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Research report

# Chronic social defeat stress leads to changes of behaviour and memory-associated proteins of young mice

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## HIGHLIGHTS

- Chronic social defeat stress lead to behavioural changes.
- Susceptible and unsusceptible mice showed different behavioural responses.
- CREB phosphorylation may play important roles.

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## ABSTRACT

It is well known that social defeat stress can induce depressive behaviours and cognitive impairment. However, the molecular mechanism by which only a minority of stress-exposed individuals are affected is not clear. In this study, thirty 3-week-old male c57BL/6 mice were exposed to 30 days of social defeat stress, following which susceptible (socially avoidant) and unsusceptible (socially interactive) mice were identified using social investigation. Twenty-four hours after the last episode of defeat, separate groups of mice were tested in the sucrose preference, open field, elevated plus-maze and Morris water maze behavioural assays. Also, the levels of memory-associated proteins in the hippocampus were examined, including postsynaptic density 95 (PSD95), postsynaptic density 93 (PSD93), and Protein kinase A (PKA). The levels of PSD95, PSD93, and PKA were significantly lower in susceptible mice. We also found that the upstream regulatory factor of these proteins, phosphorylated Camp-Responsive Element-Binding Protein (CREB), was reduced after social defeat in the susceptible group only, while the level of histone deacetylase 6 (HDAC6) was significantly elevated. These data suggest that memory-associated proteins and phosphorylated CREB may play important roles in memory impairment and behavioural responses to chronic stress.



## 1. Introduction

Depression is a heterogeneous disease that is primarily characterized by anhedonia and/or symptoms such as social avoidance, helplessness, sleep disturbance, and changes in appetite [1,2]. An estimated 3–7% of the population is thought to have suffered from depression at some point, and there is a high relapse rate, with approximately 80% of patients having a history of two episodes [3]. Stressful life events play an important role in the precipitation of depression [4]. However, some individuals maintain normal psychological and cognitive function after major stress. It is this distinction – between stress-resilient (unsusceptible) and susceptible individuals – that is of current interest. Specifically, the molecular and cellular mechanisms of stress-induced depression warrant further thorough investigations.

The learned helplessness model, chronic mild stress model, and social defeat stress model are established models of animal depression [5–8]. Although animals that are exposed to learned helplessness or chronic mild stress tend to show obvious changes in their long-term behaviour, neurosecretory function, and neurobiology, it is difficult to infer the social effect and to draw a similar conclusion about the molecular mechanisms of stress resistance. Since most environmental stressors associated with depression, including acute or chronic life events, are social in nature [9], the social defeat paradigm is considered to be one of the most robust animal models of stress-induced mood-related illnesses [10]. Compared to other animal models of depression, the social defeat paradigm has higher face, predictive, and ethological valid-

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ity, which results in enduring behavioural and neurobiological changes that mimic several symptoms of the human condition [11].

After prolonged exposure to stress, animals often show an altered brain structure and cognition [12,13], which, in humans, is said to increase the risk of developing neuropsychiatric disorders [14,15]. At the molecular level, brain-derived neurotrophic factor (BDNF) has been reported to play an important role in social defeat stress [10]. As the key downstream regulatory factor, Cyclic AMP response element binding protein (CREB) is a constitutively expressed regulatory nuclear transcription factor involved in not only stress, but also individual development and synaptic plasticity [16,17]. Another transcription factor, namely Histone Deacetylase 6 (HDAC6) has been implicated in stress resilience. Both CREB and HDAC6 have been associated with enhanced stress resilience via regulation of the glucocorticoid receptor (GR) gene and protein modification [18,19]. In addition, most synaptic proteins in the hippocampus that are regulated by CREB and HDAC6, such as postsynaptic density 95 (PSD95), postsynaptic density 93 (PSD93), and Protein kinase A (PKA), have been linked with spatial memory formation [19,20]. Until now, the effect of social defeat stress on CREB and HDAC6 and their substrate proteins has not been elucidated.

Therefore, the aim of the present study was to investigate the effect of chronic social defeat stress on various behavioural responses, including social interaction, anhedonia, anxiety-like behaviours, and spatial memory. This allowed us to identify mice as either 'susceptible' or 'unsusceptible' (i.e. stress-resilient). We then measured the levels of phosphorylated CREB and HDAC6, as well as some of their substrate proteins (PSD95, PSD93, and PKA) in susceptible and unsusceptible young mice.

## 2. Materials and methods

### 2.1. Antibodies

Polyclonal antibodies (pAb; PKAcαt against PKA catalytic α-subunit (1:1000), pAb CREB against total CREB (1:1000), and p-CREB against phosphorylated CREB at Ser133 site (1:1000)) were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). The pAb PSD95 (1:1000) against PSD95 protein, pAb PSD93 (1:1000) against PSD93 protein, Syn-1 against synapsin 1 (1:1000), pAb NR1 against *N*-methyl-D-aspartate receptor (NMDAR) 1, and pAb HDAC6 against histone deacetylase 6 (HDAC6) were from Bioworld Technology Inc. (St. Louis Park, MN, USA). IRDye 800CW conjugated goat (polyclonal) anti-rabbit IgG (1:10000) and IRDye 800CW Conjugated Goat (polyclonal) anti-mouse IgG (1:10000) were from LI-COR Biosciences (Lincoln, NE, USA).

### 2.2. Animals

Forty-five young male *C57BL/6* mice (3 weeks old, 13 ± 3 g) and fifty adult male *Kunming* mice (13 weeks old, 35 ± 5 g) were purchased from Hubei Provincial Disease Prevention and Control Center. All animal experiments were performed according to the "Policies on the Use of Animals and Humans in Neuroscience Research", revised and approved by the Society for Neuroscience in 1995. On arrival, animals were acclimatized to our animal facility for four days. All mice were housed in polypropylene cages (27.8 × 7.5 × 13 cm) with a vivarium at a temperature of 22 °C, under a 12:12 h light/dark cycle (lights on at 8 a.m.), and with access to food and water.

### 2.3. Chronic social defeat stress

The social defeat stress procedure was performed as has been previously reported [10,21]. Every day, each *C57BL/6* mouse (experimental mouse) was put into the home cage of an unfamiliar resident for 5 min and was physically defeated. Resident mice were *Kunming* mice selected for their attack latencies reliably shorter than 30 s upon 3 consecutive screening tests. After 5 min of physical interaction, residents and intruders were maintained in sensory contact for 24 h using a perforated plexiglass partition dividing the resident home cage in two halves. Every day experimental mice were exposed to a new resident home cage. Control animals were housed by pair, one on each side of a perforated plexiglass partition, and were handled daily. A series of behavioural tests were carried out at 9:00 every day after the 30 day period, both groups underwent extensive behavioural testing. The whole experimental procedure is shown in Fig. 1.

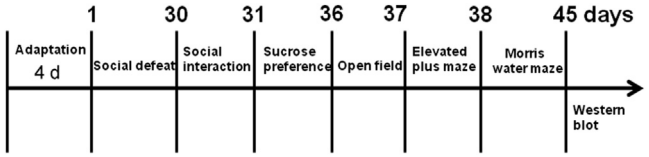


**Fig. 1.** Time-line of experimental procedures for studying the effects of social defeat stress on the behaviour of mice.

### 2.4. Social investigation

The social investigation was used to assay avoidance behaviours toward an aversive social cue [10]. The apparatus was an open-field arena (42 × 42 cm) with a wire-mesh cage (10 × 6 cm) on one side, and the task consisted of two trials of 2.5 min each. In the first ("target absent") trial, the *C57BL/6* mouse was placed in the corner furthest from the cage and was permitted to move freely around the apparatus. In the second ("target present") trial, the mouse was reintroduced into the same apparatus, but an unfamiliar *Kunming* retired breeder mouse was now present in the adjoining cage. During the trials, ANY-maze recording software was employed to quantify the time spent in the corner zones and the "interaction zone" (14 × 26 cm). Interaction ratios (IRs) were calculated by dividing the duration spent in the interaction zone in the target-present condition by that spent in the target-absent condition, multiplied by 100 (expressed as a percentage). This allowed susceptible (IR < 100) and unsusceptible mice (IR ≥ 100) to be identified [7].

### 2.5. Sucrose preference test

The sucrose preference test was performed as has been previously described but with minor modifications [22]. Before the test, all the mice were trained to adapt to sucrose solution. First, mice were presented with two bottles filled with water (water/water) for 24 h, and then one bottle of water was replaced with sucrose solution (1% w/v) for the next 24 h. After a total of 2 days selective drinking, mice were given a bottle of water and a bottle of 1% sucrose solution following a 24 h period of water and food deprivation. The weight of bottles was recorded daily in order to assess the amount of sucrose solution consumed. To avoid a side bias, bottle position was alternated daily. The test was carried out over 2 days and the sucrose preference was calculated using the following equation: sucrose consumption in mL/(water + sucrose consumption in ml) × 100 (expressed as a percentage).

### 2.6. Open field test

For the open field test, mice were placed individually in the centre of a box (40 × 40 × 35 cm). This box was divided 16 equal square sections; 4 inner sections (central area) and 12 outer sections (peripheral area). The latency of the first movement from the