaline/adrenaline levels were measured by HPLC as previously described by Trentani et al (Trentani et al., 2002). For quantification of the corticosterone concentration, dexamethasone was used as internal standard. Plasma was extracted with 3ml of diethyl ether, vortexed for 5 min and then centrifuged for 5 min at 3000 g. The extraction procedure was repeated twice. The organic phase was evaporated to dryness in a 50 °C waterbath. The detection limit of corticosterone was 10 nM. Noradrenaline (NA) and adrenaline (A) were extracted from plasma using liquid/liquid extraction with 3,4-dihydroxybenzylamine as internal standard (Smedes et al., 1982). Briefly, plasma NA and A was bound to diphenylborate-ethanolamine at pH 8.6. The extraction was performed with n-heptane (containing 1 % octanol and 25 % tetraoctylammoniumbromide). Finally, NA and A were extracted from the organic phase with diluted acetic acid. The detection limit of NA and A was 0.1 nM.
Lesion evaluation

To evaluate the exact location of the electrolytic lesion a silver staining was done according to an application of a Gallyas procedure described earlier (Ter Horst et al., 1995). In brief, coronal cryostate sections of 40 μm collected in 4 % paraformaldehyde were washed with pretreatment solution, consisting of 9 % NaOH and 1.2 % NH₄NO₃ (1:1, pH 11.9). Thereafter the sections were incubated for 10 minutes in impregnation medium, consisting of 9 % NaCl and 16 % NH₄NO₃ (1.5:1) and 50 % AgNO₃. After rinsing with a Na₂CO₃ solution (5 g Na₂CO₃ in 700 ml aquadest and 300 ml 96 % ethanol) the sections were placed in the developer solution (0.57 g C₆H₈O₇·H₂O in 700 ml aquadest with 15 ml 37 % formaldehyde and 100 ml 96 % ethanol, pH 6.0) for 4 minutes. Subsequent to the development reaction, the silver deposits in the sections were fixed a thiosulphate solution (37.5 % Na₂S₂O₃·5H₂O) and mounted on gelatin-coated slides, air dried, dehydrated and coverslipped with DEPEX. The location of the lesion was evaluated by use of the Swanson Atlas (Swanson, 1992).

Immunocytochemistry

Coronal cryostate sections of 40 μm were collected in 0.01 M phosphate buffered saline (PBS, pH 7.4) and rinsed 3x10 minutes. After pre-incubation with 0.3 % H₂O₂ (20 minutes, in 0.01 M PBS, pH 7.4) the sections were washed with 0.01 M PBS (3x10 min, pH 7.4) and incubated with an rabbit polyclonal antibody raised against c-fos (Ab-5 Oncogene Research Products, Calbiochem, 1:10.000 in 0.01 M PBS-Triton 0.25 %, 3 % normal goat serum) for 48-60 hrs at 4 ºC. Subsequently the sections were washed in 0.01 M PBS (3x10 min, pH 7.4) and incubated for 2 hours at room temperature with biotinylated goat anti Rabbit IgG (Vector, 1:1000 in 0.01 M PBS). After rinsing with 0.01 M PBS (3x10 min, pH 7.4) the immunoreactivity was visualized with a standard ABC method (Vectastain ABCkit, Vector, 1 drop A + 1 drop B)/ 10 ml PBS for 2 hrs). After washing with PBS 0.01 M (3x10 min, pH 7.4) the peroxidase reaction was developed with a DAB-nickel solution and 0.3 % H₂O₂ (20mg DAB, 1.5 g NAS, 0.8203 g NaAC in 50 ml H₂O). To stop the reaction the sections were washed with 0.01 M PBS (3x10 min, pH 7.4) and mounted on gelatin-coated slides, air dried, dehydrated and coverslipped with DEPEX.

Numbers of c-Fos positive cells in the DMH and PVN were analyzed by an investigator unaware of the experimental protocol. The area of the region of interest was measured and, after background correction, the number of immunopositive nuclei was quantified using a computerized image analysis system (Leica Qwin version 2.3, Leica Microsystems Imaging Solutions). The average number of Fos immunoreactive cells in the DMH and the PVN was calculated and expressed as number of positive nuclei/mm².
Statistical analysis

Results are expressed as the mean ± SEM. Statistical analyses were done with SPSS (version 8.0); p<0.05 was considered significant. Locomotor activity in the open field was analyzed with a repeated measures ANOVA, with the sequential open field tests as within subject variables and lesion (non-lesioned or lesioned) and stress (control or stress) as between subject variables. Sphericity assumed modeling, with Greenhouse-Geisser and Huynh-Feldt adjustments, was applied. Weight gain, adrenal weight, hormone concentrations and the number of c-Fos positive cells were analyzed with an ANOVA with lesion (non-lesioned or lesioned) and stress (control or stress) as between subject variables. An LSD post-hoc test was used for the pairwise comparisons. Due to failure in the analysis one sample was lost in the open field and immunocytochemical data.

Results

Figure 1 shows a schematic representation of the PFC lesions. The cytoarchitectonic borders and nomenclature was adopted from the Swanson Brain Atlas. Examinations of the silver staining revealed that most of the lesions were small (approximately 0,13 μm3) and bilaterally located in the prelimbic area of the mPFC and did not include the corpus callosum. Except for one rat in which the lesions were located more rostral in the dorsal part of the anterior cingulate area.

Table 1: Average values of body weight gain (BW), absolute adrenal weights and plasma corticosterone concentrations (CORT) ± SEM in non-lesioned and mPFC lesioned rats after control and chronic stress conditions.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Non-lesioned</th>
<th>Lesioned</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Stress</td>
</tr>
<tr>
<td>BW gain (g)</td>
<td>18.2 ± 3.4</td>
<td>23.8 ± 6.7</td>
</tr>
<tr>
<td>Adrenal (mg)</td>
<td>73.3 ± 4.2</td>
<td>78.7 ± 2.7</td>
</tr>
<tr>
<td>CORT (µg/dl)</td>
<td>4.1 ± 0.7</td>
<td>4.0 ± 0.7</td>
</tr>
</tbody>
</table>
Open field

Repeated measures ANOVA with Huynh-Feldt correction showed a significant effect of the sequential open field tests ($F_{1,29}=8.550$, $p=0.001$) on the total distance moved. In non-lesioned stressed female rats, the total distance moved was slightly increased after 20 days (OF3) compared to the first day (OF1; $p=0.051$; Figure 2B). Non-lesioned non-stressed animals did not show significant changes in their locomotor behavior after repeated open field exposure. The increased total distance moved after 20 days of chronic stress is strongly influenced by a significantly increased distance moved in the inner zone of the arena (Figure 2C). After 20 days of stress (OF3) the animals covered a significant greater distance in the inner zone compared to OF1 and OF2 ($p=0.006$ and $p=0.05$ respectively) and significantly reduced the time the animals spent in the tube area (in, on and around; $p=0.026$ and $p=0.033$ respectively; Figure 2D).

In contrast, stressed female rats with bilateral mPFC damage showed increased locomotor activity 13 days after the start of the protocol (OF2) compared to their own behavior on the first day (OF1; $p=0.006$; Figure 2B). In particular the inner zone activity was significantly increased at this time-point compared to the first day ($p<0.001$; Figure 2C).

Figure 2: Open field behavior after 1 day (OF1), 13 days (OF2) and 20 days (OF3) of footshock stress. The results are expressed as group means ± SEM. A) Zone definition of the arena. At the start of each trial the rat is placed in the tube. B) Total distance moved in the arena. C) Distance moved in the inner zone (see A). D) Time spent in the tube area (in, on and around the tube, see A). The behavior did not change in subsequent trials during control conditions, while after stress behavioral changes were recorded in non-lesioned rats after 20 days and in mPFC lesioned rats already after 13 days. * $p<0.05$. 
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These effects persisted and were still significant after 20 days (p<0.001 and p<0.001 respectively). Moreover, in the mPFC lesioned animals a significant interaction occurred between open field and stress indicating increased locomotor behavior after stress exposure (total distance moved, F_{1,20}=4.399, p=0.019; distance moved in inner zone, F_{1,20}=4.305, p=0.023). After 13 days of stress (OF2) the time spent in the tube area was significantly reduced compared to the first day (OF1), and was still reduced after 20 days (OF3; p<0.001 and p=0.001 respectively; Figure 2D). Non-stressed animals with bilateral mPFC damage did not show significant changes in their locomotor behavior after repeated open field exposure.

Bodyweight gain and adrenocortical function

Plasma corticosterone levels in mPFC lesioned rats were slightly increased (F_{1,30}=3.309, p<0.001), although neither bilateral mPFC lesions nor chronic stress exposure significantly affected the growth rate, adrenal size or corticosterone concentrations of female rats (Table 1).

Catecholamine concentrations

There was a significant lesion effect on the plasma catecholamine levels (NA, F_{1,30}=9.515, p=0.004; A, F_{1,30}=12.889, p=0.001). mPFC lesioned rats showed increased levels of plasma noradrenaline and adrenaline after 3 weeks of footshock stress compared to non-lesioned stressed rats (F_{1,30}=7.336, p=0.011 and F_{1,30}=16.201, p<0.001 respectively; Figure 3). However, there was no significant lesion-stress interaction (NA, F_{1,30}=0.467; A, F_{1,30}=0.440). Noradrenaline and adrenaline concentrations of individual animals were strongly correlated (Pearson’s correlation coefficient 0.877, p<0.001).

![Figure 3: Plasma levels of noradrenaline (A) and adrenaline (B) measured 2 hrs after stimulation with the conditioned stimulus in the shock box. Chronic stress caused increased levels of noradrenaline and adrenaline in the mPFC lesioned rats compared to the non-lesioned rats.](image-url)
C-Fos expression

Chronic stress significantly affected the c-Fos immunoreactivity in the DMH ($F_{1,29}=11.295$, $p=0.002$) and the PVN ($F_{1,29}=10.665$, $p=0.003$). Female rats with a damaged mPFC showed a significantly increased number of c-Fos positive cells in the DMH and the PVN after 3 weeks of stress ($F_{1,29}=16.445$, $p<0.001$ and $F_{1,29}=12.916$, $p=0.001$ respectively) compared to control conditions, while in non-lesioned females this did not reach statistical significance (Figure 4). However, the lesion-stress interaction was not significant (DMH, $F_{1,29}=1.747$; PVN, $F_{1,29}=0.782$).

Figure 4: C-Fos activity expressed as mean numbers of c-Fos positive cells per mm$^2$ ± SEM. Chronic stress increased the c-Fos immunoreactivity in A) the dorsomedial nucleus of the hypothalamus (DMH) and B) the paraventricular nucleus of the hypothalamus (PVN) in the PFC lesioned rats. C) c-Fos immunostaining of the PVN.
Discussion

In this study we showed that small electrolytic lesions in the mPFC of cyclic female rats influenced the effects of stress on behavior, catecholamine release and brain activity. The locomotor activity of the cyclic female rats in this study was slightly increased after chronic stress. Moreover, the stressed female rats show a preference for the center of the arena, which corroborates other data from our lab. Most studies reported decreased activity in the open field after stress (Ferretti et al., 1995; Willner, 1997; D’Aquila et al., 2000; Bowman et al., 2002). However, these studies included male instead of female rats. Male rats react to stress with a fight-or-flight response while female rats seem to have a different strategy. Female responses to stress are marked by a pattern of tend-and-befriend behavior (Taylor et al., 2000) and the increased open field activity of female rats after stress might be characterized as a type of friend-seeking (Westenbroek et al., 2003). Moreover, most studies are performed during the day when these nocturnal animals sleep. The open field test in this study was performed repeatedly, in the active early dark period under red light conditions and 12 hours after the footshocks session of the previous day to investigate the course of the behavioral changes.

Although stress-induced behavior is changed in rats with a mPFC lesion, notably the mPFC lesion on itself did not influence open field behavior. This is contrary to the results of Jinks et al (Jinks et al., 1997) who described decreased time spent in the center of the open field in male rats with lesions in the prelimbic and infralimbic cortex. However, this contradiction most likely is explained by the gender difference, the size of the lesions, or the time of the day of the open field test.

After 3 weeks of chronic footshock stress female rats spent significant less time around a familiar object, which was completely opposite to male open field behavior (unpublished results of our own lab). This suggests that female rats respond to stress in an opposite way than male rats do, which is in accordance with other studies that showed gender specific responses to stress in the central nervous system (Galea et al., 1997; Taylor et al., 2000; Shors et al., 2001; Trentani et al., 2001).

Bodyweight gain, adrenal size and plasma corticosterone levels were not affected by a mPFC lesion in this study. Moreover, chronic stress did not alter these parameters in either non-lesioned or mPFC lesioned rats. This corroborates studies in female rats that were not able to find stress-induced changes in these parameters as well (Ono et al., 1995; Bowman et al., 2001; Duncko et al., 2001; Westenbroek et al., 2003). However, studies in male rats showed a strong stress-induced reduction in bodyweight gain, adrenal hypertrophy and increased levels of plasma corticosterone (Ziegler et al., 1999; Mizoguchi et al., 2001; Trentani et al., 2002).

A limitation of the current study is that the female rats were not synchronized for the phase of their estrus cycle. Feeding behavior, and therefore weight gain, is related to fluctuations in levels of plasma estrogens (Laviano et al., 1996; Geary et al., 1999). Additionally, the size of the adrenal glands is also sensitive to circulating estrogens (Hata et al., 2000; own unpublished results). Therefore, if the animals are not matched by the phase of their estrus...
cycle, subtle changes in parameters sensitive to circulating estrogens might mask delicate differences between experimental groups.

Emotional stress information from higher brain areas such as the amygdala, hippocampus, septum and PFC is converged on the hypothalamus. The PVN provides the main output of the hypothalamus to the neuroendocrine system and the autonomic nervous system. The PVN activity is strongly regulated by the DMH. The DMH receives direct input from the mPFC (Onat et al., 2002) and has direct GABA-ergic and galaninergic projections to the parvocellular PVN (Ter Horst et al., 1986; Buijs et al., 2000). As shown in the current experiment, the reactivity of the PVN and DMH to chronic stress as measured with c-Fos expression is increased when the controlling function of the mPFC is disturbed.

Activation of the hypothalamus by stress elicits the activation of two different response pathways, namely the autonomic nervous system and the HPA system. When facing stress, the hypothalamus will initiate an immediate response through the autonomic nervous system. Subsequently, it coordinates a delayed but long-lasting neuroendocrine response involving HPA-axis activation and release of corticosteroids.

The parvocellular PVN projects to preganglionic autonomic neurons located in the brainstem and the spinal cord that control temperature, heart rate, blood pressure, respiration and the secretion of plasma catecholamines (Luiten et al., 1985; Kandel et al., 1991; Ter Horst et al., 1996; Buijs et al., 2000). The metabotropic glutamate receptors in the DMH have been shown to modulate sympathoexcitatory mechanisms like heart rate (DiMicco et al., 1996). Plasma adrenaline and noradrenaline levels of mPFC lesioned stressed female rats were significantly increased after 3 weeks compared to non-lesioned stressed rats. Moreover, stress in non-lesioned animals did not affect plasma catecholamine levels, which corresponds with the findings of Konarska et al (Konarska et al., 1989). Since catecholamine concentrations were measured 2 hours after the final stress exposure, we hypothesize that the elevated catecholamine levels represent a blunted regulation of the autonomic responses in mPFC damaged animals. The role of the mPFC in the regulation of the autonomic nervous system has been described previously by Van Eden et al (Van Eden et al., 2000). Except this indirect regulation of the activation of the autonomic nervous system through the PVN and DMH, a direct sympathomodulatory role has been shown for the mPFC (Terreberry et al., 1987; Bacon et al., 1993; Buijs et al., 2000; Van Eden et al., 2000). Verberne (Verberne, 1996) reported that mPFC stimulation leads to reduced sympathetic activity.

Several nuclei of the limbic system, like the PFC, the PVN, and the hippocampus, contain glucocorticoid and mineralocorticoid receptors that can initiate feedback mechanisms as part of regulation of the HPA-axis (Diorio et al., 1993; Lopez et al., 1999). However, in the current study we could not reveal increased plasma corticosterone levels 2 hour after the final stress exposure. Therefore, increased activation or disturbed feedback mechanisms of the HPA-axis are not likely to underlie the altered stress response in mPFC lesioned females.
In summary, this study showed that the activation of the DMH and PVN provoked by 3 weeks of unpredictable and inescapable stress is more pronounced in mPFC lesioned female rats than in non-lesioned rats. Moreover, mPFC damage in female rats markedly increased the catecholamine concentrations after chronic stress. Additionally, a mPFC lesion significantly reduced the time of appearance of stress-induced behavioral changes in the open field.

Chronic severe stress affects brain activity and is a cause of structural changes and neuronal damage in human (Post, 1992; Kaufman et al., 2000). Moreover, postmortem studies of depressed patients have demonstrated a decreased number and size of neurons and glia cells and disturbed cerebral blood flow and metabolic activity in the PFC (Drevets et al., 1997; Ongur et al., 1998; Galynker et al., 1998; Drevets, 2000; Rajkowska, 2000b). Therefore, severe sequential life events in human, especially in women, may affect the PFC and make the brain more vulnerable to cope properly with a subsequent stressor. The present study demonstrates that already very small lesions of the prelimbic cortex can exacerbate processing of chronic stress. This suggests that dysfunction of the PFC in patients, supposedly caused by stressful life events, may disarrange the processing of emotional stimuli resulting in aberrant behavioral and hormonal stress responses and neuronal activity that contribute to onset or maintenance of a depressive episode.

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