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# Lithium attenuates the proconvulsant effect of adolescent social isolation stress via involvement of the nitrergic system

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ABSTRACT

In this study, we tested whether acute administration of lithium mitigates the deleterious effect of adolescent social isolation stress (SIS) on seizure susceptibility. In comparison with socially conditioned (SC) mice, isolated conditioned (IC) mice exhibited an increase in seizure susceptibility to pentylenetetrazole. Acute administration of lithium (10 mg/kg) reversed the proconvulsant effect of SIS in IC mice, but this effect was not observed in SC mice. Coadministration of subthreshold doses of lithium (3 mg/kg) with nitric oxide synthase (NOS) inhibitors reversed the effect of SIS on seizure susceptibility and decreased hippocampal nitrite levels in IC animals. In addition, a subthreshold dose of a nitric oxide precursor reduced the protective effect of lithium on seizure susceptibility and increased nitrite levels in the hippocampus of IC mice. These results suggest that lithium exerts a protective influence against the proconvulsant effect of adolescent SIS via a nitrergic system that includes activation of neuronal NOS in the hippocampus.



## 1. Introduction

Experiencing aversive events in early stages of life affects the normal development of the brain and increases the risk of vulnerability to neuropsychiatric disorders in later life [1–4]. Adolescence is considered as a period in which exposure to stressful stimuli (i.e., psychosocial stress) promotes disturbances in mental health including mood and anxiety disorders [5,6]. Social isolation stress (SIS) in adolescent animals that are normally social induces behavioral and neurochemical changes [7]. It has been proposed that SIS is a valid animal model to investigate the neurobehavioral changes in psychiatric disorders that are observed in humans [8,9]. Recently, we showed that applying SIS to adolescent mice is associated with an increase in seizure susceptibility to pentylenetetrazole (PTZ) in adulthood [10]. We also demonstrated that the nitrergic system plays a role as an underlying mechanism in mediating the proconvulsant effect of SIS [10]. A recent study showed that PTZ induces seizure activity and this effect is mediated by overproduction of nitric oxide (NO) in certain areas of the brain [11]. Under physiological circumstances, NO has a modulatory effect on seizure susceptibility, while excessive production of NO in pathological conditions (such as exposure to chronic stress) has been reported to decrease the seizure threshold induced by PTZ [10,11]. Prolonged stressful conditions induce neuronal changes via increase of excitatory amino acids, and consequently, overproduction of nitric oxide (NO) is triggered by over-expression of nitric oxide synthase (NOS), mainly inducible (iNOS) and neuronal (nNOS) isoforms, in different areas of the brain including the hippocampus (HIPP) [12–14]. Accumulating data indicate that HIPP plays an important role in the development of epileptogenesis [15].

Lithium is a well-known mood stabilizer that possesses antioxidant and neuroprotective properties [16]. It has been also used as an antiepileptic agent in preclinical [17,18] and clinical studies [19,20]. Our previous studies using the pentylenetetrazole (PTZ) model of clonic seizures have shown that the anticonvulsant effect of lithium is at least partly mediated through NO [21,22]. Stressful events in early life are associated with an increased risk of epileptogenesis [15], and it has been

*Abbreviations:* SIS, social isolation stress; PTZ, pentylenetetrazole; SC, social condition; IC, isolation condition; NO, nitric oxide; NOS, nitric oxide synthase; iNOS, inducible NOS; nNOS, neuronal NOS; HIPP, hippocampus; PND, postnatal day; L-NAME, NG-nitro-L-arginine methyl ester; AG, aminoguanidine; 7-NI, 7-nitroindazole; L-arg, L-arginine; FST, forced swimming test; OFT, open-field test; HBT, hole-board test; i.p., intraperitoneal.

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shown that lithium exerts protective properties against the negative impact of stress on the HIPP [16,23]. In addition, previous studies have reported that lithium mitigates the impact of exposure to chronic stress on promoting depressive-like behaviors and aggression and the deficits in memory associated with chronic stress [24–26].

Considering the protective properties of lithium in modulating the negative effects of stress, we examined the effect of lithium on seizure susceptibility in mice exposed to SIS during adolescence. We also investigated the possible involvement of the nitrergic system in mediating the effect of lithium on seizure vulnerability in socially isolated animals.

## 2. Materials and methods

### 2.1. Animals

In the current study, male NMRI mice (Pasteur Institute, Tehran, Iran), weighing 10–14 g and on postnatal day (PND) 21-25, were used. Animals were housed under standard conditions (temperature: 23 ± 2 °C; 12-h light–dark cycle; and free access to food and water) for four weeks. Animals were housed under two different conditions: 1) social condition (SC) and 2) isolated condition (IC). Socially conditioned mice were housed in groups (6 mice per cage) in Plexiglas boxes (25 × 25 × 15 cm), and IC mice were housed individually in Plexiglas boxes (24 × 17 × 12 cm). Isolated conditioned mice were housed in a separate room and had olfactory and visual contacts. The cages of IC mice were cleaned weekly by the same experimenter to minimize handling and social contact. 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 (HHS publication 85–23, 1985), guide for the Care and Use of Laboratory Animals (1996, published by National Academy Press, 2101 Constitution Ave. NW, Washington, DC 20055, USA), and institutional guidelines for animal care and use (Department of Pharmacology, School of Medicine, TUMS). Also, each experimental group contained 6 to 8 animals.

### 2.2. Drugs

The following drugs were used in this work: lithium chloride (Merck, Darmstadt, Germany), NG-nitro-L-arginine methyl ester (L-NAME), aminoguanidine (AG), 7-nitroindazole (7-NI), L-arginine (L-arg) (Sigma, St Louis, MO, USA) and pentylenetetrazole (PTZ; Sigma, UK). Except for the 7-Nitroindazole, which was suspended in 1% aqueous solution of Tween80, all other drugs were dissolved in saline and were administered in the volume of 5 ml/kg mouse weight. To assess clonic seizure in the subjects, we administered PTZ intravenously (0.5%, i.v.) and all other drugs, intraperitoneally (i.p.). Doses of each drug were chosen according to the pilot treatments, which were published in our previous studies [21,27].

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

The open-field test was used to elucidate the effects of SIS and treatments on motor function and anxiety behavior [28]. The apparatus consisted of a white opaque Plexiglas box measuring 50 × 50 × 30 cm, which was dimly illuminated. The ground of the box was separated into 16 equal squares. Each mouse was placed gently on the central zone (25 × 25 cm), and its 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.

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

The hole-board test was used to evaluate the anxiety-like behaviors of animals as described in our previous study [29]. The apparatus was a white Plexiglas panel (50 cm × 50 cm, 2 cm thick) with 16 equal holes each with 3 cm in diameter. The board 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.

### 2.5. Forced swimming test (FST)

We used FST as a widely used behavioral test in which the prolonged immobility time presents the despair behavior reflecting the depressive-like behaviors [30,31]. Mice were individually placed in an open glass cylinder (diameter: 10 cm; height: 25 cm) containing 19 cm of water at 23 ± 1 °C. Mice were allowed to swim for 6 min, and the immobility time was recorded during the last 4 min of the test. Immobility behavior was considered when the animal remained floating motionless in the water and made only those movements necessary to keep its head above water.

### 2.6. Splash test

The splash test was carried out by spraying a 10% sucrose solution on the dorsal coat of animal in a familiar cage. The sucrose solution dirtied the coat and induced a grooming behavior. The grooming activity time was recorded for 5 min as an index of self-care and motivational behavior. Moreover, a decrease in grooming activity time is associated with reduced hedonic reactivity in the sucrose preference test and increased immobility in the FST [32–34].

### 2.7. Determination of clonic seizure threshold

In order to measure the clonic seizure threshold in animals, we used the method that was previously described [21,35]. Briefly, a winged infusion set (30-gauge) was used to infuse the PTZ (0.5%) at a constant rate of 1 ml/min into the tail vein of the freely moving subject. Infusion was halted when forelimb clonus followed by full clonus of the body was observed. The minimal dose of PTZ (mg/kg mouse weight) needed to induce a clonic seizure was considered as the index of seizure threshold. As such, seizure threshold is dependent on the dose and time of PTZ administration.

### 2.8. Hippocampal nitrite assay

To determine the NO levels in the hippocampus, we measured nitrite levels as the result of the NO end product [36]. The animals were decapitated under mild anesthesia, and then, the hippocampi were dissected on an ice-cold surface and immediately immersed in liquid nitrogen. Tissue homogenates were prepared, and nitrite levels were measured by using a colorimetric assay based on the Griess reaction. Briefly, each well was loaded with 100-μl samples, which were then mixed with 100-μl Griess reagent. Following 10-minute incubation at room temperature, 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.

### 2.9. Experiment design and treatments

To determine the effect of SIS on the animal behaviors after exposure to different housing conditions (SC or IC), behavioral experiments were carried out. Firstly, in order to validate the SIS paradigm, we applied a variety of behavioral tests including FST and splash test (for depressive-like behaviors) as well as OFT and HBT (for anxiety-like responses) using different sets of animals for each test. We assessed the effect of SIS on seizure threshold using the PTZ model of clonic seizure.

We then investigated the effect of lithium (3, 5, 10, and 25 mg/kg, i.p., 30 min before tests) on seizure threshold in different sets of SC

and IC mice. Moreover, we investigated whether the nitrergic system plays a role in mediating the effects of lithium on seizure susceptibility. In this regard, we treated both SC and IC mice with lithium and the following drugs: L-NAME (10 mg/kg, i.p., 45 min before the tests), AG (50 mg/kg, i.p., 45 min before the tests), 7-NI (15 mg/kg, i.p., 30 min before the tests), and L-arg (25 mg/kg, i.p., 45 min before the tests). Doses of each drug were chosen according to the pilot treatments, which were published in previous studies [10,21]. After administration of drugs, seizure threshold was evaluated using the PTZ model of clonic seizure. To exclude the possible effect of saline/Tween80 1% administration, SC and IC groups were injected with 5-ml/kg physiological saline/Tween80 1%. Applying the same treatments (as mentioned above) to SC and IC mice, we also measured the nitrite levels in the HIPP of both treated and nontreated experimental groups.

### 2.10. Statistical analysis

Comparison between the groups was analyzed using *t*-test and one-way ANOVA 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.

## 3. Results

### 3.1. Effects of SIS on mice behavior

Social isolation stress increased the locomotor activity (both horizontal and vertical) in IC mice compared with that in SC animals in the OFT ($p < 0.01$ and $p < 0.001$, respectively, Fig. 1a and 1b). Also, SIS induced despair behavior in the FST, in which a significant increase in immobility time was observed in comparison with SC mice ($p < 0.001$; Fig. 1c). Additionally, SIS caused a significant reduction in the grooming activity time in IC mice in the splash test (which reflects self-care disturbances) when compared with that in SC animals ($p < 0.001$; Fig. 1d). In the HBT, SIS induced a significant decrease in the number of head dips in IC animals in comparison with that in SC mice ($p < 0.001$; Fig. 1e).

### 3.2. Effects of SIS on seizure threshold in PTZ model of clonic seizures

Using the PTZ model of clonic seizures, SIS significantly decreased the seizure threshold in IC mice. *t*-Test revealed a significant difference between IC and SC animals in relation to seizure threshold ($p < 0.001$; Fig. 2).

### 3.3. Lithium administration attenuated the proconvulsant effect of SIS

One-way ANOVA analysis showed that there were significant differences between lithium-treated groups in both the SC ($F(5, 42) = 3.939$; $p < 0.05$; Fig. 3a) and IC ($F(4, 35) = 21.45$; $p < 0.05$; Fig. 3b) groups. Fig. 3a shows that the administration of lithium (3, 5, and 10 mg/kg) had no effect on the seizure threshold of SC mice ($p > 0.05$; Fig. 3a), while administration of higher dose of 25 mg/kg increased the seizure threshold in SC mice ($p < 0.05$; Fig. 3a).

Post hoc test analysis shows that administration of lithium (5 and 10 mg/kg) significantly reversed the proconvulsant effect of SIS on IC mice ($p < 0.01$ and $p < 0.001$, respectively; Fig. 3b). However, administration of lithium (3 mg/kg) did not alter the seizure threshold of IC mice in comparison with the saline-treated IC group ($p > 0.05$). Also, the result shows that saline treatment had no effect on seizure threshold of both IC and SC mice ($p > 0.05$; Fig. 3a and 3b).

### 3.4. Role of nitrergic system in anticonvulsant effect of lithium in socially isolated animals

Figs. 4–6 show the effects of subeffective doses of NOS inhibitors on seizure threshold in SC and IC animals. Administration of L-NAME (10 mg/kg) as a nonspecific NOS inhibitor, 7-NI (15 mg/kg) as a selective

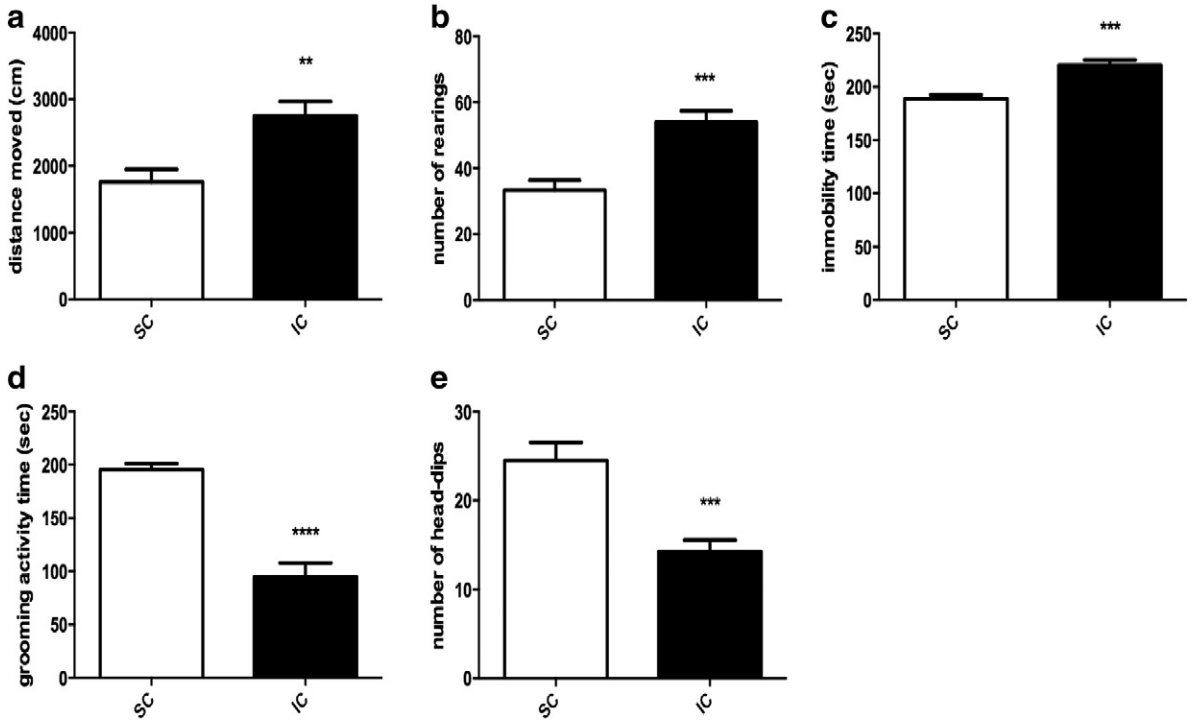


**Fig. 1.** Effect of different housing conditions, social condition (SC) and isolated condition (IC), on distance moved (horizontal activity) (a) and number of rearings (vertical activity) (b) in the open-field test; duration of immobility time in the forced swimming test (c); grooming activity time in the splash test (d); number of head dips in the hole-board test (e). Values are expressed as the mean ± S.E.M from 8 animals and were analyzed using *t*-test. $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$ compared with the SC group.

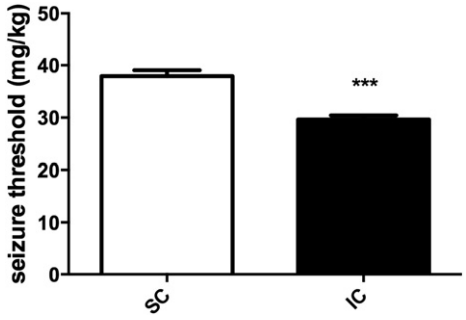


**Fig. 2.** Effect of different housing conditions, social condition (SC) and isolated condition (IC), on the seizure threshold in the PTZ model of clonic seizures. Values are expressed as the mean ± S.E.M from 8 animals and were analyzed using *t*-test. ***$p < 0.0001$ compared with the SC group.

nNOS inhibitor, and AG (50 mg/kg) as a selective iNOS inhibitor had no effect on seizure threshold of SC and IC mice ($p > 0.05$).

Next, the effects of coadministration of lithium (3 mg/kg) with subeffective doses of NOS inhibitors were determined on seizure threshold in SC and IC mice. The result shows that coadministration of L-NAME (10 mg/kg) and lithium (3 mg/kg) significantly reversed the proconvulsant effect of SIS on IC mice ($F$ (3, 28) = 10.02; ***$p < 0.001$; Fig. 4b). By comparison, the same results were not observed following coadministration of L-NAME (10 mg/kg) and lithium (3 mg/kg) in SC mice (F (3, 28) = 0.85; $p > 0.05$; Fig. 4a). In addition, unlike SC mice (F (3, 28) = 0.2612; $p > 0.05$; Fig. 5a), administration of 7-NI (15 mg/kg) significantly augmented the anticonvulsant effect of lithium (3 mg/kg) in IC animals ($F$ (3, 28) = 25.21; $p < 0.0001$; Fig. 5b). However, coadministration of AG (50 mg/kg) and lithium (3 mg/kg) did not change the seizure threshold in IC mice ($F$ (3, 28) = 0.06; $p > 0.05$; Fig. 6b) as well as SC mice ($F$ (3, 28) = 0.21; $p > 0.05$; Fig. 6a). Except for 7-NI-treated animals, all experimental groups were compared with saline-treated SC/IC mice as control groups. Mice treated with 7-NI were compared with Tween80 1%-treated SC/IC mice as control groups. Additionally, our data showed that treatment with saline and Tween80 1% (45 and 30 min before the test) had no effect on the seizure threshold of experimental groups ($p > 0.05$).

Fig. 7 shows the effect of a subeffective dose of L-arg, an NO precursor, on the seizure threshold in SC and IC animals. Fig. 7b demonstrates the effect of coadministration of lithium and L-arg on seizure threshold in SC and IC mice. Our data demonstrated that L-arg (25 mg/kg) significantly abrogated the anticonvulsant effect of lithium (10 mg/kg) in the IC mice ($F$ (3, 28) = 16.57; **$p < 0.01$; Fig. 7b). However, these treatments had no effect on the seizure threshold of SC mice ($F$ (3, 28) = 0.1214; $p > 0.05$; Fig. 7a).

### *3.5. Hippocampal nitrite levels: in absence and presence of lithium /NOS inhibitors/NO precursor*

In comparison with SC mice, analysis revealed that IC mice had higher levels of nitrite in the HIPP ($p < 0.001$). Lithium injection (10 mg/kg) significantly reduced ($p < 0.001$) the hippocampal nitrite levels in IC mice when compared with saline-treated IC group ($F$ (2, 21) = 29.43; $p < 0.05$; Fig. 8a). However, a lower dose of lithium (3 mg/kg) did not affect the nitrite levels in the HIPP of IC animals ($p > 0.05$). None of these treatments altered the nitrite levels of HIPP in SC mice ($F$ (2, 21) = 0.23; $p > 0.05$; Fig. 8a).

We then assessed the effects of NOS inhibitors/NO precursor and lithium coadministrations on hippocampal nitrite levels. Analysis of variance (ANOVA) showed that there were significant differences in HIPP nitrite levels between treated groups in IC animals ($F$ (4, 35) = 14.85; $p < 0.05$; Fig. 8c). Our data revealed that L-NAME (10 mg/kg) ($p < 0.05$) and 7-NI (15 mg/kg) ($p < 0.001$) coadministration with lithium (3 mg/kg) in IC mice significantly decreased the nitrite levels in HIPP comparing with saline/Tween80-treated IC animals. However, coadministration of AG (50 mg/kg) and lithium (3 mg/kg) to IC mice had no effect on the nitrite levels of HIPP ($p > 0.05$). Subeffective doses of NOS inhibitors did not change the nitrite levels in the HIPP when they were applied without lithium ($p > 0.05$). On the other hand, none of these treatments altered the hippocampal nitrite levels in SC animals ($F$ (4, 35) = 0.0589; $p > 0.05$; Fig. 8b).

Fig. 8 also shows that L-arg (25 mg/kg) injection abolished the effect of lithium (10 mg/kg) in reducing nitrite levels in the HIPP ($p < 0.001$; Fig. 8e). The L-arg did not alter the nitrite levels in the HIPP when it was injected alone ($p > 0.05$). Also, none of these treatments changed the hippocampal nitrite levels in SC animals ($p > 0.05$; Fig. 8d).

## 4. Discussion

In the current study, our results showed that SIS in adolescence causes a variety of behavioral deficits as well as an increase in the

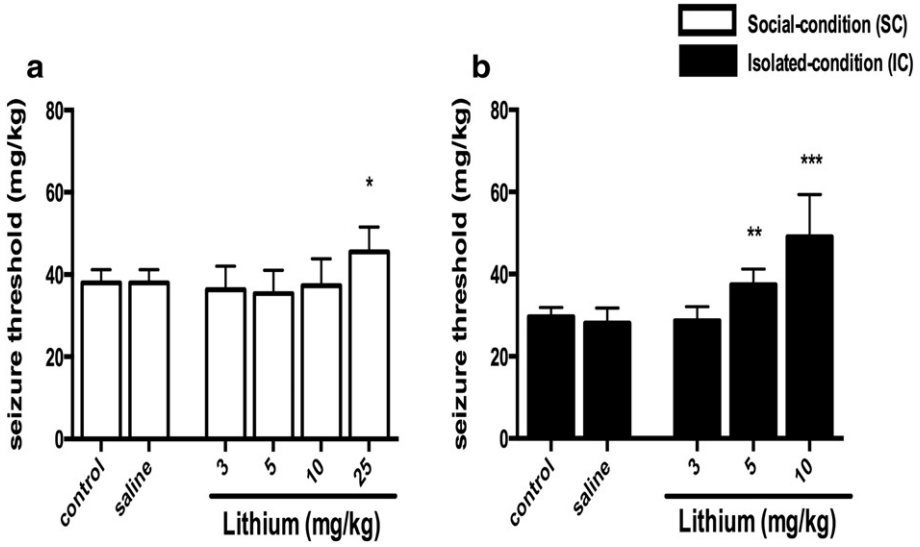


**Fig. 3.** (a) Effect of lithium chloride (3, 5, 10, and 25 mg/kg) on seizure threshold of SC animals in the PTZ model of clonic seizure. (b) Effect of lithium chloride (3, 5, and 10 mg/kg) on the proconvulsant effect of IC in the PTZ model of clonic seizure. Values are expressed as the mean ± S.E.M from 8 animals and were analyzed using one-way ANOVA followed by Tukey's post hoc test. *$p < 0.05$ compared with the SC saline-treated group. **$p < 0.01$ and ***$p < 0.001$ compared with the IC saline-treated group.

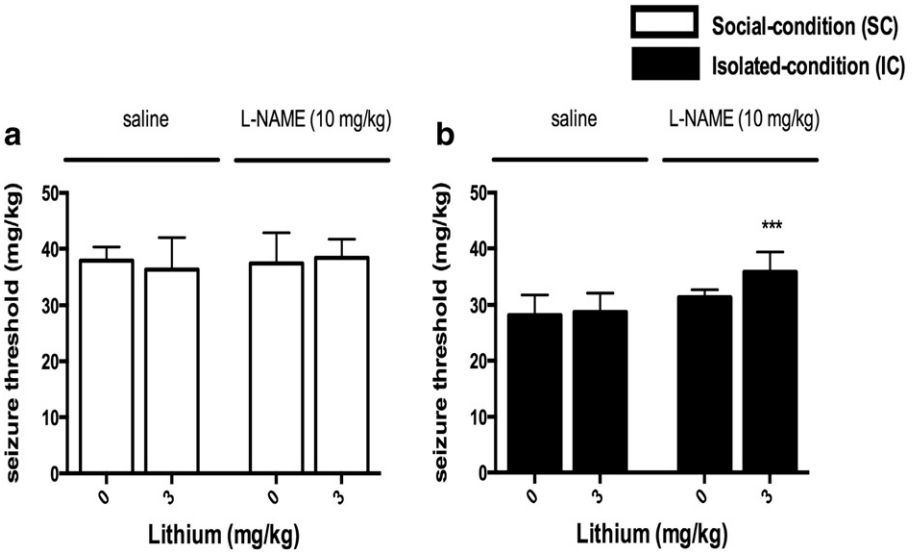


**Fig. 4.** Effect of lithium chloride (3 mg/kg) and L-NAME (LNM) (10 mg/kg) coadministration on the seizure threshold in the PTZ model of clonic seizure in the SC (a) and IC (b) animals. Values are expressed as the mean ± S.E.M from 8 animals and were analyzed using one-way ANOVA followed by Tukey's post hoc test. ***$p < 0.001$ compared with the IC saline-treated group.

seizure susceptibility in the PTZ model of clonic seizures. We showed that lithium administration significantly reversed the proconvulsant effect of SIS. In addition, the results of this study demonstrated that the nitrergic system mediates the anticonvulsant effect of lithium in socially isolated mice.

### *4.1. Social isolation stress induced seizure risk and behaviors relevant to depression and anxiety*

Experiencing aversive life events in adolescence contributes to the development of a variety of psychiatric disorders [5]. In this study, we used a number of behavioral tasks to validate the negative effects of SIS in mice. In comparison with SC mice, IC mice exhibited behaviors associated with depression and anxiety in the FST (increase in the immobility time), HBT (decrease in number of head dips), and splash test (decrease in grooming activity time). The splash test and FST have been reported as valid tests to evaluate depressive-like behaviors including self-care disturbances, hedonic loss, and behavioral despair in rodents [32,37,38]. Also, HBT is considered a suitable test for evaluation of anxiety-like behaviors including emotional changes [29]. In the present study, IC animals showed an increase in locomotion in the OFT (both horizontal and vertical activities) when compared with SC animals. Our results are in line with previous studies, which have reported that SIS induces a variety of emotional responses including anxiety- and depressive-like behaviors [7,39]. Additionally, in comparison with the SC group, IC mice had a remarkably lower seizure threshold indicating that SIS has a proconvulsant effect on socially isolated animals. This result agrees with the earlier studies that reported a proconvulsant effect of SIS on rodents [1,2]. In this regard, Matsumoto et al. reported that long-term social isolation for seven weeks increased seizure

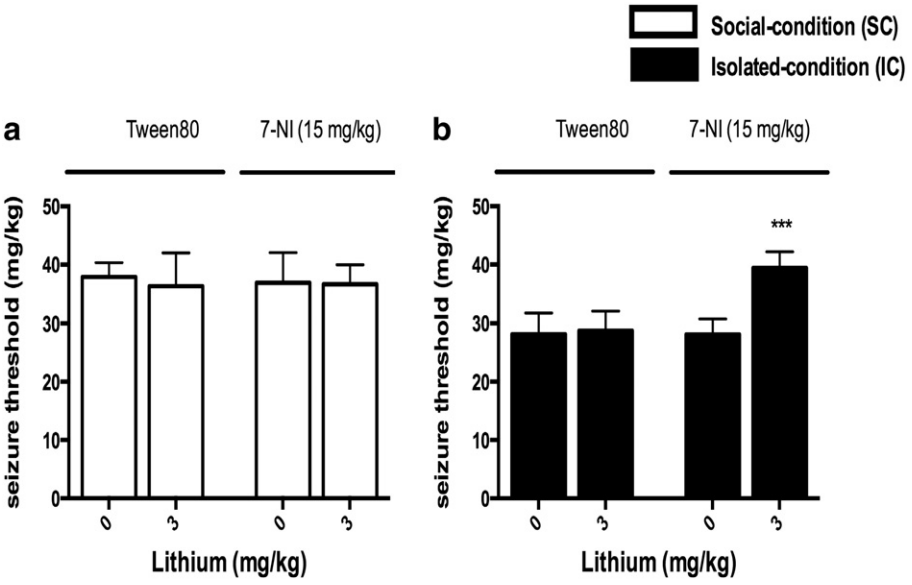


**Fig. 5.** Effect of lithium chloride (3 mg/kg) and 7-nitroindazole (7-NI) (15 mg/kg) coadministration on the seizure threshold in the PTZ model of clonic seizure in the SC (a) and IC (b) animals. Values are expressed as the mean ± S.E.M from 8 animals and were analyzed using one-way ANOVA followed by Tukey's post hoc test. ***$p < 0.001$ compared with the IC Tween80 1%-treated group.

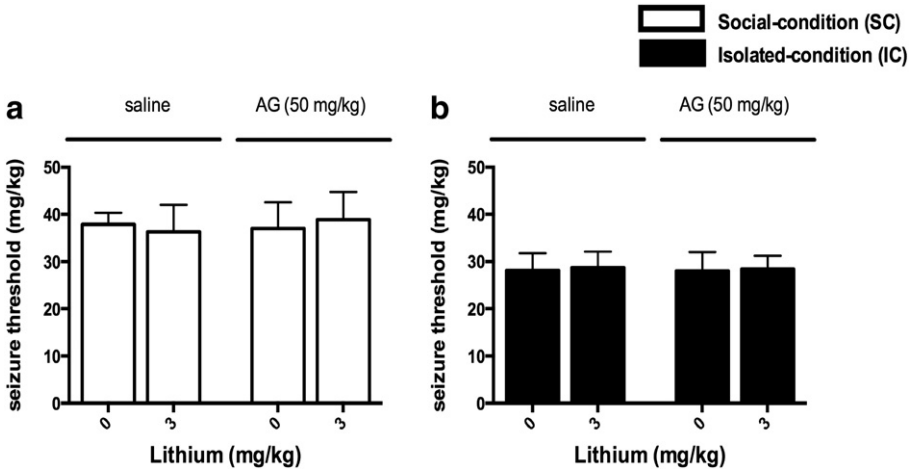


**Fig. 6.** Effect of lithium chloride (3 mg/kg) and aminoguanidine (AG) (50 mg/kg) coadministration on the seizure threshold in the PTZ model of clonic seizure in the SC (a) and IC (b) animals. Values are expressed as the mean ± S.E.M from 8 animals and were analyzed using one-way ANOVA followed by Tukey's post hoc test.

susceptibility to picrotoxin in mice [2], and a study by Russo et al. illustrated that chronic mild stress in combination with PTZ kindling can be used as a model of epilepsy and mood disorder comorbidity [40].

### *4.2. Nitrergic system mediated the anticonvulsant effect of lithium in socially isolated mice*

Animal models of stress provide conditions to investigate the effects of stress on behavioral and neurochemical changes, which are similarly observed in human psychiatric disorders [9,41]. Evidence is accumulating that lithium is not only an effective treatment for mood disorders but is also able to ameliorate additional detrimental outcomes of stress on the brain [16,42]. For example, an antiepileptic effect of lithium has been reported in a number of previous studies [18,43]. In this study, our results showed that acute administration of lithium to SC (25 mg/kg, but not lower doses) and IC (5 and 10 mg/kg, but not 3 mg/kg) mice had an anticonvulsant property. These results were in agreement with similar studies which have reported the anticonvulsant effects of lithium in nonstressed animals [18,21,22]. In addition, we showed that, in stressed animals, a lower dose of lithium reversed the effect of SIS on seizure susceptibility to PTZ. Considering the anticonvulsant effect of lithium in this study, there is evidence about the proconvulsant effect of lithium in animals [44,45]. However, consistent with our results, there are also studies that have reported the anticonvulsant properties of lithium [18,21,22]. Discrepancy between findings may be related to the different methodologies between studies because each seizure paradigm has its specific characteristics and, also, different drugs have divergent effects on different paradigms of seizures [46,47]. Furthermore, previous studies used nonstressed animals, while in this study, we investigated the effect of lithium on seizure susceptibility under pathophysiological conditions by applying an animal model of chronic stress.

Recent studies demonstrated that seizure vulnerability is associated with NO levels in the brain (mostly hippocampus) and that the PTZ model of clonic seizures is a sensitive method to modulate NO levels in the brain [11,14]. Moreover, a large body of evidence indicates that exposure to stress enhances nNOS expression in the HIPP and other areas in the brain, which consequently, leads to overproduction of NO [48,49]. Also, there is a large body of evidence showing that nNOS plays a role in modulating the seizure activity in the HIPP [10,11,21,50]. In this regard, we tested our hypothesis that the nitrergic system contributes to the anticonvulsant effect of lithium in stressed animals. Our results revealed that administration of a subeffective dose of lithium (3 mg/kg) along with subeffective doses of 7-NI and L-NAME (but

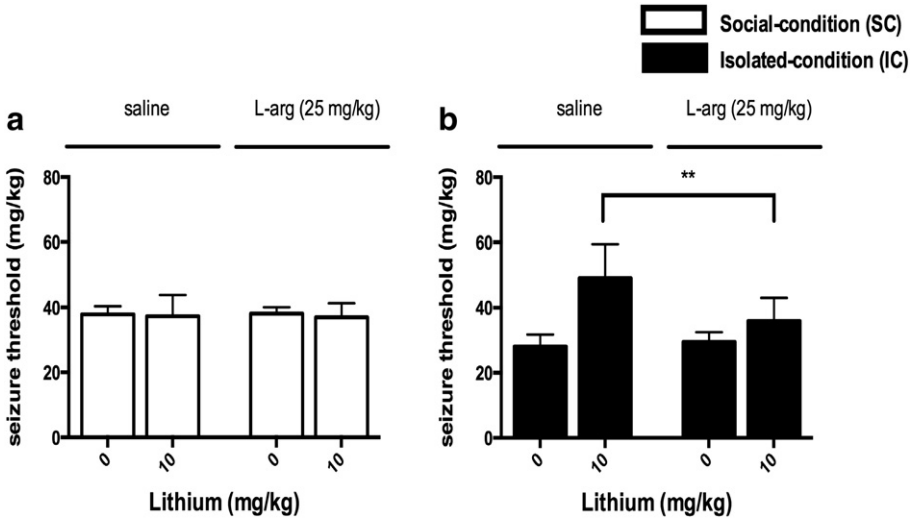


**Fig. 7.** Effect of lithium chloride (10 mg/kg) and L-arginine (L-arg) (25 mg/kg) coadministration on the seizure threshold in the PTZ model of clonic seizure in the SC (a) and IC (b) animals. Values are expressed as the mean ± S.E.M from 8 animals and were analyzed using one-way ANOVA followed by Tukey's post hoc test. $^{**}p < 0.01$ compared with the IC lithium chloride (10 mg/kg)-treated group.

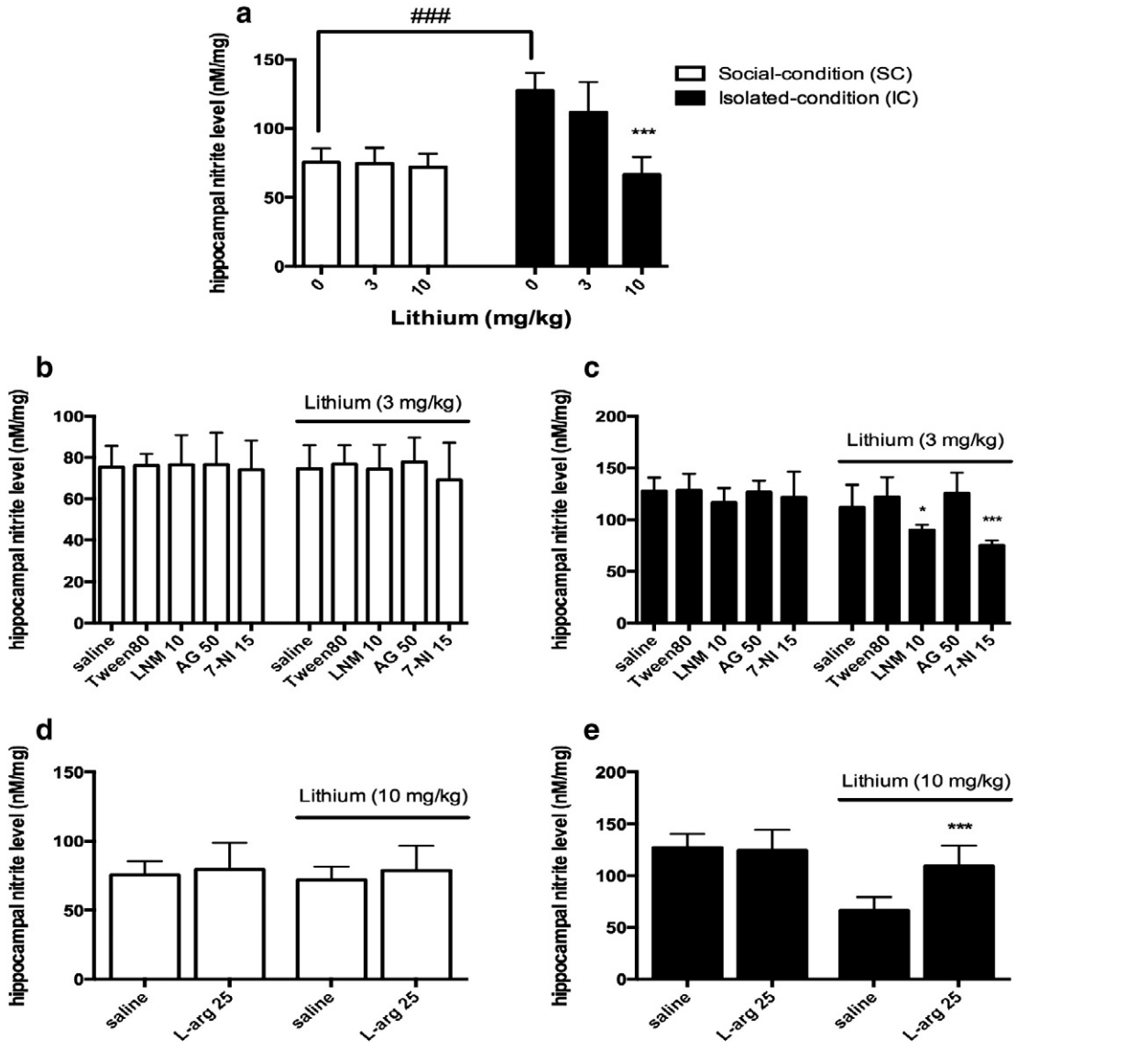


**Fig. 8.** Effect of different housing conditions (SC and IC), lithium chloride (3 and 10 mg/kg) administration and lithium chloride (3 mg/kg) coadministration with NO agents on hippocampal nitrite level. Values are expressed as the mean ± S.E.M from 8 animals and were analyzed using *t*-test and one-way ANOVA followed by Tukey's post hoc test. $^{###}p < 0.001$ 0.001 compared with the SC group. $^{***}p < 0.001$ compared with the IC saline-treated group (a). $^{***}p < 0.001$ compared with the IC lithium chloride (3 mg/kg) and Tween80 1%-treated group (c). $^{***}p < 0.001$ compared with the IC lithium chloride (10 mg/kg) and saline-treated group (e).

not AG) reversed the proconvulsant effect of SIS in IC mice, while administration of a subeffective dose of L-arg abolished the anticonvulsant effect of lithium (10 mg/kg). These findings showed that the anticonvulsant effect of lithium is partly mediated by the nitrergic system. To support this, NO levels in the HIPP of SC and IC mice were also evaluated after the same treatments. Lithium (10 mg/kg, but not 3 mg/kg) decreased the hippocampal nitrite levels. Also, lithium (3 mg/kg) along with subeffective doses of 7-NI and L-NAME decreased the NO levels in HIPP. Similarly, administration of a subeffective dose of L-arg blocked the effect of lithium (10 mg/kg) on hippocampal NO levels. Therefore, we conclude that the effects of lithium are related to nNOS, but not iNOS, activity in the HIPP of IC mice.

## 5. Conclusion

In conclusion, this study revealed that lithium has a protective role against the proconvulsant effect of SIS. In addition, we showed that the anticonvulsant effect of lithium is mostly mediated by nNOS in the HIPP.

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## Conflict of interest

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

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