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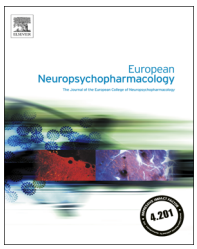

Social *vs.* environmental stress models of depression from a behavioural and neurochemical approach

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Abstract
Major depression is a mental disorder often preceded by exposure to chronic stress or stressful life events. Recently, animal models based on social conflict such as chronic social defeat stress (CSDS) are proposed to be more relevant to stress-induced human psychopathology compared to environmental models like the chronic mild stress (CMS). However, while CMS reproduces specifically core depressive symptoms such as anhedonia and helplessness, CSDS studies rely on the analysis of stress-induced social avoidance, addressing different neuropsychiatric disorders. Here, we study comparatively the two models from a behavioural and neurochemical approach and their possible relevance to human depression. Mice (C57BL/6) were exposed to CMS or CSDS for six weeks and ten days. Anhedonia was periodically evaluated. A battery of test applied during the fourth week after the stress procedure included motor activity, memory, anxiety, social interaction and helplessness. Subsequently, we examined glutamate, GABA, 5-HT and dopamine levels in the prefrontal cortex, hippocampus and brainstem. CMS induced a clear depressive-like profile including anhedonia, helplessness and memory impairment. CSDS induced anhedonia, hyperactivity, anxiety and social avoidance, signs also common to anxiety and posttraumatic stress disorders. While both models disrupted the excitatory inhibitory balance in the prefrontal cortex, CMS altered importantly this balance in the brainstem. Moreover, CSDS decreased dopamine in the prefrontal cortex and brainstem. We suggests that while depressive-like behaviours might be associated to altered aminoacid neurotransmission in cortical and brain stem areas, CSDS induced anxiety behaviours might be linked to specific alteration of dopaminergic pathways involved in rewarding processes.

1. Introduction

Living organisms have developed a series of adaptive mechanisms to cope with stressful situations. This is an invaluable advantage for the healthy organism, which, can

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adapt and overcome the unfavourable conditions. However, when stress response is uncontrollable, as a consequence of the exposure to severe or long periods of stress, it can seriously compromise the individual's health. In the last decades, an important line of research in neurobiology has shown that chronic stress induces a wide range of adverse effects on the central nervous system and is associated to different neuropsychiatric disorders (Mazure et al., 2000; Collimore et al., 2010; Friedman et al., 2011).

Major depression is a mental illness very often described as a stress-related disorder since there is good evidence that both onset and relapse of depressive disorders can be precipitated by repeated stress or severe stressful experiences (Kessler, 1997; Kendler et al., 1999, 2001; Mazure et al., 2000; Monroe et al., 2006; Hammen, 2005; Pittenger and Duman, 2008). Based on this hypothesis, mouse specific models have been developed in an attempt to mimic some of the environmental factors contributing to the induction of depressive disorders in humans. Among them, the chronic mild stress (CMS) model reproduces core clinical symptoms such as long-lasting anhedonia and helplessness (Elizalde et al., 2008) and has demonstrated a good predictive validity as a model of depression in the short and the long-term (Willner, 2005; Elizalde et al., 2008; Tordera et al., 2011).

Nevertheless, in recent years, models based on social conflict have been proposed to have clear advantages over environmental models because they involve a social form of stress, which may be relevant to stress-induced psychopathology in humans (Krishnan et al., 2008). For instance, the chronic social defeat stress (CSDS) model is based in the induction of "social subordination" caused by short periods of struggle and fellowship continued with a dominant animal. A number of hierarchical relations studies show that animals that have been "subordinated" by the dominant individuals of the same species suffer signs of stress (Lagerspetz et al., 1961; Koolhaas et al., 1997) including social avoidance, anxiety, decreased grooming, hyperactivity and increased vulnerability to addiction (Krishnan et al., 2007; Rossi et al., 2008; Denmark et al., 2010). Moreover, significant changes in brain function, neurotransmitters and hormone levels have been reported (Bjorkqvist, 2001; Rohde, 2001; Allen and Badcock, 2003; Lutter et al., 2008; Wagner et al., 2011).

Yet, CSDS studies rely on the analysis of stress-induced social avoidance, which is a psychiatric phenomenon common to different neuropsychiatric disorders such as depression, general anxiety, social phobia and post-traumatic stress disorder (Berton et al., 2006). On the other hand, few studies have examined the long-term effects of CSDS in behaviours more closely related to depression such as reactivity to rewards or helplessness (Krishnan et al., 2007). Here, we have studied comparatively the environmental CMS and the social CSDS stress models from a behavioural and neurochemical approach and have discussed their possible relevance to human depression.

2. Experimental procedures

2.1. Animals

Male C57BL/6 mice (Harlan, France, 8–10 weeks of age) were used. Food and water were available *ad libitum* for the duration of the experimental procedures unless otherwise specified. Animals were maintained in a temperature (21 ± 1 °C) and humidity-controlled room (55 ± 2%) on a 12-h light–dark cycle (lights on at 8:00 h).

Male CD1 retired breeders (Charles River, older than 5 months of age) were used as residents for the CSDS experiment.

Experimental procedures and animal husbandry were conducted according to the principles of laboratory animal care as detailed in the European Communities Council Directive (2003/65/EC), Spanish legislation (Real decreto 1201/2005) and approved by the Ethical Committee of University of Navarra.

2.2. Experimental design for the CMS and CSDS procedures

Before starting the stress models, mice were assigned to the different groups in a way that there were not differences on body weight and sucrose consumption among the groups. Mice (C57BL/6 male, 8–10 weeks of age) were exposed to the CMS procedure for 6 weeks. During this time, control and CMS mice (n=15/group) were singly housed. On the fourth week after the termination of the CMS procedure, a battery of behavioural test was applied. Concomitantly, we studied the long-term behavioural alterations induced by chronic social defeat stress (CSDS). Mice (C57BL/6 male, 8–10 weeks of age) were exposed to the CSDS procedure for 10 day. During this time, CSDS mice (n=15) were housed individually in cages with two compartments separated by a metallic mesh being a dominant mouse the partner. During this time, control mice (n=15), were singly housed in similar cages with a mouse of the same strain in the other half of the cage. Twenty-four hours after the last session, both control and defeated mice were housed individually during the following 4 weeks. During the fourth week, a battery of behavioural tests was applied (Figure 1).

Tests were performed in the following order over 9 day: spontaneous motor activity (day 1), novel object recognition (days 2 and 3), tests of anxiety (novelty suppression feeding and elevated plus-maze) (days 5 and 6), social interaction test (day 7), and test of depression (forced swimming test, FST) (day 8). Tests were performed from 9:00 to 1:00 p.m. In addition, anhedonic-like behaviour was evaluated by monitoring of sucrose intake every 10 day as well as the body weight gain (Elizalde et al., 2008).

Mice were killed in a randomized order two days after the last behavioural test, the brains were removed, and different brain areas were dissected and kept at −80 °C for posterior neurochemical studies.

2.3. Chronic mild stress procedure

Unpredictable repeated mild stressors were applied for 6 weeks following the protocol described by Elizalde et al. (2008) with minor modifications. Briefly, the following stressors (two–three in any 24 h period) were applied: low intensity stroboscopic illumination (in dark 8 h), intermittent bell (10 db, 1/10 s) or white noise (an untuned radio, 4 h), rat odour (saw dust from rat cages; 8 h), cage tilt 45° (8 h), soiled bedding (200 ml of water per cage; 6 h), paired housing (with new partner 2 h), placement of novel object in the home cage (3 h), water and food deprivation (8 h, before sucrose intake test), overnight illumination and removal of nesting material (12 h), and confinement in a small cage (80 cm^3, 1 h).

2.4. Chronic social defeat stress procedure

Chronic social defeat stress (CSDS) procedure was carried out using a similar method described by Tsankova et al. (2006). Briefly, experimental mice were submitted to social defeat stress for 10 consecutive days. Every day, each mouse was introduced into the home cage of an unfamiliar resident. Resident mice were CD1