Neurosci. Bull. February, 2016, 32(1):92–98
DOI 10.1007/s12264-015-0002-9

www.neurosci.cn
www.springer.com/12264

REPORT

Opposite Sex Contact and Isolation: A Novel Depression/Anxiety Model

Liu Yang[2] · Li-Jun Shi[1] · Bei Tang[4] · Qiu-Qin Han[3] · Jin Yu[3] · Gen-Cheng Wu[3] · Yu-Qiu Zhang[1]

Received: 10 May 2015 / Accepted: 3 August 2015 / Published online: 19 January 2016

Abstract To mimic human mood disorders, traditional chronic stresses and social defeat stress have been developed and widely applied. However, these active stresses do not mimic the emotional flaws induced by stresses, and their input levels vary greatly. Also, emotional stresses resulting from social unobtainability remain largely elusive due to the lack of useful animal models. In this study, we developed a mouse model named "opposite sex contact and isolation" (OSCI) and found that OSCI induced significant social avoidance, anhedonia, and anxiety. These behavioral defects developed differently after 7 days of OSCI. The social avoidance behavior was self-curable while anxiety gradually worsened but was alleviated by re-pairing with the same female partner. Corresponding to the behavior changes, the plasma corticosterone and phosphorylated cAMP response element binding protein levels were decreased in the nucleus accumbens of the mice that experienced isolation. Together, this study has developed a novel strategy for depression/anxiety modeling and shows that OSCI may be a useful tool for studying the lovelorn/lovesick type of depression.

Keywords Social isolation · Depression · Anxiety · Opposite sex contact · Social stress · Lovelorn

✉ Liu Yang
ribaike@163.com

✉ Yu-Qiu Zhang
yuqiuzhang@fudan.edu.cn

1 Institute of Neurobiology, Institutes of Brain Science and State Key Laboratory of Medical Neurobiology, Collaborative Innovation Center for Brain Science, Shanghai 200032, China

2 Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China

3 Department of Integrative Medicine and Neurobiology, Shanghai Medical College, Fudan University, Shanghai 200032, China

4 Liver Cancer Institute, Zhongshan Hospital, Shanghai Medical College, Fudan University, Shanghai 200032, China

Introduction

Stress-based animal models have been developed to mimic and study human mood disorders. Among them, chronic unpredictable stress (CUS) and chronic mild stress (CMS) are widely applied. Besides, as the main source of stress stimuli in humans is of a social nature, models such as social defeat have been developed. However, the models using social defeat stress have the following shortcomings. First, the stressor is derived from the aggressivity of another male animal, but there are large variations in aggressivity among individuals. Second, these active stresses do not mimic the emotional flaws induced by passive stresses, which would be much more uniform in terms of the stress level. Moreover, many types of emotional stresses are the results of social unobtainability (such as isolation, and being lovelorn or lovesick). Currently, there is no useful animal model to study these mood disorders.

In this study, we developed a prospective mouse model called "opposite sex contact and isolation" (OSCI), and studied its anxiety/depression-like behaviors, as well as related cerebral and hormonal changes.

Materials and Methods

Animals

Male C57BL/6J mice (8 weeks old, Shanghai Laboratory Animal Center, Shanghai, China) were used for model

establishment. The mice were housed in a temperature- and humidity-controlled room with a 12:12 h light–dark cycle (lights on at 07:00) and water *ad libitum*. Behavioral procedures were approved by the Experimental Animal Ethics Committee of Shanghai Medical College, Fudan University. In behavioral tests, 8–18 animals were used in each group; for corticosterone and immunofluorescence analysis, 5–8 animals were used in each group.

OSCI Procedure

The OSCI procedure is shown in Fig. 1. The mouse home cage was divided into two parts by a transparent plastic wall with 20 holes (0.5 cm in diameter), which allowed olfactory, visual, and auditory communication but prevented tactile contact. Each day involved direct contact (DC) and/or indirect contact (IC). In the DC session, a female mouse was introduced into the cage of a male mouse (the modeling mouse), allowing complete contact; in the IC session, the female partner was transferred to the other side of the double-chamber cage. During the 7-day OSCI period, the DC time was 24, 6, 1, 0.5 h, 10, 0, and 0 min from days 1 to 7, with the remaining time in each day being for IC. The difference between days 6 and 7 was that the female mouse was removed on day 7 (complete isolation). Tests were performed on day 8 (i.e. 24 h after removal of the female). In the control group, the male and female mice were kept in different cages for 7 days and never contacted each other.

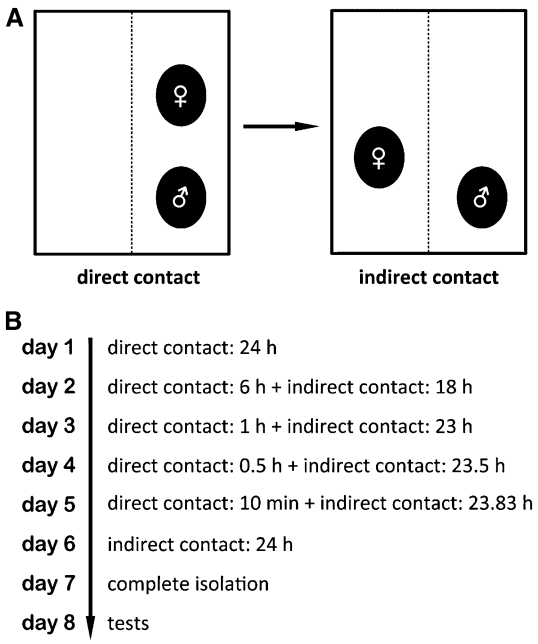

Fig. 1 Schematic representation of the experimental protocol and plan. **A** The resident male was housed on one side of the perforated divider, and the female partner was introduced into the cage for a session of direct contact (DC) and then isolated (indirect contact, IC) by the divider which allowed olfactory, visual, and auditory communication but prevented tactile contact. **B** In the 7-day period of OSCI, DC and IC were repeated but the DC was gradually shortened while IC was gradually lengthened.

Acute OSCI Procedure

A female C57BL/6J mouse was introduced into the cage and allowed to stay with the male mouse for 2 days (36–40 h), then acutely isolated before behavioral tests.

Re-Pairing (RP) Procedure

RP was performed only for the male mice exposed to OSCI stress (Fig. 3A). After 7 days of OSCI, the same female partner was re-introduced into the cage and stayed for 2 days. Then behavioral tests were performed on the experimental mouse.

Social Interaction Test

The social interaction test was conducted on day 8 in a clean open arena (42 cm × 42 cm) as previously described [1]. Each mouse was observed for two sessions (2.5 min each, the first without a target mouse and the second with a target). A mesh cage (10 cm × 6 cm, with or without an unfamiliar male C57BL/6J mouse as the target) was put on the wall of the open field, with a defined "interaction-zone" area 8 cm around it (14 cm × 26 cm). The social interaction ratio was the interaction time of the second session divided by that of the first session.

Sucrose Preference

Sucrose preference is a classical test for anhedonia. Two bottles containing different liquids (boiled water or 2% sucrose solution) were placed in parallel in the cage. The weight loss of the two bottles in 6 h was measured. The percentage of sucrose intake (sucrose solution intake/the total liquid intake × 100%) was calculated to represent the anhedonia level of each animal (decreased sucrose preference indicating increased anhedonia). The test was performed on day 8 or 9.

Elevated Plus Maze

The elevated plus maze was used to assess the anxiety level. The maze consisted of two open arms (without walls) crossed by two closed arms (with 20-cm opaque walls). Mice were placed in the center area facing the open arms and recorded for 5 min. The percentages of entries into the open arms (open-arm entries/total-arm entries × 100%) and the time spent in the open arms (open-arm time/total

time in four arms × 100%) were calculated. This test was performed on day 8, after the social interaction test with at least a 4-h interval to allow sufficient relaxation in the home cage.

Immunofluorescence

For immunofluorescence analysis, the brain was fixed in 4% paraformaldehyde and cut into 30-μm sections. The sections were permeabilized/blocked with 0.3% Triton X-100/5% donkey serum for 2 h, incubated overnight (4 °C) with rabbit anti-p-CREB antibody (Cell Signaling, Danvers, MA), and then incubated for 2 h with the secondary antibody (Alexa 488-labeled donkey anti-rabbit, Life Technologies, USA). Staining for p-CREB in the nucleus accumbens (NAc) was visualized under a confocal microscope (FV1000, Olympus, USA).

Plasma Corticosterone Level Assays

Blood samples were collected at 17:30–18:30 after different stress periods. Mice were anesthetized with ether, rapidly decapitated, and trunk blood was collected. Plasma was separated by centrifugation (3000 rpm, 15 min) and stored at −80 °C. The corticosterone concentrations were determined using a corticosterone ELISA kit (Abcam, UK).

Statistical Analysis

Data are presented as mean ± SEM and were analyzed with Student's *t* test and one-way ANOVA followed by the *post hoc* Student–Newman–Keuls test. $P < 0.05$ was considered statistically significant.

Results

OSCI Induced Anxiety/Depression-Like Behavioral Defects

After 7 days of OSCI stress, the social interaction test was used to assess social behavior, sucrose preference to assess anhedonia, and the elevated plus maze to assess anxiety-like behavior. As expected, the OSCI group exhibited a significantly decreased social interaction ratio (Student's *t* test, $t_{0.05,\ 32} = 2.567$, $P < 0.05$) (Fig. 2A) and sucrose preference (Student's *t* test, $t_{0.05,\ 23} = 2.310$, $P < 0.05$) (Fig. 2B) compared with the control group. In the elevated plus maze test, the OSCI-exposed mice showed lower percentages of open-arm entries (Student's *t* test, $t_{0.05,\ 24} = 2.252$, $P < 0.05$) (Fig. 2C) and time (Student's *t* test, $t_{0.05,\ 24} = 2.553$, $P < 0.05$) (Fig. 2D). These results suggested that OSCI is a novel model of anxiety/depression. Most depression models (such as CUS and CMS) do not induce social avoidance behaviors except for the social defeat model. Here, we found that 7 days of OSCI impaired the level of social interaction. This model combines both characteristics of traditional depression models (like CUS or CMS) and social-derived stress models (like social defeat). However, unlike the anxiety/depression-like behaviors, this behavioral defect (social avoidance) was not maintained for the long term (see below).

RP Relieved OSCI-Induced Anxiety While Social Avoidance was Self-Curable

Given these OSCI-induced behavioral defects, we further investigated whether RP with the same isolated female mouse would alleviate them. As shown in Fig. 3A, one group (OSCI RP) was re-paired for another 2 days and the other was kept alone as control (OSCI no-RP). Meanwhile, another "acute OSCI" group was set to assess what effect would be exerted by just acute isolation rather than a chronic and repeated protocol (here, "being tested" actually served as being acutely isolated). Interestingly, the social interaction ratios of the three groups were all similar to that of the control group (Fig. 3B), suggesting that

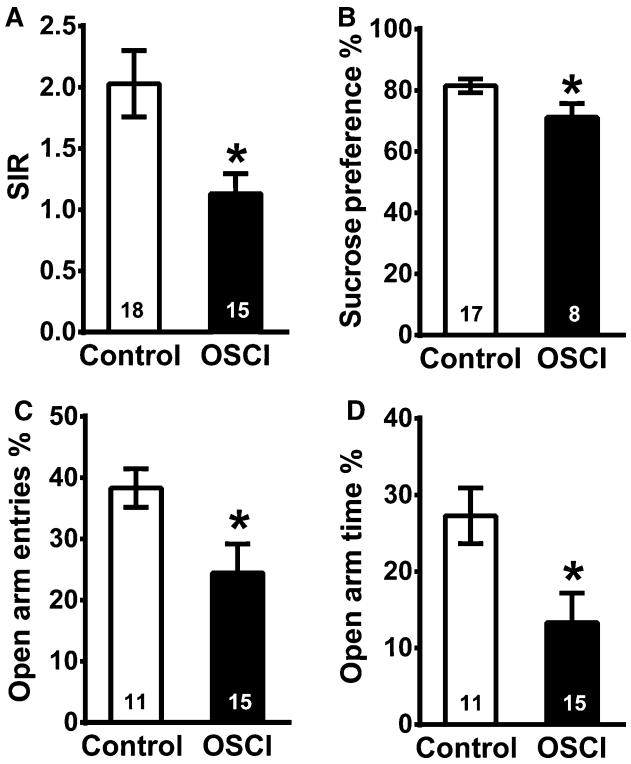

Fig. 2 OSCI induced behavioral defects (social avoidance, anhedonia, and anxiety). **A** The social interaction ratio (SIR) decreased in the social interaction test. **B** The sucrose preference was reduced. **C**, **D** The percentages of open arm entries and time were reduced in the elevated plus maze. *$P < 0.05$.

2 days after OSCI, the social avoidance behavior regressed to the normal level spontaneously, and acute OSCI stress did not induce social avoidance behavior. In the elevated plus maze, the anxiety level of the no-RP group became even worse after two days of further isolation; compared to the no-RP group, RP significantly relieved the anxiety (Fig. 3C, D; one-way ANOVA, for open arm entries, $F_{2,\ 25} = 3.907$, $P < 0.05$, OSCI RP *vs* OSCI no-RP, $P = 0.032$; for open arm time, OSCI RP *vs* OSCI no-RP, $P = 0.089$). Interestingly, acute OSCI also induced significant anxiety-like behaviors (with a level similar to the no-RP group, Fig. 3C, D). This suggested that merely isolating the animal from a familiar opposite-sex partner is sufficient for the development of an anxiety phenotype, and this phenotype lasts at least 2–3 days. Together, OSCI-induced anxiety might be relieved by RP, or else continue and become severe, but OSCI-induced social avoidance is self-curable.

Corticosterone Changes and p-CREB Expression in the NAc of Isolation-Experienced Animals

In contrast to depression/anxiety models, the OSCI group, as well as the other three isolation-experienced groups, exhibited not increased but decreased plasma corticosterone levels (Fig. 4B; one-way ANOVA, $F_{4,\ 22} = 8.633$, $P < 0.01$, OSCI, acute OSCI, no-RP and RP *vs* control, $P < 0.01$). This indicated that the depression/anxiety-like behaviors are not a consequence of stress-induced elevation of plasma corticosterone. The down-regulation of corticosterone may be due to the female partner contact. Besides, the p-CREB immunoreactive cells in both the core and shell zones of the NAc (Fig. 4C) significantly decreased in the OSCI animals compared with controls (Fig. 4D–F; one-way ANOVA, in the core, $F_{4,\ 23} = 8.903$, $P < 0.01$, OSCI *vs* control: $P < 0.01$; in the shell, $F_{4,\ 23} = 4.229$, $P < 0.05$, OSCI *vs* control: $P < 0.01$). No changes were found in the acute OSCI animals. However, two days after OSCI (the no-RP group), these changes disappeared. Interestingly, RP induced a second decrease of p-CREB immunoreactive cells in the shell, which was significantly different from the no-RP group (Fig. 4F; RP *vs* no-RP: $P < 0.05$).

Discussion

This work introduces a novel depression/anxiety model. Similar to the social defeat model, the OSCI model is also social-derived, but a major difference is that the stress of

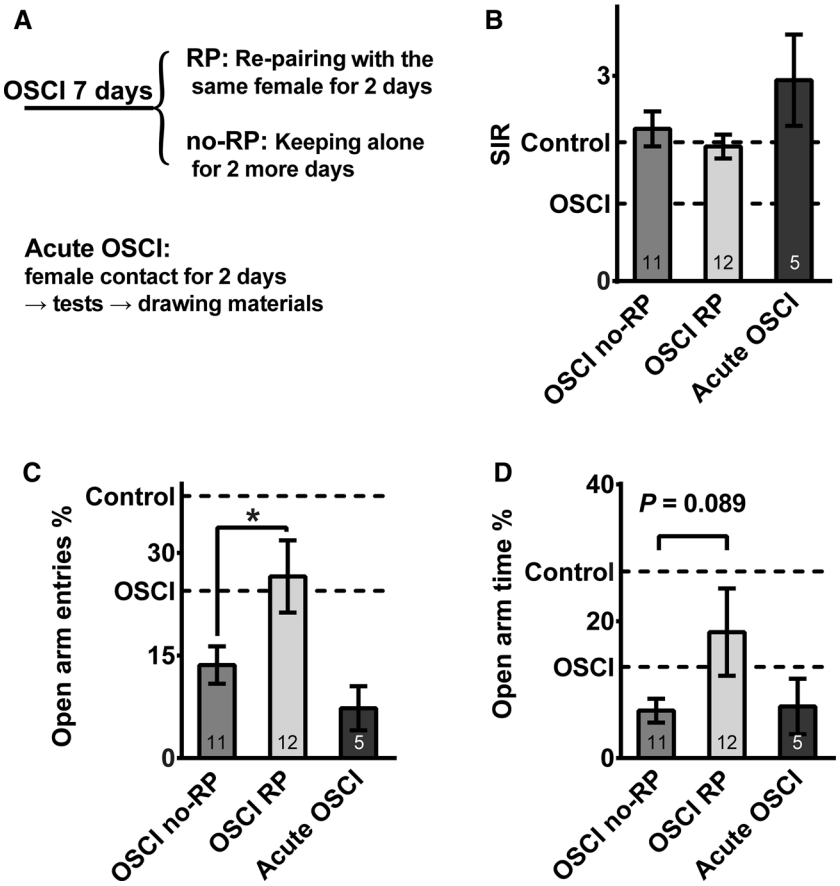

Fig. 3 Re-pairing (RP) relieved OSCI-induced anxiety and social avoidance was self-curable. **A** Schematic representation of the RP and acute OSCI protocols. **B** The social interaction ratio (SIR) showed no difference between the RP and no-RP groups. Acute OSCI did not induce social avoidance, indicating that the decreased SIR was mainly caused by repeated isolation experiences. **C**, **D** RP increased the open arm preference (an expression of an anti-anxiety role) compared to the no-RP group. Simply, the acute OSCI stress induced significant anxiety-like behavior. *$P < 0.05$.

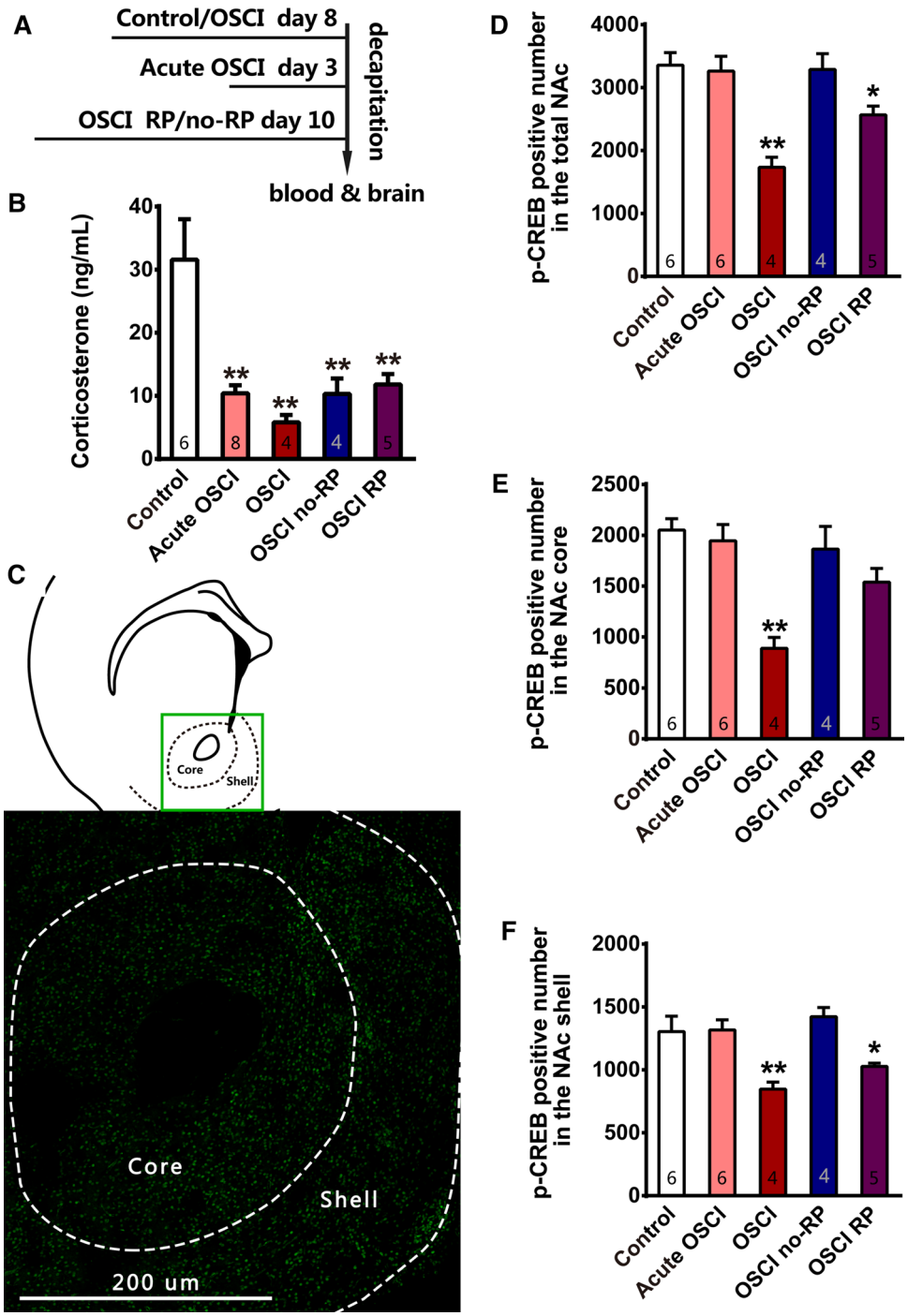

Fig. 4 Corticosterone changes and p-CREB expression in the nucleus accumbens (NAc) of isolation-experienced animals. **A** Time points of taking blood and brain samples. **B** Plasma corticosterone in different groups. **C** p-CREB staining in the NAc zone (the immunofluorescence image shows the area of the green box in the schematic). **D–F** p-CREB immunoreactive cells in the whole (**D**), core (**E**), and shell (**F**) of the NAc. $*P < 0.05$, $**P < 0.01$ compared to control.

social defeat model is induced by active insult while that of OSCI is by passive deprivation of rewards (contact with the opposite sex). Passive types of stresses have the advantage that the error of stress infliction is minimal, which avoids unpredictable variations from the social-interaction partner (e.g. aggressivity) in the positive stress types. Here, we demonstrated that partner isolation is also sufficient to induce depression/anxiety-like behaviors.

To date, the effects of sex hormones on anxiety and depressive-like behaviors are widely known [2], but very few clinical studies have reported lovelorn/lovesick effects in the brain, and this is still a blank slate in animal model research. Clinically, Song *et al*. have reported a negative correlation of NAc functional connectivity with lovelorn duration [3]. Our results are consistent with this finding. It is well known that social isolation increases anxiety-like

behaviors [4, 5]. Nevertheless, social isolation paradigms are overwhelmingly modeled by early-life stress. OSCI has many similarities to the social isolation model and therefore it is not surprising that OSCI also induces anxiety (but compared to social isolation, OSCI induces the additional defect of anhedonia). Travis E. Hodges developed a rat model including social isolation/reunion with the familiar partner (but the same sex) and found that adult rats exhibited decreased social contact after isolation [6].

In terms of mechanism, we demonstrated a change in CREB activation in the NAc, a region possibly responsible for the OSCI-induced behavioral deficits. Some reports have also shown a relationship between sexual behaviors and anxiety, which involves CREB in the NAc. Michel Barrot and his colleagues found that CREB inhibition in the NAc profoundly disrupts the initiation of sexual behavior [7], and decreases in CREB activity in the NAc also increase anxiety-like behaviors [8]. Similarly, John M. Tenayuca and Arbi Nazarian showed that hydrocodone and morphine possess rewarding properties in the conditioned place preference paradigm and reduce the phosphorylation levels of ERK and CREB in the NAc [9]. These conclusions are well supported by ours. In addition, the dopaminergic system in the NAc controls drug addiction, which has similarities to the OSCI model [10]. Moreover, the NAc receives inputs from several regions of the limbic system (e.g. hippocampus, amygdala, and medial prefrontal cortex) [11, 12]. Changes in these regions may participate in the OSCI-induced mood disorders and decreased activity of the NAc. Lesions in the hippocampus lead to depressive-like disorders and decreased social behavior, as well as reduced dendritic length of the medium spiny neurons in the NAc [13]. The hippocampus-NAc circuit is also regulated by the medial prefrontal cortex [14]. Further, some silent synapses in the amygdala-NAc become 'unsilenced' during cocaine withdrawal [15], which also may be one of the mechanisms underlying OSCI-induced anxiety. Besides the CNS changes, we found a decreased corticosterone level in the four groups of OSCI animals, and this may be due to the fact that after sexual behavior the corticosterone release was moderated (*versus* control). Another possible reason is that the control group had a dramatic corticosterone release in behavioral tests while the socially-interacting mice showed habituation and reduced corticosterone release [6]. It was reported years ago that social interaction and chronic nicotine administration attenuate stressor-induced corticosterone [16, 17]. This, combined with our findings, suggests that reward down-regulates corticosterone (but does not necessarily down-regulate anxiety). And our findings are in line with the commonly-accepted view that corticosterone is associated with the suppression of sexual behavior [18–20].

Together, we have developed a novel mouse model of depression/anxiety, which promises to be a useful tool for studying the lovelorn/lovesick type of depression.

Acknowledgments This work was supported by the National Natural Science Foundation of China (31420103903, 31421091, 81471130, and 31300931) and the Development Project of Shanghai Peak Disciplines Integrated Chinese and Western Medicine.

References

1. Han Q, Yang L, Liu Y, Lv N, Yu J, Wu G, *et al.* Resiliency to social defeat stress relates to the inter-strain social interaction and is influenced by season variation. Neurosci Lett 2014, 561: 13–17.
2. Filova B, Malinova M, Babickova J, Tothova L, Ostatnikova D, Celec P, *et al.* Effects of testosterone and estradiol on anxiety and depressive-like behavior via a non-genomic pathway. Neurosci Bull 2015, 31: 288–296.
3. Song H, Zou Z, Kou J, Liu Y, Yang L, Zilverstand A, *et al.* Love-related changes in the brain: a resting-state functional magnetic resonance imaging study. Front Hum Neurosci 2015, 9: 71.
4. Bledsoe AC, Oliver KM, Scholl JL, Forster GL. Anxiety states induced by post-weaning social isolation are mediated by CRF receptors in the dorsal raphe nucleus. Brain Res Bull 2011, 85: 117–122.
5. Chappell AM, Carter E, McCool BA, Weiner JL. Adolescent rearing conditions influence the relationship between initial anxiety-like behavior and ethanol drinking in male Long Evans rats. Alcohol Clin Exp Res 2013, 37 Suppl 1: E394–E403.
6. Hodges TE, McCormick CM. Adolescent and adult male rats habituate to repeated isolation, but only adolescents sensitize to partner unfamiliarity. Horm Behav 2015, 69: 16–30.
7. Barrot M, Wallace DL, Bolanos CA, Graham DL, Perrotti LI, Neve RL, *et al.* Regulation of anxiety and initiation of sexual behavior by CREB in the nucleus accumbens. Proc Natl Acad Sci USA 2005, 102: 8357–8362.
8. Barrot M, Olivier JD, Perrotti LI, DiLeone RJ, Berton O, Eisch AJ, *et al.* CREB activity in the nucleus accumbens shell controls gating of behavioral responses to emotional stimuli. Proc Natl Acad Sci USA 2002, 99: 11435–11440.
9. Tenayuca JM, Nazarian A. Hydrocodone and morphine possess similar rewarding effects and reduce ERK and CREB phosphorylation in the nucleus accumbens. Synapse 2012, 66: 918–922.
10. Hou H, Wang C, Jia S, Hu S, Tian M. Brain dopaminergic system changes in drug addiction: a review of positron emission tomography findings. Neurosci Bull 2014, 30: 765–776.
11. Gill KM, Grace AA. Differential effects of acute and repeated stress on hippocampus and amygdala inputs to the nucleus accumbens shell. Int J Neuropsychopharmacol 2013, 16: 2013–2025.
12. Warner-Schmidt JL, Schmidt EF, Marshall JJ, Rubin AJ, Arango-Lievano M, Kaplitt MG, *et al.* Cholinergic interneurons in the nucleus accumbens regulate depression-like behavior. Proc Natl Acad Sci USA 2012, 109: 11360–11365.
13. Vazquez-Roque RA, Ramos B, Tecuatl C, Juarez I, Adame A, de la Cruz F, *et al.* Chronic administration of the neurotrophic agent cerebrolysin ameliorates the behavioral and morphological changes induced by neonatal ventral hippocampus lesion in a rat model of schizophrenia. J Neurosci Res 2012, 90: 288–306.
14. Belujon P, Grace AA. Critical role of the prefrontal cortex in the regulation of hippocampus-accumbens information flow. J Neurosci 2008, 28: 9797–9805.

15. Lee BR, Ma YY, Huang YH, Wang X, Otaka M, Ishikawa M, *et al.* Maturation of silent synapses in amygdala-accumbens projection contributes to incubation of cocaine craving. Nat Neurosci 2013, 16: 1644–1651.
16. Terranova ML, Cirulli F, Laviola G. Behavioral and hormonal effects of partner familiarity in periadolescent rat pairs upon novelty exposure. Psychoneuroendocrinology 1999, 24: 639–656.
17. Faraday MM, Blakeman KH, Grunberg NE. Strain and sex alter effects of stress and nicotine on feeding, body weight, and HPA axis hormones. Pharmacol Biochem Behav 2005, 80: 577–589.
18. Lombana K, Middleton N, Coddington E. Suppression of sex behavior by kappa opiates and stress steroids occurs via independent neuroendocrine pathways. Gen Comp Endocrinol 2015, 210: 81–86.
19. Boyd SK, Moore FL. Evidence for GABA involvement in stress-induced inhibition of male amphibian sexual behavior. Horm Behav 1990, 24: 128–138.
20. Moore FL, Miller LJ. Stress-induced inhibition of sexual behavior: corticosterone inhibits courtship behaviors of a male amphibian (*Taricha granulosa*). Horm Behav 1984, 18: 400–410.