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Original Article

Lithium carbonate attenuates behavioral alterations and partially reverses reduced prefrontal BDNF mRNA expression induced by post-weaning social isolation in male rats

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Abstract

Social isolation is an environmental stressor that can adversely affect behavioral function and neuroplasticity-related pathways. Brain-derived neurotrophic factor (BDNF) is one of the key molecules implicated in these changes. Lithium has been reported to influence neuroplasticity-related signaling and may modulate BDNF expression. In this study, we investigated the effects of lithium carbonate on behavioral alterations induced by chronic post-weaning social isolation in male rats and assessed BDNF mRNA expression in the prefrontal cortex. Rats were subjected to social isolation from postnatal day (PND) 21 to PND 70. Lithium carbonate (20 mg/kg, i.p.) was administered once daily during PND 64-70. Behavioral performance was evaluated using the open field test, hot plate test, marble burying test, and forced swim test. Compared with controls, socially isolated rats showed hyperlocomotion, increased rearing, lower pain threshold, altered immobility behavior in the forced swim test, obsessive-compulsive-like behavior, and reduced prefrontal BDNF mRNA expression. Lithium treatment attenuated several isolation-induced behavioral alterations and was accompanied by a partial reversal of reduced prefrontal BDNF mRNA expression toward control levels. Interpretation of molecular findings should be considered preliminary because gene expression analysis was conducted on a limited number of biological samples (n = 3 per group) and only BDNF mRNA, not BDNF protein, was measured. In conclusion, lithium carbonate improved several behavioral abnormalities associated with chronic social isolation and was associated with changes in prefrontal BDNF mRNA expression in male rats. Further studies are needed to clarify the molecular pathways and causal mechanisms underlying these effects.

Keywords: Brain-derived neurotrophic factor (BDNF); Lithium carbonate; Social isolation; Prefrontal cortex; Behavior; Gene expression.

1. Introduction

Exposure to severe stress during early life can disrupt normal brain development and increase the risk of mental and neuropsychiatric disorders [1–3]. For example, early-life stressors such as maternal separation or prolonged social isolation have been shown to adversely affect brain development and can precipitate various neuropsychiatric conditions [4, 5]. Social isolation, in particular, is a chronic environmental stressor known to impair cognitive function and mood. Social deprivation during critical developmental periods has been linked to neurodevelopmental disorders including autism spectrum disorder (ASD), attention-deficit/hyperactivity disorder (ADHD), and schizophrenia [4, 5].

It is well documented that isolating rats during adolescence leads to a range of behavioral and cognitive abnormalities. Adolescent social isolation can cause pronounced hyperlocomotion along with changes in pain sensitivity and anxiety-like behavior in adulthood [6]. Another study reported that adolescent isolation in rats increased locomotor activity but did not produce consistent changes in anxiety-like behaviors [7]. These findings indicate that while hyperactivity is a robust outcome of adolescent isolation, its effects on anxiety-related behavior may depend on specific experimental conditions.

Brain-derived neurotrophic factor (BDNF) is one of the most important and well-studied neurotrophins in the central nervous system, playing a pivotal role in synaptic plasticity and synaptogenesis [8]. Alterations in BDNF signaling have been implicated in a wide range of neuropsychiatric disorders, including depression, anxiety, post-traumatic stress disorder (PTSD), obsessive–compulsive disorder (OCD), and schizophrenia [9–14]. Chronic social isolation may disrupt BDNF expression in the brain, although findings have been inconsistent across studies. Adolescent isolation has been reported to decrease BDNF levels in the rat hippocampus [6], and similar hippocampal BDNF downregulation in isolated rats has been observed by others [15]. One study found that social isolation significantly reduced BDNF levels in the nucleus accumbens

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[16]. In contrast, some reports found no significant change in BDNF in the hippocampus or plasma following adolescent isolation [17]. Intriguingly, isolation during early adolescence was shown to increase BDNF protein levels in the medial prefrontal cortex while simultaneously decreasing it in the hippocampus [18]. Thus, the impact of social isolation on BDNF expression appears to depend on factors such as brain region and experimental conditions, and remains somewhat inconsistent. Given the critical role of the prefrontal cortex in executive function and mood regulation, changes in BDNF within this region are particularly relevant. Previous studies have shown that adolescent social isolation can alter BDNF expression in the medial prefrontal cortex [18, 52].

Lithium is a classic mood stabilizer that has been used for decades in the treatment of neuropsychiatric disorders, particularly bipolar disorder, and it can influence neuroplasticity-related signaling. In addition to its clinical mood-stabilizing role, lithium is known to interact with multiple intracellular pathways, including glycogen synthase kinase-3 beta (GSK-3β), Akt, and cAMP response element-binding protein (CREB)-related signaling, all of which are relevant to neuronal survival, synaptic plasticity, and transcriptional regulation of neurotrophin-related genes [57, 58]. Evidence from animal models suggests that lithium treatment modulates BDNF expression in the brain, although results vary with the model and context. For example, in a rat model of mania induced by rapid eye movement (REM) sleep deprivation, lithium administration elevated hippocampal BDNF levels and concurrently reversed mania-like and OCD-like behaviors [14]. Lithium also upregulated hippocampal BDNF in a rat model of depression induced by unpredictable mild stress [19], and chronic lithium treatment has been reported to increase BDNF expression in rat brain tissue [59]. However, in a fear-conditioning model of PTSD, lithium had no effect on hippocampal BDNF levels [9]. Likewise, in an amphetamine-induced mania model, lithium did not alter BDNF levels in serum [20]. Although lithium has been shown to influence BDNF signaling in several stress-related and mood-related models, its effects on prefrontal BDNF expression following post-weaning social isolation remain poorly understood. Prior work has examined social isolation models, lithium-treated stress models, and lithium-BDNF interactions separately; however, fewer studies have linked lithium treatment, post-weaning social isolation-induced behavioral disruption, and prefrontal BDNF mRNA expression within the same experimental framework.

Accordingly, the present study aimed to explore whether lithium treatment can ameliorate the behavioral alterations caused by post-weaning social isolation in rats and whether such treatment is accompanied by changes in BDNF gene expression in the prefrontal cortex. We hypothesized that lithium would attenuate isolation-induced behavioral abnormalities and partially reverse reduced prefrontal BDNF mRNA expression. Because the molecular assessment was limited to BDNF mRNA, the study was designed to test an association rather than to establish a causal mechanism.

2. Materials and methods

2.1. Animals

Male Wistar rat pups (postnatal day 21), along with their dams, were obtained from the Cognitive Neuroscience Lab, Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran, and housed under standard laboratory conditions (12:12 h light–dark cycle, lights on at 07:00; temperature 22 ± 1 °C; relative humidity ~50%).

All rats had ad libitum access to food and water. After weaning at PND 21, pups were assigned to control or isolation conditions using a computer-generated random sequence. Allocation was performed before treatment by an investigator not involved in behavioral scoring. Cages were coded, and group codes were concealed from investigators who performed behavioral scoring and molecular analyses until the primary analyses were completed. Control rats were housed 3-4 per cage, whereas isolated rats were housed individually in cages of identical dimensions (30 × 50 × 40 cm). Rats in the isolation condition had no physical contact with other rats for the remainder of the experiment. Isolated rats had free access to food and water, and the housing area was kept free of external disturbances throughout the isolation period [6]. All behavioral tests were conducted during the light phase (between 09:00 and 16:00). The order of behavioral testing was kept identical across groups.

The behavioral battery was performed on PND 70 in the following fixed order: open field test, hot plate test, marble burying test, and forced swim test. This order was selected to begin with the least physically demanding and least aversive assay and to place the forced swim test last because it is the most stressful procedure. All behavioral tests were completed on the same day to avoid repeated handling and repeated re-exposure of isolated animals across multiple testing days; however, the possibility of cumulative stress or carryover effects cannot be fully excluded and is acknowledged as a limitation.

2.2. Ethics Statement

All experimental procedures complied with institutional and international guidelines for animal welfare. Ethical approval for this study was obtained from the Ethics Committee of Islamic Azad University, Tehran Medical Sciences (IR.IAU.TMU.REC.1403.112). The experiments were performed in accordance with the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals (8th edition, 2011) [21] and the ARRIVE guidelines for reporting animal research. Animals were monitored daily, humane endpoints were predefined, and every effort was made to minimize suffering and use the minimum number of animals necessary. Behavioral scoring, RNA extraction, qPCR setup, Ct extraction, and statistical processing were performed using coded samples so that investigators remained blinded to group allocation during data generation and initial analysis.

2.3. Injections

Lithium carbonate (Tehran Darou, Tehran, Iran) was administered via intraperitoneal (i.p.) injection at a dose of 20 mg/kg once daily during PND 64-70 (the final week of the isolation period). The injection regimen and dosage of lithium were based on previous rodent studies [22-25] and were selected as a behaviorally active dose range that has been used without overt toxicity in experimental models. Serum lithium concentrations were not measured in the present study; therefore, direct equivalence between this

regimen and therapeutic serum lithium levels in humans cannot be inferred. The translational relevance of the dose should therefore be interpreted cautiously. Control rats received i.p. saline injections (0.5 mL per rat) on the same schedule. All injections were given in the morning (approximately 09:00-10:00) to maintain consistency.

2.4. Open Field Test

The open field test was used to assess locomotor activity and anxiety-like behavior. The apparatus (Tajhiz-Gostar Omid Iranian Co., Tehran, Iran) consisted of a clear Plexiglas box (floor area 30 cm × 30 cm, wall height 40 cm) subdivided into 16 equal squares. Each rat was placed in the center of the arena and allowed to explore freely for 5 minutes. Locomotor activity was quantified as the number of floor grid squares the rat crossed during the 300-second trial [26]. Anxiety-like behavior was evaluated by counting the number of rearing events (standing on the hind legs) during the trial [27]. The open field test was performed 60 minutes after the last lithium or saline injection on PND 70. All open field sessions were video-recorded, and an observer blind to the group assignments later scored the locomotion and rearing counts to reduce observer bias.

2.5. Hot Plate Test

The hot plate test was used to measure pain threshold (nociceptive sensitivity). The hot plate apparatus (Tajhiz-Gostar Omid Iranian Co., Tehran, Iran) consisted of a metallic surface maintained at 50 °C. Each rat was gently placed on the heated surface, and the latency to the first pain response (hind paw lick or jump) was recorded in seconds as the pain threshold. A cutoff time of 100 s was set to prevent tissue injury [28]. The hot plate test was conducted 30 minutes after the open field test.

2.6. Marble Burying Test

The marble burying test was employed to assess obsessive–compulsive-like (OCD-like) behavior. Ten glass marbles (approximately 15 mm in diameter, 5–6 g each) were placed evenly on top of the bedding in the rat's home cage in two rows of five. The rat was then left undisturbed in the cage for 30 minutes. A marble was considered buried if at least 50% of it was covered by bedding [29, 30]. The number of marbles buried at the end of the 30 minutes was recorded as an index of compulsive behavior [14, 30]. The marble burying test took place 30 minutes after the hot plate test.

2.7. Forced Swim Test (FST)

Immobility behavior was assessed using the forced swim test. Rats were individually placed in a transparent cylindrical container filled with water (20–22 °C) to a depth that prevented them from touching the bottom (water filled about two-thirds of the cylinder's height). Each rat remained in the water for 5 minutes with no possibility of escape. During this period, immobility time was recorded as the duration of passive floating with only the minimal movements necessary to keep the head above water [9]. The FST was conducted 30 minutes after the marble burying test. After the test, rats were immediately removed from the water, dried with a towel, and returned to their home cage.

2.8. Tissue Collection and RNA Preparation

Immediately following the conclusion of behavioral testing on PND 70, rats were euthanized for brain collection. Each animal was deeply anesthetized with an overdose of sodium pentobarbital (150 mg/kg, intraperitoneal) and then decapitated. The brain was rapidly removed, and the prefrontal cortex was dissected on ice. Brain tissues were immediately frozen in liquid nitrogen and stored at –80 °C until RNA extraction.

The prefrontal cortex was selected a priori because this region is critically involved in executive control, stress-related behavioral regulation, and mood-related processes, and previous studies have reported social isolation-related alterations in medial prefrontal BDNF expression [18, 52]. The present molecular analysis was therefore focused on a region directly relevant to the behavioral outcomes under investigation.

Total RNA was extracted from prefrontal cortex tissue using a standard phenol–chloroform extraction protocol (TRIzol reagent), followed by DNase I treatment to remove any genomic DNA contamination. The quantity and purity of the extracted RNA were assessed by spectrophotometry (260/280 nm ratio), and RNA integrity was checked via agarose gel electrophoresis.

2.9. Real-Time PCR

Synthesis of cDNA was performed using a high-capacity cDNA reverse transcription kit (Thermo Fisher Scientific), following the manufacturer's instructions. Gene-specific primer pairs were designed to amplify BDNF and the reference gene GAPDH from cDNA, with each primer set spanning an exon–exon junction to avoid amplification of genomic DNA. Primer sequences were verified using NCBI Primer-BLAST to ensure specificity to the target genes and to avoid off-target binding. The primers were optimized to have melting temperatures of ~58–60 °C to promote efficient and specific annealing while minimizing primer–dimer formation. The specific primer sequences for BDNF and GAPDH are listed in Table 1.

Real-time PCR reactions were prepared in 10 µL volumes containing 5 µL of 2× SYBR Green master mix (Applied Biosystems), 2 µL of template cDNA, 0.5 µL of forward primer (10 µM), 0.5 µL of reverse primer (10 µM), and 2 µL of nuclease-free water. Amplification was performed in a StepOnePlus Real-Time PCR System (Applied Biosystems) with the following cycling program: an initial enzyme activation and template denaturation step at 95 °C for 15 min, followed by 40 cycles of 95 °C for 15 s (denaturation) and 60 °C for 30 s (combined annealing/extension).

Upon completion of the 40 PCR cycles, a melting curve analysis was carried out to verify the specificity of the amplified products. This involved gradually increasing

Table 1. Primer sequences for BDNF and GAPDH were used for quantitative real-time PCR.

Gene	Forward primer	Reverse primer
BDNF	AGCCTCCTCTGCTCTTTCTG	ATGGGATTACACTTGGTCTCG
GAPDH	TAAAGAACAGGCTCTTAGCACA	AGTCTTGGAAATGGATTGTCTC

the temperature from 60 °C to 95 °C while continuously monitoring fluorescence. A single distinct melting peak for each reaction confirmed that a specific product had been amplified, with no evidence of primer–dimer artifacts or non-specific amplification.

No-template controls were included to monitor contamination. Amplification specificity was evaluated by melt-curve profiles and by the presence of a single amplification product for each primer pair. Formal standard-curve-based PCR efficiency values and R^2 estimates were not available for all assays; this reporting limitation is acknowledged, and the BDNF mRNA data are interpreted as preliminary supportive molecular evidence rather than definitive mechanistic proof. GAPDH was used as the reference gene because of its common use in rat brain qPCR studies; however, independent reference-gene stability testing across the present experimental groups was not performed and is acknowledged as a limitation.

Cycle threshold (Ct) values were obtained for BDNF and GAPDH from each sample. GAPDH was used as the housekeeping gene to normalize target gene expression. Relative gene expression levels were calculated using the 2^–ΔΔCt comparative method. For each sample, the ΔCt was determined by subtracting the GAPDH Ct from the BDNF Ct. ΔΔCt values for the experimental groups were then obtained by subtracting the average ΔCt of the control group (calibrator) from the ΔCt of each sample. Fold-changes in BDNF expression relative to controls were expressed as 2^–ΔΔCt. All qPCR reactions were run in technical triplicate for each biological sample, and the mean Ct value for each animal was used for calculation. Statistical analyses of gene expression were performed using ΔCt values, whereas 2^–ΔΔCt values were used for relative-expression presentation in the figure.

2.10. Experimental Groups

Behavioral analyses were performed using all five experimental groups (n = 7 per group), whereas molecular analyses were conducted on a smaller predefined subset of biological samples. For gene expression analysis, four predefined groups were included: Control + Saline, Control + Lithium, Isolated (no treatment), and Isolated + Lithium, with n = 3 biological replicates per group. The Isolated + Saline group was included in the behavioral analyses but was not included in the qPCR analysis because molecular measurements were performed on the predefined subset allocated for gene expression assessment.

The rats were divided into five groups (n = 7 per group) as follows:

Group 1 – Control + Saline: Non-isolated control rats receiving intraperitoneal saline injections (0.5 mL/rat) once daily for seven days (PND 64–70).

Group 2 – Control + Lithium: Non-isolated control rats receiving lithium carbonate injections (20 mg/kg i.p.) once daily for seven days (PND 64–70).

Group 3 – Isolated (no treatment): Rats subjected to chronic social isolation from PND 21 to 70, with no injections during PND 64–70.

Group 4 – Isolated + Saline: Rats socially isolated from PND 21 to 70, receiving saline injections (0.5 mL/rat i.p.) once daily during PND 64–70.

Group 5 – Isolated + Lithium: Rats socially isolated from PND 21 to 70, receiving lithium carbonate injections (20 mg/kg i.p.) once daily during PND 64–70.

2.11. Statistical analysis

Data were analyzed using SPSS software (version 26). Before one-way analysis of variance (ANOVA), behavioral data were inspected for outliers and evaluated for distributional assumptions using Shapiro-Wilk tests for normality and Levene tests for homogeneity of variance. When assumptions were considered acceptable, behavioral outcomes were analyzed using one-way ANOVA followed by Tukey post hoc tests for multiple comparisons. Omnibus ANOVA effect sizes were reported as eta squared (η^2), calculated as (df_between × F)/(df_between × F + df_within). Because the primary statistical framework involved multi-group one-way ANOVA, η^2 was selected as the main effect-size metric. Pairwise Cohen's d values were not calculated for all possible post hoc contrasts because no a priori two-group contrasts were prespecified. Gene expression data were statistically analyzed using ΔCt values for the predefined groups included in the molecular analysis, whereas relative expression values (2^-ΔΔCt) were used for graphical presentation. Because the molecular analysis was conducted on a small subset of biological samples (n = 3 per group), formal distributional assessment has limited interpretive power; therefore, the gene expression findings were interpreted cautiously. Data are presented as mean ± SD, and $p < 0.05$ was considered statistically significant. Behavioral analyses were performed with n = 7 animals per group, whereas molecular analyses were performed with n = 3 biological replicates per group. A formal a priori power analysis was not conducted; therefore, the limited molecular sample size is acknowledged as a limitation.

3. Results

3.1. Open Field Test

Locomotor activity: One-way ANOVA revealed a significant difference in locomotor activity among the groups ($F(4,30) = 51.18$, $p < 0.001$, $\eta^2 = 0.87$). Post hoc Tukey tests indicated that lithium-treated control rats had significantly lower locomotor counts compared to saline-treated controls ($p < 0.001$). In contrast, socially isolated rats (with or without saline injections) exhibited markedly higher locomotor activity than non-isolated controls ($p < 0.001$). Importantly, isolated rats that received lithium showed significantly reduced locomotion compared with isolated rats without lithium ($p < 0.001$), indicating that lithium attenuated the isolation-associated increase in locomotor activity (Figure 1).

Rearing: Rearing frequency (an indicator of exploratory/anxiety-like behavior) also differed significantly across groups ($F(4,30) = 48.06$, $p < 0.001$, $\eta^2 = 0.86$). Lithium-treated controls reared less often than saline-treated controls ($p < 0.001$), whereas isolated rats (with or without saline) showed significantly higher rearing counts compared to controls ($p < 0.001$). Lithium treatment during isolation led to a significant reduction in rearing relative to saline-treated isolated rats ($p < 0.001$), indicating partial normalization of this behavior (Figure 2).

3.2. Hot Plate Test

The latency to paw lick on the hot plate (pain threshold) differed significantly among groups ($F(4,30) = 20.27$, $p < 0.001$, $\eta^2 = 0.73$). Socially isolated rats, whether untreated or given saline, had a significantly shorter withdrawal latency compared to control rats ($p < 0.001$), indicating in-

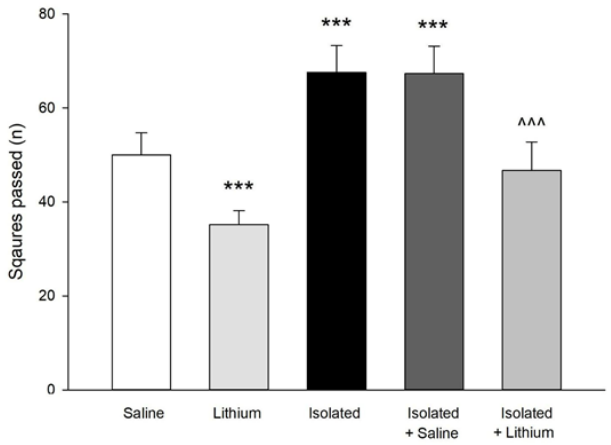


Fig. 1. Locomotor activity (number of squares crossed in the open field) in all experimental groups: Control + Saline, Control + Lithium, Isolated (no treatment), Isolated + Saline, and Isolated + Lithium. Data are presented as mean ± SD (n = 7 rats per group). *p < 0.001 vs. Control + Saline; ^^^p < 0.001 vs. Isolated (no treatment).

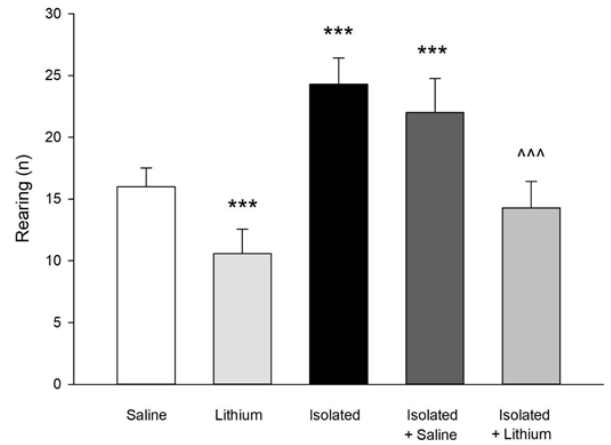


Fig. 2. Rearing behavior in the open field test, shown for all groups (group definitions as in Figure 1). Data are presented as mean ± SD (n = 7 rats per group). *p < 0.001 vs. Control + Saline; ^^^p < 0.001 vs. Isolated (no treatment).

creased pain sensitivity (hyperalgesia). Lithium administration to isolated rats significantly prolonged the response latency relative to isolated rats given saline ($p < 0.001$), partially counteracting the isolation-induced hyperalgesia (Figure 3). Lithium-treated control rats did not differ significantly from saline-treated controls in pain latency ($p > 0.05$).

3.3. Forced Swim Test (FST)

Immobility time in the forced swim test differed significantly among groups ($F(4,30) = 22.86$, $p < 0.001$, $\eta^2 = 0.75$). Rats exposed to chronic social isolation (with or without saline) showed significantly lower immobility times than control rats ($p < 0.001$). Given the concurrent hyperlocomotor phenotype observed in the isolated groups, this finding should be interpreted cautiously and is best considered as altered immobility behavior rather than a straightforward antidepressant-like effect. Lithium treatment in isolated rats significantly increased immobility time compared with isolated rats without lithium ($p < 0.001$), shifting values toward the control range (Figure 4). No significant difference was observed between the control + lithium and control + saline groups ($p > 0.05$).

3.4. Marble Burying Test

The number of marbles buried (an index of OCD-like behavior) showed a significant group effect ($F(4,30) = 116.12$, $p < 0.001$, $\eta^2 = 0.94$). Socially isolated rats (with or without saline) buried significantly more marbles than control rats ($p < 0.001$), indicative of compulsive-like behavior. Lithium treatment during isolation markedly reduced the marble-burying count compared to isolated rats given saline ($p < 0.001$), substantially attenuating the isolation-induced OCD-like behavior (Figure 5). There was no difference in marble burying between lithium- and saline-treated controls ($p > 0.05$).

3.5. BDNF mRNA Expression in Prefrontal Cortex

BDNF mRNA expression in the prefrontal cortex differed significantly among the four groups included in the molecular analysis ($F(3,8) = 7.75$, $p < 0.01$, $\eta^2 = 0.74$). Compared with the control + saline group, the isolated

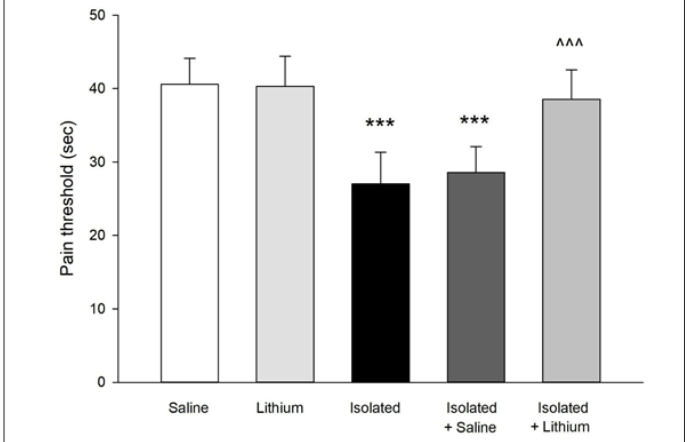


Fig. 3. Pain threshold measured by the hot plate test (latency to paw lick at 50 °C, in seconds) in all groups. Data are presented as mean ± SD (n = 7 rats per group). *p < 0.001 vs. Control + Saline; ^^^p < 0.001 vs. Isolated (no treatment).

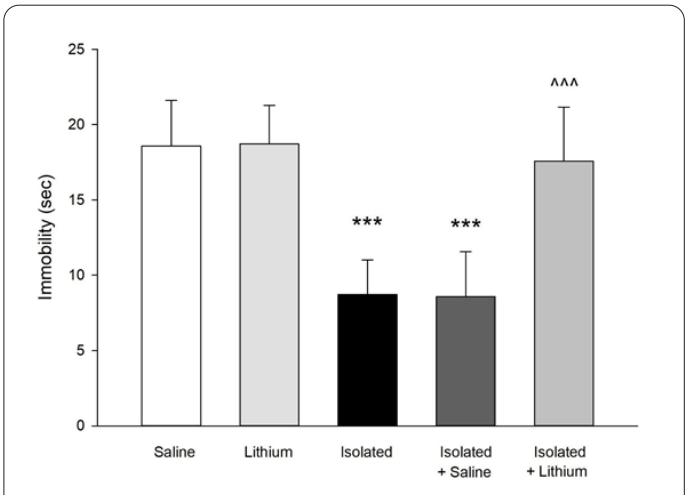


Fig. 4. Immobility behavior in the forced swim test, measured as total immobility time (seconds) over 5 minutes, for all groups. Data are presented as mean ± SD (n = 7 rats per group). *p < 0.001 vs. Control + Saline; ^^^p < 0.001 vs. Isolated (no treatment).

group showed significantly lower BDNF mRNA expression ($p < 0.05$). Lithium treatment in isolated rats significantly increased prefrontal BDNF mRNA expression relative to the isolated group ($p < 0.05$), shifting values toward the control range (Figure 6). Because this analysis was performed with n = 3 biological replicates per group and measured mRNA rather than protein expression, these molecular findings should be considered preliminary and interpreted as associative evidence.

4. Discussion

In this study, we found that lithium treatment on its own reduced locomotor activity and rearing behavior in non-isolated (control) rats. Meanwhile, chronic post-weaning social isolation was associated with hyperactivity (increased locomotion and rearing), heightened pain sensitivity (lower pain threshold), decreased immobility in the forced swim test, OCD-like behavior, and reduced BDNF mRNA expression in the prefrontal cortex. Importantly, lithium administration attenuated several behavioral alterations induced by social isolation and was accompanied by a partial reversal of reduced prefrontal BDNF mRNA expression.

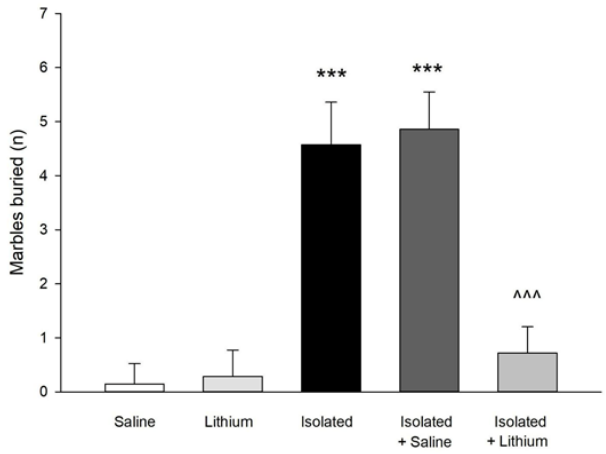


Fig. 5. Obsesssive-compulsive-like behavior assessed by the marble burying test, measured as the number of marbles buried during the test session, for all groups. Data are presented as mean ± SD (n = 7 rats per group). *$p < 0.001$ vs. Control + Saline; ^^^$p < 0.001$ vs. Isolated (no treatment).

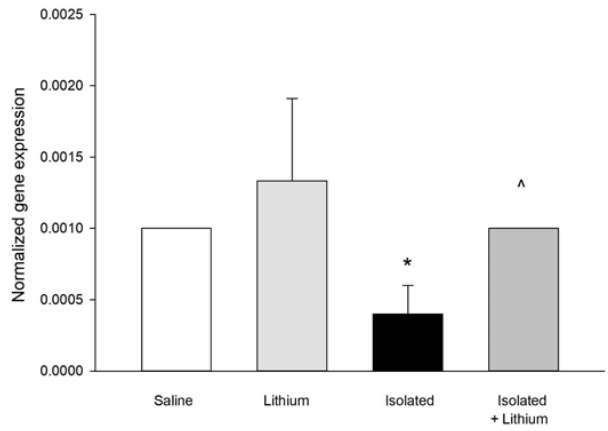


Fig. 6. Prefrontal BDNF mRNA expression relative to GAPDH in the four groups included in the molecular analysis: Control + Saline, Control + Lithium, Isolated (no treatment), and Isolated + Lithium. Data are presented as mean ± SD (n = 3 biological replicates per group). *$p < 0.05$ vs. Control + Saline; ^$p < 0.05$ vs. Isolated (no treatment). Error bars represent SD; where not visually apparent, error bars are smaller than the plotted bar resolution. Because of the limited molecular sample size and mRNA-only endpoint, these findings should be interpreted cautiously.

Chronic social isolation during developmental periods has long been recognized as a severe environmental stress that may contribute to a broad range of cognitive deficits and mood disturbances [31, 32]. In rodent models, prolonged social isolation can precipitate schizophrenia-like behaviors [33], and it significantly increases the risk for developing psychopathologies such as anxiety, depression, schizophrenia, and drug addiction [34]. One prominent outcome of adolescent isolation in rodents is altered locomotor activity. Consistent with our findings, previous studies have reported that social isolation increases locomotor activity in both female and male rats. For example, female rats isolated during adolescence show heightened locomotion in the open field test [35]. Similarly, adolescent isolation in male rats leads to pronounced hyperlocomotion in the open field [6], and other researchers have also observed increased locomotor activity following isolation [36]. In our study, isolated rats demonstrated both increased locomotion and elevated rearing behavior in the open field, reflecting a hyperactive response to the novel environment. The increase in rearing is likely a component of this overall hyperactivity. Changes in baseline locomotor activity are a common feature of many neuropsychiatric and neurodevelopmental disorder models [37, 38]. In fact, abnormally high locomotor activity in rodents is often used to identify psychostimulant effects or to screen for potential antipsychotic treatments, as it provides insight into underlying psychiatric conditions [38].

We also observed that socially isolated rats spent less time immobile in the forced swim test. At face value, reduced immobility might suggest an antidepressant-like effect; however, we interpret this finding cautiously. The decreased immobility is likely a consequence of isolation-induced hyperactivity rather than an indication of reduced despair. The literature on social isolation and FST behavior is mixed. Some studies have shown that chronic social isolation increases depressive-like behavior, evidenced by longer immobility times [16, 39]. By contrast, other studies—including those focusing on adolescent isolation—have reported a decrease in immobility [6, 40], which aligns with our results. Thus, the influence of isolation on FST performance may depend on the specifics of the isolation protocol (duration, timing, and context) and the resultant locomotor activity levels. Our finding of reduced immobility should not be misconstrued as a beneficial effect of isolation; rather, it likely reflects the overall elevated activity and stress response in isolated animals.

Chronic isolation in our study also produced OCD-like behavior (increased marble burying) and hyperalgesia (as evidenced by a lower pain threshold on the hot plate). Evidence regarding the effects of social isolation on compulsive behavior and pain perception is relatively sparse and somewhat contradictory. Our results are in line with those of Kamaei et al. (2023), who reported that adolescent social isolation led to a decreased thermal pain threshold and increased marble-burying behavior in rats [6]. Similarly, Perić et al. have shown that even a three-week isolation

period can induce OCD-like increases in marble burying [41, 42].

There is also evidence that social isolation heightens sensitivity to certain pain modalities: for example, isolation rearing for 8 weeks was found to lower the threshold for visceral pain [43], and even short-term acute isolation stress can increase visceral pain sensitivity [44]. On the other hand, not all studies have found such effects. For instance, Kinley et al. (2021) observed no significant change in marble burying following peri-adolescent isolation in rats [45], and other reports have noted that isolation can decrease pain sensitivity under some conditions [46, 47]. In fact, a 21-day isolation protocol was reported to raise the pain threshold (i.e., produce hypoalgesia) in rats [48]. These discrepancies likely arise from differences in isolation duration, the developmental stage during isolation, the type of pain test used, and the sex or strain of the animals. In our experimental conditions (a relatively prolonged isolation from weaning through adolescence in male Wistar rats), chronic isolation was associated with hyperalgesia and compulsive-like behavior, supporting the view that prolonged social deprivation may be detrimental to both emotional regulation and sensory processing.

At the molecular level, our data showed that social isolation was associated with reduced BDNF mRNA expression in the prefrontal cortex. Because BDNF is involved in neuroplasticity-related processes, this reduction may be relevant to the behavioral alterations observed in the isolated animals; however, the present data do not establish a direct causal mechanism. Importantly, only BDNF mRNA was measured; therefore, these findings cannot be assumed to reflect parallel changes in BDNF protein or downstream TrkB signaling. Our findings are supported by several other studies reporting decreased BDNF after isolation stress. For instance, Kamaei et al. (2023) found lower BDNF levels in the hippocampus of isolated rats [6], and Fanaei et al. (2024) reported that serum BDNF was significantly reduced by isolation [49]. Other studies have also demonstrated hippocampal BDNF downregulation with social isolation [50, 51], as well as decreases in BDNF in the nucleus accumbens [16]. Notably, Li et al. (2019) observed that adolescent social isolation decreased BDNF protein in the medial prefrontal cortex [52], which is in agreement with the direction of our mRNA findings. On the other hand, some inconsistencies exist in the literature: for example, one study found no significant change in BDNF levels in the hippocampus or serum of isolated female rats [17]. Such differences could be due to variations in experimental design, including sex of the animals, brain region analyzed, molecular endpoint, and duration of isolation. Overall, the available evidence suggests that extended social isolation may negatively affect BDNF-related markers in key brain areas associated with mood and cognition, with the prefrontal cortex being a relevant locus of these effects.

In the present study, lithium treatment attenuated the behavioral alterations induced by social isolation and was accompanied by increased prefrontal BDNF mRNA expression relative to isolated rats. To our knowledge, this is among the first studies to examine this pattern in a post-weaning social isolation model. Although our findings are consistent with previous reports showing that lithium or other neuroactive interventions can improve behavioral outcomes together with changes in BDNF-related markers in experimental models [19, 53-56, 59], the present data support an association rather than a definitive mechanism. Because only BDNF mRNA expression was measured and no downstream signaling or protein assays were performed, further mechanistic studies are required before concluding that BDNF upregulation mediates lithium's therapeutic effects in this context.

The observed pattern may be discussed in the context of established lithium-sensitive signaling pathways. Lithium is known to inhibit GSK-3β directly and indirectly and to interact with Akt- and CREB-related pathways that regulate neuronal survival, synaptic plasticity, and neurotrophin-related transcription [57, 58]. CREB-related signaling is particularly relevant because it can influence BDNF transcription, and previous experimental work has reported lithium-associated increases in BDNF expression [59]. Thus, one plausible interpretation is that lithium-induced behavioral improvement was accompanied by modulation of neuroplasticity-related transcriptional processes. However, the present study did not measure GSK-3β activity, Akt or CREB phosphorylation, TrkB signaling, corticosterone, or BDNF protein concentrations. Therefore, these pathways should be regarded as biologically plausible explanations for future testing rather than demonstrated mechanisms in the present dataset.

The lithium dose also requires cautious translational interpretation. The selected 20 mg/kg i.p. regimen was based on previous preclinical studies and was sufficient to modify behavioral outcomes in the present model. However, serum lithium concentrations were not measured, and rodent intraperitoneal dosing cannot be directly equated with clinical oral dosing or therapeutic serum ranges in humans. Accordingly, the present findings should be interpreted as preclinical evidence of lithium responsiveness in this model rather than as a direct clinical dose-equivalence statement.

Limitations

This study has several limitations that should be acknowledged. First, the molecular analysis was conducted on a smaller predefined subset of biological samples (n = 3 per group) than the behavioral experiments (n = 7 per group). A formal a priori power analysis was not conducted for the molecular endpoint, and the small sample size limits statistical power, robustness of distributional assessment, and confidence in the BDNF mRNA findings. Accordingly, the molecular results should be interpreted as preliminary associative evidence rather than definitive mechanistic proof. Second, BDNF was measured only at the mRNA level; BDNF protein, TrkB signaling, and downstream pathway markers were not assessed. Third, standard-curve-based PCR efficiency values and R^2 estimates were not available for all assays, melt-curve plots are not presented as supplementary validation figures, and GAPDH stability was not independently validated across the experimental groups; future studies should validate amplification efficiency, melt-curve documentation, and reference-gene stability using dedicated qPCR validation procedures. Fourth, the molecular analysis focused only on the prefrontal cortex. This region was selected a priori because of its relevance to executive function, stress-related regulation, and previous evidence of prefrontal BDNF alterations after social isolation [18, 52], but the absence of parallel hippocampal or nucleus accumbens measure-

ments prevents conclusions about regional specificity. Fifth, behavioral testing was conducted in a single day with tests spaced 30-60 minutes apart. Although the order was arranged from less stressful to more stressful procedures, cumulative stress or carry-over effects cannot be excluded. Sixth, corticosterone or other stress biomarkers were not measured; such measures would have strengthened interpretation of the stress component of the isolation model and lithium response. Seventh, serum lithium levels were not measured, limiting direct translational interpretation of the selected dose. Eighth, only male rats were used; therefore, the findings may not generalize to females, particularly because sex differences can influence social isolation effects and lithium responsiveness [9]. Future studies should use larger molecular cohorts, include both sexes, measure BDNF protein and lithium-sensitive signaling pathways, validate qPCR efficiency and reference-gene stability, and incorporate endocrine stress markers.

Despite these limitations, the present findings indicate that lithium attenuated several behavioral abnormalities associated with chronic social isolation and was accompanied by changes in prefrontal BDNF mRNA expression. These results support the relevance of neuroplasticity-related pathways in this model, while also underscoring the need for larger and more mechanistically detailed studies.

5. Conclusion

In conclusion, lithium carbonate attenuated several behavioral alterations induced by chronic post-weaning social isolation and was accompanied by changes in prefrontal BDNF mRNA expression in male rats. These findings suggest that lithium-induced behavioral improvements are accompanied by changes in prefrontal BDNF mRNA expression, although causality remains to be established. Because only BDNF mRNA was assessed and the molecular analysis was performed on a limited number of biological samples, further studies with larger molecular cohorts and protein- and pathway-level analyses are required.

Graphical Abstract: A schematic summary of the experimental design and main findings.

Conflict of Interests

The authors declare no conflicts of interest.

Consent for publications

Not applicable.

Ethics approval and consent to participate

The study protocol was approved by the Islamic Azad University Ethics Committee (IR.IAU.TMU.REC.1403.112). All procedures were carried out in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (2011) and in accordance with ARRIVE guidelines. Humane endpoints were used to minimize suffering.

Informed Consent

Not applicable.

Availability of data and material

The datasets generated and analyzed during this study are available from the corresponding author upon reasonable request.

Authors' contributions

H. M. D.: Performed all experiments and data collection. B. G. Y.: Conceived and designed the study, supervised the project, and is the corresponding author. M. A.: Assisted with project progression and provided laboratory support. M. G.: Assisted with project progression and provided laboratory support.

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