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Age-Related Alterations of Spatial Memory in Rat Model of Autism Induced by Valproic Acid

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The present study was designed to evaluate age-related alterations of spatial memory in the valproic acid (VPA) rat model of autism. To induce autism-like animal model, the pregnant rats were intraperitoneally injected 500 mg/kg NaVPA at the gestation day 12.5. Experiments were carried out on three age groups of male offspring: prepubertal adolescence (1 month), adult (6 months) and old (22 months) rats at the start of experimentation. The learning process and long-term spatial memory was assessed in the Morris water maze (MWM). Both control and VPA-treated rats of all age groups met the learning criteria by the end of the training period. During the probe test, which was performed 24 h after task acquisition, the control and VPA-treated adolescent and adult rats as well as VPA treated aged rats showed normal spatial memory abilities in the MWM task, however rats within the control aged group exhibited a retention deficit 24 h after training. In conclusion, we found that spatial memory function in VPA induced rat model of autism is relatively age-resistant and we hope that further research will expand our understanding of age-related changes in cognitive function in the abnormal central nervous system. *© 2022 Bull. Georg. Natl. Acad. Sci.*

rat model of autism, valproic acid, aging, spatial memory

Autism (autism spectrum disorders – ASD) is a lifelong disability affecting the functioning of the brain. Despite progress in understanding of autism, relatively little attention has been paid to date to the process of aging. The largest bulk of research on ASD focuses on children and adolescents, with very few studies focusing specifically on adults, older adults, or on longitudinal data [1]. Since the life cycle of rats is much shorter, the study of the behavioral and neurobiological features of the aging process should be effective both in healthy animals and in animal models of diseases. The valproic acid (VPA) rat model is an environmentally triggered model with strong construct and clinical validity [2].

The term ASD is used to describe a group of behaviorally defined neurodevelopmental disorders that are characterized by impaired communication and social interaction [3], repetitive behaviors, stereotypies, and a limited repertoire of interests and activities [4, 5]. Comorbidities are prevalently seen in individuals with ASD, whereas studies in

animal models are rather focused on the actual core symptoms of the disease [6]. The most frequent associated symptoms in ASD include anxiety, epileptic seizures and cognitive impairments [6]. VPA-exposed rodents have been largely tested in learning and memory tasks but studies had mixed results; some found support for impaired memory [7, 8], others found evidence enhanced [9] memory function. In addition, while the autistic-like behavioral phenotype of the young VPA rat model has been studied extensively, the effects of age on this ASD-like rat model have not been elucidated.

The present study was designed to evaluate age-related alterations of spatial memory in rats prenatally exposed to VPA. Studying the effect of aging on memory function in an animal model of autism will be important to shed light on the full developmental trajectory of some cognitive functions in this neurodevelopmental disorder.

Materials and Methods

Animals. The animals were procured from the Laboratory Animal Division of Ivane Beritashvili Center of Experimental Biomedicine. The day of detection of spermatozoa in the vagina of female rats after night mating was designated as the first day of pregnancy. On the 12.5th day after conception, half of the female rats received a single intraperitoneal injection of 500 mg/kg sodium valproate (Sigma-Aldrich, 250 mg/ml, dissolved in saline), and the other half (control group) received saline. The male offsprings of both groups of females (valproate-treated and control) were used in behavioral experiments. Experiments were carried out in three age groups: prepubertal adolescence (1 month), adult (6 months) and old (22 months). Accordingly, experiments were performed on 3 groups of control [C1 (n=9), C6 (n=12), C22 (n=15)] and 3 groups of prenatally VPA exposed [V1 (n=9), V6 (n=11), V22 (n=11)] rats. 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.

Morris water maze. Acquisition and retention of spatial memory was assessed using the Morris water maze (MWM) task as described previously [10]. Briefly, the maze apparatus consisted of a circular tank (1.5 m in diameter and 0.5 m in height) filled with opaque (white-colored) water. An escape platform was hidden 2 cm below the surface of the water in a fixed location. The rats were placed and allowed to swim in the pool to find a hidden platform. On each four training days, the animals received a block of four consecutive trials one from each of four equidistantly located start locations (N, S, E, W) in a randomized sequence. The retention was tested in a probe trial 24 h after task acquisition, during which the platform was removed from the pool. Rats were allowed to swim from a new start location for 60 sec and the time spent in each of the four quadrants was recorded for further analysis. Tracking the animal movements in water maze, also collection of other numeric data was made with an aid of video tracking system designed and installed in our laboratory.

Statistical analysis. Statistical analysis was performed using SigmaStat statistical software. The effect of treatment on the learning process were analyzed (separately for each age group) by Two Way Repeated Measures ANOVA (one factor repetition), with group and training days (1-4 days) as factors. Further post hoc comparisons or two-tailed t-tests were used, where appropriate. A one-sample t-test was used to compare the time spent in the test (Qtest) and opposite (Qopp) quadrants and to compare the time spent in the test quadrant with a chance level (15 s – the quadrant time expected from a random search of the water maze) during the probe test for each group. All the data is presented as a mean ± standard error of the mean (SEM). Differences were considered significant when $P < 0.05$.

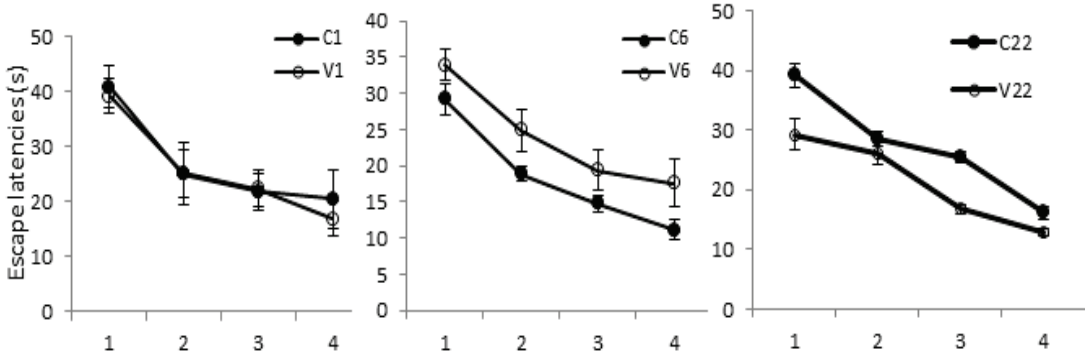

Fig. 1. The average escape latencies of control (C1, C6, C22) and prenatally VPA treated (V1, V6, V22) rats in days 1-4 of the training phase. The rats of all groups showed significantly decreased latency reaching the hidden platform. The data is given as a mean ± SEM.

Results

The effect of VPA treatment on the learning process were analyzed (separately for each age group) by Two Way Repeated Measures ANOVA. The decrease in the escape latency between 1-4 training days in MWM training task was considered to be a measure of the learning process. At the age of 1 month ANOVA showed no significant effect of treatment ($F_{1.71}$=0.0648, P<0.802), but showed significant effect for training days (days 1, 2, 3 and 4; $F_{3.71}$=16.234, P<0.001). There is not a statistically significant interaction between treatment and days ($F_{3.71}$=0.207, P=0.891). At the age of 6 months ANOVA showed significant effect of treatment ($F_{1.91}$=6.825, P<0.016), and significant effect for training days ($F_{3.91}$=31.759, P<0.001). There is not a statistically significant interaction between treatment and days ($F_{3.91}$=0.109, P= 0.954). At the age of 22 months ANOVA showed significant effect of treatment ($F_{1.103}$=56.665, P<0.001), significant effect for training days ($F_{3.103}$=77.776, P<0.001) and statistically significant interaction between treatment and days ($F_{3.103}$=3.970, P=0.011). Both control and VPA-treated rats met the learning criteria by the end of the training period. When analyzing training phase days 1 and 4 (Tukey Test), animals of all groups showed significantly decreased latency in reaching the hidden platform (P<0.001, for all groups), although rats in the V6 group exhibited a longer latency reaching the hidden platform in comparison to the C6 group and rats in the C22 exhibited a longer latency in comparison to the rats of V22 group indicating that they had poorer learning ability (Fig. 1).

A probe test was performed 24 hours after task acquisition to assess spatial reference memory. Spatial memory of the hidden platform location is indicated by preference for a Qtest over the Qopp. The results of the T-test analysis of the differences for time spent in Qtest and Qopp identified a significant difference between quadrants in the animals of all VPA treated groups (V1: t=-2.711, P=0.017; V6: t=-2.541, P=0.019; V22: t=-7.807, P=<0.001) and in the control rats at the age of 1 and 6 months (C1: t=-6.453, P=<0.001; C6: t=-10.085, P=< 0.001). There was no significant difference between time spent in the quadrants for the control aged rats (t=-1.221, P=0.232; Fig. 2).

In order to determine whether rats in each groups learned the location of the hidden platform, the time spent in the target quadrant was compared to 15 s (chance level), using a one-sample t-test. During the probe test performed 24 hours after task acquisition, the trained rats in the C1, C6, V1, V6, and V22 groups spent significantly longer than chance (15 s, the dotted lines on Fig. 2) in the target quadrant where the hidden platform was located during the training trials (C1: 23.331 ± 1.684, t = -4.947, P<0.001; C6: 21.724±1.161, t=-5.792, P=<

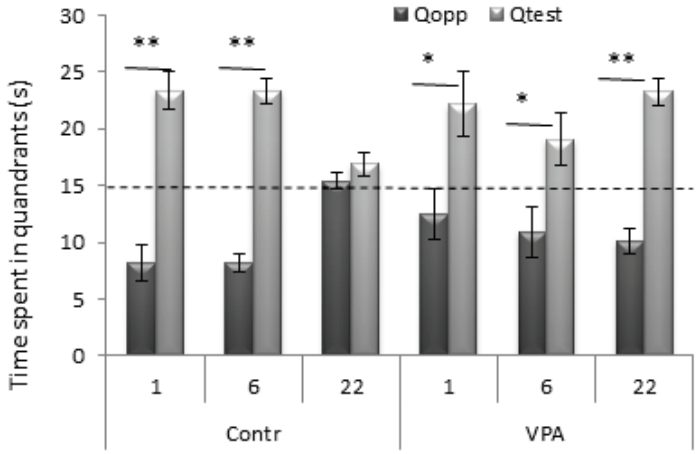

Fig. 2. Probe-test performance of control and prenatally VPA treated rats from different age (1, 6 and 22 months) groups. The dotted line indicates the chance level -15 s (the quadrant time expected from a random search of the water maze). Long-term spatial memory for the location of the hidden platform is indicated by preference for the Q_{test} over Q_{opp}. Note: The rats in the control aged group exhibited a retention deficit 24 hours after training. The data are given as a mean ± SEM. * $p < 0.01$; ** $p < 0.001$.

0.001; V1: 22.192±2.816, t=-2.553; P=0.023; V6: 20.613±2.065, t=-2.718, P=0.013; V22: 23.238± 1.236, t=-6.664, P=<0.001). In contrast, in target quadrant the rats of the C22 group spent no longer than chance (16.915±1.007, t=-1.619, P=0.118). As all rats learned the location of the hidden platform during training trials, the results suggest that the rats of the C22 group could not remember the information acquired during training 24 h later. There were no treatment effects seen on cued trials in the MWM indicating that treatment had no effects on sensorimotor abilities or escape motivation (data not presented).

Discussion

The results of MWM experiments showed that all rats exhibited decreased latency in finding the hidden platform across the training trials. Both control and VPA-treated rats met the learning criteria by the end of the training period. During the probe test, which was performed 24 h after task acquisition, the control and VPA-treated adolescent and adult rats as well as VPA treated aged rats showed normal spatial learning and memory abilities in the MWM task, however rats within the control aged group exhibited a retention deficit 24 h after training. Thus, rats from C22 group showed impaired spatial reference memory in the maze. Although control aged rats exhibited a longer latency in reaching the hidden platform compared to rats in VPA treated aged groups, learning does in fact take place. This result is consistent with studies that have reported the poorer performance of naturaly aged rats in MWM tasks [11, 12]. In human studies very few data and statistics on aging people with autism are available yet. Most behavioral research focused on adolescent or adult high-functioning individuals with ASD on the grounds that these individuals have “pure autism” uncontaminated by linguistic or intellectual impairments: results show a heterogeneous cognitive profile with a specific pattern of intact and compromised processes in memory [13]. To the best of our knowledge the age-related changes in spatial memory in rats prenatally exposed to VPA have not been investigated. Our results is consistent with studies that have reported a robust maintenance of cognitive ability (assessed in the novel object preference test of learning and memory) despite advanced age in BTBR T+tf/j mouse, a well characterized and widely used mouse model that displays an ASD-like phenotype [14].

It is interesting to note that during the aging process, the connectivity of the nervous system usually decreases due to accumulated damage. However, one of the characteristic features of the ASD observed in the brains of both animal models and humans with ASD is the hyperconnectivity of neuronal circuits [15, 16]. It is quite possible that this factor is important to maintain memory function in the aging process in VPA treated rat. However, there is also the possibility that some other factors play a role in neuroprotection, allowing the maintenance of spatial memory function in VPA-treated old rats; for example, some memory functions can be more easily processed with compensatory strategies that make them easier for VPA-treated old rats. The successful use of compensatory strategies by VPA treated rats may derive from their developmental condition. These types of compensations may be harder to acquire when a memory disorder is acquired in adulthood.

In conclusion, we found that spatial memory function in VPA induced rat model of autism is relatively age-resistant and it is our hope that further research will enhance our understanding of aging in an abnormal central nervous system.

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ადამიანისა და ცხოველის ფიზიოლოგია

სივრცითი მეხსიერების ასაკთან დაკავშირებული ცვლილებები ვალპროის მჟავათი გამოწვეულ აუტიზმის ვირთაგვას მოდელში

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

წარმოდგენილ ნაშრომში კვლევის მიზანს წარმოადგენდა ასაკთან დაკავშირებული სივრცითი მეხსიერების ცვლილებების შეფასება ვალპროის მჟავათი (VPA) ინდუცირებულ ვირთაგვას აუტიზმის მოდელში. აუტიზმის ცხოველური მოდელის მისაღებად ვირთაგვების მაკეობის მე-12,5 დღეს ტარდებოდა 500მგ/კგ NaVPA-ს ინტრაპერიტონეულური ინექცია. ექსპერიმენტები ტარდებოდა მამრობითი სქესის ნაყარის სამ ასაკობრივ ჯგუფზე: პუბერტატამდელ მოზარდ (1 თვის), ზრდასრულებსა (6 თვის) და ბებერ (22 თვის) ვირთაგვებზე. დასწავლის პროცესი და ხანგრძლივი სივრცითი მეხსიერება ფასდებოდა მორისის წყლის აუზში. დასწავლის ფაზის დასრულებისას საკონტროლო, ასევე VPA-ს ზემოქმედების მქონე ყველა ასაკობრივი ჯგუფის ვირთაგვები აკმაყოფილებდნენ დასწავლის კრიტერიუმებს. სატესტო სინჯის დროს, რომელიც ტარდებოდა დასწავლიდან 24 საათის შემდეგ, საკონტროლო ჯგუფის მოზარდი და ზრდასრული, ასევე VPA-ს ზემოქმედების მქონე ყველა ასაკობრივი ჯგუფის ვირთაგვები აჩვენებდნენ ნორმალურ სივრცითი მეხსიერების ხანგრძლივად შენახვის უნარს მორისის წყლის აუზის ამოცანაში. დასწავლიდან 24 საათის შემდეგ საკონტროლო ჯგუფის ბებერ ვირთაგვებში გამოვლინდა მეხსიერების დეფიციტი. ამრიგად, გამოვლინდა, რომ VPA-ით გამოწვეული აუტიზმის ვირთაგვას მოდელში სივრცითი მეხსიერების ფუნქცია შედარებით მდგრადია ასაკობრივი ცვლილებების მიმართ და ვიმედოვნებთ, რომ შემდგომი კვლევა გაამდიდრებს ჩვენს ცოდნას დაბერების პროცესში კოგნიტური ფუნქციების ცვლილებების შესახებ ცენტრალური ნერვული სისტემის პათოლოგიის პირობებში.

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