Physiology & Behavior 145 (2015) 38–44

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

# Co-occurrence of anxiety and depressive-like behaviors following adolescent social isolation in male mice; possible role of nitrergic system ☆

Shayan Amiri [a,b], Arya Haj-Mirzaian [a,b], Maryam Rahimi-Balaei [c], Ali Razmi [d], Nastaran Kordjazy [a,b], Armin Shirzadian [a,b], Shahram Ejtemaei Mehr [a,b], Hamed Sianati [e], Ahmad Reza Dehpour [a,b,*]

[a] Experimental Medicine Research Center, Tehran University of Medical Sciences, Tehran, Iran
[b] Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, P.O. Box, 13145-784 Tehran, Iran
[c] Department of Human Anatomy and Cell Science, College of Medicine, Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada
[d] Pharmacology and Applied Medicine Department of Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran
[e] School of Medicine, Iran University of Medical Sciences, Tehran, Iran

## HIGHLIGHTS

- Social isolation stress induced affective responses in adolescent Swiss male mice.
- Nitrergic system is involved in the behavioral deficits caused by social isolation.
- Social isolation induced nitric oxide overproduction in the cortex and hippocampus.
- Aminoguanidine and L-NAME, but not 7NI, reversed the behavioral difficulties.
- Decrease in nitric oxide production by nNOS did not change the affective responses.

## ARTICLE INFO

*Article history:*
Received 2 December 2014
Received in revised form 28 February 2015
Accepted 19 March 2015
Available online 26 March 2015

*Keywords:*
Social isolation stress
Adolescence
Depression
Anxiety
Inducible NOS
Nitric oxide

## ABSTRACT

Approximately more than 50% of patients with depression have the co-occurrence of anxiety, which complicates the treatment of disease. Recently, social isolation stress (SIS) paradigm has been suggested as an animal model to investigate the underlying mechanism involved in depression–anxiety co-occurrence. In this study, applying six weeks of SIS to adolescent mice, we tested whether nitrergic system plays a role in co-occurrence of depression and anxiety. In this study, comparisons between socially and isolated conditioned (SC and IC) animals showed that SIS induces behaviors relevant to depression and anxiety in IC mice and in addition, nitrergic system is involved in mediating the negative outcomes of SIS. Administration of subeffective doses of aminoguanidine (a specific inducible nitric oxide synthase inhibitor or iNOS, 50 mg/kg) and L-NAME (non-specific inhibitor of NOS, 10 mg/kg) significantly reversed the negative effects of SIS on behavioral profile as well as nitrite levels in the cortex of IC mice, Although administration of subeffective dose of 7-nitroindazole (a specific neuronal NOS inhibitor, 25 mg/kg) decreased the nitrite levels in the hippocampus, but had no effect on depressant and anxiogenic effects of SIS. Results of this study confirmed that SIS is an appropriate animal model to investigate the potential mechanisms in depression–anxiety co-occurrence. We also showed that nitrergic system has contributed to co-occurrence of depression and anxiety in IC mice as an underlying mechanism.



## 1. Introduction

Mood and anxiety disorders have a large variety of similar pathophysiological characteristics and co-occur in nearly 50–60% of clinical subjects [1–3]. Evidence from both human and animal studies indicates that early life exposure to environmental and social stressors plays a putative role in development of affective disorders [4,5]. In animal studies, using chronic stress paradigms has provided conditions to investigate the underlying mechanisms, which are involved in the pathogenesis of mood and anxiety disorders [6]. A large body of evidence indicates that applying social isolation stress (SIS) in adolescence is able to induce profound behavioral and neurochemical changes in rodents [7,8]. Also, it is has been shown that SIS evokes symptoms similar to those observed

☆ Please note that the first two authors are considered as the first authors.

* Corresponding author at: Experimental Medicine Research Center, Tehran University of Medical Sciences, P.O. Box 13145-784, Tehran, Iran.
*E-mail addresses:* dehpoura@sina.tums.ac.ir, dehpour@yahoo.com (A.R. Dehpour).

http://dx.doi.org/10.1016/j.physbeh.2015.03.032
0031-9384/

in patients with depression and anxiety [9,10] and also, can be used as an animal model for investigating of mechanisms involved in anxiety–depression co-occurrence [11]. Stress negatively affects regions of the brain, which play substantial role in regulation of emotion including the hippocampus (HIPP) and cortex [12,13]. Under stressful conditions, these areas of brain undergo various neurochemical changes including nitric oxide (NO) overproduction [14,15].

Nitric oxide is a signaling molecule, which regulates key functions in the central nervous system (CNS) as both a neurotransmitter and a neuromodulator. This molecule is synthesized in the brain from oxidation of L-arginine by nitric oxide synthase (NOS) [16]. Nitric oxide synthases are a family of three proteins consisting of endothelial (eNOS), neuronal (nNOS) and inducible NOS (iNOS) which play a modulatory role in biological processes such as cardiovascular regulation and neurotransmission [17]. In this context, eNOS and nNOS are constitutive isoenzymes, which account for generation of low levels of NO, while it has been shown that iNOS is responsible for overproduction of NO mostly under pathological conditions [16]. Previous studies have shown that nitrergic system is involved in the pathogenesis of mood and anxiety disorders [18]. Administration of NOS inhibitors, such as aminoguanidine (a specific iNOS inhibitor), 7-nitroindazole (a specific nNOS inhibitor), and NG-nitro-L arginine methyl ester (a nonspecific NOS inhibitor) have been shown to have antidepressant [19,20] and anxiolytic [21,22] properties. Also, NO plays a role in the mechanism of action of some antidepressant and anxiolytic drugs [23,24].

This study was aimed to investigate the hypothesis whether nitrergic system has contributed in the co-occurrence of anxiety and depressive-like behaviors provoked by adolescent social isolation in mice.

## 2. Materials and methods

### 2.1. Animals and conditions

Swiss albino male mice weighting 10–14 g in postnatal day (PND: 21–25) (Pasteur Institute, Tehran, Iran) were used throughout the study. Animals were randomly housed for six weeks under two different conditions: 1) Social Condition (SC), and 2) Isolated Condition (IC). Socially conditioned mice were housed (6 per cage) in Plexiglas boxes (25 × 25 × 15 cm) and IC mice were housed individually in Plexiglas boxes (24 × 17 × 12 cm) and were able to have visual contact. Wood shavings were used as bedding for animals and cages of isolated animals were cleaned weekly by the same experimenter to avoid minimum handling and social contact. All behavioral experiments were conducted during the period between 10:00 and 13:00 under dim light (12-hour regular light/dark cycle) and temperature (22 ± 1 °C). All procedures in this study were carried out in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH publication #80-23) and institutional guidelines for animal care and use (Department of Pharmacology, School of Medicine, TUMS). Also, each experimental group contains 6 to 8 animals.

### 2.2. Drugs

The following drugs were used in this study: 1) $N^G$-L-arginine methyl ester (L-NAME), a non-selective NOS inhibitor, 2) aminoguanidine (AG), a selective iNOS inhibitor, 3) 7-nitro indazole (7-NI), a selective nNOS inhibitor, and 4) L-arginine (L-arg), an NO precursor (all drugs were purchased from Sigma, St Louis, MO, USA). All drugs were freshly dissolved in physiological saline except for 7-NI which was dissolved in Tween 80 1% solution. All injections were through intraperitoneal (*i.p.*) route and with a volume of 5 mL/kg body weight.

### 2.3. Forced swimming test (FST)

The test was directed using the method of Porsolt et al. [25,26]. In brief, mice were separately placed in an open cylinder-shaped flask (diameter: 10 cm, height: 25 cm), containing 19 cm water at 23 ± 1 °C. Mice were permitted to swim for 6 min and the immobility time was recorded throughout the last 4 min of the test. Each mouse was judged to be immobile when it ceased struggling and stayed floating motionless in the water, making only those movements necessary to keep its head above water.

### 2.4. Splash test

This test was used to evaluate the self-care behavior in animals. In this test, grooming behavior of mice, which can be considered as an indirect measure of palatable solution intake, was measured. A 10% sucrose solution was squirted on the dorsal coat of animals in their home cage and mice were videotaped for 5 min. The total grooming activity time was recorded during 5 min after the sucrose vaporization. Grooming activity consists of nose/face grooming, head washing and body grooming [27].

### 2.5. Open-field test (OFT)

The open-field test was used to evaluate the locomotion and anxiety behavior of animals in response to SIS [28]. The open-field apparatus was made of white opaque Plexiglas (50 cm × 50 cm × 30 cm), which was dimly illuminated. Each mouse was placed gently on the center square (30 cm × 30 cm), and behaviors were recorded by a camera for 5 min and were analyzed by EthoVision software version 8 (Noldus, Netherlands). The surface of the apparatus was cleaned with 70% ethanol after each experiment. The distance moved (horizontal activity), time spent in the central zone, and the number of rearings (vertical activity) were evaluated.

### 2.6. Hole-board test (HBT)

The hole-board test was used to evaluate the anxiety of subjects [29]. The apparatus consisted of a white Plexiglas square (50 cm × 50 cm) with 16 equidistant holes (3 cm in diameter) and was positioned 50 cm above the floor. Mice were placed in the center of the board and the number of head-dips was counted in a 5-min period. The apparatus was cleaned with 70% ethanol after each experiment.

### 2.7. Nitrite assay

To determine the nitrite levels in both HIPP and cortex, nitrite levels were measured as the result of NO end product [30]. The animals were sacrificed under mild anesthesia and HIPP and cortex was dissected on ice-cold surface and immediately immersed into liquid nitrogen. Tissue homogenates were prepared and nitrite levels were assessed by a colorimetric assay based on the Griess reaction. First, each well was loaded by 100 μL samples and mixed with 100 μL Griess reagent. Following 10 min of incubation in room temperature the absorbance was measured at 540 nm in an automated plate reader. Concentration of nitrite was determined by reference to a standard curve of sodium nitrite (Sigma, USA) and normalized to the weight of each sample.

### 2.8. Experiment design and treatments

After 6 weeks of housing under isolation or social conditions, animals (PND: 63–67) were subjected to behavioral assessments. In the first part of study, effects of housing conditions on the anxiety and depressive-like behaviors were investigated in the FST, OFT, HBT and splash test (4 groups IC + 4 groups SC). As behavioral tests like FST and OFT are considered as acute stressors and socially isolated animals