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THE EFFECTS OF SOCIAL DEFEAT ON BEHAVIOR AND DOPAMINERGIC MARKERS IN MICE

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Abstract—The present study investigated the effects of chronic social defeat stress on several behavioral parameters, and the expression of dopaminergic markers, i.e., dopamine D_1 receptors (D1Rs), dopamine D2 receptors (D2Rs), and dopamine and cyclic adenosine 3′,5′-monophosphate-regulated phosphoprotein-32 (DARPP-32), in the prefrontal cortex (PFC), amygdala (AMY), and hippocampus (HIP) of mouse brains. After 10 days of social defeat stress, the defeated mice were divided into two groups: one group underwent a series of behavioral tests. The other group was sacrificed on the 11th day and tissue samples were collected for Western blotting. The behavioral tests comprised tests of locomotion, light/dark preference, social interaction, as well as the novel object recognition test (NORT), Morris water maze, and forced swimming test (FST). We measured the expression of D1Rs, D2Rs, total DARPP-32, phospho-Thr34 or Thr75-DARPP-32 using Western blotting. The defeated mice showed increased anxiety- and depression-like behaviors, and impaired cognition. No significant differences in D1Rs and D2Rs expression were shown between defeated and control mice in any area studied. A significantly increased expression in total DARPP-32, and phospho-DARPP-32 was observed in the PFC or AMY of defeated mice. These data suggest that alterations in dopaminergic markers may be involved in anxiety- and depression-like behaviors, and cognitive impairment induced by social defeat stress.

Key words: social defeat, behavioral tests, dopamine D_1 receptors, dopamine D2 receptors, dopamine and cyclic adenosine 3′,5′-monophosphate-regulated phosphoprotein-32 (DARPP-32).

INTRODUCTION

Social defeat is defined as losing a confrontation among conspecific animals, or any type of hostile dispute among humans, in either a dyadic or group context. Adapting to social defeat stress is part of social homeostasis and maladaptation to this may have pathological sequelae. There are two main animal models for social defeat stress: the resident/intruder paradigm and the colony model. The resident/intruder paradigm, which is more popular, consists of introducing an intruder male into the home cage of another male, the resident, with the former being defeated by the latter (Martinez and Pico-Alfonso, 1998). Social defeat stress obtained in the resident/intruder paradigm has been reported to cause a variety of molecular, physiological, and behavioral changes (Blanchard et al., 2001; Buwalda et al., 2005). In particular, social defeat stress in animals has been reported to induce depression-like behaviors, such as reduced sucrose preference (Krishnan et al., 2007; Yu et al., 2011). In addition, it induces anxiety-like behaviors, such as more time spent in the dark box in the light/dark preference test (Kinsey et al., 2007), enhanced and prolonged response to acoustic startle (Pulliam et al., 2010), and behavioral sensitization to a wide variety of drugs of abuse including cocaine, amphetamine, and morphine (Covington and Miczek, 2001; Miczek et al., 2004). Hence, the social defeat paradigm has been considered an animal model for depression, anxiety disorder, and drug abuse. Interestingly, substantial evidence suggests that the social defeat paradigm can be further extended as a model of psychosis. Related key findings are a deficit of prepulse inhibition (Adamcio et al., 2009), enhanced mesocorticolimbic dopamine response (Tidey and Miczek, 1996; Cabib et al., 2000), increased phasic activity of ventral tegmental area (VTA) dopamine neurons (Razzoli et al., 2011a), reduced striatal dopamine transporter (DAT) binding (Isovich et al., 2001), and behavioral and neuronal cross-sensitization to amphetamine (Nikulina et al., 2004). Selten and Cantor-Graae (2005) have proposed that social defeat is a risk factor for schizophrenia based on a review of the preclinical and clinical

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Abbreviations: AMY, amygdala; D1Rs, dopamine D1 receptors; D2Rs, dopamine D2 receptors; DARPP-32, dopamine and cyclic adenosine 3′,5′-monophosphate-regulated phosphoprotein-32; EDTA, ethylenediaminetetra acetic acid; EGTA, ethyleneglycoltetra acetic acid; HIP, hippocampus; HRP, horse-radish peroxidase; IgG, immunoglobulin G; NAC, nucleus accumbens; NORT, novel object recognition test; PFC, prefrontal cortex; PKA, protein kinase A; RI, recognition index; TTBS, Tween Tris-Buffered Saline.

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findings that relate to the sensitization of the mesolimbic dopamine system by social defeat or social discrimination. However, it should be noted that these findings are mainly from behavioral experiments, or electrophysiological and neurochemical studies at the presynaptic and synaptic cleft levels, not from studies at the postsynaptic level. These would include dopamine receptors and intracellular dopamine signaling, which are other key targets underpinning the pathogenesis of psychosis.

Currently, only a few studies have investigated the effects of social defeat stress on changes in dopamine receptors. For example, there are dynamic changes over time in the sensitivity of dopamine D_1 receptors (D1Rs) in the frontal cortex of losers (Avgustinovich and Alekseyenko, 2010) and the development of pharmacological desensitization of D1Rs in both losers and winners (Kudryavtseva et al., 2008). In addition, there is increased D1R binding in the caudate and putamen (Novick et al., 2011), and amphetamine-induced blocking of the down-regulation of dopamine D2 receptors (D2Rs) in the nucleus accumbens (NAC) of adult rats exposed to social defeat stress during adolescence (Burke et al., 2011). In our study, we were especially interested in dopamine and cyclic adenosine 3′,5′-monophosphate-regulated phosphoprotein-32 (DARPP-32) within the intracellular dopamine signaling pathway, DARPP-32 is a cytosolic protein that is selectively enriched in medium-sized spiny neurons in the neostriatum (Ouimet et al., 1984). It plays a key role in mediating the effects of dopamine (Svenningsson et al., 2002). It has the unique property of being a dual-function protein, acting either as an inhibitor of protein phosphatase-1 (PP-1) or protein kinase A (PKA). Albert et al. (2002) have provided evidence for decreased DARPP-32 in the prefrontal cortex (PFC) of schizophrenic subjects. Leucocytes expressing DARPP-32 are also reduced in patients with schizophrenia and bipolar disorder (Torres et al., 2009). No study has been conducted to explore the effects of social defeat stress on the expression of DARPP-32 in the brain. We chose the PFC, amygdala (AMY), and hippocampus (HIP) to investigate effects of social defeat stress on dopamine receptors and DARPP-32 for the present study. This was because the PFC and AMY are major target structures of the mesolimbic dopamine system, forming a fear circuit (Pezze and Feldon, 2004). The HIP has strong reciprocal connections with the medial PFC (Goldman-Rakic et al., 1984), and is involved in the detrimental effects of social stress on cognitive performance (Buwalda et al., 2005) and fear extinction (Quirk and Mueller, 2008). The present study aimed to investigate the effects of chronic social defeat on several behavioral parameters including memory performance, and dopaminergic markers, D1Rs, D2Rs, and total- and phosphorylated-DARPP-32 (phospho-DARPP-32), in the PFC, AMY, and HIP in mice.

EXPERIMENTAL PROCEDURES

Animals

Male C57BL/6J and male CD1 mice (Central Lab Inc., Hamamatsu, Japan), aged seven and 14 weeks and weighing 22–25 and 40–44 g, respectively, were used throughout the study. These animals were housed on a 12-h light–dark cycle with access to food and water *ad libitum*, with a constant temperature maintained at 22 °C. All the procedures were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at Chonbuk National University Medical School and all possible efforts were made to minimize the suffering and number of animals used.

Social defeat procedure

The procedure for inducing social defeat stress was carried out as previously reported (Berton et al., 2006). Male CD1 mice were screened for levels of aggressiveness by measuring the latency to attack a naïve C57BL/6 mouse; only mice that attacked in less than 30 s on three consecutive tests were used, which amounted to ~15% of the aggressors screened. C57BL/6 mice were introduced into the home cage of an unfamiliar CD1 aggressor mouse and allowed to interact for 10 min. During this exposure, all subject mice were defeated and showed signs of subordination (i.e., lying on their back, freezing or upright submissive postures). If the aggressor did not attack the intruder, the aggressor was removed and replaced by a new aggressor mouse. After 10 min of full interaction, the defeated mouse was separated from the aggressive resident by introducing a perforated Plexiglas divider into the cage to allow sensory contact for the rest of the day. On the next day, the C57BL/6 mouse was exposed to a new resident CD1 aggressor mouse to prevent habituation. The social defeat procedure lasted for 10 consecutive days. Non-defeated C57BL/6 control mice were placed into equivalent cages with members of the same strain, which were changed daily. After 10 days of social defeat stress, the defeated mice were divided into two groups: the first group was sacrificed for Western blotting on the following day, and the second group were housed individually and underwent a series of behavioral tests (Fig. 1).

Behavior tests

Locomotion test. An automated recording of locomotor activity was conducted in an open acrylic box (30 × 40 × 50 cm) using a video tracking system with SMART software (Panlab, Barcelona, Spain). Mice were allowed to habituate to the testing room for 30 min. Following this, they were put into the testing apparatus and their activity (time and distance moved) was measured for 30 min. Additionally, the time spent in the center (calculated as 25% of total box area) was determined.

Light/dark preference test. The light/dark preference test was performed in a quiet and darkened room. Mice were habituated in the test room for at least 1 h before the session. The apparatus consisted of a rectangular acrylic box (46 × 27 × 30 cm) which was divided into one small (18 × 27 cm) and one large (27 × 27 cm)