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# Neonatal NMDA receptor blockade alters anxiety- and depression-related behaviors in a sex-dependent manner in mice

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ABSTRACT

There is increasing evidence that *N*-methyl-D-aspartate (NMDA) receptor blockade in the neonatal period has a long-lasting influence on brain and behavior development and has been linked to an increased risk for neuropsychiatric disorders in later life. We sought to determine whether postnatal NMDA receptor blockade can affect normal development of body weight, corticosterone levels, anxiety- and depression-related behaviors in male and female mice in adulthood. For this purpose, male and female NMRI mice were treated with either saline or phencyclidine (PCP; 5 and 10 mg/kg, s.c.) on postnatal days (PND) 7, 9, and 11, and then subjected to different behavioral tests, including open field, elevated plus-maze, elevated zero-maze, light-dark box, tail suspension test and forced swimming test in adulthood. The results indicated that neonatal PCP treatment reduced body weight during neonatal and adulthood periods, and did not alter baseline corticosterone levels in both male and female mice. Moreover, this study obtained some experimental evidence showing the PCP at dose of 10 mg/kg increases stress-induced corticosterone levels, anxiety- and depression-related behaviors in males, while decreasing levels of anxiety without any significant effect on depression in female mice in adulthood. These data support the argument that neonatal NMDA receptor blockade can lead to behavioral abnormalities and psychiatric diseases in adulthood. Collectively, our findings suggest that neonatal exposure to PCP may have profound effects on development of anxiety- and depression-related behaviors in a sex- and dose-dependent manner in mice.



## 1. Introduction

The neonatal period is an important neurodevelopmental stage to the understanding of the etiology and developmental trajectory of neuropsychiatric disorders such as anxiety and depression in later life. Epidemiological reports indicate that these behavioral dysfunctions are complex and multifactorial disorders involving both genetic and environmental factors (Anisman et al., 2008). It was found that anxiety is often associated with depression (Beuke et al., 2003) which is not surprising, considering the notion that these disorders share a common genetic pathway (Kendler et al., 2007; Williamson et al., 2005). There are fundamental differences between male and female in many psychological and behavioral aspects, and also the structure and morphology of the brain in both sexes are highly similar. Hence, there are consistent differences between them with subsequent important implications for each sex (Bao and Swaab, 2011; Ngun et al., 2011). For example, anxiety and depression are almost twice as common in females as in males (McLean and Anderson, 2009); however the factors mediating these differences are not well understood.

A number of recent studies have demonstrated that *N*-Methyl-D-aspartate (NMDA) receptor plays an important role in normal brain development and plasticity during early life (du Bois et al., 2009a; du Bois and Huang, 2007; Lim et al., 2012). It has been documented

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that developing neurons strongly depend on NMDA receptor activation for neuronal survival, differentiation, migration, and synaptogenesis (du Bois and Huang, 2007). In this respect, multiple lines of evidence suggest that perinatal exposure to NMDA receptor antagonists such as MK-801, Ketamine and phencyclidine (PCP) leads to behavioral abnormalities including learning and memory dysfunctions, hyperlocomotion, as well as impaired prepulse inhibition (PPI) and sensorimotor gating which are particularly related to schizophrenia symptoms and these antagonists can also alter other psychological aspects of brain function in adult laboratory animals (Andersen and Pouzet, 2004; du Bois and Huang, 2007; du Bois et al., 2008b; Lim et al., 2012; Nakatani-Pawlak et al., 2009; Turner et al., 2012; Viberg et al., 2008; Wang et al., 2001; Wiley et al., 2003). In the first three weeks after birth, especially postnatal days (PND) 7–14, exposure to NMDA receptor antagonist increases the normal apoptotic neurodegeneration process, because this stage is corresponding to the brain developmental synaptogenesis and peak expression of NMDA receptors (du Bois and Huang, 2007). These evidences demonstrate the important role of NMDA receptor in the brain development, so that brain is highly vulnerable and sensitive to the NMDA receptor blockade in the limbic system during early development.

Furthermore, there is robust evidence to support the links between neonatal manipulation and lifelong changes in hypothalamic–pituitary–adrenal (HPA) axis function, which can play a key role in the regulation of anxiety- and depression-related behaviors (Doosti et al., 2013; Millstein and Holmes, 2007; Ros-Simó and Valverde, 2012). Along with these lines, many studies have shown that different neurotransmitter systems such as serotonin, gamma-aminobutyric acid (GABA), dopamine and glutamate are involved in control of anxiety and depression in the brain (Dunlop and Nemeroff, 2007; Graeff et al., 1996; Möhler, 2012). In addition, there is very strong evidence that early postnatal NMDA receptor blockade alters some neurotransmitter networks in the brain (du Bois et al., 2009a, 2008a; du Bois and Huang, 2007; Lim et al., 2012). In this regard, several studies concluded that postnatal NMDA receptor blockade correlates with an increased risk for schizophrenia (du Bois and Huang, 2007; Lim et al., 2012). However, little attention has so far been devoted to the evaluation of the long-term impacts of neonatal PCP treatment on development of anxiety- and depression-related behaviors in both male and female mice in adulthood. Therefore, the aim of the current study was to investigate the effects of early postnatal PCP exposure on development of body weight, corticosterone (COR) levels, anxiety- and depression-related behaviors in both male and female mice in adulthood.

## 2. Materials and methods

### 2.1. Animals

Adult male and female albino NMRI mice (10–12 weeks of age) were obtained from the animal house of Pasteur Institute of Iran. Animals were housed in standard polycarbonate cages (4 per cage; same sex) in a room with a 12:12 h light/dark cycle (lights on 08:00–20:00 h), controlled temperature (23 ± 1 °C) and had free access to food and water. These conditions were kept as a standard housing condition in all stages of experiments. After a 2-week period of acclimatization to the new animal holding room, in order to facilitate of mating, male and female mice were kept together one-by-one in a cage. Animals were closely monitored every day for the presence of common mouse diseases; all checks were negative. Female mice were visually examined daily for confirmation of pregnancy, when it was confirmed the female mice were removed from the breeding cages and housed individually in standard cages. All pregnant mothers were allowed for normal delivery. The first day of birth was considered postnatal day (PND) 0. One day after the birth, all litters were culled to 6 pups per mother (3 female and 3 male). On the day 21, litters were weaned by removal of the mother and then were housed with the same sex littermates (3 animals per cage). A total of forty litters were used during this study in four stages, each of which included 10 litters. Only one mouse per sex per dam was selected for each of the group to avoid the litter-effect. All procedures described in the current study had been approved by the Research and Ethics Committee of Ardabil University of Medical Sciences, and are in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institute of Health (NIH; Publication No. 85-23, revised 1985).

### 2.2. Experimental design

A summary of the experimental design is shown in Fig. 1. During this research project, pups were divided into 4 clusters (each cluster only used for two tests, with a 7-days interval between each test), each of which consisted of three main groups: control group (in which pups were subjected to the same treatment conditions, but injected with saline 1 ml/kg), and two PCP groups. Eight male or female mice were included in each group. In the present study the pups received subcutaneously (s.c.; in the interscapular region) injections of PCP hydrochloride (Tocris Co., UK) at doses of 5 and 10 mg/kg or vehicle solution at a volume of 1 ml/kg once daily on the PNDs 7, 9 and 11. The PCP was dissolved in sterile saline (0.9% NaCl) and injections were performed between 10:00 and 11:00 A.M. Each injection was performed through a 27-gauge needle connected by polyethylene tubing to a 10-μl Hamilton syringe. In this study, each male or female mouse was tested only once in the one test.

### 2.3. Body weight

The body weights of male and female mice (0.01 g) were regularly monitored at 13:00 h every 10 days from PND 7 to 11 or 60.

### 2.4. Corticosterone

Baseline and stress-induced COR levels were measured on the PND 65, respectively, five days after the last weight measurement and following the experiment of the EPM test. Blood in male and female mice was collected using cardiac puncture method as previously described (Enayati et al., 2012). Circulating levels of COR (Bio-Medical Assay Company, 20635) in mice serums were evaluated using the

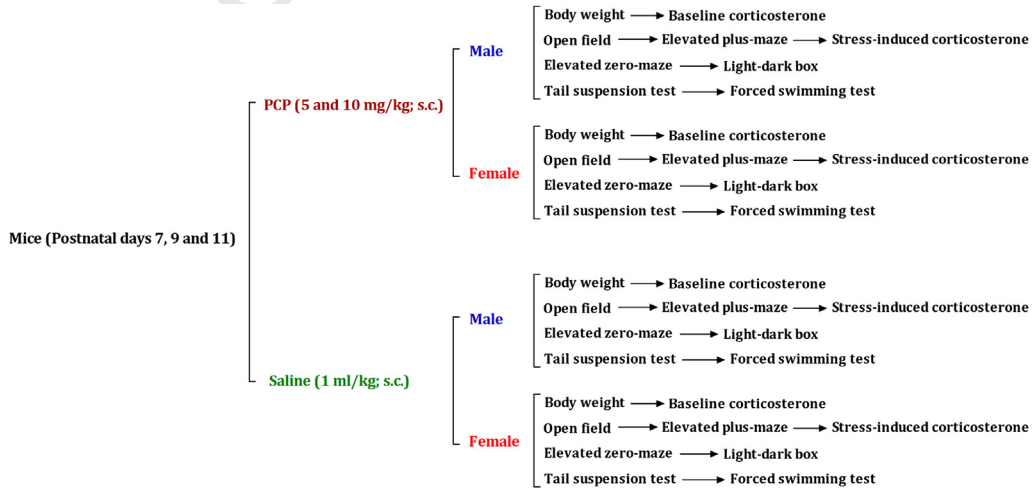


**Fig. 1.** Experimental design: the effects of neonatal PCP treatment on body weight, corticosterone, anxiety- and depression-related behaviors in adult female and male mice.

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