Research Article

Pamukkale Medical Journal
doi:https://dx.doi.org/10.31362/patd.1657975

Effects of different social interactions on hippocampal neurogenesis and neuron numbers in juvenile rats: a histological and behavioral study

Juvenil sıçanlarda farklı sosyal etkileşimlerin hipokampal nörogenez ve nöron sayıları üzerindeki etkileri: histolojik ve davranışsal bir çalışma

Senem Esin Yavaş, Semiha Ersoy

Posted date:14.03.2025 Acceptance date:08.07.2025

Abstract

Purpose: Adolescence is a period of development affected by numerous hormonal and neurochemical changes. During this period, time spent with peers and experiences are known to have neurodevelopmental benefits. The hippocampus is a brain region where the production of new neurons after birth continues throughout life and is sensitive to changing social interactions. In this study, we aimed to investigate the effects of changing social interactions on behavioral parameters, doublecortin (DCX) expression, and total neuron numbers in the hippocampus of rat pups.

Materials and methods: 21-day-old male and female Wistar albino rats were divided into three groups: control, social isolation, and environmental enrichment. Social isolation and environmental enrichment were applied for 4 weeks. Behavioral parameters were analyzed using ball burying, social interaction, and open-field tests. Following the behavioral tests, the rats were euthanized, and their hippocampal tissues were extracted. Cresyl violet staining was performed on paraffin sections, and DCX immunohistochemistry was performed on floating sections.

Results: Increased social interactions had positive effects on exploratory behavior in adolescent rats. Decreased social interactions caused anxiety, depression behaviors, and increased locomotor activity. Histological analysis revealed a significant increase in the total number of neurons and DCX-positive neuroblasts in the dentate gyrus in the environmental enrichment group. In the social isolation group, a significant decrease in the total number of neurons and DCX-positive neuroblasts was observed.

Conclusion: These results implied that changing social interactions during the weaning period have an effect on neurogenesis and the number of mature neurons in the DG, and this effect is regulated by different survival mechanisms.

Keywords: Hippocampus, neurogenesis, doublecortin protein, social isolation, social interaction.

Yavas SE, Ersoy S. Effects of different social interactions on hippocampal neurogenesis and neuron numbers in juvenile rats: a histological and behavioral study. Pam Med J 2025;18:783-793.

Öz

Amaç: Ergenlik, çok sayıda hormonal ve nörokimyasal değişimden etkilenen bir gelişim dönemidir. Bu dönemde akranlarla geçirilen zamanın ve deneyimlerin nörogelişimsel faydaları olduğu bilinmektedir. Hipokampus, doğumdan sonra yeni nöron üretiminin yaşam boyunca devam ettiği ve değişen sosyal etkileşimlere duyarlı bir beyin bölgesidir. Bu çalışmada, değişen sosyal etkileşimlerin sıçan yavrularının hipokampusundaki davranışsal parametreler, doublecortin (DCX) ekspresyonu ve toplam nöron sayıları üzerindeki etkilerini araştırmayı amaçladık.

Gereç ve yöntem: 21 günlük erkek ve dişi Wistar albino sıçanlar kontrol, sosyal izolasyon ve çevresel zenginleştirme olmak üzere üç gruba ayrıldı. Sosyal izolasyon ve çevresel zenginleştirme 4 hafta boyunca uygulandı. Davranışsal parametreler bilye gömme, sosyal etkileşim ve açık alan testleri kullanılarak analiz edildi. Davranış testlerinin ardından sakrifiye edilen sıçanların hipokampüsleri çıkarıldı. Parafin kesitlerde Crezil violet boyaması ve yüzen kesitlerde DCX immünohistokimyası yapıldı.

Bulgular: Artan sosyal etkileşimlerin ergen sıçanlarda keşif davranışı üzerinde olumlu etkileri olmuştur. Azalan sosyal etkileşimler anksiyete, depresyon davranışları ve lokomotor aktivitede artışa neden olmuştur. Histolojik analiz, çevresel zenginleştirme grubunda dentat girustaki toplam nöron ve DCX-pozitif nöroblast sayısında önemli bir artış olduğunu ortaya koymuştur. Sosyal izolasyon grubunda ise toplam nöron ve DCX-pozitif nöroblast sayısında anlamlı bir azalma gözlenmiştir.

Sonuç: Bu sonuçlar, sütten kesme döneminde değişen sosyal etkileşimlerin nörogenez ve DG'daki olgun nöron sayısı üzerinde etkisi olduğunu ve bu etkinin farklı hayatta kalma mekanizmaları tarafından düzenlendiğini göstermiştir.

Senem Esin Yavaş, M.S. Bursa Uludag University, Medical Faculty, Department of Histology and Embryology, Bursa, Türkiye, e-mail: senemesin@uludag.edu.tr (https://orcid.org/0000-0002-6949-1210)

Semiha Ersoy, Prof. Bursa Uludag University, Medical Faculty, Department of Histology and Embryology, Bursa, Türkiye, e-mail: semihaersoy@uludag.edu.tr (https://orcid.org/0000-0002-6419-0304) (Corresponding Author)

Anahtar kelimeler: Hipokampus, nörogenez, doublecortin proteini, sosyal izolasyon, sosyal etkileşim.

Yavaş SE, Ersoy S. Juvenil sıçanlarda farklı sosyal etkileşimlerin hipokampal nörogenez ve nöron sayıları üzerindeki etkileri: histolojik ve davranışsal bir çalışma. Pam Tıp Derg 2025;18:783-793.

Introduction

Social bonds are crucial for the survival of juveniles [1]. Restricting social interaction in conditions such as social isolation (SI) is detrimental to physical and cognitive health [2]. SI causes numerous behavioral, morphological, and functional abnormalities in the central nervous system (CNS) [3]. Stress is associated with increased proinflammatory cytokines, [4] decreased neurotrophin expression, [5, 6] and glucocorticoid signaling, resulting in decreased hippocampal neurogenesis [7]. Social isolation is also a form of stress that affects some hippocampus-related functions, such as learning and memory, and can lead to emotional disorders [3].

Environmental enrichment (EE) is a term used to describe exposing laboratory animals to more physical and/or social stimulation than standard housing conditions. It is classified in two ways: Physical environmental enrichment includes structural changes such as increased floor space and exercise, toys (plastic tunnels, wooden objects to gnaw on, ropes, swings, balls, and ramps) [8, 9]. Social enrichment involves housing animals in groups and dynamic interaction between the animals and the caregiver. Ideally, social and physical enrichment elements are preferred [10]. It has been shown that EE affects neurotrophic factors such as Brain-Derived Neurotrophic Factor, Glial cell line-derived Neurotrophic Factor, and Nerve Growth Factor, which provide neuronal proliferation and synaptic function [11-13].

Neurogenesis continues in the brains of many animal species, including humans, after birth and into adolescence. It occurs particularly prominently in the hippocampal formation's dentate gyrus (DG) [14]. Doublecortin (DCX) is a microtubule-associated protein critical for regular neuronal migration during the developmental period. The adult mammalian brain is a reliable marker for distinguishing post-mitotic immature neurons [15]. However, in the literature, the effect of different social interactions on juvenile neurogenesis has yet to be previously evaluated in DCX-expressing neuroblasts.

Distance and social isolation (SI) have recently been proposed to prevent the spread of the massive pandemic caused by the Severe Acute Respiratory Syndrome Coronavirus-2 [16]. However, long-term SI is known to disrupt brain physiology and cause problems in different cognitive and emotional development contexts [17]. This study aimed to investigate the effects of social isolation or environmental enrichment during the weaning period on neurogenesis by doublecortin immunohistochemistry in the hippocampus, total number of DG neurons, and anxiety in rats.

Materials and methods

Animals and experimental design

A total of 30 male and female Wistar albino rat pups, aged 20–21 days (weaning age) and weighing 50–60 grams, were used as experimental animals. The rats were kept on a 12 h dark/12 h light cycle with free access to water and feed. The pups were housed with their parents in standard birth cages until weaning. Then, the animals were randomly divided into three groups as: 1.) control (C) (n=10), 2.) social isolation (SI) (n=10), and 3.) environmental enrichment (EE) (n=10). Based on previous similar research, the number of animals per group was determined by an a priori power analysis assuming Cohen's f=0.60 (large effect) and conducted at the 95% confidence level (α=0.05). Animals weaned at 21 days of age were exposed to different social interaction treatments for 4 weeks. After the social interaction phase, behavioral tests were performed, and then the subjects were sacrificed, and their brains were used for histological analyses.

Wistar rats from the same facility were used as "external peers" for social interaction experiments.

To perform our experiments, ethical approval was obtained from the Local Ethics Committee for Animal Experiments of Bursa Uludag University on 05.04.2022, decision number 2022-03/03. All experiments were performed in the research laboratories of Bursa Uludag University Experimental Animal Breeding Application and Research Center (DEHYUAM) and Bursa Uludag University Faculty of Medicine, Department of Histology and Embryology.

Social isolation and environmental enrichment model

Male and female rat pups randomly selected at weaning were housed in single rat cages to create a social isolation model. The socially isolated rats were kept in dark-colored plastic cages with only a top wire and light access so that they could only hear other rats and not see them in other cages. They were prevented from seeing the researchers except for daily feed and water monitoring. In the environmental enrichment model, pups were housed in groups of 3 or 4 in large cages containing thick litter containing rodent exercise balls, toys for gnawing, and tunnels. Control group rats were housed in standard laboratory cages with 3 or 4 rats per cage. The researchers handled rats in the EE group for 20 minutes each daily during the experimental period.

Behavior tests

MBT: Marble Burying Test

This test is used to assess depression and anxiety in rodents. Standard polycarbonate rat cages (20 cm x 35 cm x 17 cm) were used as the test arena. Fresh sawdust 5 cm thick was placed in each cage, and the surface was leveled. Standard glass toy balls were placed on the sawdust surface in 3 columns x 5 rows = 15 rows. The balls were washed with 10% ethanol, rinsed thoroughly in distilled-deionized water, and dried before each use. The rat was placed in a corner of the cage with the balls, as far away from the balls as possible, and left in the cage undisturbed for 30 minutes. After the time was up, the subject was removed from the cage and returned to the nest without moving or removing the balls. The number of balls with two-thirds of the surface area covered with chips was scored as 1 point. Mean values were calculated using the number of embedded balls per subject.

OFT: Open Field Test

The open field test is widely used to test rodents' exploratory behavior, anxiety state, and general locomotor activity. The open field consists of a plastic cabinet (57 cm x 57 cm x 47 cm) surrounded by black plastic walls. Rats were placed in the cabinet's center and allowed to explore the open arena for 5 minutes. A video tracking system recorded the time and movements spent in the center, periphery, and corners of the arena (EthoVision XT, Noldus). Between experiments, the chamber was cleaned with 10% ethanol. Time spent in the center and peripheral part of the booth was assessed to assess voluntary exploration of unsafe areas.

Open field - social interaction test

Following the open field test, rats were subjected to a social interaction test. The test arena consisted of modifying the open field enclosure [18]. First, each rat was placed in an open field arena containing an empty wire cage for 5 min and allowed to recognize the empty cage. The rat was removed, and a young, non-pubertal rat was added to the wire cage. The subject rat was placed back in the arena and spent 5 minutes with the stranger rat. Social interaction was automatically calculated as time spent in the 'social interaction zone' while the rat was in the arena.

Sacrification and tissue collection

16-24 hours after the last behavioral test, rats were anesthetized by high dose ether inhalation, and perfusion fixation with intracardiac buffered 4% paraformaldehyde was performed. After fixation, the removed brains were postfixed in 4% paraformaldehyde at +4°C for 24 hours.

The brain tissue of half of the rats (n=15) in the experimental group was preserved as paraffin-embedded tissue for subsequent histological analysis. The remaining half of the rats (n=15) underwent postfixation, after which 40 µm-thick serial free-floating sections were obtained from the brains using a vibratome. These sections were collected in glass vials to form sets of five serial sections. Sections containing the hippocampal region were identified and selected for immunohistochemical analysis.

Nissl staining

Coronal 5µm serial paraffin sections taken from the hippocampus were stained with 0.5% cresyl violet stain. The total number of neurons in the dentate gyrus was determined by evaluating three fields at 40x objective in 5 sections of each subject.

Immunohistochemistry and cell counting

By doublecortin immunohistochemistry applied to coronal 40µm serial floating sections obtained from the hippocampus, the differences between SI and CE groups were evaluated according to the changes in expression levels in the hippocampal tissue. In immunohistochemistry applications, the sections removed from the freezer were brought to room temperature and washed in TRIS (pH 7.6) buffer for 3x5 min. After protein blocking, the sections were incubated with doublecortin antibody (Santa Cruz DCX, 1:200) for 2 nights at +4°C and then with donkey anti-mouse biotinylated secondary antibody (1:300, Jackson) for 2 hours at room temperature. Sections were then ABC complexed and visualized with DAB chromogen.

Cell counts were performed in 5 coronal sections per rat obtained from different levels of the hippocampus (Bregma between, -2.80 and -4.30), which were labeled with anti-Doublecortin antibody, according to the Rat Brain Atlas [19]. Images were photographed with an Olympus BX50 photomicroscope. DCX (+) cell counts were performed using Qupath software.

Statistical analyses

For statistical analyses, the mean±standard deviation of the data and the difference between groups were performed using the SPSS 26.0 program (IBM). Before comparing all groups in terms of statistical differences, the normal distribution of the data was evaluated by the Shapiro-Wilk test. The data were normally distributed if the value obtained with the Shapiro-Wilk test was more significant than 0.05. The One-Way ANOVA test evaluated the differences between normally distributed data. Firstly, whether the variances in the groups were homogeneously distributed was analyzed by Levene's test; if they were homogeneously distributed, the One-Way ANOVA was used; if they were not, the same groups were analyzed by the Kruskal Wallis test. The evaluation of differences between those not customarily distributed was also analyzed using the Kruskal-Wallis test. A value of $p<0.05$ was accepted for statistical significance.

Results

To examine the effects of social isolation and environmental enrichment on depression, anxiety, and locomotor activity, rats were subjected to the Open Field Test (Figure 1A). According to the data of the ball burying test, one of the tests in which anxiety behavior was evaluated, the increased burying behavior observed in the social isolation group (SI: 11.33±0.91) rats was significant compared to the other two groups (C: 6.28±1.14, $p=0.04$; EE: 6.33±0.84, $p=0.014$) (Figure 1B). In terms of the time spent in the center of the open field arena, another test parameter that allows the evaluation of anxiety behavior, a decrease was determined in the isolation group rats compared to the other two groups. Still, it was not statistically significant (C: 24.60±5.04; SI:16.85±2.72; EE: 28.72±5.56; $p=0.280$) (Figure 1C). The rats in the EE group entered the center of the open field arena in higher (EE vs C, $p=0.044$) numbers than the control group (C: 8.20±1.01; EE: 14.00±1.22; SI: 8.50±2.39) (Figure 1D). The total distance (C: 1343.52±118.01; EE: 1791.27±189.53; SI: 1894.93±152.12, C vs SI, $p=0.016$) traveled and total movement time (C: 183.24±8.49; EE: 203.00±11.85; SI: 219.52±5.45, C vs SI, $p=0.036$) in the open field arena were significantly increased in the social isolation group compared to the control group (Figure 1E-F).

A social interaction test involving the modification of the open field arena was performed to examine the effect of EE and SI on social interaction in rats. Figure 2A shows the representative heat maps of the rats of the experimental groups, indicating the time they were in the arena during the experimental period. Rats in the SI group exhibited a significant reduction in social interaction behavior with a stranger rat compared to both the control and EE groups (C: 157.57±12.87; EE: 161.65±18.75; SI: 85.42±7.27, C vs SI, $p=0.005$ and EE vs SI, $p=0.003$). However, no statistically significant difference in social interaction was observed between the EE group and the control group ($p=1.00$) (Figure 2B).

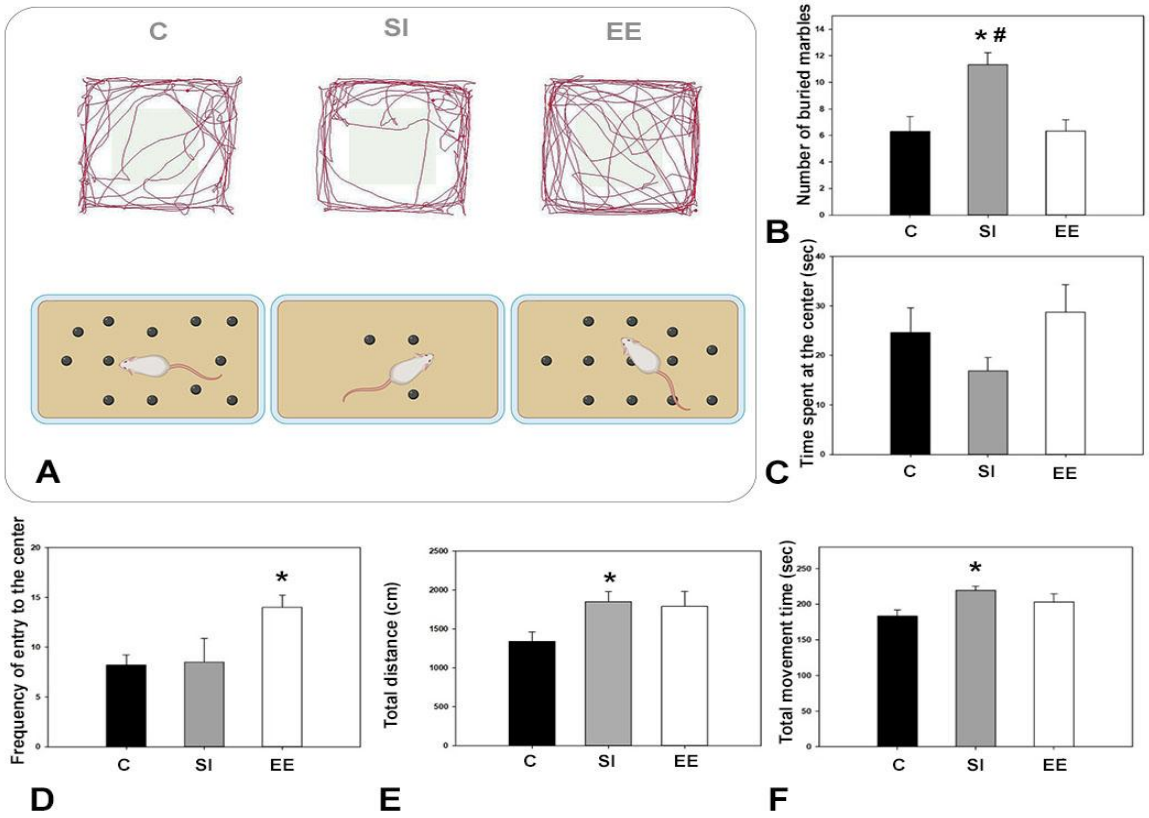

Figure 1. Effects of social isolation and environmental enrichment on depression, anxiety, and locomotor activity

Open Field Test, representative images showing typical exploratory behavior of groups (A)
Ball burying test Kruskall Wallis: $x^2(2, N:19)=8.49$, $p=0.014$ (B)
Time spent at the center in Open field test area, ANOVA:$F(2.11)=1.43$ $p=0.280$, (C)
Frequency of entry to the center, ANOVA:$F(2.11)=4.748$, $p=0.033$ (D)
Total distance in test areas, Kruskall Wallis test: $x^2(2)=6.15$, $p=0.046$, (E)
Total movement time ANOVA:$F(2.12)=4.08$, $p=0.044$, (F). *: C and SI; #: SI and EE

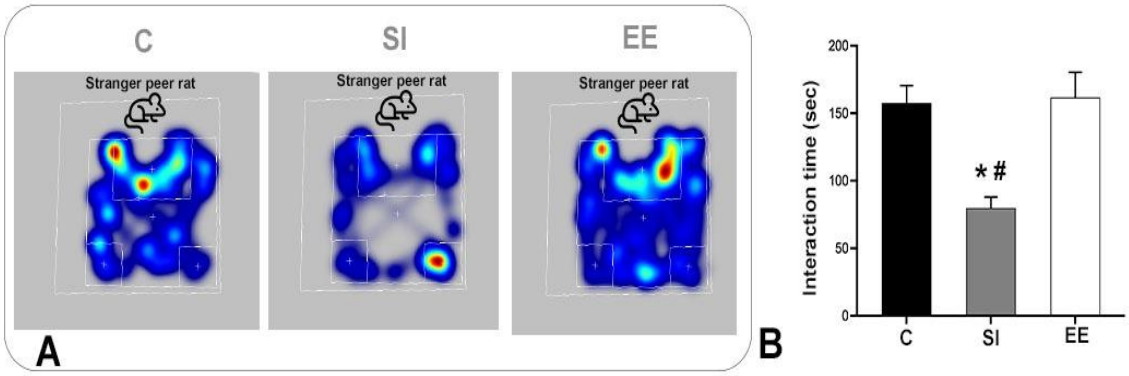

Figure 2. Effects of social isolation and environmental enrichment on the open field-social interaction test

Representative heat maps of time spent in the social interaction area in the test arena (A)
Comparison of social interaction time between groups (B). *: C and SI, #: SI and EE. ANOVA: $F(2.18)=9.68$, $p<0.001$

When the number of DCX (+) neuroblasts in the DC was evaluated between groups (C: 3054.63±162.47; EE: 3748.25±198.81; SI: 2.148.83±171.38) (Figure 3A-C), it was found that the 4-week SI treatment caused a statistically significant decrease compared to the control group (C vs SI, p=0.023),. Increased social interaction with EE caused a significant increase in the number of DCX (+) neuroblasts (C vs EE, p=0.008; SI vs EE, p=0.001) (Figure 3-D). It was also observed that some of the DCX (+) neuroblasts in the isolation group had shorter apical dendrites.

When the number of neurons stained with cresyl violet in the dentate gyrus was evaluated between the groups (C: 68.59±2.04; EE: 79.55±2.28; SI: 60.90±1.94) (Figure 4A-C), a significant decrease in the total number of granule neurons was found in the SI group (C vs SI, p=0.034), and a significant increase was seen with EE (C vs EE, p=0.006; SI vs EE, p<0.001) (Figure 4D).

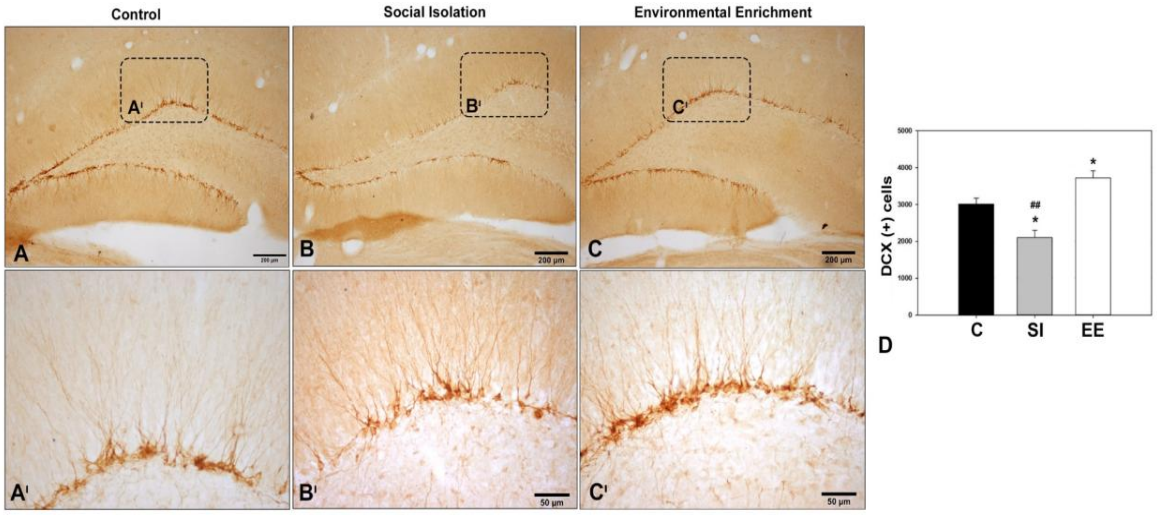

Figure 3. Effect of social isolation and environmental enrichment on neuroblast numbers according to DCX immunohistochemistry in the subgranular zone

Representative photographs of DCX (+) stained neuroblast expressions in the subgranular zone of the groups (A-C). High-magnification images of neuroblasts in the subgranular zone of the groups (A-I-CI). Comparison of the DCX (+) cell number between groups (D)
Kruskall Wallis Tests: $x^2(2, N=75)=28.24$, p<0.001

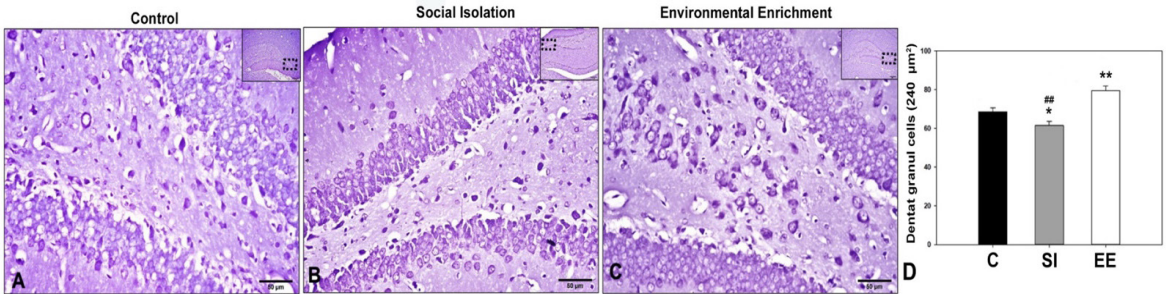

Figure 4. Effects of social isolation and environmental enrichment on dentate gyrus granular neuron numbers

Representative photographs of the histomorphologic appearance of the groups dentate gyrus and granule neurons, cresyl violet (A-C). Comparison of the number of granule neurons per field between groups (D). *: C and SI, #: SI and EE. Kruskall Wallis Tests: $x^2(2, N=360)=29.12$, p<0.001

Discussion

In this study, the effects of isolation, which is a negative social experience, and environmental enrichment, which is considered a positive social experience, on anxiety, movement, and social interaction in Wistar rats during the weaning period were investigated by behavioral tests, and DC neurogenesis was examined by the immunohistochemical method. According to the results of numerous studies in humans, post-weaning social isolation is a risk factor for mental health problems in adolescence [2, 3, 20]. In contrast to SI, EE involves many social stimuli. Previous studies have generally focused on environmental enrichment in adulthood. Still, adolescence is a critical period for the development of mood disorders in humans, and most mood disorders are first diagnosed between the ages of 12-18 [21]. During adolescence, neuronal circuits affecting emotional, social, and cognitive pathways go through critical periods and are shaped by environmental factors [22-25].

Social isolation is used as a model of depression in rodents because this condition is caused by hypofunction of the serotonergic system [26, 27]. In our study, the social isolation stress that 21-day-old Wistar rat pups were exposed to during the weaning period caused them to exhibit significant behavioral impairment in the ball-burying test. Although there was no statistical significance, a decrease was also observed in the evaluations of entry to the center of the test arena in the open field test. Similar results were consistent with a study conducted in male Sprague Dawley rats exposed to prolonged isolation stress for 10 and 29 weeks at weaning [28].

The findings of locomotor activity in the open field arena showed that this activity increased significantly after SI compared to the control group. This suggests that social isolation may cause locomotor hyperactivity in rats. Some recent studies also confirm our results of social isolation-induced increased locomotor activity [29-30]. This finding seems to be consistent with the childhood Attention Deficit Hyperactivity Disorder (ADHD) phenotype in humans [31]. Although not statistically significant, the increase in activity observed in the EE group was thought to be related to the increase in the impulse to explore.

Social experiences during adolescence play a fundamental role in rats' social, cognitive, and emotional development [32]. Social behavior can be measured by observing specific social interactions. The enriched environment reversed the decreased interest in initiating social contacts caused by chronic methylphenidate ingestion in Wistar pups [33]. In our study, the subject rat was compared with an unfamiliar peer rat in a test arena where it could see and smell the rat. Time spent in the social interaction zone did not change with increased social interaction. However, rats exposed to social isolation showed less interest in social interaction with the new rat.

The hippocampus is a critical brain region involved in learning, memory, and emotional control. The hippocampal neurogenesis that continues into adulthood also plays a vital role in emotional regulation. Impairments in neurogenesis and neuronal integration are recognized as pathological features of neurodegenerative disorders [34, 35]. DCX (+) neuroblasts represent early stages of new neurons that play a crucial role in the regulation of learning, memory, and emotional functions [36]. The effects of environmental stimuli and experiences on these cells are critical for understanding the dynamic nature of brain plasticity. Increased DCX expression indicates stimulated neurogenesis and support of brain functions, whereas a decrease is associated with cognitive decline, particularly in stress-related and neurodegenerative processes [37]. Given the demonstrated association between anxiety and altered neurogenesis, [38] we evaluated whether the hippocampal neurogenic process causes anxiety behavior induced by social changes. Numerous experimental studies in recent years have shown that social isolation causes behavioral changes resembling depression, can increase microglial and astrocyte activation in the prefrontal cortex and hippocampus, and reduces adult hippocampal neurogenesis [39-42]. Studies show that chronic stress affects the development and maturation of the dendritic tree of immature neurons and dendritic remodeling in mature neurons in the adult brain [43-46]. Behavioral deficits and impairments after stress may also be related to morphological damage with short dendritic trees, which may be effective in the later stages of the development and maturation of DCX (+)

cells. In our study, the decrease in the total number of neurons by cresyl violet staining and the decrease in the number of DCX (+) neuroblasts by immunohistochemistry in the SI group suggest that isolation negatively affects neurogenesis. The decrease in the number of DCX (+) neuroblasts and total neurons due to social isolation indicates the adverse effects of stress on the hippocampus. Social isolation may negatively impact neurogenesis during developmental periods through elevated glucocorticoid levels [47]. These findings of our study are consistent with previous research demonstrating that chronic stress disrupts the structural and functional integrity of the hippocampus and inhibits the formation of new neurons.

Studies reported that hippocampal cell layers were more extensive, and there were 15% more granule cell neurons in dentate gyri in mice with environmental enrichment modification. In addition, it has been stated that the learning ability of the rodents administered ES was also increased compared to controls [48]. Environmental enrichment in 5-week-old Swiss Webster mice did not alter ball-burying behavior induced by chronic toluene exposure. Still, it reversed the toluene-induced reduction in DCX (+) neuroblast numbers [26].

Environmental enrichment includes stimuli such as an environment enriched with objects, physical exercise, and social interaction. These stimuli enhance brain plasticity and promote the generation of new neurons in the dentate gyrus [50]. This stimulating environment may contribute to the maintenance and improvement of hippocampal functions by supporting neurogenesis. In our study, the increase in DCX (+) neuroblasts following environmental enrichment appears to be consistent with the neurogenesis-enhancing effects reported in the literature. However, most studies have focused on the effects of environmental enrichment on DCX (+) neuroblast numbers in older people and adults. According to our results, 4 weeks of positively enhanced social interactions that we administered to Wistar rats at 21 days of weaning caused an increase in DCX (+) neuroblast numbers and total dentate gyrus neuron numbers, indicating that similar effects are also observed in the early stages of life, as well as being in parallel with studies of the same period.

In this study, male and female rat pups were subjected to social enrichment together, and both sexes were exposed to social isolation conditions; however, analyses were not conducted separately by sex. Similar experimental approaches have also been reported in the literature [50]. Although potential effects of sex differences on experimental outcomes in neuroscience are acknowledged, due to the limited sample size, analyses had to be performed by combining data from both sexes. Furthermore, the primary aim of our study was to reach general conclusions rather than to identify sex-specific differences. Therefore, the data were analyzed and presented collectively. Nonetheless, future studies with larger sample sizes investigating sex effects in more detail would further elaborate on these general findings. Accordingly, the inability to evaluate sex effects in detail should be considered a limitation of this study.

In conclusion, neurogenesis is a multi-step dynamic process. For neurogenesis to be successful, the processes of proliferation, migration, differentiation, and survival must be complete. Our results may provide insight into the activity of the hippocampal stem cell niche in the adult neurogenesis of positive and negative social interactions. We also revealed that these interactions interfere with various stages of neurogenesis in the hippocampal dentate gyrus, affecting neuroblast numbers, social interaction, and anxiety behavior (Figure 5). Mandatory social restrictions under the recent COVID-19 measures and social isolation, especially for children, have been a major problem worldwide. This has led research to focus on the molecular mechanisms of the effects of social isolation. In conclusion, our results will contribute to understanding molecular mechanisms and environmental factors that impact behavior.

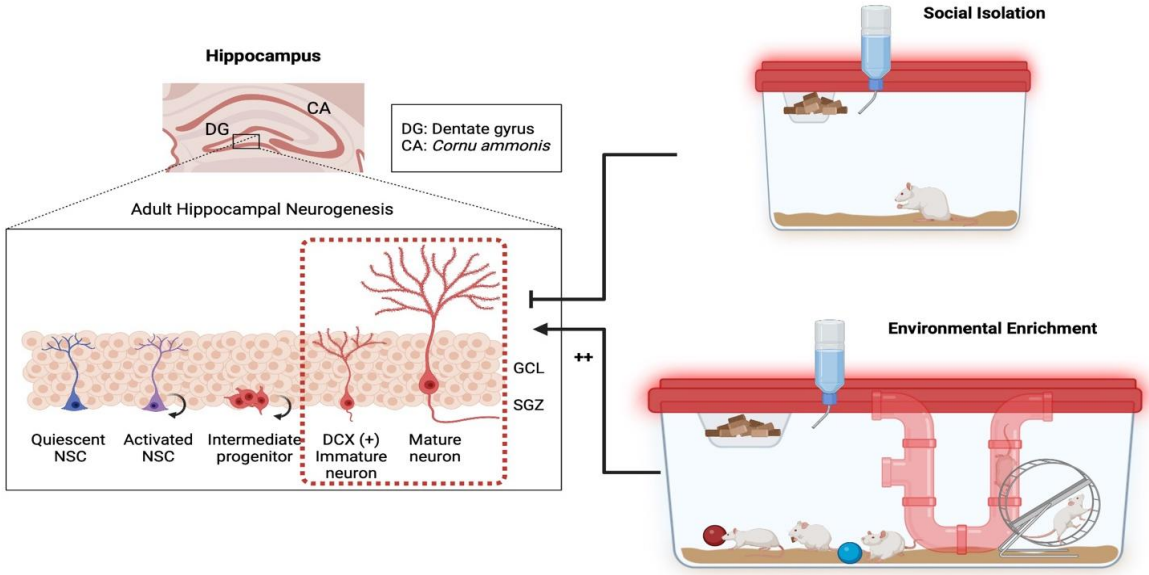

Figure 5. Social isolation applied to weaning rats for 4 weeks decreased the number of dentate gyrus mature neurons and DCX (+) immature neurons, whereas environmental enrichment increased the number of dentate gyrus mature neurons

NSC: Neural Stem Cell, GCL: Granular Cell Layer, SGZ. Subgranular Zone

Data availability statement: The data supporting this study's findings are available from the corresponding author, [Prof. Dr. Semiha ERSOY], upon reasonable request.

Some of the data of this study were presented at the 15th National and 1st International Congress of Histology and Embryology (May 26-28, 2022).

Funding statement: This study was supported by grants from the Bursa Uludag University Scientific Research Projects Unit (BAP, THIZ-2022-1073).

Acknowledgements: We appreciate Dr. Gökhan Göktalay, faculty member of the Department of Pharmacology, for supporting us to use the facilities of the Behavioral Laboratory.

Authors contributions: S.E. have constructed the main idea and hypothesis of the study. S.E.Y. and S.E.: They developed the theory and arranged/edited the material and method section. S.E. and S.E.Y. have done the evaluation of the data in the results section. The discussion section of the article was written by S.E. and S.E.Y. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

Conflict of interest: The authors declare that they have no conflict of interest.

References

1. Harlow HF, Zımmermann RR. Affectional responses in the infant monkey; orphaned baby monkeys develop a strong and persistent attachment to inanimate surrogate mothers. *Science*. 1959;130(3373):421-432. doi:10.1126/science.130.3373.421
2. House JS, Landis KR, Umberson D. Social relationships and health. *Science*. 1988;241(4865):540-545. doi:10.1126/science.3399889
3. Holt Lunstad J. Why Social Relationships Are Important for Physical Health: A Systems Approach to Understanding and Modifying Risk and Protection. *Annu Rev Psychol.* 2018;69:437-458. doi:10.1146/annurev-psych-122216-011902
4. Koo JW, Duman RS. IL-1β is an essential mediator of the antineurogenic and anhedonic effects of stress. *PNAS*. 2008;105(2):751-756. doi:10.1073/pnas.0708092105
5. Duman RS, Monteggia LM. A neurotrophic model for stress-related mood disorders. *Bio Psych*. 2006;59(12):1116-1127. doi:10.1016/j.biopsych.2006.02.013
6. Jacobsen JPR, Mork A. Chronic corticosterone decreases brain-derived neurotrophic factor (BDNF) mRNA and protein in the hippocampus, but not in the frontal cortex, of the rat. *J Brain Res*. 2006;11(10):221-225. doi:10.1016/j.brainres.2006.06.077

7. Wong EY, Herbert J. Roles of mineralocorticoid and glucocorticoid receptors in the regulation of progenitor proliferation in the adult hippocampus. *Eur J Neurosci.* 2005;22(4):785-792. doi:10.1111/j.1460-9568.2005.04277
8. Rosenzweig MR, Bennett EL. Psychobiology of plasticity: effects of training and experience on brain and behavior. *Behav Brain Res.* 1996;78(1):57-65. doi:10.1016/0166-4328(95)00216-2
9. Zaias J, Queeney TJ, Kelley JB, Zakharova ES, Izenwasser S. Social and physical environmental enrichment differentially affect growth and activity of preadolescent and adolescent male rats. *J Am Assoc Lab Anim* Sci. 2008;47(2):30-34.
10. Johansson BB, Ohlsson AL. Environment, social interaction, and physical activity as determinants of functional outcome after cerebral infarction in the rat. *Exp Neurol.* 1996;139(2):322-327. doi:10.1006/exnr.1996.0106
11. Branchi I, Francia N, Alleva E. Epigenetic control of neurobehavioural plasticity: the role of neurotrophins. *Behav Pharmacol.* 2004;15(5):353-362. doi:10.1097/00008877-200409000-00006
12. Kang H, Schuman EM. Long-lasting neurotrophin-induced enhancement of synaptic transmission in the adult hippocampus. *Science.* 1995;267:1658-1662. doi:10.1126/science.7886457
13. Pham TM, Ickes B, Albeck D, et al. Changes in brain nerve growth factor levels and nerve growth factor receptors in rats exposed to environmental enrichment for one year. *Neuroscience*. 1999;94(1):279-286. doi:10.1016/s0306-4522(99)00316-4
14. Jacobs BL, van Praag H, Gage FH. Adult brain neurogenesis and psychiatry: a novel theory of depression. *Mol Psychiatry*. 2000;5:262-269. doi:10.1038/sj.mp.4000712
15. Liu JYW, Matarin M, Reeves C, et al. Doublecortin-expressing cell types in temporal lobe epilepsy. *Acta Neuropathol Commun.* 2018;6:60. doi:10.1186/s40478-018-0566-5
16. Banerjee D, Rai M. Social isolation in Covid-19: The impact of loneliness. *Int J Social Psychiatry.* 2020;66(6):525-527. doi:10.1177/0020764020922269
17. Mumtaz F, Khan MI, Zubair M, Dehpour AR. Neurobiology and consequences of social isolation stress in animal model—A comprehensive review. *Biomed Pharmacother.* 2018;105:1205-1222. doi:10.1016/j.biopha.2018.05.086
18. Hisey EE, Fritsch EL, Newman EL, Ressler KJ, Kangas BD, Carlezon WA. Early life stress in male mice blunts responsiveness in a translationally-relevant reward task. *Neuropsychopharmacol*. 2023;48(12):1752-1759. doi:10.1038/s41386-023-01610-7
19. Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 6th edition. San Diego: Academic Press; 2009.
20. Holt Lunstad J, Smith TB, Layton JB. Social relationships and mortality risk: a meta-analytic review. *PLoS Med.* 2010;7(7):e1000316. doi:10.1371/journal.pmed.1000316
21. Lee FS, Heimer H, Giedd JN, et al. Adolescent mental health—Opportunity and obligation. *Science*. 2014;346(6209):547-549. doi:10.1126/science.1260497
22. Aoki C, Romeo RD, Smith SS. Adolescence as a critical period for developmental plasticity. *Brain Res.* 2017;1654:85-86. doi:10.1016/j.brainres.2016.11.026
23. Crews F, He J, Hodge C. Adolescent cortical development: A critical period of vulnerability for addiction. *Pharmacol Biochemis Behav.* 2007;86(2):189-199. doi:10.1016/j.pbb.2006.12.001
24. Fuhrmann D, Knoll LJ, Blakemore SJ. Adolescence as a Sensitive Period of Brain Development. *Trends Cogn Sci.* 2015;19(10):558-566. doi:10.1016/j.tics.2015.07.008
25. Foulkes L, Leung JT, Fuhrmann D, Knoll LJ, Blakemore SJ. Age differences in the prosocial influence effect. *Dev Sci.* 2018;21(6):e126666. doi:10.1111/desc.12666
26. Paez Martinez N, Flores Serrano Z, Ortiz Lopez L, Ramirez Rodriguez G. Environmental enrichment increases doublecortin-associated new neurons and decreases neuronal death without modifying anxiety-like behavior in mice chronically exposed to toluene. *Behav Brain Res.* 2013;256(1):432-440. doi:10.1016/j.bbr.2013.09.007
27. Chumley MJ, Catchpole T, Silvany RE, Kernie SG, Henkemeyer M. EphB receptors regulate stem/progenitor cell proliferation, migration, and polarity during hippocampal neurogenesis. *J Neurosci.* 2007;27(49):13481-13490. doi:10.1523/jneurosci.4158-07.2007
28. Vrankova S, Galandakova Z, Benko J, Cebova M, Riecansky I, Pechanova, O. Duration of social isolation affects production of nitric oxide in the rat brain. *Int J Mol Sci.* 2021;22(19):10340. doi:10.3390/ijms221910340
29. Ieraci A, Mallei A, Popoli M. Social isolation stress induces anxious-depressive-like behavior and alterations of neuroplasticity-related genes in adult male mice. *Neural Plast.* 2016:1-13. doi:10.1155/2016/6212983
30. Begni V, Sanson A, Pfeiffer N, et al. Social isolation in rats: Effects on animal welfare and molecular markers for neuroplasticity. *PLOS One*. 2020;15(10):e0240439. doi:10.1371/journal.pone.0240439
31. Sapkale B, Sawal A. Attention deficit hyperactivity disorder (ADHD) causes and diagnosis in adults: A review. *Cureus.* 2023;15(11):e49144. doi:10.7759/cureus.49144
32. Hodges TE, Baumbach JL, McCormick CM. Predictors of social instability stress effects on social interaction and anxiety in adolescent male rats. *Dev Psychobiol*. 2018;60(6):651-663. doi:10.1002/dev.21626

33. Herrera Isaza L, Zárate Guerrero S, Corredor K, Gómez Fonseca Á, Escobar Cornejo G, Cardenas FP. Role of environmental enrichment on social interaction, anxiety, locomotion, and memory in Wistar rats under chronic methylphenidate intake. *Front Behav Neurosci.* 2023;17:1251144. doi:10.3389/fnbeh.2023.1251144
34. Brinton RD, Wang JM. Therapeutic potential of neurogenesis for prevention and recovery from Alzheimer's disease: allopregnanolone as a proof of concept neurogenic agent. *Current Alzheimer Res.* 2006;3(3):185-190.doi:10.2174/156720506777632817
35. Petrik D, Lagace DC, Eisch AJ. The neurogenesis hypothesis of affective and anxiety disorders: Are we mistaking the scaffolding for the building? *Neuropharmacol.* 2012;62(1):21-34. doi:10.1016/j.neuropharm.2011.09.003
36. Kempermann G, Jessberger S, Steiner B, Kronenberg G. Milestones of neuronal development in the adult hippocampus. *Trends Neurosci.* 2004;27(8):447-452. doi:10.1016/j.tins.2004.06.013
37. Encinas JM, Enikolopov G. Identifying and quantitating neural stem and progenitor cells in the adult brain. *Methods Cell Biol.* 2008;85:243-272. doi:10.1016/S0091-679X(08)85011-4
38. Revest JM, Dupret D, Koehl M, et al. Adult hippocampal neurogenesis is involved in anxiety-related behaviors. *Mol Psychiatry*. 2009;14(10):959-967. doi:10.1038/mp.2009.15
39. du Preez A, Onorato D, Eiben I, et al. Chronic stress followed by social isolation promotes depressive-like behavior, alters microglial and astrocyte biology, and reduces hippocampal neurogenesis in male mice. *Brain Behav Immun.* 2021;91:24-47. doi:10.1016/j.bbi.2020.07.015
40. Cinini SM, Barnabe GF, Galvão Coelho N, et al. Social isolation disrupts hippocampal neurogenesis in young non-human primates. *Front Neurosci*. 2014;27(8):45. doi:10.3389/fnins.2014.00045
41. Ausas MS, Mazzitelli Fuentes L, et al. Social isolation impairs active avoidance performance and decreases neurogenesis in the dorsomedial telencephalon of rainbow trout. *Physiol Behav*. 2019;198(1):1-10. doi:10.1016/j.physbeh.2018.10.006
42. Chan JN, Lee JC, Lee SS, et al. Interaction effect of social isolation and high dose corticosteroid on neurogenesis and emotional behavior. *Front Behav Neurosci*. 2017;11:18. doi:10.3389/fnbeh.2017.00018
43. Dioli C, Patrício P, Sousa N, et al. Chronic stress triggers divergent dendritic alterations in immature neurons of the adult hippocampus, depending on their ultimate terminal fields. *Transl Psychiatry*. 2019;9(1):143. doi:10.1038/s41398-019-0477-7
44. Vyas A, Mitra R, Shankaranarayana Rao BS, Chattarji S. Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. *J Neurosci*. 2002;22(15):6810-6818. doi:10.1523/jneurosci.22-15-06810.2002
45. Radley JJ, Sisti HM, Hao J, et al. Chronic behavioral stress induces apical dendritic reorganization in pyramidal neurons of the medial prefrontal cortex. *Neuroscience.* 2004;125(1):1-6. doi:10.1016/j.neuroscience.2004.01.006
46. Gould E, McEwen BS, Tanapat P, Galea LA, Fuchs E. Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation. *J Neurosci.* 1998;18(16):7330-7338. doi:10.1523/JNEUROSCI.18-16-07330.1998
47. Mirescu C, Peters JD, Gould E. Early life experience alters response of adult neurogenesis to stress. *Nat Neurosci*. 2004;7(8):841-846. doi:10.1038/nn1283
48. Kempermann G, Kuhn HG, Gage FH. More hippocampal neurons in adult mice living in an enriched environment. *Nature*. 1997;386(6624):493-495. doi:10.1038/386493a0
49. van Praag H, Kempermann G, Gage FH. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nat Neurosci.* 1999;2(3):266-270. doi:10.1038/6368
50. Branchi I, D'Andrea I, Fiore M, Di Giovanni G, Alleva E. Early social enrichment shapes social behavior and brain development in rats. *Dev Psychobiol.* 2013;55(7):623-635. doi:10.1002/dev.21069