საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, ტ. 17, №3, 2023
BULLETIN OF THE GEORGIAN NATIONAL ACADEMY OF SCIENCES, vol. 17, no. 3, 2023

Human and Animal Physiology

Environmental Enrichment Improves Behavioral Abnormalities in Rats Prenatally Exposed to Valproic Acid

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In the present paper, the effects of environmental enrichment at two different developmental stages on behavioral abnormalities in valproic acid (VPA) induced rat model of autism (autism spectrum disorders – ASD) are investigated. To study the effects of environmental enrichment at different stages of development 3 weeks before behavioral testing half of the offspring from the control and VPA-exposed rats were housed in an enriched environment and the other half were reared in the standard environment. Behavioral experiments were carried out on control and VPA-exposed rats in two age groups: prepubertal adolescence – postnatal day 30 (PND 30) and late adolescence (PND 60). Locomotor activity, exploratory behavior, anxiety level and stereotyped/ repetitive behavior were evaluated by an open-field test. The results indicated that in standard environmental condition the anxiety level and stereotyped/repetitive behavior was increased, the exploratory behaviour was reduced in VPA-exposed rats compared to control rats. Environmental enrichment at both developmental stages cause decrease of anxiety leveland stereotyped/repetitive behavior in VPA-exposed rats but does not cause significant changes in the exploratory behaviour. In conclusion, our study showed that an enrichment environment improves behavioral abnormalities in prenatally VPA-exposed rats. Our results bring further support to the validity of the proposed VPA animal model of autism and reinforce the importance of this model for the preclinical investigation of new therapeutic approaches. *© 2023 Bull. Georg. Natl. Acad. Sci.*

rat model of autism, valproic acid, behavior, environmental enrichment

Autism spectrum disorders (ASD) represent a lifelong, neurodevelopmental disorder manifesting early in development and characterized by repetitive behaviors, difficulties in social interactions, also impairments in verbal and nonverbal communication [1,2]. In the last years, an increasing number of ASD became heavy burden for society [3]. Despite many researches, there is no agreement about the causation of autism and the etiopathogenetic factors of ASD are unknown. Animal models produced by exposure to environmental factors are crucial for understanding the pathogenic mechanisms of autism. For example, valproic acid (VPA) is an

antiepileptic drug and mood stabilizer that is widely used in humans. It has become evident that VPA exposure in the first trimester of gestation during neural tube closure represents the highest risk for the child to develop ASD [4]. Rodents prenatally exposed to a single dose of VPA on embryonic day 12.5 show neuroanatomical and behavioral deficits similar to human autism. Remarkable, that an animal model does not show the whole picture of human disorder, but it allows us to represent core symptoms and increase our understanding of the neurobiological mechanism of autistic behavior [5] and determine treatment strategies. Currently, no pharmaceutical compound is approved to alleviate the core symptoms of ASD. Still, environmental enrichment remains promising for ASD. It has been known that environmental factors make influence-brain development [6] and induce alterations at neuroanatomical, neurochemical and behavioral levels. However, the effect of environmental enrichment on VPA-induced behavioral, structural and morphological alterations remains contradictory. In the present study, we investigated the effects of environmental enrichment at two different developmental stages on behavioral alterations in rats prenatally exposed to VPA.

Materials and Methods

Animals. The animals were procured from the Laboratory Animal Division of Ivane Beritashvili Center of Experimental Biomedicine. Female wistar rats were mated overnight and, in the morning, if spermatozoa were found in vaginal secretion it was designated as the first day of gestation (embryonic day 0.5). Females received a single intraperitoneal injection of 500 mg/kg sodium VPA (Sigma-Aldrich, USA) dissolved in saline at a concentration of 250 mg/ml on the 12.5 day after conception, and control females were injected with physiological saline at the same time. Pregnant female rats were housed individually in the plastic home cage and had access to food and water *ad libitum*. Behavioral experiments were carried out on offspring of the females described above in two age groups: prepubertal adolescence – postnatal day 30 (PND 30) and late adolescence (PND 60). To study the effects of environmental enrichment at different stages of development 3 weeks before behavioral testing half of the offspring from the control and VPA-exposed groups were housed in an enriched environment (EE) and the other half were reared in the standard environment (SE). Accordingly, experiments were carried out on 4 subgroups in two age (PND 30, PND 60) groups. The offspring were weaned on a postnatal day (PND) 21. The number of animals in each experimental group was 20. Both male and female offspring were included in the study. All experimental procedures were conducted in accordance with the European Communities Council Directive Guidelines for the care and use of Laboratory animals (2010/63/EU – European Commission) and approved by the animal care and use committee at the Ivane Beritashvili Center of Experimental Biomedicine.

Enriched environment. The enriched chamber (100 cm in diameter and 70 cm high) was installed in the same room where the animals were kept since birth. The arena contained different types of objects (plastic tunnels, plastic blocks, shelter, running wheel, ladder, platform and wooden blocks which were of different colors and sizes) and nesting material. The positions of the shelter and running wheel rotated daily to maintain novelty, and the items completely changed out weekly. During behavioral experiments, animals were in an enriched environment.

Behavioral apparatus and procedure. Locomotor activity, exploratory behavior, anxiety and stereotyped/repetitive behavior were evaluated by an open field (60 × 60 × 40 cm) test. The floor of the open field was divided into 25 equals sized, 9 central squares from 25 defined as an inner zone. The light intensity was 40 lux. For habi-

tuation, each rat was placed into the box for 5 minutes the day before the experiment. For subsequent analysis, the main section of the experiment was recorded for 5 minutes by an overhead camera and a video recorder. Each animal was placed individually in an inner zone of the open field box. The measures recorded were number of grids crossed (with the four paws), number of times that the rats crossed the central area, number of grooming episodes and rearing. Behavior was scored by the researcher blind to the animal's status. Between the trial, the open field box was cleaned with 70% ethanol.

Statistical analysis. Statistical analysis was performed using Prism 9.5.0 (GraphPad statistical software). For two-group comparison used Unpaired t-test and three-way ANOVA was used to determine statistical differences between the groups, followed by Tukey's multiple comparisons tests. All data are presented as mean ± standard error of the mean. Differences were considered significant when $p < 0.05$.

Results

Locomotor activity was assessed in the open field by counting the number of crossed grids. Three-way ANOVA showed significant effect of age ($F_{(1,159)} = 120.263$; $P < 0.001$), but no significant effect of treatment ($F_{(1,159)} = 0.237$; $P=0.627$), group ($F_{(1,159)} = 3.810$; $P=0.053$) and significant interaction between group and age ($F_{(1,159)} = 4.527$, $P=0.035$). Multiple comparison procedures (Tukey Test) showed that there was significant difference ($P=0.004$) in locomotor activity between VPA exposed and control rats at PND 60, while no such difference was observed between groups at PND 30 ($P=0.901$). The results showed that locomotor activity increased significantly with age in both – VPA-exposed and control rats (PND 30 *vs* PND 60 – $P < 0.001$; Fig. 1).

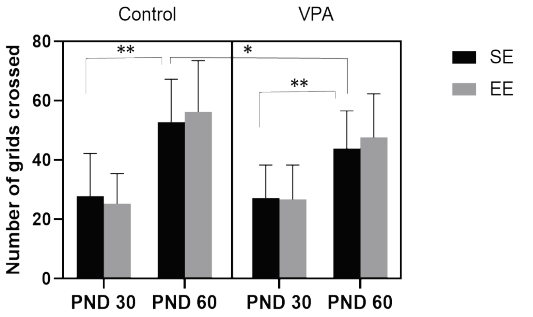

Fig. 1. Locomotor activity in the open field in control and VPA-exposed rats at two different ages (PND 30 and PND 60). Locomotor activity increased significantly with age in both – VPA-exposed and control rats (PND 30 vs PND 60 – **P<0.001). There was significant difference in locomotor activity between VPA-exposed and control rats (*P<0.01) at PND 60. EE – environmental enrichment, SE – standard environment. Data expressed as mean ± SEM (n = 20 per group).

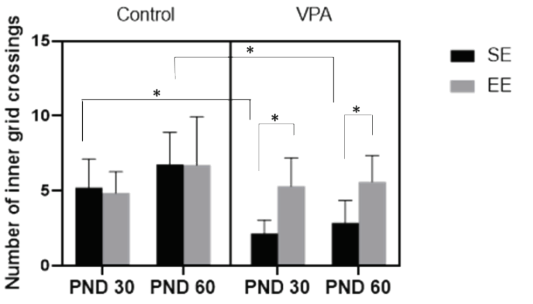

Fig. 2. The effect of environmental enrichment on anxiety behavior in the open field (evaluated by counting the number of crossed inner grids) in control and VPA-exposed rats at two different ages (PND 30 and PND 60). Level of anxiety was increased in VPA-exposed rats compared to controls (*P <0.001) and environmental enrichment at both developmental stages cause decrease anxiety level in VPA-exposed rats (*P <0.001). EE – environmental enrichment, SE – standard environment. Data expressed as mean ± SEM, (n = 20 per group).

The anxiety level was evaluated by counting the number of crossed inner grids. Three-way ANOVA has shown significant effects of group ($F_{(1,159)} = 37.987$; $P < 0.001$), age ($F_{(1,159)}=12.732$; $P < 0.001$), treatment ($F_{(1,159)}=19.894$, $P < 0.001$) and significant interaction between group and treatment ($F_{(1,159)}=26.103$; $P < 0.001$), but no interaction between the factors age x treatment and group x age x treatment. The results indicated that in the standard environmental condition, the number of

inner crossed grids was decreased in VPA-exposed rats compared to controls ($p < 0.001$), and environmental enrichment at both developmental stages cause an increase in the number of inner crossed grids in VPA-exposed rats (SE *vs* EE – $p < 0.001$; Fig. 2).

The exploratory activity was assessed by the number of rearing (rat stood on their hind legs, not touching the cage floor with their forepaws). Three-way ANOVA showed significant effects of group ($F_{(1,152)} = 37.409$; $P < 0.001$) and age ($F_{(1,152)} = 32.864$; $P < 0.001$), but no significanteffects of treatment ($F_{(1,152)} = 0.763$; $P < 0.384$). The interaction between factors were not significant (group x age – $P = 0.432$, group x treatment – $P = 0.086$, age x treatment – $P = 0.949$, group x age x treatment – $P = 0.298$). The results showed that the number of rearing in VPA-exposed rats was reduced at both developmental ages compared to age-matched control rats. Also, the number of rearings was found to be age-dependent: in animals of both groups, it was higher at PND 60 than at PND 30 (Fig. 3). No effect of the enriched environment was detected in either the control or VPA-exposed groups.

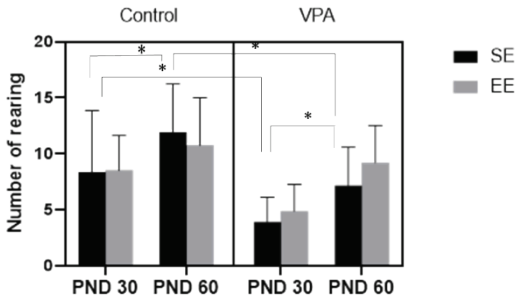

Fig. 3. The exploratory behavior in the open field (evaluated by counting the number of rearing) in control and VPA-exposed rats at two different ages (PND 30 and PND 60). Exploratory behavior in VPA-exposed rats was reduced at both developmental ages compared to age-matched control rats (*P <0.001). Exploratory behavior was higher at PND 60 than at PND 30 (*P <0.001) in both - control and VPA-exposed groups. No effect of the enriched environment was detected in either the control or VPA-exposed groups. EE - environmental enrichment, SE – standard environment. Data expressed as mean ± SEM, (n = 20 per group).

Stereotyped/repetitive behavior was assessed by self-grooming behavior. Three-way ANOVA showed significant effects of group ($F_{(1,159)} = 20.440$; $P < 0.001$) and treatment ($F_{(1,159)} = 27.539$; $P < 0.001$), but no significant effect of age ($F_{(1,159)} = 0.0261$; $P = 0.872$) and significant interaction between factors group and treatment ($F_{(1,159)} = 10.429$; $P = 0.002$). Results showed that in standard environment the number of grooming in VPA-exposed rats was increased compared to control rats and environmental enrichment at both developmental ages cause a decrease in the number of grooming (SE *vs* EE - $p < 0.001$; Fig. 4).

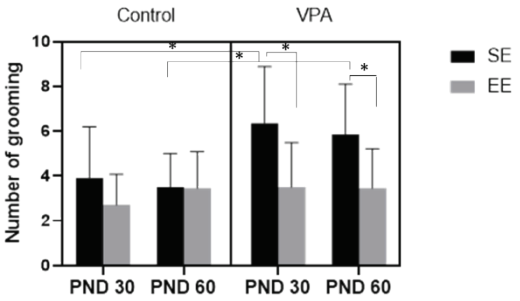

Fig. 4. The effect of environmental enrichment on repetitive/stereotyped behavior in the open field (evaluated by counting the number of grooming) in control and VPA-exposed rats at two different ages (PND 30 and PND 60). The repetitive/stereotyped behavior in VPA-exposed rats was increased compared to control rat (*p<0.001) and environmental enrichment at both developmental ages cause a decrease in the repetitive/stereotyped behavior (*p<0.001). EE – environmental enrichment, SE – standard environment. Data expressed as mean ± SEM, (n=20 per group).

Discussion

In this study, were assessed the effect of an enrichment environment at two developmental stages in control and prenatally VPA-exposed rats. The obtained results showed that locomotor activity was increased at the PND 60 compared to the PND 30 groups, but at the same age of development, the difference was not observed between the control and VPA treated groups. In the study of Kinjoa, et al [7] male offspring of pregnant rats treated with VPA showed locomotor hyperactivity. However, in

this study, a long-term VPA dosing regimen was used: VPA was administered daily for 4 weeks. Accordingly, this discrepancy between results may reflect differences in VPA administration schedules across studies. In addition, only male rats were used in their experiments, while both male and female rats were used in our study. It should be noted that enriched environmental conditions at any stage of development do not cause significant changes in locomotor activity in the open field, neither in the control nor in rats exposed to VPA.

Anxiety is one of the associated symptoms of ASD [8]. Our study confirms the results of other studies in which a rat model of autism created by prenatal exposure to VPA showed higher levels of anxiety-like behavior compared to controls [9]. The results indicated that in standard environmental condition the level of anxiety was increased in VPA-exposed rats compared to controls and environmental enrichment at both developmental stages cause a decrease of anxiety level in VPA-exposed rats.

In this study, exploratory behaviour was assessed by a number of rearing and the results showed that the number of rearing in VPA-exposed rats was reduced at both developmental ages compared to age-matched control rats. Also, the number of rearings was found to be age-dependent: in animals of both (control, VPA-exposed) groups, it was higher at PND 60 than at PND 30. No effect of the enriched environment was detected in either the control or VPA-exposed groups. Stereotyped/repetitive behavior was assessed by self-grooming behavior. Results showed that in standard environment the number of grooming in VPA-exposed rats was increased compared to control rats and environmental enrichment at both developmental ages cause a decrease in the number of grooming.

Environmental enrichment represents an alternative way (no pharmaceutical), which can change or improve behavioral alterations in animal models of psychiatric disorders and neurodegenerative diseases [10]. Remarkable that this alternative way may also be promising for ASD; our study showed that enriched environment improves some behavioral abnormalities in rat model of ASD. One of the goals of this study was to determine which developmental stage is more sensitive to environmental enrichment in a rat model of ASD. The result showed that environmental enrichment has a positive effect at both ages of development.

In conclusion, our study showed that an enrichment environment improves behavioral alteration in prenatally VPA-exposed rats, and decreases anxiety and repetitive/stereotyped behavior. Our results bring further support to the validity of the proposed VPA animal model of autism and reinforce the importance of this model for the preclinical investigation of new therapeutic approaches.

This work was supported by Shota Rustaveli National Science Foundation of Georgia (SRNSFG): Grant # YS-19-1737.

ადამიანისა და ცხოველთა ფიზიოლოგია

გამდიდრებული გარემო აუმჯობესებს ქცევით დარღვევებს ვალპროის მჟავათი გამოწვეულ აუტიზმის ვირთაგვას მოდელში

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**აკადემიის წევრი, ივანე ბერიტაშვილის ექსპერიმენტული ბიომედიცინის ცენტრი, ქცევისა და კოგნიტურ ფუნქციათა ლაბორატორია, თბილისი, საქართველო

ნაშრომში წარმოდგენილია გამდიდრებული გარემოს ეფექტები ვალპროის მჟავათი (valproic acid – VPA) გამოწვეულ აუტიზმის ვირთაგვას მოდელის ქცევით დარღვევებზე განვითარების ორ განსხვავებულ ეტაპზე. ამ მიზნით ქცევითი ექსპერიმენტების დაწყებამდე 3 კვირით ადრე საკონტროლო და VPA-ს ზემოქმედების მქონე ცხოველების ნახევარი გადაგვყავდა გამდიდრებულ გარემოში, ხოლო მეორე ნახევარი იზრდებოდა სტანდარტულ გარემოში. ქცევითი ექსპერიმენტები ტარდებოდა ორ ასაკობრივ ჯგუფში: პრეპუბერტატული მოზარდობის (დაბადებიდან 30-ე დღე) და გვიანი მოზარდობის - 60 დღის ასაკში. ლოკომოტორული აქტიურობა, კვლევითი ქცევა, შფოთვის დონე და სტერეოტიპული/განმეორებითი ქცევა ფასდებოდა ღია ველში. მიღებული შედეგებით გამოვლინდა, რომ სტანდარტულ გარემო პირობებში საკონტროლო ჯგუფის ცხოველებთან შედარებით, VPA-ს ზემოქმედების მქონე ცხოველებში შფოთვის დონე და სტერეოტიპული/ განმეორებითი ქცევა იზრდება, ხოლო კვლევითი ქცევა მცირდება. განვითარების ორივე სტადიაში გამდიდრებული გარემოს ზემოქმედება ამცირებს შფოთვის დონეს და სტერეოტიპულ/განმეორებით ქცევას VPA-ს ზემოქმედების მქონე ცხოველებში, თუმცა არ იწვევს კვლევითი ქცევის მნიშვნელოვან ცვლილებას. ამრიგად, მიღებული შედეგების თანახმად გამოვლინდა, რომ გამდიდრებული გარემო აუმჯობესებს ქცევით დარღვევებს VPA-ს ზემოქმედების მქონე ცხოველებში. მიღებული შედეგები დამატებით ამყარებს VPA-ს ზემოქმედებით მიღებული აუტიზმის ცხოველური მოდელის ვალიდურობას და აძლიერებს ამ მოდელის მნიშვნელობას ახალი თერაპიული მიდგომების პრეკლინიკური გამოკვლევისთვის.

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Received April, 2023