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Chronic stress leads to long-lasting deficits in olfactory-guided behaviors, and to neuroplastic changes in the nucleus of the lateral olfactory tract

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ABSTRACT

A recent study reported that the integrity of the nucleus of the lateral olfactory tract (nLOT) is required for normal olfaction and for the display of odor-driven behaviors that are critical for species survival and reproduction. In addition to being bi-directionally connected with a key element of the neural circuitry that mediates stress response, the basolateral nucleus of the amygdala, the nLOT is a potential target for glucocorticoids as its cells express glucocorticoid receptors. Herein, we have addressed this hypothesis by exploring, first, if chronic variable stress (CVS) disrupts odor detection and discrimination, and innate olfactory-driven behaviors, namely predator avoidance, sexual behavior and aggression in male rats. Next, we examined if CVS alters the nLOT structure and if such changes can be ascribed to stress-induced effects on the activity of the main output neurons, which are glutamatergic, and/or of local GABAergic interneurons. Finally, we analyzed if the stress-induced changes are transient or, conversely, persist after cessation of CVS exposure. Our data demonstrate that CVS leads to severe olfactory deficits with inability to detect and discriminate between odors and to innately avoid predator odors. No effects of CVS on sexual and aggressive behaviors were observed. Results also showed that CVS leads to somatic hypertrophy of pyramidal glutamatergic neurons, which likely results from neuronal disinhibition consequent to the loss of inhibitory inputs mediated by GABAergic interneurons. Most of the CVS-induced effects persist beyond a 4-week stress-free period, suggesting long-lasting effects of chronic stress on the structure and function of the olfactory system.

1. Introduction

Prolonged exposure to stress affects virtually all bodily systems and increases the vulnerability to numerous health problems (Chrousos, 2009). In particular, it has been implicated in the pathophysiology of neuropsychiatric disorders such as post-traumatic stress, major depression and anxiety due to the adverse nature of its cumulative effects on neuroplasticity, which ultimately lead to cognitive and behavioral deficits (Chrousos, 2009; Kim et al., 2015; Lupien et al., 2009; Roozendaal et al., 2009). The effects of prolonged stress have been mostly studied, and are more apparent, in brain regions that have been implicated in neuropsychiatric disorders and are sensitive to stress hormones, i.e., the hippocampus, prefrontal cortex and amygdala (reviewed in Radley et al., 2015). Interestingly, the pattern of the stress-induced structural and functional alterations in the amygdala differs completely from that observed in the hippocampus and prefrontal cortex. Moreover, and also in contrast to these regions, different stress paradigms and different durations of stress exposure provoke distinct structural and functional responses in the amygdala (reviewed in Lupien et al., 2009; Radley et al., 2015; Wilson et al., 2015). Also important is that, in contrast to the hippocampus, the morphological and functional changes in the amygdala last for weeks after termination of chronic stress (Sousa et al., 2000; Vyas et al., 2004).

Most studies addressing the effects of stress on the amygdala have focused on the basolateral complex and central nucleus. The basolateral complex receives the majority of the inputs to the amygdala. Here, the information is processed before being transmitted to the central nucleus, which gives rise to projections to the behavioral, autonomic and

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endocrine effector systems (reviewed in McDonald, 1998). Afferents to the basolateral complex derive mostly from cortical areas and convey sensory inputs of all types (McDonald, 1998). Considering the recognized role of olfaction in emotional and cognitive processes (Song and Leonard, 2005; Yuan and Slotnick, 2014), it is intriguing that the flow of olfactory information to the basolateral complex is not direct, but instead mediated by afferents originating in the cortical pallial amygdala, a target area of the main olfactory bulb and piriform cortex (McDonald, 1998). In this context, the nucleus of the lateral olfactory tract (nLOT) is of particular interest as it projects strongly, and in a topographically organized way, to the basolateral amygdaloid complex from where it receives reciprocal connections. Moreover, it also projects to the prefrontal cortex (Jolkkonen et al., 2001; Luskin and Price, 1983; McDonald, 1991; Price, 1973; Santiago and Shammah-Lagnado, 2004), which is bi-directionally connected to the basolateral amygdaloid complex (McDonald, 1998). Last but not the least, it is known that lesions of the nLOT are associated with a complete loss of the sense of smell and disruption of olfactory-guided behaviors (Vaz et al., 2017).

Due to the reasons mentioned above and to likelihood that the nLOT is a stress-sensitive nucleus, as it possesses a large number of cells expressing glucocorticoid receptor mRNA and protein (Morimoto et al., 1996), we thought of particular interest to examine whether the nLOT displays structural and neurochemical alterations that might contribute to explain the functional and behavioral effects of chronic stress, particularly those in which the olfactory system plays a relevant role. To accomplish this, we have used a chronic variable stress paradigm and examined its effects on olfactory functions and innate olfactory-driven behaviors, namely predator avoidance, sexual behavior and aggression. In addition, we have estimated the number and size of nLOT neurons and of its different classes of interneurons, and quantified the local levels of GABA. Finally, to analyze whether the stress-induced alterations would be reversible or permanent, we performed the same studies 4–7 weeks after the end of the stress protocol.

2. Material and methods

2.1. Animals and treatments

The experiments were conducted in male Wistar rats (Charles River, France). After acclimation to laboratory conditions for at least 1 week, rats were housed in groups of 3 and maintained in standard environmental conditions (12-h light/dark cycles with lights on at 0700 h, ambient temperature of 21 ± 1 °C, 45 ± 5% relative humidity) with ad libitum access to food and water, unless specifically noted. All experiments were carried out in accordance with the guidelines of the European Communities Council Directives of 22 September 2010 (2010/63/EU) and Portuguese Act n°113/13, and approved by ORBEA, the internal committee of the Faculty of Medicine, University of Porto (Portugal). At 3 months of age (Fig. 1), rats were randomly assigned to a group receiving chronic variable stress (CVS; $n = 18$), also referred to as chronic unpredictable, mild or intermittent stress (Willner, 2017), to a stress-recovery group (n = 18) or to a control group (n = 18). Starting at 4 months of age, rats of the stressed group were exposed daily, over 4 weeks, to one of four stressors: i.p. injection of hypertonic saline (9% NaCl; 1.0 ml/100 g), overcrowding (1 h), restraint (30 min) and placement on a vibrating/rocking platform (1 h). To maximize unpredictability, stressors were applied at various times during the light phase. Rats of the recovery group started to be exposed to the same CVS paradigm 4 weeks before, i.e., about the age of 3 months and, thereafter, they were left undisturbed for an additional 4 weeks. At the end of the experiments, rats of both groups were submitted to a series of behavioral studies and then killed. Control rats were handled daily before being submitted to the same behavioral evaluation. During the experiments, rats were weighed every morning. Post-mortem weights of adrenal glands and thymus, which were quickly removed after euthanasia, were also recorded as indicators of the efficacy of the stress protocol. Testis were also collected and immediately weighed. For determination of corticosterone levels, blood samples were collected from the dorsal tail vein at the end of CVS exposure, directly from the heart immediately before perfusion or from the trunk after decapitation. The last samples were also used for measurement of testosterone concentrations.

2.2. Behavioral studies

All tests were performed during the standard light phase, starting at 1400 h, except for sexual behavioral and aggression tests that were started 1 h after the beginning of the dark phase. Three days after the end of the experimental periods, rats ($n = 18$/group) were submitted to the buried food test followed by the olfactory habituation/cross-habituation test (Fig. 1). Then, the three main groups were divided in two independent cohorts. One cohort ($n = 9$/group) was submitted to open-field and elevated plus-maze before being tested for olfactory preference tests. The second cohort (n = 9/group) was tested for sucrose preference, and sexual and aggressive behaviors. All tests were applied with at least 1-day inter-trial intervals. In order to maintain a constant lag time between the tests performed in stressed and in recovery rats, the sequence of the tests was the same for all main groups. Rats were killed 3–4 days after the end of behavioral testing (Fig. 1).

2.2.1. Open-field test

To assess general exploratory locomotion and anxiety-like behaviors, an open-field apparatus that consisted of a white acrylic arena (100 × 100 × 40 cm) was used. As previously described (Cardoso et al., 2016; Vaz et al., 2017), the inner zone was defined as 60 × 60 cm square in the center of the arena, leaving a 20 cm broader

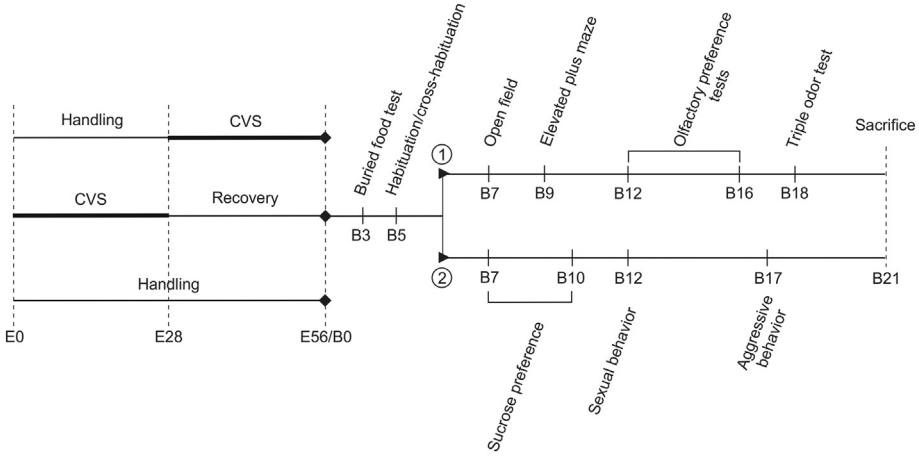

Fig. 1. Experimental timeline of the stress paradigm protocol and sequence of behavioral tests. The beginning of chronic variable stress (CVS) was used as day 0 (E0) of the experiments. After day 28 (E28), rats of the recovery group were left undisturbed for an additional 28 days (Recovery). Rats of the stressed group started to be exposed to CVS at E28. Control rats were handled during the total experimental period (E0-E56). The end of the experimental period (E56) corresponds to day 0 (B0) of behavioral testing. B3-B18, indicate the days at which the different behavioral tests were performed and B21, day of sacrifice.