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Pre-weaning Environmental Enrichment Reduced Hippocampal Level of BDNF and Symptoms Severity in VPA Rat Model of Autism

Rahimian Shohreh[1], **Amin Yazdi Seyed Amir**[2], **Bigdeli Imanollah**[1], **Edalatmanesh Mohammad Amin**[3]
1. Department of Psychology, Faculty of Psychology and Education, Ferdowsi University of Mashhad, Mashhad, Iran
2. Department of Counseling and Educational Psychology, Faculty of Psychology and Education, Ferdowsi University of Mashhad, Mashhad, Iran
3. Department of Physiology, College of Sciences, Shiraz Branch, Islamic Azad University, Shiraz, Iran

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Abstract

Background & Objective: Due to the lack of early diagnosis, it is unclear how the environmental stimulations in infancy would affect the underlying structures of Autism Spectrum Disorder (ASD), as well as the onset or severity of symptoms. The study aimed to investigate the effects of receiving pre-weaning environmental enrichment on the severity of ASD symptoms and the hippocampal level of brain-derived neurotrophic factor (BDNF) in the valproic acid (VPA) rat model.

Materials & Methods: Male rats exposed to valproic acid (VPA) or normal saline (Sal) embryonally (E12.5) were randomly assigned to 4 environments: Standard (ST), pre-weaning environmental enrichment (PEE), secondary environmental enrichment (SEE) and PEE+SEE (EE). Behavioral tests were repeated at postnatal day (PND)30 and PND60, in the light phase with a blinded examiner. The BNDF level was determined at PND68.

Results: In VPA rats, receiving PEE, increased social interactions and decreased anxiety, pain sensitivity even in early adulthood. Also, it reduced repetitive behavior but with no significant differences. The BDNF level in VPA-PEE and VPA-SEE was lower than VPA-ST, VPA-EE and saline groups. The biggest improvement in symptom severity was seen in EE.

Conclusion: Reduction of symptoms severity in VPA-PEE and the best performance in VPA-EE showed that rich and sensory overflew environment in infancy can change the formation of ASD. Finding might point to hyperactivity or a lack of regulation of BDNF levels in ASD. PEE most likely reduced hyperactivity, and continued environmental enrichment in EE, regulated the level of BDNF in the hippocampus.

Keywords: Pre-weaning environmental enrichment, Post-weaning environmental enrichment, Autism Spectrum Disorder, Brain-derived neurotrophic factor, Valproic acid.

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Introduction

Health professionals are concerned about the prevalence of autism spectrum disorder (ASD), which is 1 in 44 people (1). Its diagnosis is extremely challenging due to the heterogeneity of symptoms, associated disorders, as well as developmental factors like cognitive and linguistic abilities (2). The main symptoms of this neurodevelopmental disorder, as they occur in early childhood, are persistent deficits in social interaction, as well as repetitive or restricted interests, activities, and behavioral patterns that affect a child's daily activities (3-8).

Numerous factors have been implicated in the etiology of ASD, including genetics, deficiencies

✉ **Corresponding Author: Amin Yazdi Seyed Amir,** Department of Counseling and Educational Psychology, Faculty of Psychology and Education, Ferdowsi University of Mashhad, Mashhad, Iran
Email: yazdi.amin@gmail.com

in neurotransmitter systems and neuropeptide oxytocin, immunodeficiency, infections, and structural abnormalities of the cerebellum, brainstem nucleus, prefrontal cortex, and some areas of the cerebral cortex (9–13). But today, differences in the development and function of the underlying structures, particularly the amygdala and hippocampus, have been taken into consideration because of new discoveries in the field of neuroscience, particularly based on simulated animal models with ASD (14, 15).

These findings indicate that changes in conception, attention, and memory in the autistic brain result from hyper-reactivity and hyper-plasticity in neural microcircuits, particularly in the hippocampus and amygdala, in contrast to earlier observations that had regarded the autistic brain as hypo-functional. Therefore, exposure to an unpredictable environment with over-stimulation leads to increased conditioned fear and anxiety, and as a result, the perception of the world becomes annoying and stressful. Consequently, the symptoms of ASD appear as a solution to cope with this world (16-19).

According to these findings, age and the richness and stability of the environment, have a significant impact on how biology and behavior are affected by environmental changes (17, 20). Early sensory stimulation in a rich, stable environment is advised in therapeutic interventions. Based on these, in order to facilitate the type of sensory overflow required for each developmental stage, they must avoid missing critical developmental periods, and influence the functional patterns of fundamental brain functions (17, 21).

Unfortunately, although there are numerous warning signs before the age of one, the majority of ASD diagnoses occur in ≥3-year-old children (22). Therefore, even while many ASD therapeutic approaches, such as sensory integration therapy, prioritize the provision of sensory stimuli, performing these methods tends to occur after this age, and doing so does not sufficiently enrich the child's sensory world (16, 23). A delayed diagnosis not only causes important developmental periods to be lost in the treatment of ASD but also causes inadequate and unpredictable stimuli in the environment to affect the function of neurotransmitters and the occurrence of ASD symptoms (24).

Simulated animal models of this illness have been widely employed because using human subjects for research would violate ethical and scientific standards. As a result, previous studies have shown that although environmental stimuli are important for enhancing learning, accelerating development, and reducing emotional reactivity in infant rats (25, 26), the majority of these studies have focused on providing post-weaning or secondary environmental enrichment (SEE). Information on the effects of pre-weaning environmental enrichment on infant rats and on critical periods that are crucial in the treatment of ASD is scarce and sometimes inconsistent (27–32).

Furthermore, Brain-derived neurotrophic factor (BDNF) is another important neurobiological factor in the cause of ASD. It is important for neurogenesis, fear learning, and memory, and its function is highly dependent on age and a critical developmental stage. Adolescence is one of the critical periods (approximately PND30 in rodents) (33). Previous studies have revealed instability and heterogeneity in the role of BDNF and in its underlying mechanisms in ASD. Most of them are based on studies that have evaluated the effects of therapies performed after childhood (34-39).

The aim of the current study was to determine how pre-weaning environmental enrichment affects the severity of autistic symptoms and anxiety in rats exposed to valproic acid prenatally, and what changes it will make in hippocampal levels of BDNF and how being in various environments with receiving environmental enrichment in different developmental periods or continuously from birth can change the ASD symptoms and

hippocampal BDNF levels in VPA rats compared to those that live in a standard environment.

Materials and Methods

Valproic Acid Model of Autism

Male and female Wistar adult rats (N=22 in each group, 10-11 week old) in good health, purchased from Production and Research Complex Pasteur Institute of Iran, were employed in this study. Each rat weighed between 250 and 300 g, and they were kept in a lab at a standard temperature of 23±2 ˚C, relative humidity levels of 50 to 55 percent, and a 12-hour light cycle from 7 am to 7 pm. All animals had access to water (from the top of the cage) and food (on standard laboratory food pallets, Pars Animal Feed Co., Iran) ad libitum. 2 rats with same sex were kept in a cage (35×13×20 cm).

All experiments and tests were performed in accordance with the ethical guidelines of the National Institutes of Health in connection with animal experimental protocols and were approved by the Ethics Committee of Ferdowsi University of Mashhad (IR. UM.REC.1398.075). Experimenters were blind to the groups during the measurement.

The rats were adapted to the laboratory environment for two weeks, and then they were mated in the Monogamous breeding scheme overnight, set by co-housing 1 male and 1 female. Observing spermatozoa in the vagina the next morning was considered the first day of pregnancy (E1) by the pap smear method. 21 pregnant mother were kept individually in cage 35×13×20 after confirmation of pregnancy. In (E12.5), 11 randomly selected mothers received 500 mg/Kg valproic acid sodium salt (NaVPA, Sigma) dissolved in normal saline (9%) (150 mg/mL) (pH 7.3), 10 randomly selected, received normal saline solution only, by intraperitoneal injection. The amount of injection was determined according to the mother's weight and was injected on the same day. This amount of valproic acid dosage causes autism like behaviors in rats (21). After injection, mothers were housed individually, so that they could raise their own litters. Day of birth was designated as PND1.

Embryos weight was measured for 10 rats, randomly selected from VAP and saline groups.

Given the higher susceptibility of male offspring to ASD (1, 5), they were chosen for this study. Male pups in VPA (N=40 from 11 litters) and Saline (Sal) (N=40 from 10 litters) groups, which were similar in number and health, were randomly divided into to 4 environments: Standard environment (ST), receiving pre-weaning environmental enrichment (PEE), receiving secondary environmental enrichment (SEE) and receiving PEE+SEE (EE). the conditions in each kind of environment were described below. Accordingly, the study consisted of 8 groups (10 rats in each group) as follows: (Sal-ST), (Sal-PEE), (Sal-SEE), (Sal-EE), (VPA-ST), (VPA-PEE), (VPA-SEE), (VPA-EE).

Behavioral assessments for all 8 groups, were conducted in 2 developmental periods, at PND30-37 (prepubertal adolescence, 130–150 gr, at the beginning of the tests) and PND60-67 (early adulthood, 225–250 gr, at the beginning of the tests), during the light phase, from 8:00 am to 15:00 pm. The rats were transferred to the behavioral test room one hour before starting the measurements. Tests were performed in the same sequence (repetitive behavior- nociception- anxiety- social interaction). Test boxes were cleaned with 70% alcohol before each test. At PND68, biochemical testing was conducted.

Standard Environment

An environment with a cage measured 35×13×20 cm, and had a woodchip bottom (2 cm thick). Access to food and water was considered ad libitum. The caregivers were different (19). Pups were kept in this environment at PND1-22 with their mothers and, at PND23, three identically sexed rats were placed in this environment.

PEE Environment

Pups in ST received the multisensory stimulation protocol, which was used in earlier

research by Schneider et al. (40). In this method, at PND 7–21, offspring were separated from mothers within a specific time between 8 am to 3 pm for 25 minutes and were individually subjected to sensory stimulation with constant pressure and rhythm. The room temperature was 23°C. The procedure involved negative geotaxis (completing turns when placed in a head-down position on an inclined surface and a box with a changing slant), placing the rats on surfaces with different temperatures and structures such as glass, paper towels, and wood, and teaching them to use their righting reflex (to stand up on all four feet from a supine position), and swimming (in a water-filled aquarium 40×20×20 with 10 cm depth and a temperature of 28–29 °C for 5 minutes). Being in ST continued at PND 23-60.

SEE Environment

In this environment, pups were kept in ST until PND 22 and, at PND 23–60 they were placed in groups of 6 in a larger cage measuring 60×40×20 cm, containing a layer of soft wood chips (2 cm thick) on the floor, as well as a running wheel, a wooden ladder, a plastic tunnel, a shelter, bells, marbles, and a spiral maze made of colored blocks. Throughout the experiment, the position, color, and substance of these objects in the cage remained consistent (19).

EE Environment

In this environment, pups were replaced in PEE at PND 7-21 and in EE at PND 23-60 (40).

Social Interaction

To evaluate social interaction, the night before the experiment, rats were separated and housed individually to increase social interaction on the following day. The tool was a white plastic box (50 × 40 × 40 cm) with a plastic tube inside that was large enough for a rat to hide in. Rats were matched based on weight and gender. Over the course of 20 minutes, pairs of treated or control rats were put into the apparatus. Indicators of social engagement included the percentage of time spent pinning (one rat lies on its back while the other stands on top of it), touching, grooming each other, sniffing any body part other than anogenital parts, following, sniffing anogenital parts, and hiding inside the tube during the 10-minute period (21).

Anxiety

The Elevated Plus Maze (EPM) was 10×60×50 cm in size and featured two open arms and two closed arms. This maze was employed to measure anxiety. To facilitate the rats' searching activities in this test, they were first left in the maze for 5 minutes. After the pretest, the rat was placed in the center of the maze, facing one of the open arms. The amount of time the rat spent in closed arms was used as an indicator of anxiety (21).

Repetitive behavior

A Y-shaped Plexiglas maze with three perpendicular arms, measuring 15×15×60 cm, was utilized to measure repetitive behaviors. In Trial 1, a rat was individually placed in the first arm and given the opportunity to choose, enter, and spend 5 seconds in a new arm. After that, it was returned to the first arm. The technique was repeated in Trial 2, and the rat's decision to choose an arm was recorded once more. The percentage of rats in each group who chose a repeating arm was also taken into account as an indicator (19).

Nociception

Thermal nociception sensitivity was assessed using a hot plate test (IITC Life Science). The device was set to a temperature of 50 °C, and the amount of time it took for the hind paw to withdraw in discomfort was noted. One minute was edited off (19).

Biochemical Test

The rat was given a chloroform gas anesthetic before the rodent's head was separated with a guillotine. The brain was swiftly and entirely removed from the skull and put on ice. After that,

stereoscope was used to meticulously separate the hippocampus from the rest of the brain. The hippocampus was cleaned with saline solution and Tris buffer (Sigma, Germany), and then the tissue was homogenized using a homogenizer at 5000 rpm for five minutes. The homogenized solution was centrifuged in a refrigerated centrifuge with the protease inhibitor phenyl methyl-sulphonyl fluoride solution (0.5 mM; Sigma-Aldrich, Germany). After centrifugation, a sampler was used to extract the supernatant solution, which was then analyzed in an Elisa Reader using a kit (E0476Ra-Bioassay Technology Laboratory) (41). The samples were stored at -80°C until this analysis.

Statistical analysis

Differences between groups were analyzed with an independent T-test (for weight), Two-way Repeated measure ANOVA (for hotplate, EPM and social interaction tests), and Chi-square test (Maze Y test). Two-way ANOVA (for BDNF level), and LSD test was used as a Post-hoc test and data in figures were reported as mean values ± SEM. The null hypothesis was rejected with $p<0.05$. Spss.28 was used for all analyses.

Results

Weight

About 47.5% of VPA infant rats (19 from 40 male VPA offspring) had tail abnormalities (Fig. 1). The results showed that rat weight at PND1 in the VPA group (mean weight ± SE =5.83 ± 0.130, n =10) was significantly lower (t =7.82, P =0,000) than in the Saline group (mean weight ± SE =7.09 ± 0.09, n =10).

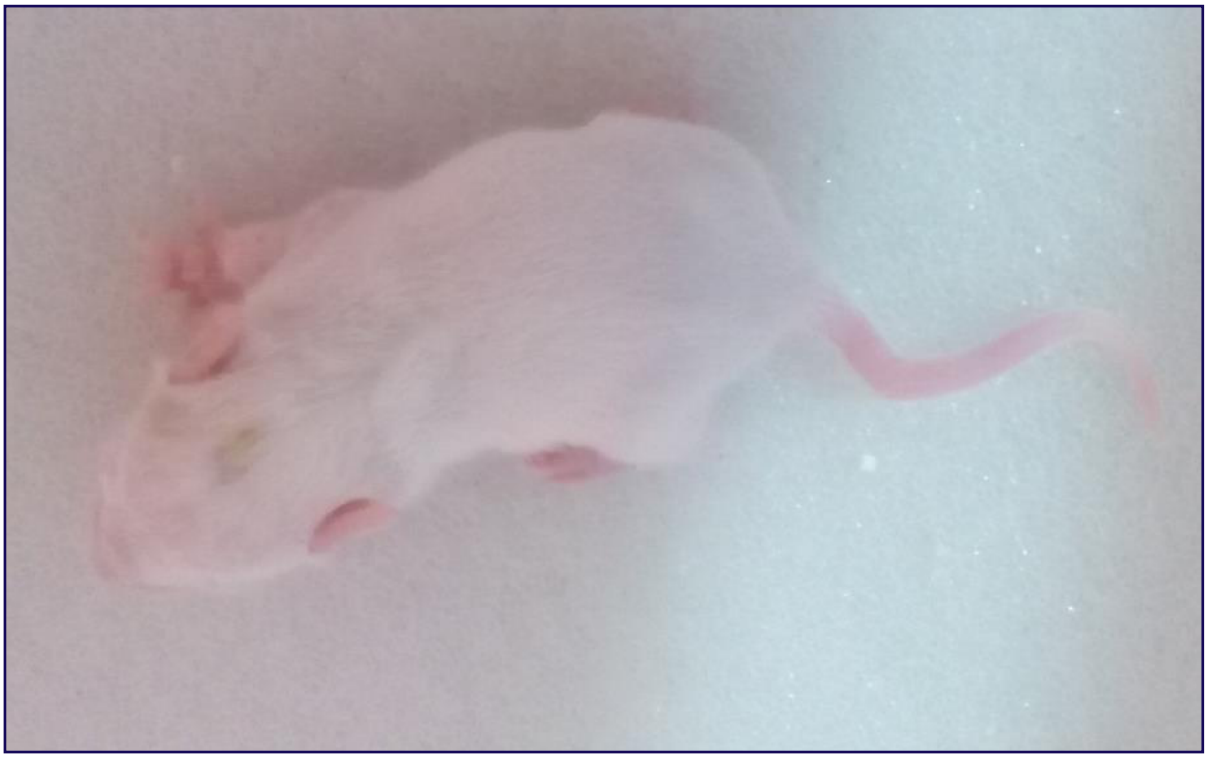

Figure1. Tail Abnormalities in VPA infant rats

Social Interaction

Significant main effect of group observed in Pinning ($F_{-(1.72)}$)= 40.57,P= 0.000), Sniffing ($F_{-(1.72)}$)= 32.64,P= 0.000), Follow ($F_{-(1.72)}$)= 7.66,P= 0.007), Touch ($F_{-(1.72)}$)= 27.03, P= 0.000) and Hiding ($F_{-(1.72)}$)= 22.66, P= 0.000). The main effect of environment was significant in Hiding ($F_{-(1.72)}$)= 3.55, P= 0.018) but no significant interaction effect was seen. Interaction effect of Time × environment ×group was significant in Pinning ($F_{-(3.72)}$)= 4.5, P= 0.006), Sniffing ($F_{-(3.72)}$)= 3.97, P= 0.011), Touch ($F_{-(3.72)}$)= 4.85, P= 0.004) and Hiding ($F_{-(3.72)}$)= 3.16, P= 0.029).

According to these results Post-hoc test showed that, at PND30, the time of pinning in VPA-PEE is higher than VPA-ST and VPA-SEE. VPA-EE's performance was lower than VPA-PEE and higher than VPA-ST and VPA-SEE but this differences were not significant. This result was repeated for Sniffing time ($p< 0.05$) too. At PND 60, VPA-PEE showed no significant improvement in pinning and sniffing time over time. Increase in pinning ($p< 0.05$) and sniffing ($p< 0.01$) time observed in VPA-SEE and VPA-EE. Lower pinning was observed in VPA-ST compared to other VPA groups. VPA-PEE had no significant difference with VPA-SEE and VPA-EE. Sal-EE showed the reduction in Pinning ($p< 0.05$) and sniffing ($p< 0.01$) time and there was no significant difference between 4 Sal groups ant PND30 nor PND60. In comparing between Sal and VPA rats in all 4 environments, only VPA-EE had no significant difference with Sal-EE at PND60 in 5 indicators of social interaction test.

The effect of time× group was significant ($F_{-(1.72)}$)= 12.10, P= 0.000). VPA-ST ($p< 0.01$) and VPA-SEE ($p< 0.05$) showed lower time compared to Sal-ST at PND30. No significant difference was observed between other groups and environments. In Touch no significant difference observed between VPA-PEE with other VPA rats at PND30 but in hiding this group had significantly less time compared to other VPA rats. At PND60 no VPA-PEE had any difference compared to other VPA rats exposed to environmental enrichment. VPA-ST had lower touching and higher hiding time compared to other VPA rats and Sal-ST at PND30 and 60. VPA-PEE, VPA-SEE and VPA-EE had no significant difference in any time. VPA-SEE and VPA-EE hid less ($p< 0.001$) at PND60.

In summary, result showed that social interaction in VPA-PEE increased in PND30 compared to VPA-ST and this improvement persisted over time. VPA-EE had the best improvement in social interaction between all VPA rats at PND60. In many indicators no significant differences were observed between VPA-PEE and VPA-SEE at PND60 (after 30 days' environmental enrichment therapy). VPA-ST had lower social interaction compared to Sal-ST at PND30 and 60 (Chart 1).

Repetitive behavior

Chi-square test resulted that receiving pre-weaning environmental enrichment did not reduce repetitive behaviors at PND30 (Value=4.86, P= 0.182). At PND60 non-repetitive behavior increased significantly in VPA groups (Value=7.96, P= 0.047). No increased non-repetitive behaviors were seen in Sal groups (Value=0.78, P= 0.781) (Table 1). in this time VPA-EE had less repetitive behaviors than VPA-ST ($p< 0.05$). No significant difference was observed between VP-PEE, VPA-SEE and VPA-EE (Chart 2).

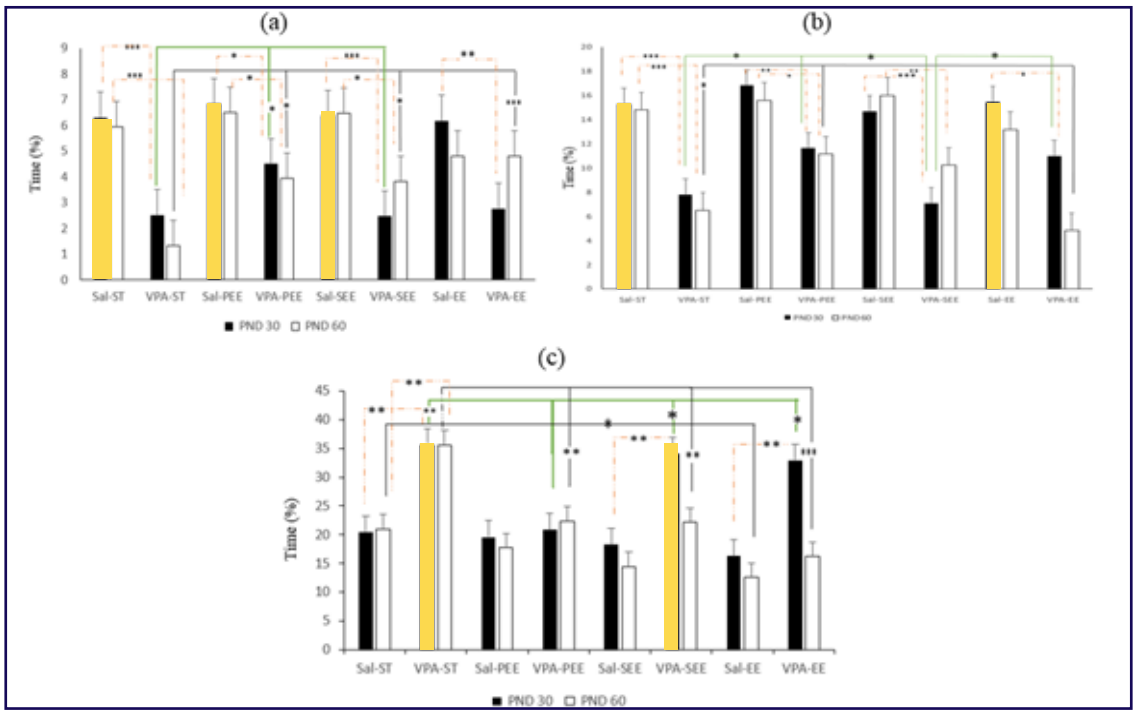

Chart 1. Comparison between all VPA and Sal groups in social interaction test in Pinning (a), Sniffing (b) and Hiding (c) in the PND30 and PND60 Graphs depict Mean ± SEM. *P≤0.05; **P≤0.01; ***P≤0.001

Table1. Changing in frequency of repetitive and non-repetitive behaviors over time between PND30-60

Group				Sal environment				VPA environment			
				ST count	PEE count	SEE count	EE count	ST count	PEE count	SEE count	EE coun
PND30	Non repetitive	PND60	Non repetitive	6	7	7	6	1	5	3	7
			repetitive	1	0	1	1	3	1	2	0
	repetitive	PND60	Non repetitive	0	1	2	3	1	2	4	1
			repetitive	3	2	0	0	5	2	1	2

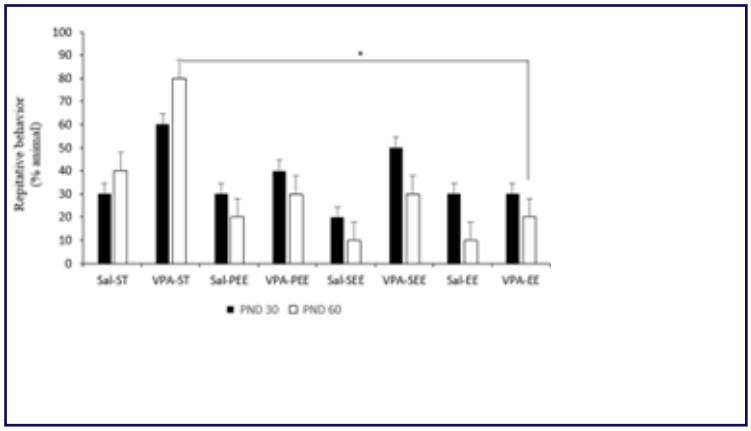

Chart 2. Comparison between all VPA and Sal groups in Y-maze test in the PND30 and PND60. Graphs depict Mean ± SEM. *P≤0.05

Nociception

According to a Two-way Repeated measure ANOVA, the interaction ($F_{-(1.72)}$)= 3.57, P= 0.018) and main effects for environment ($F_{-(3.72)}$)= 7.78, P= 0.000) and group ($F_{-(1.72)}$)= 146.64, P= 0.000) were significant. There was no difference between Sal groups. No significant time effect was seen but Time× environment ×group effect was significant ($F_{-(3.72)}$)= 12.19, P= 0.000).

At PND30, the VPA-PEE showed a statistically shorter reaction time than the VPA-ST (p< 0.01), VPA-SEE (p< 0.01) and VPA-EE (p< 0.05). At PND60, all VPA rats receiving environmental enrichment (PEE, SEE, EE) showed a significantly lower reaction time than the VPA-ST. A significant difference (p< 0.05) observed between VPA-PEE compared to VPA-EE (Chart 3a).

VPA-SEE (p< 0.001) and VPA-EE (p< 0.001) had a significant reduction, however VPA-ST (p< 0.001) had increase in reaction time over time. difference between Sal and VPA groups was seen only at PND60 in EE environment (Chart 3b).

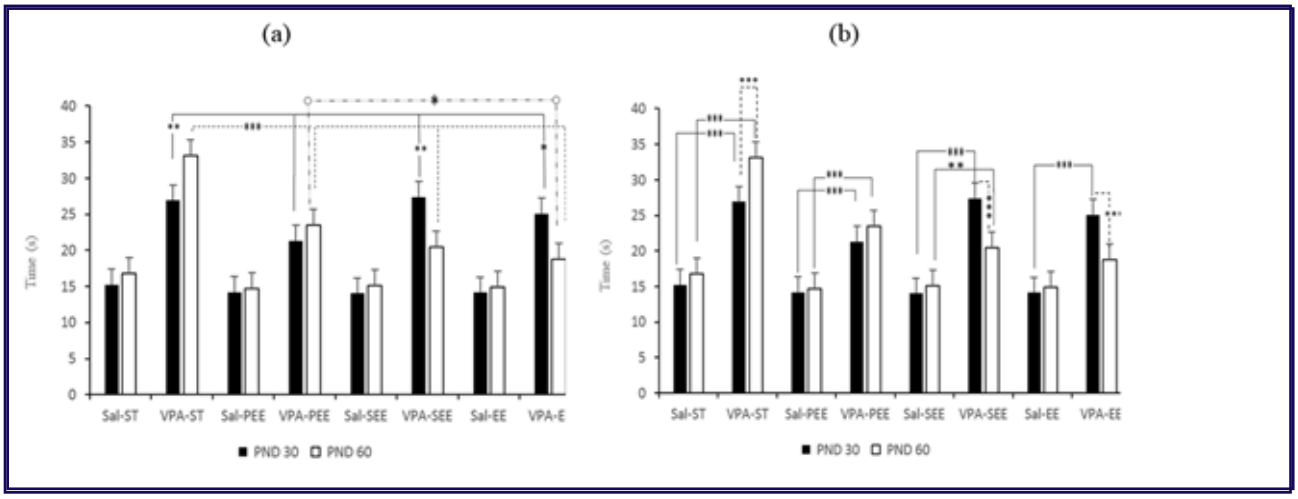

Chart 3. Comparison Sensitivity to thermal nociception in Hotplate test in Sal and VPA groups in PND30 and PND60 (a) and investigation within group changes over time (b). Graphs depict Mean ± SEM. *P≤0.05; **P≤0.01; ***P≤0.001

Anxiety

Anxiety level significantly differed, according to a Two-way Repeated measure ANOVA. there is significant interaction ($F_{-(3.72)}$)= 7.16, P=0.000) and main effect for environment ($F_{-(3.72)}$)= 8.53, P= 0.000) and group ($F_{-(1.72)}$)= 142.39, P= 0.000). Time× environment ×group effect was significant ($F_{-(3.72)}$)= 7.36, P= 0.000).

At PND30, the VPA-PEE and VPA-EE showed significantly lower levels of anxiety than the VPA-ST and VPA-SEE. At PND60, the VPA-ST showed lower anxiety (p< 0.001) compared to all enriched environment (PEE, SEE, EE). The difference between VPA-PEE and VPA-SEE was not significant. VPA-EE had a significant lower Anxiety compared to VPA-PEE (p< 0.01) and VPA-SEE (p< 0.01) (Chart 4a).

At PND30, anxiety level was significantly higher in VPA groups than Sal's. But at PND60, no significant difference was observed between the Sal-EE and VPA-EE. The results of comparing each group over time showed that (Sal) rats in none of the environments (ST, PEE, SEE, EE) had significant differences in performance over time. In the VPA which are receiving environmental enrichment experienced significantly less anxiety in PND60 compared to PND30 (Chart 4b).

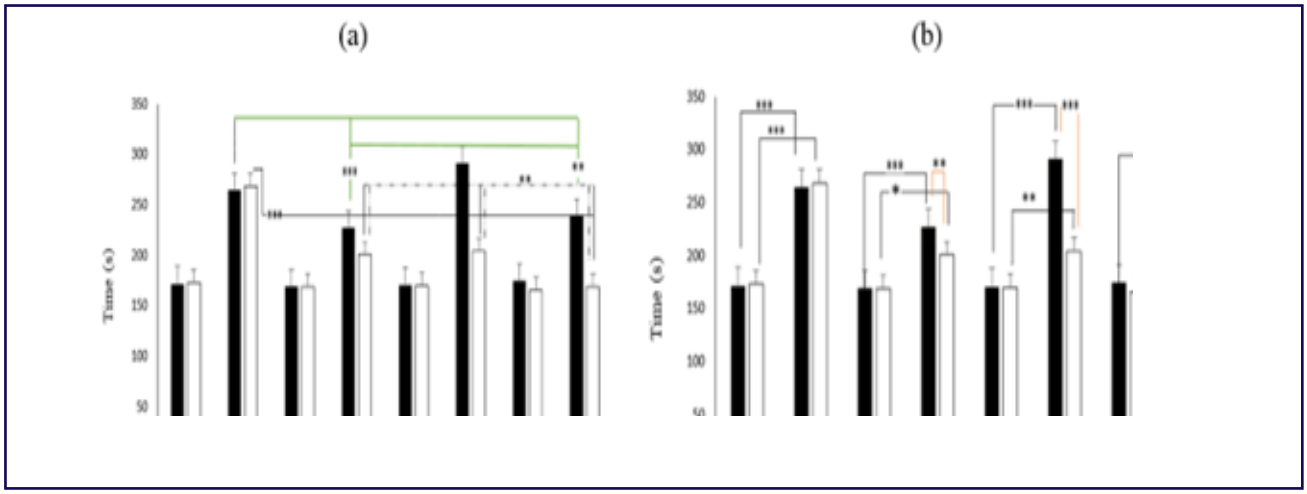

Chart 4. Comparison between Sal and VPA groups at PND30 and PND60 (a) and investigation within group changes over time (b) the time spent in the close arm of the Elevated Plus Maze test showed the level of anxiety in each group. Graphs depict Mean ± SEM. *P≤0.05; **P≤0.01; ***P≤0.001

Biochemical test

Hippocampal tissue's BDNF levels significantly differed, according to a Two-way ANOVA there is significant interaction ($F_{-(3.72)}$)= 2.94, P= 0.039) and main effect for environment ($F_{-(3.72)}$)= 4.040, P= 0.010) and group ($F_{-(1.72)}$)= 19.645, P= 0.000). In pairwise comparison, BDNF levels were considerably lower in the VPA-PEE compared to VPA-ST (P= 0.003), VPA-EE (P= 0.002) and Sal-PEE (P= 0.001). this result repeat for VPA-SEE compared to VPA-ST (P= 0.015), VPA-EE (P= 0.011) and Sal-SEE (P= 0.000). No significant differences existed between VPA-ST compared to VPA-EE and all Sal groups. Environmental enrichment increased BDNF in hippocampus in all saline groups but only significant difference observed in Sal-EE compared to Sal-ST (P= 0.015) (Chart 5).

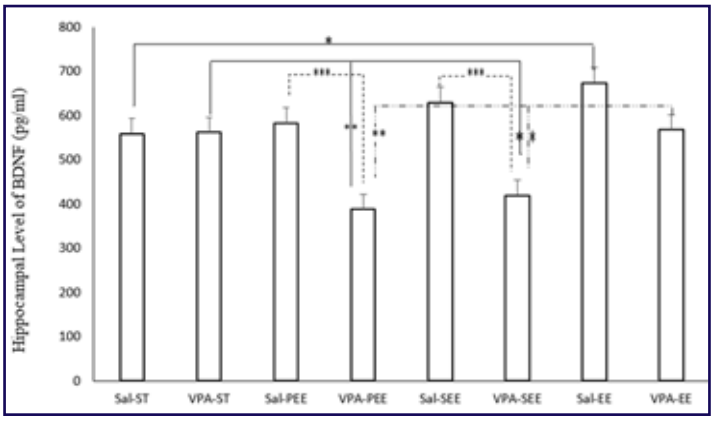

Chart 5. Hippocampal Level of BDNF in PND67 analyzed by a kit (E0476Ra - Bioassay Technology Laboratory) in an Elisa Reader. Graphs depict Mean ± SEM. *P≤0.05; **P≤0.01; ***P≤0.001

Discussion

This study found that pre-weaning environmental enrichment reduced the severity of ASD symptoms and BDNF level in the hippocampi of VPA rats. At PND30 all VPA groups had higher reaction time to pain than the Sal's. VPA-PEE exhibited a slower reaction time to nociception than other VPA groups. At PND60, VPA-PEE had significant differences with VPA-ST substantially, and no difference with VPA-SEE. VPA-EE was the only autistic group that had no significant difference with Sal group. In repetitive behavior VPA-EE which had received pre-weaning environmental enrichment, had the best improvement in performance in both time periods.

Numerous researches have shown that VPA rats exhibit higher levels of repeated behavior and reduced pain sensitivity (22, 40, 42). However, some studies found significant difference in the frequency of repetitive activities between several groups of normal and VPA rats in standard and enriched environments (19). In recent years, some documents have presented conflicting findings about the effectiveness of early enrichment in making differences among repetitive behavior (31, 43). According to the results of the current study, which found no difference between all VPA groups at PND30, it seems that repetitive behaviors and how well environmental enrichment works depend on a number of factors, such as the age and species of the lab animals, behavioral tests, methodological factors, the protocol for environmental enrichment, and the time of its presentation (28).

Authors found more anxiety in VPA-ST rats than the Sal-ST (19, 21, 40, 44). In PND30, VPA-PEE and VPA-EE had significantly less anxiety compared to VPA-ST and VPA-SEE, although at PND60 VPA-SEE showed reduction in anxiety level compared to VPA-ST too. VPA-EE was the only group that showed no difference in anxiety compared to Sal group at PND60. This result approved Schneider, Turczak and Przewlocki (2006) findings that combination of pre- and post-weaning environmental enrichment has the best effects.

Pre-weaning environmental enrichment in VPA group, in some indicators, caused the higher improvement in social interaction even more than EE environment at PND30. Also, VPA-EE's better performance compared to VPA-SEE emphasized the role of PEE environment (40).

According to research, pre-weaning environmental enrichment reduced the symptoms of Rett syndrome in mutant rats when treatment started before the second week of the MecP2 phenotype (45). The provision of pre-weaning environmental enrichment has critical periods and does not always result in obvious behavioral changes in the infant

rodents, but it may have an indirect impact on later developmental behaviors (46). This is perhaps one of the reasons for this point that non-repetitive behavior increase after 60 days, as well as the fact that combining the pre-and post-weaning enrichment has the greatest effect on improving the symptoms of ASD rats (40). Although a rat's first month of life is a particularly vulnerable time (28) and stimulation in the early days of life, especially from people, may make a newborn more anxious, these anxiety-related behaviors can act as a protective factor for the rat when it experiences stress as an adult (29). Therefore, a significant improvement in autistic symptoms and behavioral improvements in EE environment at PND60, would result from appropriate stimulation during the critical pre-weaning periods in adult rats. However, more research needs to be done in this area because it is not clear what mechanisms are underlying these changes.

One interesting point in current results, was the better performance of VPE-PEE compared to VPA-EE in some behavioral test (like in social interaction or EMP test) at PND30, despite continued environmental stimulation in EE environment. It can be stated that despite the scientific evidence supporting the impact of environmental enrichment during infancy on enhancing problem solving, social skills, play, exploration, and long-term stress protection (47–51), perhaps the abrupt change in cage environment in the post-weaning enrichment in the brains of VPA rats in the SEE and EE groups initially acted as a stress-related event, causing a brief increase in anxiety and affecting the trend of reducing the severity of ASD symptoms, or even caused an increase in the severity of symptoms, but with time and the creation of the necessary environmental stimuli, led to a further alleviation of symptoms. At 30 days of age, the behavioral incidence of reduced symptom intensity was more obvious in the PEE group than in the EE group since this stressful event did not occur in the PEE group. The results demonstrated that early life stress can affect hippocampal development, which is associated with a higher sensitivity to anxiety. As a result, pre-or post-weaning, environmental enrichment leads to an increased level of anxiety and a decreased level of social interaction in rats with pseudo-autistic behaviors (52, 53). This increased anxiety may have also affected the low level of some social interaction indicators, the higher mean value of Hiding in the VPA-EE compared to VPA-PEE, or no significant difference between the VPA-SEE and VPA-ST at PND30, proving that an increased level of fear is a cause of ASD (17).

Although BDNF levels in the hippocampus were lower in the VPA-ST compared to Sal-ST, but did not differ significantly with each other or Sal-EE. Results regarding autistic people, autistic animal models, and rats under poor breeding settings are highly contradictory, and in some cases, they indicate an increase, decrease, or no change in the blood or various parts of the brain (34, 37, 54–57). This study did not find a big difference between ST and Con, which was in line with other research (39, 50) that showed that valproic acid exposure did not significantly cause changes in the hippocampal levels of BDNF in the of 90-day-old rats or 60-day-old mice.

It is interesting to note that, according to the findings of this study, the level of BDNF in the hippocampus of VPA rats was significantly lower in VPA-PEE and VPA-SEE compared to the VPA-ST and VPA-EE. This conclusion, which likely indicates an early hyperactivity or a problem in regulating BDNF levels in the hippocampus as a primary or secondary cause of the disease must exist in ASD, was supported by the lowering and then increasing trend in the enrichment groups (54, 58). Age and critical developmental periods, the type of stimuli and the moment at which they enter the neural system, as well as the type of animal species, are all extremely influential

in determining the rate of BDNF changes (33, 37, 44, 59). Environmental enrichment affects neurogenesis in the hippocampus of normal rats during the pre-weaning stage which is crucial for the hippocampus's growth (59, 60), and then raises the level of BDNF in the hippocampus of these rats during the post-weaning stage (61–63). In the current study, it appears that pre-weaning environmental enrichment prevented an abnormal rise in hippocampus BDNF levels and reduced the severity of ASD symptoms by providing a sensory overflow, which is necessary at the critical periods of growth.

Because the findings of the present study are groundbreaking results on the effects of pre-weaning environmental enrichment or comparison between providing environmental enrichment in different periods of development on the ASD symptoms and BDNF levels in the hippocampus of VPA rats, it is advised that additional studies with similar designs be conducted on behavioral and biochemical changes in adult and normal rats. The effects of pre-weaning environmental enrichment can be explored in infants who have been in enrichment cages since birth, in addition to the multisensory stimulation protocol. It is also suggested that in other research projects, the changes in the number or density of cells in different areas of the hippocampus should be investigated. More investigations into environmental enrichment, especially in animal models, can lead to the design of easier and cheaper treatments which can apply to all ages (16, 64).

Conclusion

According to the findings, pre-weaning environmental enrichment increased social interactions while reducing repetitive behaviors, anxiety, pain sensitivity, and hippocampus BDNF levels. The most significant reduction in disorder symptoms was observed when enrichment was associated with pre- and post-weaning periods. The current findings on BDNF level changes in enrichment groups agree with earlier findings (17), which may indicate hyperactivity and a failure to regulate BDNF levels in the hippocampus of VPA rats. Pre-weaning environmental enrichment likely averted this hyperactivity in infant rats, and with continued enrichment after post-weaning, the levels of BDNF in the hippocampus were regulated, significantly reducing the symptoms of ASD.

Founding

This research was thesis for PhD in psychology for Shohreh Rahimian.

Ethical approval

The Ethics Committee of Ferdowsi University of Mashhad approved the study (IR.UM.REC.1398.075).

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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