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Research report

Attenuation of oxidative and nitrosative stress in cortical area associates with antidepressant-like effects of tropisetron in male mice following social isolation stress

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ABSTRACT

Tropisetron, a 5-HT3 receptor antagonist widely used as an antiemetic, has been reported to have positive effects on mood disorders. Adolescence is a critical period during the development of brain, where exposure to chronic stress during this time is highly associated with the development of depression. In this study, we showed that 4 weeks of juvenile social isolation stress (SIS) provoked depressive-like behaviors in male mice, which was associated with disruption of mitochondrial function and nitric oxide overproduction in the cortical areas. In this study, tropisetron (5 mg/kg) reversed the negative behavioral effects of SIS in male mice. We found that the effects of tropisetron were mediated through mitigating the negative activity of inducible nitric oxide synthase (iNOS) on mitochondrial activity. Administration of aminoguanidine (specific iNOS inhibitor, 20 mg/kg) augmented the protective effects of tropisetron (1 mg/kg) on SIS. Furthermore, L-arginine (nitric oxide precursor, 100 mg/kg) abolished the positive effects of tropisetron. These results have increased our knowledge on the pivotal role of mitochondrial function in the pathophysiology of depression, and highlighted the role of 5-HT_3 receptors in psychosocial stress response during adolescence. Finally, we observed that tropisetron alleviated the mitochondrial dysfunction through decreased nitrergic system activity in the cerebral cortex.

1. Introduction

Chronic psychological stress, mainly during the developmental timing of the brain, is considered to be a potent risk factor in the development of psychiatric disorders, including depression (Lupien et al., 2009). Major depressive disorder (MDD) is a debilitating mental disorder with high prevalence, morbidity, and costly socioeconomic burden (Mrazek et al., 2014). Emerging lines of research indicate that experiencing social adversity during adolescence is linked to the onset of mood-related psychopathologies (Andersen and Teicher, 2008). Evidence from animal studies has demonstrated that post-weaning social isolation stress (SIS) induces a wide variety of behavioral abnormalities including depressive-like behaviors (Berry et al., 2012; Fone and Porkess, 2008). Adolescence is a critical time during brain development with specific importance in the development of cortical areas (Andersen and Teicher, 2008).

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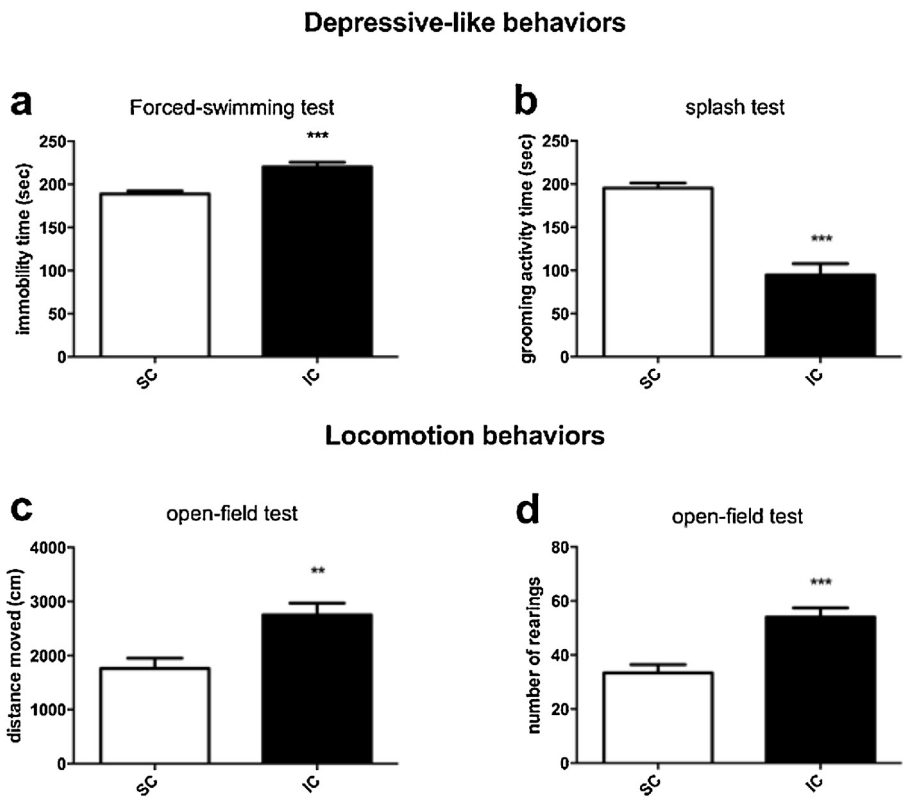

Fig. 1. Effect of housing condition on behaviors related to depression. Effect of different housing conditions, social condition (SC) and isolated condition (IC), on the immobility time in the FST (a), grooming activity time in the splash test (b), total distance moved in the OFT (c), and number of rearings in the OFT (d). Values are expressed as the mean ± S.E.M. of 8 animals and were analyzed using *t*-test. $^{**}P < 0.01$ and $^{***}P < 0.001$ compared with the SC control group.

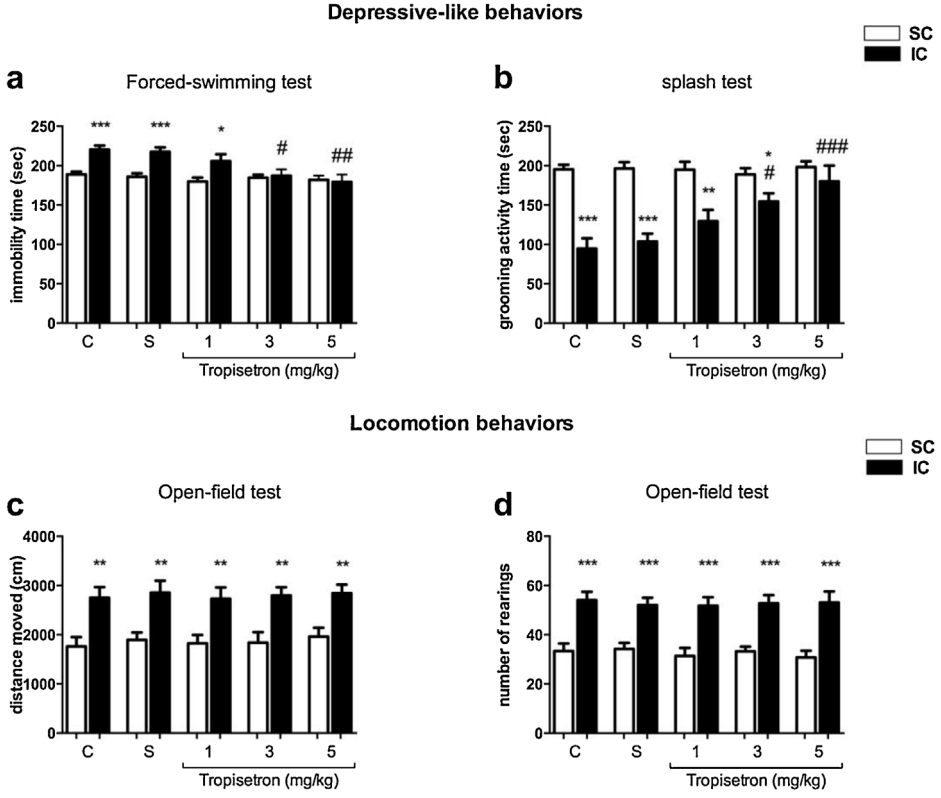

Fig. 2. Effect of tropisetron treatment on behaviors related to depression in different housing conditions. Effects of tropisetron (1, 3, and 5 mg/kg) on the immobility time in the FST (a), grooming activity time in the splash test (b), total distance moved in the OFT (c), and number of rearings in the OFT (d) in different housing conditions, social condition (SC) and isolated condition (IC). Values are expressed as the mean ± S.E.M. of 8 animals and were analyzed using one-way ANOVA followed by Tukey's post hoc test. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ compared with the SC control group. $\#P < 0.05$, $\#\#P < 0.01$, and $\#\#\#P < 0.001$ compared with the IC saline-treated group (S group). C: control group; S: saline group.

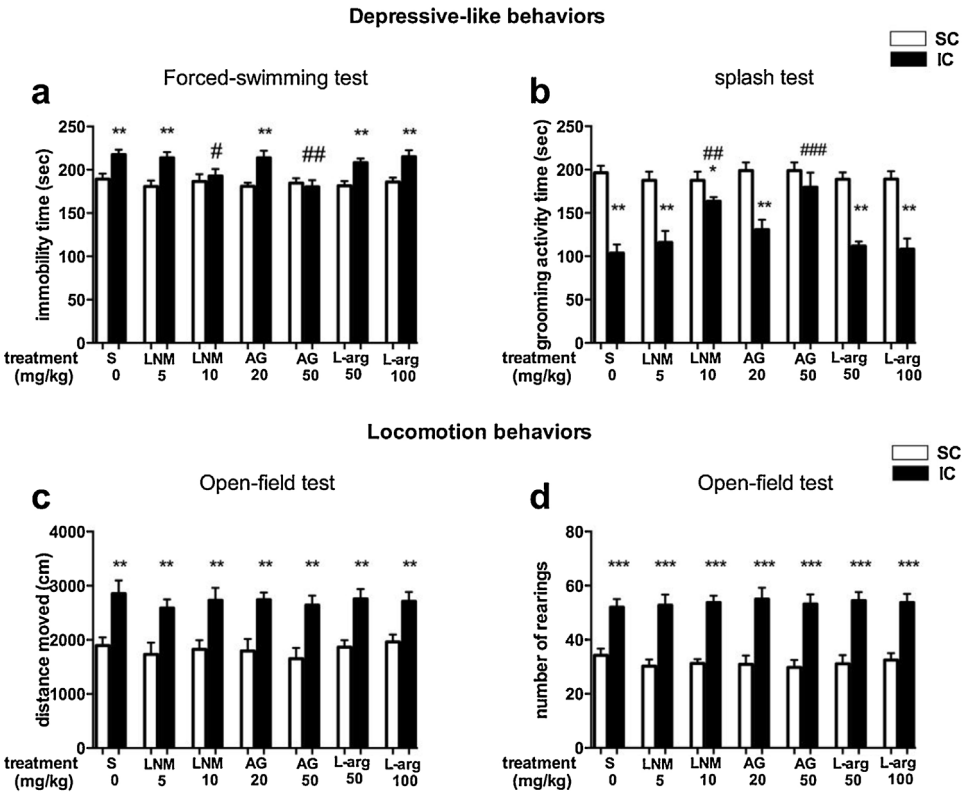

Fig. 3. Effect of NO agents on behaviors related to depression in different housing conditions. Effects of NO agents including L-NAME (LNM) 5 and 10 mg/kg, Aminoguanidine (AG) 20 and 50 mg/kg, and L-arginine (L-arg) 50 and 100 mg/kg on the immobility time in the FST (a), grooming activity time in the splash test (b), total distance moved in the OFT (c), and number of rearings in the OFT (d) in different housing conditions, social condition (SC) and isolated condition (IC). Values are expressed as the mean ± S.E.M. of 8 animals and were analyzed using one-way ANOVA followed by Tukey's post hoc test. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ compared with the SC control group. $\#P < 0.05$, $\#\#P < 0.01$, and $\#\#\#P < 0.001$ compared with the IC saline-treated group (S group).

Furthermore, the developing brain requires high levels of energy, which is provided by mitochondria (more than 90% of brain energy) (Ikonomidou and Kaindl, 2011). Recent studies suggest that mitochondrial dysfunction plays key roles in pathophysiology of MDD (Morava and Kozicz, 2013). In this regard, independent groups have demonstrated that chronic stress increases oxidative and nitrosative stress (O&NS) and excitatory neurotransmission as well as mitochondrial dysfunction in the regions of the brain related to pathophysiology of MDD (Maes et al., 2011). Under chronic stress, an increase in energy demand results in the overproduction of mitochondrial-induced reactive oxygen species (ROS) and nitric oxide (NO) which triggers a cascade of molecular alterations leading to oxidative damage to brain structures such as cortical areas (Brown and Bal-Price, 2003; Chen et al., 2015). Clinical and preclinical evidence suggest that therapeutic properties of antidepressant drugs are partly associated with their ability to modulate the O&NS and mitochondrial dysfunction (Maes et al., 2012).

Despite extensive research on new therapeutic drug treatments for depression, the efficacy of currently prescribed antidepressants is only 40–50 percent (Trivedi et al., 2014). A growing body of evidence indicates that 5-hydroxytryptamine receptors type 3 (5-HT_3) antagonists (such as tropisetron) have antidepressant-like effects in animal models of depression (Carr and Lucki, 2011; Rajkumar and Mahesh, 2010). In this context, results in our laboratory and others have revealed that tropisetron, in addition to its known anti-emetic effect, has a protective effect against O&NS challenge, excitotoxicity and inflammatory responses under pathophysiological conditions (Rahimian et al., 2011; Rahimian et al., 2013; Swartz et al., 2013).

It has been well documented that excessive NO production negatively affects mitochondrial function and contributes to the pathogenesis of a vast majority of brain disorders such as depression and anxiety (Amiri et al., 2015a; Chen et al., 2015). Results of our laboratory and others have shown that chronic stress (such as SIS) is able to induce behavioral abnormalities in mice through overproduction of NO (mainly by iNOS) and cortical areas are more susceptible to detrimental impact of stress (Amiri et al., 2015b; Filipović et al., 2011; Olivenza et al., 2000; Zlatković et al., 2014). In addition, recent clinical research revealed that cortical areas (such as frontal, parietal and cingulate regions) are more vulnerable to the effects of MDD during early neurodevelopmental stages of brain (Peng et al., 2015). In a recent study, we focused on the role of mitochondria and anti-oxidant system and their interactions with nitrergic system in the cortex. Considering that 5-HT_3 receptors have a critical role in the development of brain and nearly 30% of cortical interneurons are 5-HT_3 positive cells that regulate inhibitory and excitatory processes, we focused on the cortex and investigated whether tropisetron is able to attenuate the depressive-like behaviors in male mice following four weeks of SIS during adolescence. We used a post-weaning SIS paradigm in this study because this paradigm evokes a variety of neurobehavioral changes in the animals similar to behavioral difficulties observed in individuals with psychiatric disorders such as depression (Nestler and Hyman, 2010; Powell et al., 2012).

2. Materials and methods

2.1. Animals and conditions

Male NMRI mice weighing 10–14 g on postnatal day (PND) 21–25 were used in our study (Pasteur Institute, Tehran, Iran). PND 21–25 animals were randomly housed in either of the following two opposite conditions for 4 weeks: (1) social condition

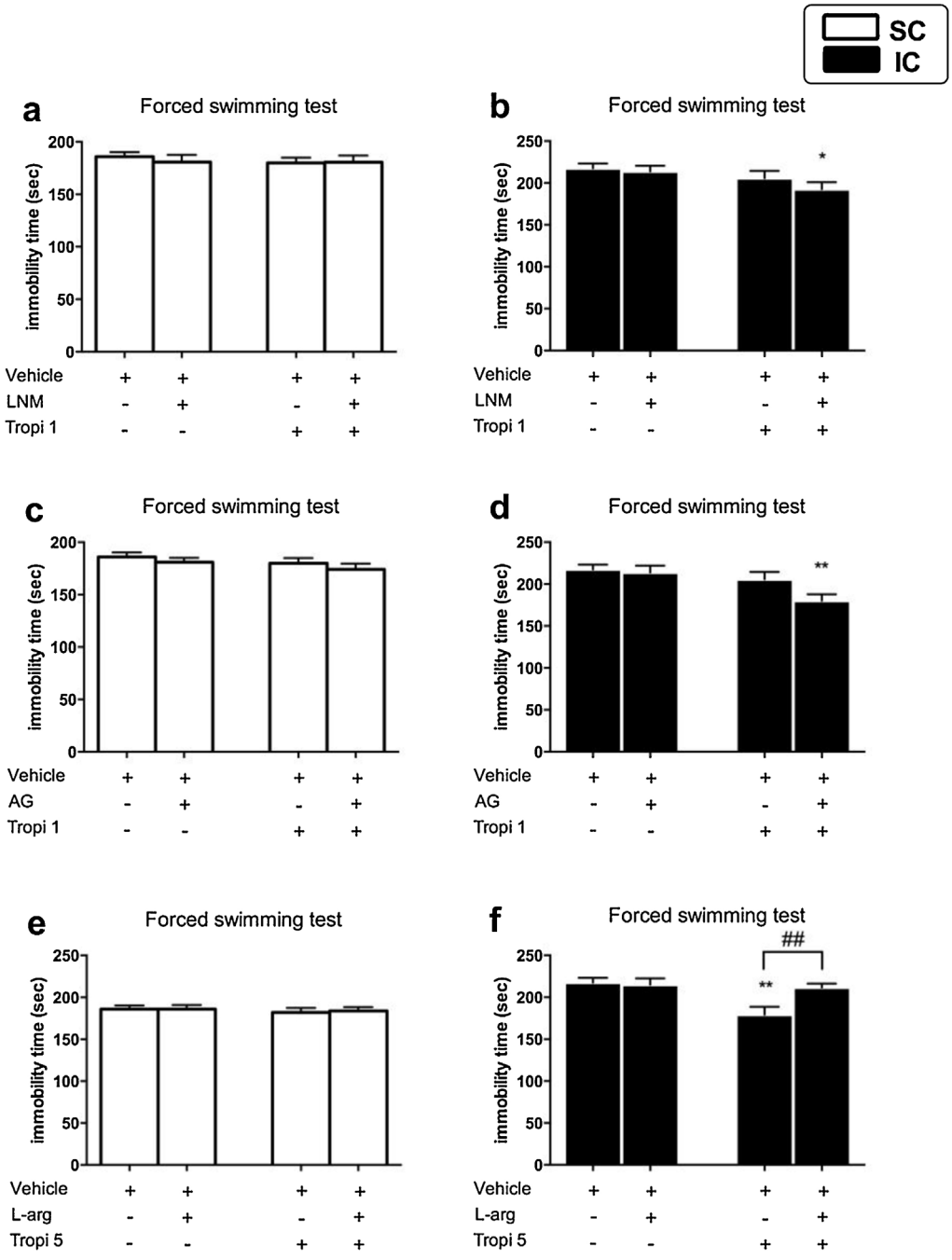

Fig. 4. Effects of tropisetron and NO agents co-administrations on immobility time in the FST. (a and b) Effect of treatment with L-NAME (5 mg/kg) on tropisetron-treated animals (1 mg/kg) in SC and IC animals. (c and d) Effect of treatment with AG (20 mg/kg) on sub-effective tropisetron-treated animals (1 mg/kg) in SC and IC animals. (e and f) Effect of treatment with L-arg (100 mg/kg) on tropisetron-treated animals (5 mg/kg, effective dose) in SC and IC animals. The duration on immobility evaluated using FST. Values are expressed as the mean ± S.E.M. of 6–8 animals and were analyses using two-way ANOVA followed by Tukey's post hoc test. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with vehicle-treated IC group. $\#\#P < 0.01$ compared with tropisetron (5 mg/kg)-treated IC group. Vehicle refers to saline administration in each parts of figure.

(SC), or (2) isolated condition (IC). The SC mice were housed (6 per cage) in Plexiglas boxes (25 cm × 25 cm × 15 cm) and IC mice were housed individually in Plexiglas boxes (24 cm × 17 cm × 12 cm) in normal day-room light (12-h regular light/dark cycle) and temperature (22 ± 1 °C). Wood shavings were used as bedding for animals, and mice were allowed unlimited access to food and water. Cages of isolated animals were cleaned weekly by the same experimenter to minimize handling and social contact. All experiments were conducted during the period between 10:00 a.m. and 2:00 p.m. Each experimental group consisted of 6–8 mice in behavioral tests and 5–8 mice in molecular evaluations. All procedures in our study were carried out in accordance with the National Institute of Health (NIH) Guide for the Care and Use of Laboratory Animals (HHS publication 85-23, 1985) and legislation for the protection of animals used for scientific purposes (Directive 2010/63/EU), and we also adhered to institutional guidelines for animal care and use (Department of Pharmacology, School of Medicine, TUMS).

2.2. Drugs and treatments

The following drugs were used in this study: (1) tropisetron, 5-HT_3 receptor antagonist. (2) N^G-L-arginine methyl ester (L-

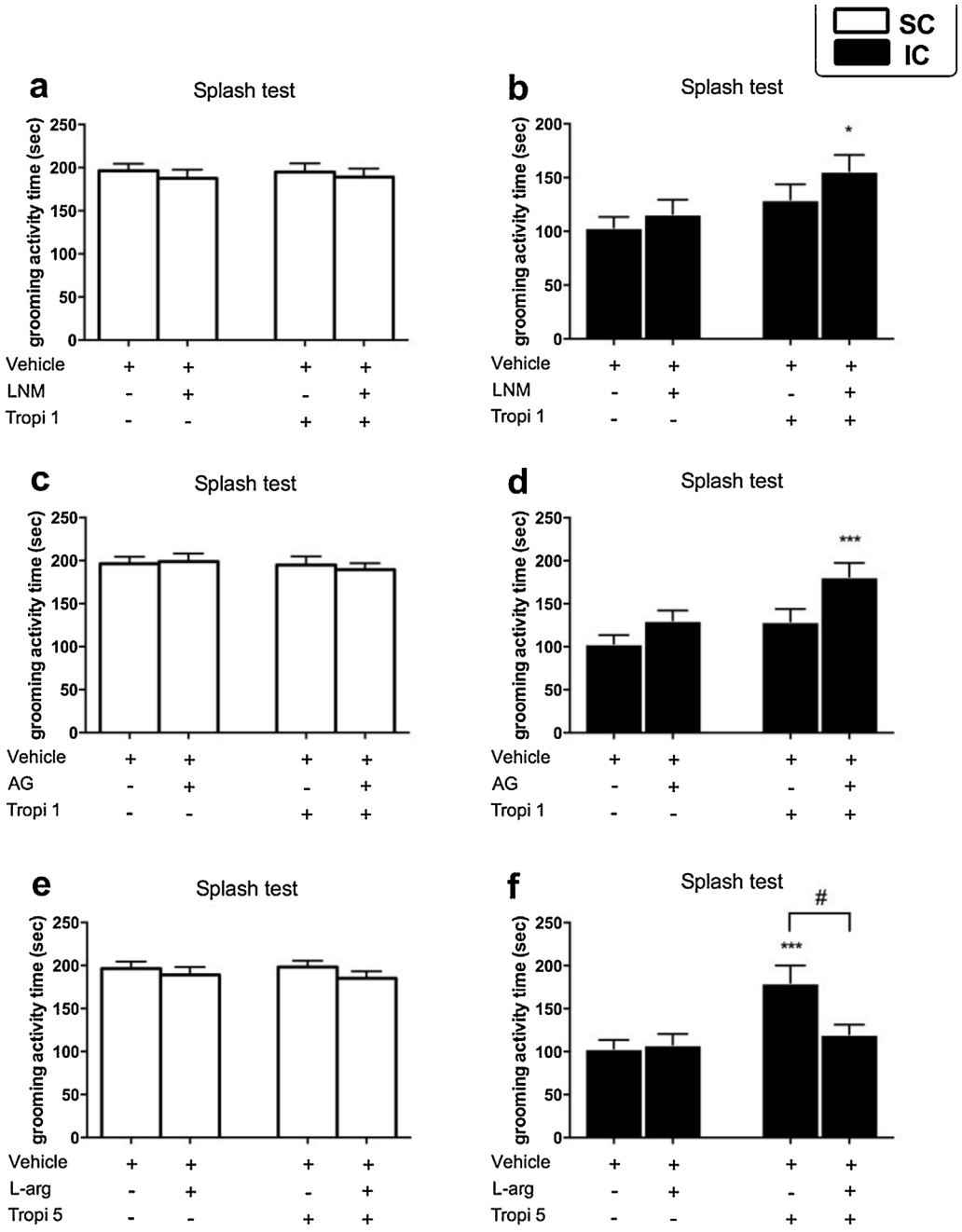

Fig. 5. Effects of tropisetron and NO agents co-administrations on grooming activity time in the splash test. (a and b) Effect of treatment with L-NAME (5 mg/kg) on tropisetron-treated animals (1 mg/kg) in SC and IC animals. (c and d) Effect of treatment with AG (20 mg/kg) on sub-effective tropisetron-treated animals (1 mg/kg) in SC and IC animals. (e and f) Effect of treatment with L-arg (100 mg/kg) on effective tropisetron-treated animals (5 mg/kg) in SC and IC animals. The duration grooming activity evaluated using splash test. Values are expressed as the mean ± S.E.M. of animals and were analyses using two-way ANOVA followed by Tukey's post hoc test. $^{*}P < 0.05$ and $^{***}P < 0.001$ compared with vehicle-treated IC group. $\#P < 0.05$ compared with tropisetron (5 mg/kg)-treated IC group. Vehicle refers to saline administration in each parts of figure.

NAME), a non-selective nitric oxide synthase or NOS inhibitor. (3) Aminoguanidine (AG), a selective inducible NOS or iNOS inhibitor. (4) L-Arginine, a NO precursor. All drugs were purchased from Sigma, St. Louis, MO, USA. All drugs were dissolved in physiological saline prior to use for each experiment. All injections were through intraperitoneal (i.p.) route and with a volume of 5 ml/kg body weight. The doses of drugs were chosen according to previous studies, as well as our pilot studies (described below). We treated mice with L-arginine (30 min), AG and L-NAME (45 min) and tropisetron (60 min) prior to behavioral or molecular experiments.

2.3. Forced swimming test (FST)

The FST was directed using the described methods by Haj-Mirzaian et al. (2015a,b), Porsolt et al. (1977a,b). In this behavioral test, extended immobility time presents the despair behavior reflecting the depressive-like symptoms (Cryan and Holmes, 2005). 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 period of immobility was recorded throughout the last 4 min of the test. Each mouse was judged to be immobile when it ceased struggling

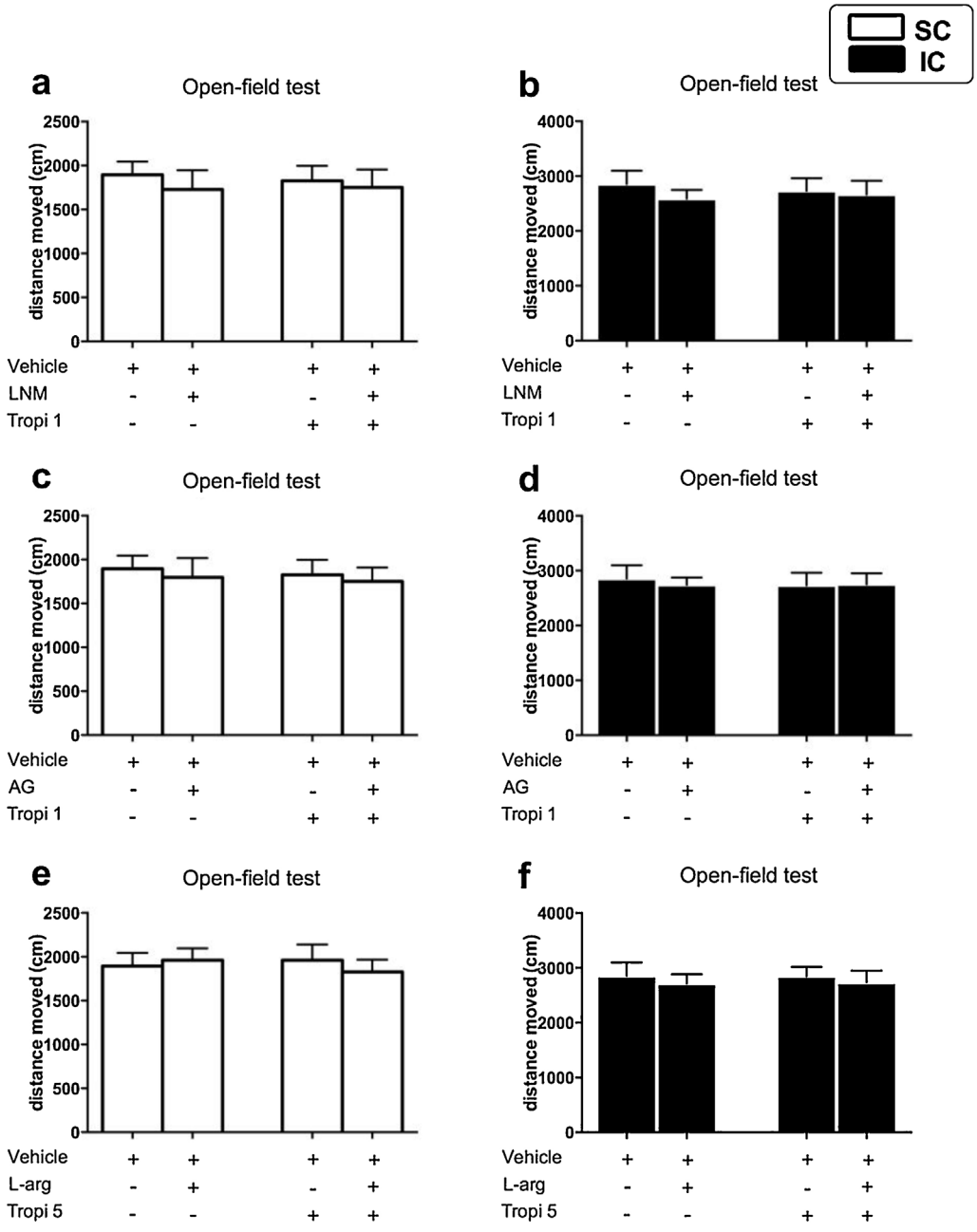

Fig. 6. Effects of tropisetron and NO agents co-administrations on total distance moved in the OFT. (a and b) Effect of L-NAME (5 mg/kg) administration on tropisetron-treated animals (1 mg/kg) in SC and IC animals. (c and d) Effect of AG (20 mg/kg) administration on sub-effective tropisetron-treated animals (1 mg/kg) in SC and IC animals. (e and f) Effect of L-arg (100 mg/kg) administration on effective tropisetron-treated animals (5 mg/kg) in SC and IC animals. The total distance moved evaluated using OFT. Values are expressed as the mean ± S.E.M. of 6–8 animals and were analyses using two-way ANOVA followed by Tukey's post hoc test. Vehicle refers to saline administration in each parts of figure.

and stayed floating motionless in the water, making only those movements necessary to keep its head above water.

2.4. Splash test

Splash test was used to assess motivational and self-care difficulties, often indicated depression disorder in animals (Ducottet et al., 2003; Haj-Mirzaian et al., 2015a; Marrocco et al., 2014; Petit et al., 2014). Lack of motivation and self-care behaviors, as the symptoms of depression, is determined by a reduction in grooming activity time. In this behavioral test, grooming activity of mice including nose/face grooming, head washing and body grooming, which considered as an indirect measure of pleasant solution intake, was recorded. A 10% sucrose solution was spurted on the dorsal coat of animals in their home cage mice and was filmed for 5 min. The total grooming activity time was recorded during 5 min after the sucrose vaporization.

2.5. Open-field test (OFT)

Open-field test was used to elucidate the effects of SIS and treatments on motor function, exploratory behavior, and to rule out possible alterations in locomotion that might affect FST. In this study, we used OFT in order to evaluate the behavioral changes in mice resulting from stress. The device consisted of a wooden box measuring 50 cm × 50 cm × 30 cm. The surface of the box was

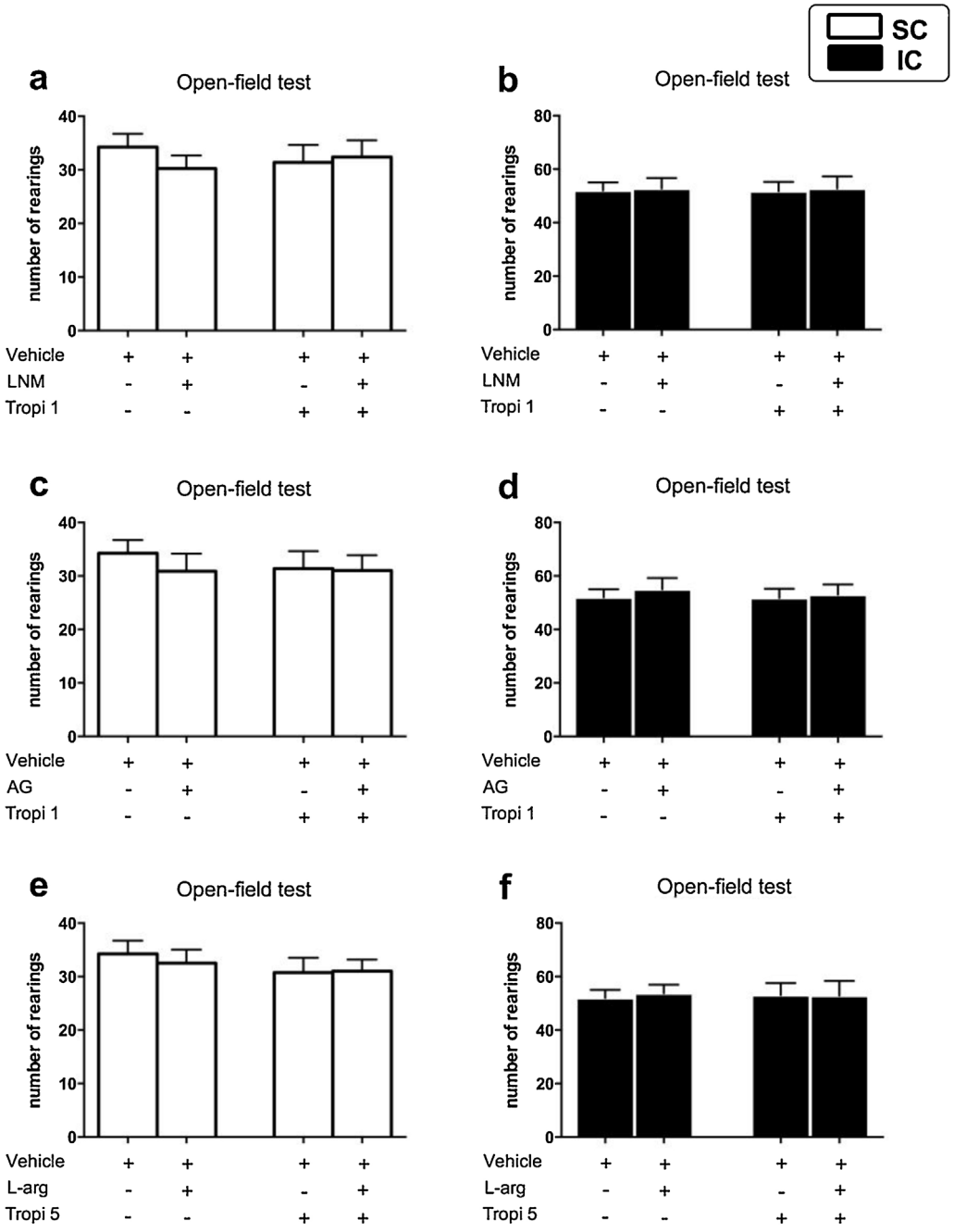

Fig. 7. Effects of tropisetron and NO agents co-administrations on number of rearings in the OFT. (a and b) Effect of L-NAME (5 mg/kg) treatment on tropisetron-treated animals (1 mg/kg) in SC and IC animals. (c and d) Effect of AG (20 mg/kg) treatment on sub-effective tropisetron-treated animals (1 mg/kg) in SC and IC animals. (e and f) Effect of L-arg (100 mg/kg) treatment on effective tropisetron-treated animals (5 mg/kg) in SC and IC animals. The number of rearings evaluated using OFT. Values are expressed as the mean ± S.E.M. of 6–8 animals and were analyses using two-way ANOVA followed by Tukey's post hoc test. Vehicle refers to saline administration in each parts of figure.

separated into 16 equal squares. Each mouse was placed gently on the center square (30 cm × 30 cm) and its behaviors were recorded by a camera for 5 min and analyzed by Ethovision software version 8 (Noldus, Netherlands) (Kordjazy et al., 2015; Kulesskaya and Voikar, 2014). The total distance moved (horizontal activity) and the number of rearings (vertical activity) were evaluated. The apparatus was cleaned with 70% ethanol after testing each mouse.

2.6. Nitrite assay

To determine the NO levels in cortex tissue, we measured nitrite levels as the result of the NO end product (Ding et al., 2010). The animals were decapitated under anesthesia using halothane, and then the whole brain cortex was dissected on ice-cold surface and immediately immersed in liquid nitrogen. Tissue homogenates were prepared, and the nitrite levels were measured by a colorimetric assay based on the Griess reaction. Firstly, 100 μl of samples were mixed with 100 μl Griess reagent. After a 10-min incubation at room temperature, the absorbance was measured at 540 nm in an automated plate reader. The concentration of nitrite was determined by reference to a standard curve of sodium nitrite (Sigma, USA) and normalized to the weight of each sample.

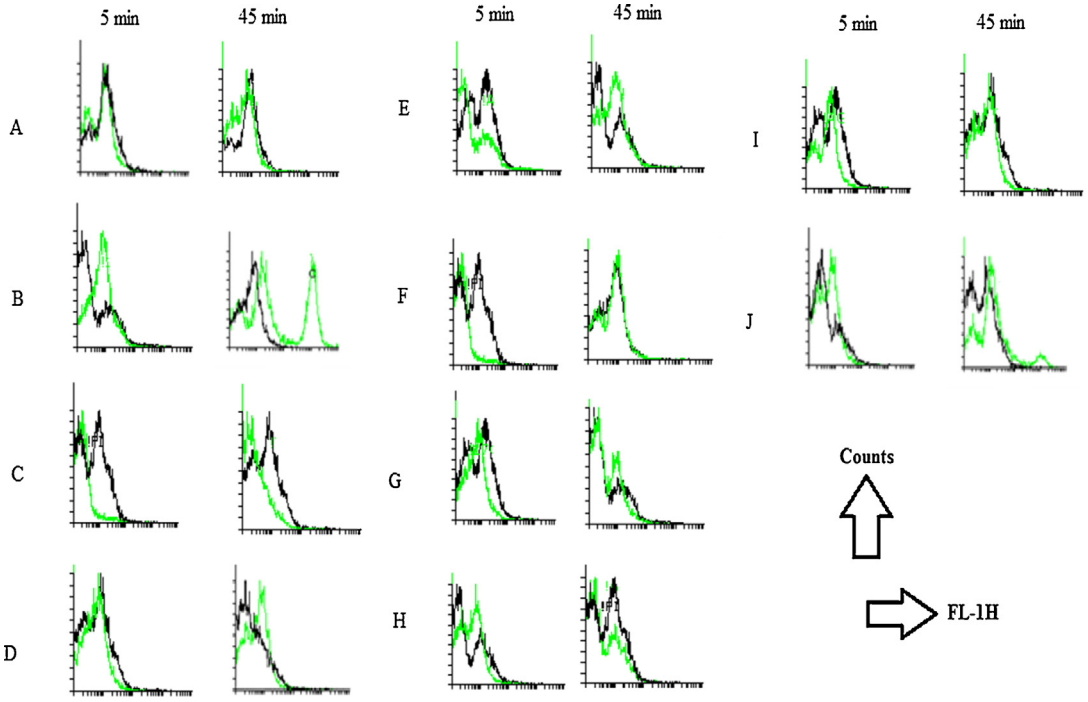

Fig. 8. Effects of different housing conditions and treatments on ROS formation in the cortex. The ROS in each sample was read with 485 nm excitation and 520 nm emission using a fluorimeter after 5 and 45 min. The signs for increased ROS formation in flowcytograms are shifting the ROS peak to the right ward not just increased AUC. (A) SC; (B) IC; (C) IC + Trop-1 mg/kg; (D) IC + Trop-5 mg/kg; (E) IC + LNM-5 mg/kg; (F) IC + AG-20 mg/kg; (G) IC + L-arg-100 mg/kg; (H) IC + L-arg-100 mg/kg + Trop-5 mg/kg; (I) IC + AG-20 mg/kg + Trop-1 mg/kg; (J) IC + LNM-5 mg/kg + Trop-1 mg/kg.

2.7. Determination of ATP and glutathione (GSH) levels

At the end of the behavioral tests, the mice were anesthetized and whole brain cortex tissue was dissected and washed in cold standard saline at 4 °C. In order to measure the ATP level, sample tissue extract of each mouse was sonicated in 250 μl of trichloroacetic acid (TCA 6%) and then centrifuged at 12,000*g* for 10 min at 4 °C. The supernatant was collected and neutralized with potassium hydroxide (KOH = 4 M) and samples were immediately stored at −80 °C for biochemical assessment (ATP and GSH). ATP levels were measured using a luciferase enzyme activity assay as described in our previous work (Eskandari et al., 2012). Bioluminescence intensity was measured using a Sirius tube luminometer (Berthold Detection System, Germany). Glutathione levels were determined by spectrophotometry method using 5,5′dithio-bis-2-nitrobenzoic acid (DTNB) as the indicator. To do this, 0.1 ml of supernatant was added into 0.1 mol L^{-1} of phosphate buffer and 0.04% DTNB in a total volume of 3.0 ml (pH 7.4). Colorimetric measurement at 412 nm was performed using a spectrophotometer (UV-1601 PC, Shimadzu, Japan). GSH content was expressed as μg/mg protein (Jayakumar et al., 2014).

2.8. Measurement of reactive oxygen spices (ROS) formation (flow cytometry analysis)

In cellular toxicology, mitochondria can be considered as one of the major sources for generation of ROS. Therefore, it decided to determine the mitochondrial ROS formation in the tissue extract of each mouse. We isolated mitochondria by ultracentrifugation, following the method according to Hosseini et al. (2014). The cortex were removed and minced with small scissors in a cold mannitol solution (containing 0.225 M D-mannitol, 75 mM sucrose and 0.2 mM EDTA), then gently homogenized and centrifuged at 10,000*g* at 4 °C. The mitochondrial protein content was determined using the Bradford assay method with bovine serum albumin as the standard (Bradford, 1976) and the mitochondrial protein concentration was adjusted to 100 μg/ml protein. ROS formation experiments were performed by incubation of 2′,7′-dichlorofluorescein diacetate (DCFH-DA) (final concentration of 10 μM) in respiratory buffer solution containing 130 mM KCl, 5 mM $MgCl_2$, 20 mM NaH_2PO_4, 1.7 mM ADP, 0.1 mM β-NADPH, 0.1 mM $FeCL_3$ (pH = 7.4) according to the method described by Mashayekhi et al. (2015). All samples were incubated at 30 °C and protected from light for 15 min. Mitochondrial fluorescence and light scattering were analyzed for at least 15000 counts by flowcytometry using the Flomax software (Partec, Deutschland). A flowcytometer (Partec, Deutschland) equipped with a 488-nm argon ion laser was used and fluorescence signals were obtained using a 530-nm band pass filter (FL-1 channel) (Gao et al., 2009; Eskandari et al., 2015).

2.9. Experimental design and treatments

To determine the effect of social isolation on animal behaviors after exposure to different housing conditions (SC or IC), animals at age PND 50–55 (early adulthood) were subjected to the experiments. In the first part of the study, the effects of SIS on depressive-like behaviors and locomotor activity were investigated. The effect of SIS on depressive-like behaviors was determined using FST and splash test, and the locomotor activity of mice was evaluated by OFT. In order to minimize the number of animals used in this study, the same mice tested using OFT was immediately evaluated using FST. Different sets of mice were evaluated using splash test.

The second part of the study was aimed to determine the possible effect of tropisetron as well as NO agents on the depressive-like behaviors evoked by adolescent SIS. In this regard, different sets of SC and IC mice were treated with different doses of following drugs: tropisetron (1, 3, and 5 mg/kg), L-NAME (5, 10 mg/kg), AG (20, 50 mg/kg), and L-arg (50, 100 mg/kg). After administration of drugs, SC animals and IC animals were subjected to the aforemen-

tioned behavioral tasks. Doses and time of administration of each drug were chosen according to the pilot treatments and pervious published studies (Amiri et al., 2015b; Amiri et al., 2014; Barzegar-Fallah et al., 2014; Bravo and Maswood, 2006; Rahimian et al., 2011). To exclude the possible impact of saline administration on animal behaviors, a number of IC and SC were injected with 5 ml/kg physiological saline.

The next part of the study was carried out to determine the possible role of the nitrergic system in mediating antidepressant-like effects of tropisetron. Therefore, sub-effective doses of L-NAME (5 mg/kg) and AG (20 mg/kg) co-administered with sub-effective dose of tropisetron (1 mg/kg) to both SC and IC mice. Also, we co-administered sub-effective dose of L-Arg (100 mg/kg) with effective dose of tropisetron (5 mg/kg).

Next, we investigated the effects of different housing conditions and drug treatments on the cortical levels of nitrite, GSH, ATP, and ROS production in the different sets of SC and IC animals. Different groups of SC and IC mice were used for molecular evaluations (these animals had not been tested by behavioral tasks), in order to avoid the possible effects of behavioral experiments and manipulations on these parameters. Further, samples were not pooled together, and each sample of each animal was evaluated separately for assessing GSH, ATP, NO levels, and ROS formation.

2.10. Statistical analysis

Comparison between groups was performed using *t*-test, one-way and two-way ANOVA analysis followed by Tukey's post hoc test in the SPSS package software (version 21) and Graph-pad prism software (version 6). $P < 0.05$ was considered statistically significant. The factors were housing (social condition (SC) and isolation condition (IC)) and treatments (control: no treatment and treatment: drug-administered animals by i.p. injection) for all assessments.

3. Results

3.1. Effects of housing conditions on depressive and locomotion behaviors

Applying SIS to mice in adolescence provoked depressive-like behaviors in IC mice when compared to SC animals. Fig. 1 shows that the immobility time increased in the IC compared to SC animals as measured by FST ($t = 5.1$, $df = 14$, $P < 0.001$, Fig. 1a). Lack of motivation and self-care difficulties in mice, as symptoms of depression, is determined by a reduction in grooming activity time in the splash test. Results show that SIS caused a significant reduction in grooming time in the splash test when compared to the SC animals ($t = 6.964$, $df = 14$, $P < 0.001$, Fig. 1b). OFT data indicates, SIS significantly increased the total distance moved (horizontal activity) ($t = 3.442$, $df = 14$, $P < 0.01$, Fig. 1c) and number of rearings (vertical activity) ($t = 4.213$, $df = 14$, $P < 0.001$, Fig. 1d) in IC mice when compared to SC mice.

3.2. Effects of tropisetron on locomotion and depressive -like behaviors

We assessed the effects of various doses of tropisetron (1, 3 and 5 mg/kg) on depressive-like and locomotion behaviors in both SC and IC mice using the aforementioned tasks. ANOVA analysis revealed that there were significant differences between tropisetron-treated groups in immobility time as measured by FST ($F(9, 70) = 6.123$, $P < 0.001$, Fig. 2a), grooming activity time assessed by splash test ($F(9, 70) = 12.50$, $P < 0.001$, Fig. 2b), distance moved in OFT ($F(9, 70) = 6.521$, $P < 0.001$, Fig. 2c), and number of rearings in OFT ($F(9, 70) = 11.20$, $P < 0.001$, Fig. 2d).

In the FST, tukey's analysis showed that administration of tropisetron (3 and 5 mg/kg, but not 1 mg/kg) to IC mice significantly decreased the immobility time in comparison with saline-treated IC mice ($P < 0.05$ and $P < 0.01$, respectively). Treatment with all doses of tropisetron produced no significant alterations in the immobility time of SC mice when compared with saline-treated SC mice as measured by FST ($P > 0.05$).

In the splash test, tukey's analysis shows that administration of tropisetron (3 and 5 mg/kg, but not 1 mg/kg) enhanced the grooming activity of IC animals when compared to IC-saline treated group ($P < 0.05$ and $P < 0.001$, respectively). However, the results showed that administration of same doses of tropisetron had no significant effect on grooming activity time of SC groups ($P > 0.05$).

In the OFT, administration of tropisetron (1, 3, 5 mg/kg) did not affect total distance moved and number of rearings of SC and IC mice in comparison with saline-treated SC and IC groups ($P > 0.05$). Further, our results showed that saline treatment did not affect the behaviors of both SC and IC mice in all applied behavioral tests ($P > 0.05$).

3.3. Effects of NOS inhibitors and NO precursor on locomotion and depressive-like behaviors

In order to assess the effect of NOS inhibitors and NO precursor on depressive-like behaviors in animals, we investigated the effects of different doses of L-NAME, AG, and L-arg on animal behaviors using the aforementioned tasks. Using ANOVA analysis, we showed that there are significant differences between L-NAME-treated groups as well as aminoguanidine-administered groups in immobility time in the FST ($F(5, 42) = 4.624$, $P < 0.01$; $F(5, 42) = 6.778$, $P < 0.001$, Fig. 3a), grooming activity time in the splash test ($F(5, 42) = 16.99$, $P < 0.001$; $F(5, 42) = 13.07$, $P < 0.001$, Fig. 3b), distance moved in OFT ($F(5, 42) = 6.503$, $P < 0.001$; $F(5, 42) = 7.954$, $P < 0.001$, Fig. 3c), and number of rearings in OFT ($F(5, 42) = 17.47$, $P < 0.001$; $F(5, 42) = 13.67$, $P < 0.001$, Fig. 3d). Also, there are significant differences between L-arginine-treated animals in immobility time ($F(5, 42) = 7.154$, $P < 0.001$, Fig. 3a), grooming activity time ($F(5, 42) = 25.53$, $P < 0.001$, Fig. 3b), distance moved ($F(5, 42) = 7.628$, $P < 0.001$, Fig. 3c), and number of rearings ($F(5, 42) = 15.24$, $P < 0.001$, Fig. 3d).

Tukey's analysis showed that administration of L-NAME (5 and 10 mg/kg) had no effect on the behavioral tests of SC animals ($P > 0.05$). But, in comparison with saline-treated IC mice, administration of L-NAME (10 mg/kg) significantly decreased the immobility time of IC mice in FST ($P < 0.05$) and increased grooming activity time in splash test ($P < 0.01$). However, administration of the lower dose of L-NAME (5 mg/kg) did not alter the immobility and grooming time of IC mice in comparison with saline-treated IC group ($P > 0.05$). Further, our results show that L-NAME treatment did not affect the locomotor activity of IC mice in the OFT ($P > 0.05$).

Additional analysis revealed that administration of AG (20 and 50 mg/kg) had no behavioral effect on SC animals when assessed by behavioral tasks ($P > 0.05$). However, in comparison with saline-treated IC mice, results showed that administration of AG (50 mg/kg, but not 20 mg/kg) significantly decreased the immobility time of IC mice in the FST ($P < 0.01$). Also, treatment with AG (50 mg/kg, but not 20 mg/kg) improved the grooming activity time of IC mice when compared to saline-treated IC group ($P < 0.001$). In the OFT, AG (20 and 50 mg/kg) did not affect both horizontal and vertical activity of IC animals ($P > 0.05$).

Furthermore, treatment with L-arg (50 and 100 mg/kg) had no effect on all behavioral tests including FST, splash test, and OFT in both SC and IC animals ($P > 0.05$).

3.4. Effects of nitrergic system on antidepressant-like properties of tropisetron

3.4.1. Depressive-like behaviors in the FST

In SC groups, statistical analysis revealed that co-administration of sub-effective doses of NOS inhibitors/NO precursor with sub-effective dose of tropisetron had no effect on the immobility time in the FST ($P > 0.05$, Fig. 4a, c and e). However, the results were different in IC groups.

Co-administration of L-NAME (5 mg/kg) along with tropisetron (1 mg/kg) decreased the duration of immobility in IC animals when compared to saline-treated IC controls ($P < 0.05$, Fig. 4b). Two-way ANOVA analysis revealed significant differences in the FST for the tropisetron treatment ($F(1, 28) = 4.73$, $P < 0.05$).

Also, injecting a sub-effective dose of AG (20 mg/kg) to tropisetron-treated IC animals (1 mg/kg) caused obvious antidepressant-like effect revealed in the FST (decrease in immobility time) when compared to saline-treated IC mice ($P < 0.01$, Fig. 4d). Two-way ANOVA analysis revealed significant differences for the tropisetron treatment ($F(1, 28) = 8.8$, $P < 0.01$).

In addition, treatment with sub-effective dose of L-arg (100 mg/kg) abolished the antidepressant-like effect of tropisetron (5 mg/kg, effective dose) by increasing the immobility time ($P < 0.01$, Fig. 4f). Two-way ANOVA analysis revealed significant differences for the tropisetron treatment ($F(1, 28) = 8.485$, $P < 0.01$), L-arg treatment ($F(1, 28) = 4.456$, $P < 0.05$), and tropisetron × L-arg interaction ($F(1, 28) = 6.064$, $P < 0.05$).

3.4.2. Depressive-like behaviors in the splash test

In comparison to saline-treated IC mice, treating IC mice with tropisetron (1 mg/kg) and L-NAME (5 mg/kg) significantly increased the grooming activity time in the splash test ($P < 0.05$, Fig. 5b). Two-way ANOVA analysis demonstrated significant differences in the grooming activity time for the tropisetron treatment alone ($F(1, 28) = 6.233$, $P < 0.05$).

In addition, co-administration of tropisetron (1 mg/kg) with AG (20 mg/kg) to IC mice induced a significant increase in the grooming activity time in the splash test ($P < 0.001$, Fig. 5d) as compared to saline-treated IC mice. Two-way ANOVA analysis showed significant differences in the splash test for the tropisetron treatment alone ($F(1, 28) = 8.615$, $P < 0.01$) and AG treatment ($F(1, 28) = 9.126$, $P < 0.01$).

Further, comparison with saline-treated IC controls showed that administration of L-arg (100 mg/kg) abolished the antidepressant effect of tropisetron (5 mg/kg) by reducing the grooming activity time in the splash test ($P < 0.05$, Fig. 5f). Two-way ANOVA analysis revealed significant differences in the splash test for the tropisetron treatment alone ($F(1, 28) = 10.09$, $P < 0.01$), L-arg treatment ($F(1, 28) = 3.9$, $P < 0.05$), and tropisetron × L-arg treatment interaction ($F(1, 28) = 5.322$, $P < 0.05$). In comparison to saline-treated SC mice, administration of the same doses of above drug combinations had no effect on the depressive-like behaviors of SC mice in the splash test ($P > 0.05$, Fig. 5a, c and e).

3.4.3. Horizontal and vertical activities in the OFT

Co-administration of L-NAME (5 mg/kg) or AG (20 mg/kg) with tropisetron (1 mg/kg) did not change either distance moved ($P > 0.05$, Fig. 6a–d) or number of rearings ($P > 0.05$, Fig. 7a–d) in the SC and IC animals in comparison with saline-treated controls. Two-way ANOVA analysis revealed no significant differences in the total distance moved and number of rearings in the OFT in the SC and IC animals ($P > 0.05$).

Furthermore, co-administration of the effective dose of the tropisetron (5 mg/kg) with sub-effective dose of L-arg (100 mg/kg) did not alter the locomotion behaviors of SC and IC mice ($P > 0.05$, Figs. 6e and f and 7e and f) and similarly, through two-way ANOVA analysis we determined there are no significant differences between tropisetron treatment, L-arg treatment, and tropisetron × L-arg for total distance moved and number of rearings in the SC and IC animals ($P > 0.05$).

3.5. Effects of different housing conditions and drug treatments on mitochondrial function and O&NS

3.5.1. Effects of housing conditions and drug treatments on GSH levels in the cortex

Two-way ANOVA analysis revealed that there were significant effects as a result of housing conditions ($F(1, 72) = 109.8$, $P < 0.001$) as well as housing conditions × treatments interaction ($F(11, 72) = 5.404$, $P < 0.001$) on cortical GSH levels in animals (Table 1). Multiple analyses show a significant decrease in the GSH levels of cortical cytosolic fraction of IC mice in comparison with SC animals ($P < 0.001$).

The administration of tropisetron (5 mg/kg) significantly restored GSH levels in comparison with saline-treated IC mice in the cortex ($P < 0.001$). However, treatment with tropisetron 1 mg/kg failed to induce any significant change in GSH levels when compared to saline-treated IC mice ($P > 0.05$).

Similarly, administration of sub-effective doses of L-NAME (5 mg/kg), AG (20 mg/kg), and L-arg (50 an 100 mg/kg) had no significant change on the cortical GSH levels in IC mice as compared to controls ($P > 0.05$). However, higher doses of L-NAME (10 mg/kg) ($P < 0.05$) and AG (50 mg/kg) ($P < 0.001$) significantly affected the cortical GSH levels in IC animals. In comparison with saline-treated IC mice, treating IC mice with AG 20 mg/kg in combination with tropisetron (1 mg/kg) significantly restored GSH levels in the cortex ($P < 0.001$). Also, co-administration of L-NAME (5 mg/kg) in combination with tropisetron (1 mg/kg) significantly restored GSH levels in the cortex ($P < 0.01$). In addition, administration of L-arg (100 mg/kg) significantly lowered the elevated levels of GSH in the IC mice treated with tropisetron (5 mg/kg) in the cortex ($P < 0.001$). This data shows that administration of all applied drugs did not change the cortical GSH levels in SC animals when compared to SC control group ($P > 0.05$).

3.5.2. Effects of housing conditions and drug treatments on ATP levels in the cortex

Results obtained from two-way ANOVA analysis show that there were significant effects due to housing conditions ($F(1, 74) = 459.4$, $P < 0.001$), treatments ($F(11, 74) = 15.79$, $P < 0.001$), and their interactions ($F(11, 74) = 27.42$, $P < 0.001$) on the cortical ATP levels (Table 1). Multiple analyses revealed that there is a significant decrease in ATP production in the cortex ($P < 0.001$) of IC mice as compared with SC controls (Table 1). Treatment of IC mice with tropisetron (5 mg/kg) increased ATP levels in the cortex ($P < 0.001$) in comparison with IC control mice. Furthermore, co-administration of sub-effective dose of L-arg (100 mg/kg) abolished the positive effect of tropisetron on ATP generation ($P < 0.001$). Conversely, co-administration of L-NAME (5 mg/kg) or AG (20 mg/kg) with tropisetron (1 mg/kg) significantly increased ATP levels in the cortex ($P < 0.001$ and $P < 0.001$ respectively) of IC mice in comparison with controls. Our data shows that administration of all applied drugs had no any significant effect on the cortical ATP levels in SC animals when compared to SC control group ($P > 0.05$).

3.5.3. Effects of housing conditions and drug treatments on nitrite levels in the cortex

Two-way ANOVA analysis revealed that there were significant effects as a result of housing conditions ($F(1, 87) = 1451$, $P < 0.001$), treatments ($F(11, 87) = 15.32$, $P < 0.001$), and their interactions ($F(11, 87) = 7.849$, $P < 0.001$) on the cortical nitrite levels (Table 1). The data indicates that IC mice have greater levels of cortical nitrite con-

Table 1
Effect of different housing conditions (SC and IC) and treatments on GSH, ATP, and nitrite levels in the cortex. Values are expressed as Mean ± S.D. of 3–6 samples and were analyses using two-way ANOVA followed by Tukey's post hoc test. * P < 0.05, ** P < 0.01, and *** P < 0.001 compared with SC control group. #P < 0.05, ## P < 0.01, and ### P < 0001 compared with IC control group in each column.

Treatments/Conditions	GSH (μg/mg protein)		ATP (nmol/mg protein)		Nitrite (nmol/mg protein)	
	SC	IC	SC	IC	SC	IC
+Saline	36.4 ± 5.9	16.58 ± 3.7***	3.37 ± 0.2	1.06 ± 0.17***	30.5 ± 4	53 ± 5***
+Tropisetron (1 mg/kg)	35.3 ± 3.3	23.5 ± 5.4**	3.1 ± 0.25	1.55 ± 0.3#***	29.1 ± 6	47 ± 6***
+Tropisetron (5 mg/kg)	31.9 ± 7.1	30.1 ± 4.8###	3.1 ± 0.2	3.1 ± 0.2###	28.4 ± 5.7	34 ± 4###
+LNM (5 mg/kg)	34.9 ± 2.8	22.8 ± 3.7*	3 ± 0.3	1.15 ± 0.3***	28.2 ± 7.6	50 ± 7***
+LNM (10 mg/kg)	30.5 ± 4.8	26.2 ± 2.9#	3.3 ± 0.4	2.16 ± 0.2###**	27.12 ± 8.7	38 ± 5##
+AG (20 mg/kg)	35.5 ± 8.1	22.9 ± 2.1**	2.9 ± 0.3	1.55 ± 0.3#***	27.7 ± 8	45 ± 6***
+AG (50 mg/kg)	34.7 ± 6.7	29.9 ± 4.2###	3.1 ± 0.3	2.96 ± 0.2###	25.9 ± 8.3	32 ± 5###
+L-arg (50 mg/kg)	37.1 ± 3.1	16.41 ± 3.1***	3.3 ± 0.4	1.04 ± 0.2***	30.1 ± 6	54 ± 6***
+ L-arg (100 mg/kg)	39.2 ± 7.3	16.1 ± 2.7***	3.45 ± 0.3	1.01 ± 0.27***	33.3 ± 7	57 ± 8***
+Tropisetron (1 mg/kg) + LNM (5 mg/kg	29.8 ± 7.1	28.3 ± 3.8##	3 ± 0.35	3.03 ± 0.25###	27.8 ± 5.7	39 ± 6##*
+Tropisetron (1 mg/kg) + AG (20 mg/kg)	33.1 ± 6.2	33.5 ± 4.5###	3.15 ± 0.3	3.22 ± 0.4###	28.1 ± 9	31 ± 4###

tent in comparison with SC animals ($P < 0.001$) (Table 1). Also, there was no significant difference in the cortical nitrite levels between IC control mice and IC mice treated with sub-effective doses of AG (20 mg/kg), L-NAME (5 mg/kg), L-arg (50 and 100 mg/kg), and tropisetron (1 mg/kg) ($P > 0.05$). In comparison with IC control mice, a significant decrease in cortical nitrite levels was observed when IC mice were treated with tropisetron (5 mg/kg) ($P < 0.001$). Also, co-administration of sub-effective doses of L-arg (100 mg/kg) blocked the effect of tropisetron (5 mg/kg) on elevated cortical nitrite levels ($P < 0.001$). Furthermore, co-administration of L-NAME (5 mg/kg) or AG (20 mg/kg) with tropisetron (1 mg/kg) significantly decreased nitrite levels in the cortex of IC mice in comparison with controls ($P < 0.01$ and $P < 0.001$ respectively). Conversely, our data shows that administration of all applied drugs did not change the cortical nitrite levels in SC animals in comparison to SC control group ($P > 0.05$).

3.5.4. Effects of housing conditions and drug treatments on ROS formation in the cortex

Assessment of ROS formation was performed at 2 time intervals (5 min and 45 min) in the cortex (Fig. 8) of animals. The signs for increased ROS formation in flow cytograms are shifting of the ROS peak to the right and not just increased the area under the curve (AUC). As shown in Fig. 8, there is a significant concentration dependent shift of 2′,7′-dichlorofluorescein diacetate (DCF) peak to the right in the cortex of IC group when compared with SC group (Fig. 8A and B). Therefore, an increase in ROS generation in the cortex of IC mice was observed at both 5 and 45 min intervals. Treating IC mice with AG (20 mg/kg), L-NAME (5 mg/kg), L-arg (50 and 100 mg/kg), and tropisetron (1 mg/kg) did not obviously affect the elevated levels of ROS formation in the cortex. Also, in comparison with IC controls, administration of tropisetron (5 mg/kg) to IC animals reversed the cortical (Fig. 8D) ROS formation to the normal state, albeit, the latter effect was abolished by administration of L-arg (100 mg/kg) (Fig. 8H). As shown in Fig. 8I and J, co-administration of L-NAME (5 mg/kg) or AG (20 mg/kg) with tropisetron (1 mg/kg) decreased the enhanced ROS production in the cortex of IC animals at both 5 min and 45 min intervals (Fig. 8I and J).

4. Discussion

4.1. Depressive-like behaviors following early SIS correlated with mitochondrial dysfunction and oxidative challenge in the cortex

Results of the current study revealed that experiencing SIS during adolescence provoked depressive-like behaviors in adult male mice. These behavioral alterations correlated with elevated levels of O&NS and mitochondrial dysfunction in the cortex of the animals. In addition, our results showed that administration of both NOS inhibitors and tropisetron (alone or in combination) mitigated the negative effects of early SIS on the behavioral profile of mice. Also, the same treatments improved the mitochondrial function and oxidative state in the cortex. Furthermore, the nitrergic system seemed to be involved in the antidepressant-like effects of tropisetron as the administration of L-arg (NO precursor) abolished the protective effects of tropisetron.

Adolescence is a determinant state in the timing of brain development during which, the cortico-limbic circuits undergo significant alterations (Andersen and Teicher, 2008; Lupien et al., 2009). The developing brain is vulnerable to environmental challenges (such as stress overload) resulting from high energy demands, number of immature cells and poor antioxidant system defense (Hagberg et al., 2014; Ikonomidou and Kaindl, 2011). Emerging lines of research suggest that mitochondrial dysfunction plays a role in the etiology of MDD owing to its role in a variety of vital cell functions including energy metabolism, response to glucocorticoids and ROS production (Morava and Kozicz, 2013; Picard et al., 2014). Recently, we have demonstrated that SIS during adolescence is associated with severe mitochondrial dysfunction in the brain (unpublished data). Considering the negative impacts of early life stress on mitochondrial function and evolution, we showed that 4-weeks of juvenile SIS induced overproduction of ROS in the cortex of IC mice. Excessive generation of ROS is associated with poor oxidative phosphorylation in the mitochondrial respiratory chain, which negatively impacts on the generation of ATP as the vital molecule required for cell survival (Picard et al., 2014). Results of this study revealed that there was a significant difference in ATP levels in the cortex between the IC and SC rats indicating the disruption of energy hemostasis in the cortical regions of the brain in IC mice. In addition, we showed that the overproduction of ROS accompanied by a decrease in GSH levels, which is considered as the main antioxidant agent in the brain. Increasing of ROS in mitochondria and neighboring of ROS production site to lipid membrane, makes more susceptible site in mitochondria which leads to oxidative stress and lipid peroxidation. Also, glutathione is an important component of non-enzymatic antioxidant system that not only modulates the deleterious effects of free radicals but also markedly plays an important role in pathophysiology of psychiatric disorders (Gawryluk et al., 2011a,b). Our results showed there was a significant reduction in the mitochondrial GSH levels between the IC and SC controls in line with previous studies reporting that mitochondrial dysfunction following chronic stress is implicated in the pathogenesis of depression (Gong et al., 2011; Leonard and Maes, 2012). We also demonstrated that adolescence SIS potently increased nitrite levels in the cortex reflecting the pres-

ence of nitrosative stress in these regions. Overproduction of NO in these regions has been reported to inhibit the ATP synthesis by mitochondria as well as considerably reducing GSH levels through *S*-nitrosylation (Almeida and Bolaños, 2001; Anand and Stamler, 2012). Collectively, these alterations following 4-weeks of SIS in the adolescent stage suggest that depressive-like behaviors in IC mice is associated with significant impairment in the mitochondrial and redox system in the cortex.

Through the application of behavioral tests to investigate the influence of early SIS on the depressive-like behaviors, we showed that adolescent SIS provoked despair behavior (increased immobility time in the FST) as well as difficulties in motivation and self-care behaviors in mice (reduction in the grooming activity time in the splash test) (Cryan et al., 2002; Petit et al., 2014). These behaviors are considered as the main symptoms of depression observed in humans indicating that early SIS induces depressive-like behaviors in IC mice. It is important to note that FST is a cogent and rapid screening test for the evaluation of novel antidepressants and is based on observations of depressed patients not being able to cope with stressful conditions and similarly, depressed rodents behave the same in the stressful conditions (cylinder filled with water) by exhibiting passive behaviors (Castagné et al., 2011; Cryan and Holmes, 2005). In this study, we also used the splash test as a suitable tool to evaluate motivation and self-care behavior based on grooming behavior (Ducottet et al., 2003; Marrocco et al., 2014). Furthermore, enhanced vertical and horizontal activity of IC mice in the OFT not only validated the FST results but showed the anxiety response of IC mice to a novel environment. These results were consistent with other studies, which demonstrate that early SIS is able to provoke behaviors relevant to depression in animals (Amiri et al., 2015b; Berry et al., 2012; Grippo et al., 2007).

4.2. Nitric oxide is involved in the protective effects of tropisetron against behavioral and mitochondrial dysfunction

Recent studies on animal models of depression have demonstrated the antidepressant-like properties of the 5-HT_3 receptor antagonists, including tropisetron (Carr and Lucki, 2011; Rajkumar and Mahesh, 2010). Normal performance of cortical areas is tightly associated with the structure of cortical circuits. 5-HT_3 receptors play a pivotal role during development of cortico-limbic circuits by regulating the neuronal migration and differentiation during early stages of life. Recent evidence indicates that experiencing early-life stress negatively affects 5-HT_3 receptor function leading to an increased risk of developmental psychiatric disorders (Vitalis et al., 2013). The results of this study show that administration of tropisetron or NOS inhibitors exerted antidepressant-like effects in IC mice subjected to FST and splash test. Behavioral improvements in IC mice were associated with significant amelioration of mitochondrial function as well as redox state in the hippocampus (data not shown) and cortex. It has been generally accepted that under chronic stress conditions that an overproduction of NO (through iNOS) is involved in pathophysiology of depression (Chen et al., 2015; Montezuma et al., 2012). In this regard, evidence indicates that high levels of NO (ir) reversibly suppresses mitochondrial respiration and ATP synthesis and induce cellular damage mainly in the cortical areas (Moncada and Erusalimsky, 2002; Sarti et al., 2012). Interestingly, it has been reported that iNOS is implicated in the pathogenesis of depression and inhibition of this enzyme is associated with improvements in mitochondrial function and depressive-like behaviors (Gałecki et al., 2010; Montezuma et al., 2012; Sarti et al., 2012). Our results are in agreement with studies, which have revealed that cortical iNOS plays a role in mediating depressive-like behaviors as determined by inhibiting iNOS using AG or 1400W induced antidepressant-like effects in animal models of chronic stress including early SIS (Amiri et al., 2015b; Montezuma et al., 2012; Peng et al., 2012). On the other hand, tropisetron showed antidepressant-like effects while administered to IC mice. Co-administration of AG or L-NAME with tropisetron at sub-effective doses reversed the deleterious impacts of early SIS as determined through neurochemical and behavioral analysis suggesting the mediatory role of the nitrergic system in protective effects of tropisetron on mitochondrial function and oxidative state. To support this, pretreatment with L-arg potently abolished the effects of tropisetron indicating that antidepressant-like effects of tropisetron are partly mediated by mitigating the adverse effects of iNOS activity on the mitochondrial function as well as anti-oxidant system in the cortical regions. It is important to note that although the antidepressant-like effects of NOS inhibitors have been well documented in the literature, only AG (but not 7-NI as a neuronal NOS inhibitor) exhibited protective properties in IC mice (data not shown). To explain the positive effects of tropisetron on behavioral and biochemical conditions of IC mice, it is important to state that the cerebral cortex contains abundant 5-HT_3 positive neurons which control the excitatory and inhibitory processes relevant to higher-order cognitive functions. Nearly 30% of cortical interneurons are 5-HT_3 positive and are located in the superficial layers of the neocortex (Engel et al., 2013; Rudy et al., 2011). 5-HT_3 receptors are implicated in the pathophysiology of depression as their activation by glucocorticoids results in calcium influx and consequently the release of glutamate from presynaptic synaptosomes in prelimbic cotical areas (Rajkumar and Mahesh, 2010; Zhang et al., 2012). Except for the inhibitory properties of GABAergic interneurons in the cortex, 5-HT_3 positive interneurons have been reported to exhibit dis-inhibitory role that cause activity of pyramidal glutamatergic neurons in the cortex (Pi et al., 2013). Thus, antidepressant-like activity of tropisetron may be associated with its ability to decrease calcium influx into cells and alleviation of mitochondrial dysfunction. Furthermore, results from our laboratory and others indicate that the protective properties of tropisetron under pathological conditions are associated with its anti-inflammatory properties (Rahimian et al., 2011, 2013; Swartz et al., 2013). Recent investigations in our laboratory demonstrated that tropisetron reduced the expression of iNOS, release of mitochondrial cytochrome c and TNF-α levels in the hippocampus following the administration of beta-amyloid (Aβ) (Rahimian et al., 2013). Similar results were observed while tropisetron efficiently diminished the oxidative stress and iNOS expression in the kidney following cisplatin toxicity (Zirak et al., 2014). Accordingly, we suggest that the antidepressant-like effects of tropisetron may be related to its modulatory effects on the NO (by iNOS) and ROS overproduction which together produce large amounts of peroxynitrite in the specific regions of the brain and initiate the inflammatory responses. Our study is not without limitation where we showed that the nitrergic system is involved in the tropisetron effects, we did not specifically determine whether the protective effects of tropisetron were related to the antagonization of 5-HT_3 receptors or partial activation of α7 nicotinic acetylcholine receptors. Considering that 5-HT_3 receptors consisted of divergent homomeric and heteromeric subtypes that are expressed in various patterns in different tissues (Faerber et al., 2007), uncertain responses evoked by tropisetron are expected through 5-HT_3 (in) dependent pathways.

5. Conclusion

In conclusion, our previous reports on the protective effects of tropisetron have indicated that in various pathological conditions such as Alzheimer's disease, anxiety-like behaviors and ischemia tropisetron may exert its effects through a variety of mechanisms such anti-inflammatory effects. In this study, we found that the antidepressant-like effects of tropisetron (5 mg/kg) in socially iso-

lated mice are associated with its ability to mitigate the negative impacts of iNOS-derived NO on mitochondrial function and redox status in brain cortical areas.

Conflicts of interest

The authors have no conflicts of interest to declare regarding the study described in this article and preparation of the article.

Authors contribution

COO and MR provided scientific input and edited the manuscript.

Acknowledgments

None.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.brainresbull.2016.04.018.

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