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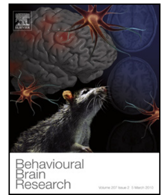

Research report

Chronic stress affects the number of GABAergic neurons in the orbitofrontal cortex of rats

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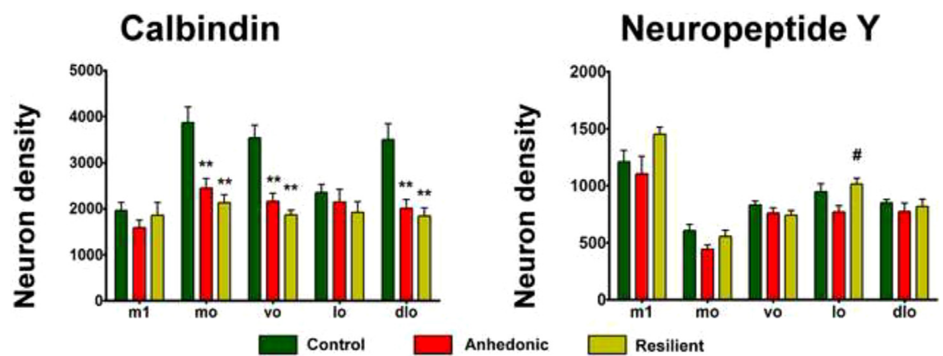
HIGHLIGHTS

- We used the chronic mild stress model for depression and investigated GABAergic neurons in the orbitofrontal cortex.
- We quantified calbindin-, calretinin-, cholecystokinin-, parvalbumin-, NPY- and somatostatin-positive neurons.
- We found pronounced stress-induced reduction of calbindin-positive neuron densities in the orbitofrontal cortex.
- Stress-resilient rats had increased densities of CCK+ and NPY+ cells in their ventral and lateral orbitofrontal cortex.

GRAPHICAL ABSTRACT



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ABSTRACT

Cortical GABAergic dysfunctions have been documented by clinical studies in major depression. We used here an animal model for depression and investigated whether long-term stress exposure can affect the number of GABAergic neurons in the orbitofrontal cortex (OFC). Adult male rats were subjected to 7-weeks of daily stress exposure and behaviorally phenotyped as anhedonic or stress-resilient animals. GABAergic interneurons were identified by immunohistochemistry and systematically quantified. We analyzed calbindin-(CB), calretinin-(CR), cholecystokinin-(CCK), parvalbumin-(PV), neuropeptide Y-(NPY) and somatostatin-positive (SST+) neurons in the following specific subareas of the OFC: medial orbital (MO), ventral orbital (VO), lateral orbital (LO) and dorsolateral orbital (DLO) cortex. For comparison, we also analyzed the primary motor cortex (M1) as a non-limbic cortical area. Stress had a pronounced effect on CB+ neurons and reduced their densities by 40–50% in the MO, VO and DLO. Stress had no effect on

Abbreviations: CB, calbindin; CCK, cholecystokinin; CMS, chronic mild stress; CR, calretinin; DLO, dorsolateral orbitofrontal cortex; GABA, *gamma*-aminobutyric acid; LO, lateral orbitofrontal cortex; M1, primary motor cortex; MO, medial orbitofrontal cortex; mPFC, medial prefrontal cortex; NPY, neuropeptide Y; OFC, orbitofrontal cortex; PFC, prefrontal cortex; PV, parvalbumin; SST, somatostatin; VO, ventral orbitofrontal cortex.

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CCK+, CR+, PV+, NPY+ and SST+ neurons in any cortical areas. None of the investigated GABAergic neurons were affected by stress in the primary motor cortex. Interestingly, in the stress-resilient animals, we observed a significantly *increased* density of CCK+ neurons in the VO. NPY+ neuron densities were also significantly different between the anhedonic and stress-resilient rats, but only in the LO. Our present data demonstrate that chronic stress can specifically reduce the density of calbindin-positive GABAergic neurons in the orbitofrontal cortex and suggest that NPY and CCK expression in the OFC may relate to the stress resilience of the animals.

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1. Introduction

The prefrontal cortex (PFC) of rats is typically divided into two main subregions: the orbitofrontal cortex (OFC) and the medial prefrontal cortex (mPFC) [1,2]. These two areas are functionally and anatomically distinct, but both of them play a role in the stress response and in emotional regulation [3–5]. Stress is known to affect the morphology and functioning of limbic structures including the PFC. Extensive amount of experimental data demonstrate this concept in the mPFC [4–8], but much less is known on the stress-induced cellular changes affecting the OFC.

Surprisingly, a recent study, which investigated chronic stress-induced changes in gene expression in the PFC and hippocampus, reported the greatest changes in the OFC [9]. Earlier, Liston and colleagues found that chronic stress substantially *increase* apical dendritic length and spine density of layer II–III pyramidal neurons in the OFC [10]. These findings were in sharp contrast to the data available from the mPFC where layer II–III pyramidal neurons *reduce* their apical dendritic length and spine density in animals subjected to repeated stress [11–13]. Another study investigating cellular responses to stress in the OFC reported *decreased* spine density [14] and similar effect was seen after chronic corticosterone exposure [15]. Complementing these preclinical findings, clinical investigations also demonstrated reduced volumes of the prefrontal areas in response to severe stress [16].

Animal models which involve chronic stress exposure are valuable tools to study the pathophysiology of mood disorders [17,18]. Here we used one of the best validated animal models for depression and examined GABAergic changes in the frontal cortex of the animals. The reason for such a study was that clinical findings report on cortical inhibitory deficits in major depressive disorder [19–21]. It has been hypothesized that fronto-cortical GABAergic deficits play a key role in the pathophysiology of mood disorders [21–23]. Furthermore, clinical imaging studies demonstrate reduced volumes of the orbitofrontal cortex of depressed patients [24,25] and post mortem neuropathological studies show neuronal and glial cell pathology in this brain area of depressed patients [26–29].

The aim of the present study was to examine whether GABAergic neurons of the orbitofrontal cortex are susceptible to the effect of chronic stress in an animal model for major depression. We used the chronic mild stress (CMS) protocol, which is one of the best validated animal models of depression [30,31]. During the experiment we behaviorally phenotyped the animals into anhedonic or resilient rats, based on their sucrose consumption. At the end of the experiment we did a post mortem quantitative histopathological analysis and examined whether stress affects the number or calbindin-, cholecystokinin-, calretinin-, neuropeptide Y-, parvalbumin- or somatostatin-positive neurons in the OFC. These immunohistochemistry markers identify separate subpopulations of GABAergic interneurons, but all together label almost the entire population of GABAergic cells in the neocortex [32–34]. We counted GABAergic neurons in the different subareas of the OFC, *i.e.* the medial orbital cortex (MO), the ventral orbital cortex (VO), the lateral orbital cortex (LO) and the dorsolateral orbital cortex (DLO). Our hypothesis was that chronic stress will selectively reduce the number of specific types of GABAergic interneurons in the OFC of the anhedonic animals. We also included the primary motor cortex (M1) in our analysis as a "control", non-limbic cortical area, because we expected that stress will affect GABAergic cell numbers only in the limbic OFC cortex.

2. Materials and methods

2.1. Ethics

All animal experiments were conducted in accordance with the guidelines of the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were accepted by the Danish National Committee Ethics in Animal Experimentation (2008/561-477).

2.2. Animals

Adult male Wistar rats (5–6 weeks old, with a body weight about 120 g) were obtained from Taconic (Denmark). The initial experimental group contained 330 rats. 250 rats were submitted to CMS while the rest (80 rats) were used as unchallenged control rats. Out of this large cohort of animals eighteen rats were used in the present histopathological study: $n = 6$ controls, $n = 6$ anhedonic and $n = 6$ stress-resilient rats. The animals were singly housed, except when grouping was applied as a stress parameter. Food and water was available *ad libitum* except when food and/or water deprivation was applied as a stress parameter. The standard 12-h light/dark cycle was only changed in course of stress regime.

2.3. The chronic mild stress (CMS) protocol

The CMS procedure has been described in detail in our earlier publications [35–38]. Briefly, rats were submitted to one period of intermittent illumination, stroboscopic light, grouping, food or water deprivation; two periods of soiled cage and no stress; and three periods of 45° cage tilting. During grouping, rats were housed in pairs with different partners, with the individual rat alternately being a resident or an intruder. All the stressors lasted from 10 to 14 h. Control and stressed animals were kept in separate rooms. Stressed rats were exposed to these mild stressors every day for seven weeks while control rats remained unchallenged. All animals were food and water deprived 14 h before the sucrose consumption test.

2.4. Sucrose consumption test

Sucrose consumption analysis was done as described previously [37]. Initially, the animals were trained to consume a palatable sucrose solution (1.5%). The training lasted for five weeks, with sucrose test conducted twice a week during the first two weeks and once a week during the last three weeks. Animals were food and water deprived 14 h before the test, which was performed

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