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# Effects of adolescent social stress and antidepressant treatment on cognitive inflexibility and *Bdnf* epigenetic modifications in the mPFC of adult mice

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ABSTRACT

Adolescent social stress (ASS) can increase susceptibility to depression in adulthood. However, the underlying psychological and neural mechanisms remain unclear. Cortically mediated cognitive dysfunctions are increasingly recognized as an independent and important risk factor of depression. Using social defeat stress, a classical animal model of depression, our previous studies found that mice subjected to this form of stress during early adolescence displayed cognitive inflexibility (CI) in adulthood. This change was accompanied by a down-regulation of *Bdnf* gene expression in the medial prefrontal cortex (mPFC); this gene encodes a key molecule involved in depression and antidepressant action. In the present paper, we identified epigenetic modification of *Bdnf* as a possible mechanism underlying the behavioral and molecular changes. ASS induced a set of depressive phenotypes, including increased social avoidance and CI, as well as reduced levels of total *Bdnf* and isoform IV but not isoform I or VI transcripts in the mPFC. In parallel with changes in *Bdnf* gene expression, previously stressed adult mice showed increased levels of dimethylation of histone H3 at lysine K9 (H3K9me2) immediately downstream of the *Bdnf IV* promoter. On the other hand, no differences were found in trimethylation of histone H3 at lysine K4 (H3K4me3) or in acetylation of histone H3 at lysine K9 (H3K9ac) or at K4 (H3K4ac) in the *Bdnf IV* promoter. Likewise, no alterations were found in DNA methylation of the *Bdnf IV* promoter. Additionally, treatment with the chronic antidepressant tranylcypromine reversed *Bdnf* epigenetic changes and related gene transcription while also reversing CI, but not social avoidance, in previously stressed adult mice. These results suggest that epigenetic changes to the *Bdnf* gene in the mPFC after adolescent social adversity may be involved in the regulation of cognitive dysfunction in depression and antidepressant action in adulthood.

## 1. Introduction

Persistent depressive mood state is the core symptom of major depression. It is increasingly realized that this emotional bias is closely connected with cognitive inflexibility (CI), leading to an exaggerated attentional bias toward – and difficulty shifting attention away from – negative emotional information (Beck, 2008). Lack of improvement in CI after antidepressant treatment increases the remission risk of depression (Chang et al., 2012). Moreover, the existence of CI before the onset of depression can predict first-episode depression and the therapeutic effects of antidepressants (Fossati et al., 2004; Goeldner et al., 2013; Marazziti et al., 2010). Therefore, cognitive dysfunction is increasingly recognized as an independent and important risk factor for depression. The establishment of a transferable animal cognitive model of depression will provide a valuable tool to further investigate the underlying neurobiological mechanisms and develop new therapeutic strategies.

Stress, especially during early life, can increase susceptibility to depression throughout later life (Lupien et al., 2009). It has been shown that various forms of social adversity during adolescence, including bulling and social isolation, significantly increase depressive and anxiety symptoms in adolescents as well as in adults (Buwalda et al., 2011; Sachser et al., 2013). A stronger and more prolonged HPA axis response to stressor exposure during adolescence compared with

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adulthood may explain why adolescence is such a vulnerable period for stress-induced structural and functional effects on brain (McCormick and Green, 2013). This is further emphasized by the fact that several brain areas are still undergoing development alterations during this period, especially the prefrontal cortex (PFC), a key area mediating emotion and cognitive function (Spear, 2000). The "resident-intruder" paradigm is an ethologically valid animal model of depression analogous to bullying in humans and can be used to mimic some aspects of symptoms found in depressive patients (Golden et al., 2011). Recently, we reported that this form of social stress during early adolescence had a delayed effect on cognitive flexibility, inducing cortically mediated cognitive inflexibility in adult but not adolescent mice (Xu et al., 2016; Zhang et al., 2017). Concomitant with the cognitive impairment, the mRNA and protein expression of brain-derived neurotrophic factor (BDNF), a key molecule in depression and antidepressant action, showed a time-dependent decrease, particularly in the medial PFC (mPFC) of adult mice (Xu et al., 2016). However, that study did not address the regulatory mechanisms underlying these long-term behavioral and molecular effects. Increasing clinical and basic evidence has demonstrated that early stress can leave persistent epigenetic marks, which alter gene transcription without altering the DNA sequence, mediating the lasting effects of stress on brain and behavioral functioning throughout adulthood (Lutz and Turecki, 2014).

Epigenetic mechanisms are most commonly regulated by DNA methylation and posttranslational modification of histones (Moore et al., 2013). In general, DNA methylation and histone acetylation are considered to inhibit and activate gene transcription, respectively, while histone methylation can achieve both gene activation and repression depending on the lysine or arginine residue methylated and the number of methyl groups incorporated (Peter and Akbarian, 2011). Data from depressive patients, as well as from animal models, have indicated that epigenetic changes in the *Bdnf* gene may be involved in depression and antidepressant action in a regionally specific manner (Duclot and Kabbaj, 2015; Tadic et al., 2014; Boulle et al., 2012; Covington et al., 2011). For example, epigenetic modifications of the *Bdnf* gene and its expression in the hippocampus and nucleus accumbens exert different effects on depressive behaviors induced by chronic social stress in adult mice (Tsankova et al., 2006; Covington et al., 2011; Koo et al., 2016). In addition, epigenetic mechanisms underlying experience-dependent neural changes during development exert vital roles in the dynamic regulation of gene expression in the developing and adult brain (Fagiolini et al., 2009). Thus, the developmental stage when the stressor occurred may be a factor relevant to profiles of epigenetic change after early stress (Lutz and Turecki, 2014; Morrison et al., 2014; Roth et al., 2009). For example, prenatal stress induces decreased transcription levels and increased DNA methylation levels at promoter IV of the *Bdnf* gene in the hippocampus and amygdala of neonatal and adult mice (Boersma et al., 2014). A recent study by our group showed that adolescent social isolation increased and decreased *Bdnf* total mRNA levels in the mPFC and hippocampus, respectively, of adult mice, accompanied by changes in acetylated histone H3 levels in these areas (Li et al., 2016). We aimed to further identify the profile of epigenetic changes in the *Bdnf* gene induced by adolescent social stress, a classical model of depression, as well as whether these changes underlie the emotional and cognitive symptoms of depression. Finally, we aimed to investigate whether antidepressants could normalize the behavioral and molecular changes induced by adolescent social stress.

Using adolescent social stress model to induce depressive symptoms during adulthood, this study first assessed the expression of total *Bdnf* mRNA and three *Bdnf* splice variants (*Bdnf* I, *Bdnf* IV and *Bdnf* VI) in the mPFC, as these three *Bdnf* variants are the main variants expressed in the brain (Dong et al., 2015; Roth et al., 2009; Fuchikami et al., 2011; Rousseaud et al., 2015). Having observed a consistent reduction in the levels of *Bdnf* total and IV but not I or VI mRNA in previously stressed adult mice, we next examined the levels of DNA methylation and histone 3 (H3) modification around the promoter of *Bdnf IV*. These epigenetic targets were chosen because DNA methylation and histone 3 modifications, especially acetylation and methylation at histone 3 are extensively modified in stress response, neural plasticity and cognitive activity (Anier et al., 2014; Day and Sweatt, 2011; Gupta-Agarwal et al., 2012; Zovkic et al., 2013). Furthermore, we investigated whether the observed behavioral and molecular alterations could be ameliorated by chronic treatment with the antidepressant drug tranylcypromine (TCP), a nonselective monoamine oxidase inhibitor (MAOI) that has been reported to be effective against emotional symptoms in major depression and bipolar disorder (Heijnen et al., 2015). Through this combination of experiments, the present study aimed to investigate the epigenetic modifications involved in depression-related cognitive dysfunction and its response to antidepressant treatment.

## 2. Materials and methods

### 2.1. Animals

Male C57BL/6J mice to be used as intruders were obtained at weaning (postnatal day [PND] 21) from our in-house breeding program (Center for Experimental Animal, Institute of Psychology, Chinese Academy of Sciences). For the next week, siblings were housed in groups of 2–4 mice/cage with free access to water and food. Male CD1 mice to be used as residents were obtained from Vitalriver and housed singly until 3–4 months old. The room temperature was maintained at 20 ± 2 °C with a 12 h/12 h light–dark cycle (lights on at 07:00 a.m.).

Experimental procedures were performed with the approval of the Institutional Review Board of the Institute of Psychology, Chinese Academy of Sciences, and according to the guideline of the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

### 2.2. Social stress

On PND28, male litters were assigned to stress (STR) and control (CON) groups and exposed to social defeat stress using the "resident-intruder" paradigm or control manipulation for 10 consecutive days as described previously (Golden et al., 2011; Xu et al., 2016; Zhang et al., 2016). Briefly, STR mice were directly confronted with a novel CD1 aggressor for 5 min daily and then separated from the aggressor by a transparent, perforated divider to maintain sensory contact for the remainder of the day. During the 5 min of physical contact, at least one visible gesture of subordination occurred. During the stress period, CON mice were housed with a novel C57BL/6J male mouse daily on opposite sides of the divider and never allowed physical contact with the novel mouse.

After the last social defeat episode, STR mice were singly housed, while CON mice were returned to group housing with former cagemates for a 6-week recovery period leading up to adulthood behavioral tests. Our previous study has shown that single housing after the stress is required for the expression of cognitive inflexibility in adult mice subjected to adolescent social defeat, and this effect can be ameliorated by social housing (with siblings) after the stress (Zhang et al., 2016). Therefore, a protocol that we designate "adolescent social stress" (ASS, consisting of adolescent social defeat followed by single housing) was used in the present study.

### 2.3. Drug administration

Tranylcypromine (TCP, Sigma–Aldrich) was dissolved in saline (0.9%) at a concentration of 10 mg/ml. The drug or saline was intraperitoneally injected once daily in a quantity of 0.1 ml/10 g body weight for 14 days before behavioral tests in adulthood (PND65-78). This dose was selected on the basis of its stable antidepressant-like effects reported in other studies (Altar et al., 2003; Ivy et al., 2003).