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Sodium valproate exposure during the brain growth spurt transiently impairs spatial learning in prepubertal rats

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Abstract

The brain is extremely vulnerable to teratogenic insults during the brain growth spurt, a period that starts during the third trimester of human gestation and is characterized by synaptogenesis establishment of neuronal circuits. While the treatment of epilepsy during pregnancy increases the risk of neurodevelopmental disorders in offspring, the consequences of exposure to anticonvulsants during the brain growth spurt remain poorly known. Here we investigate whether exposure to sodium valproate (VPA) during a similar period in rats impairs spatial learning of juvenile rats. Long-Evans rats were exposed to VPA (200mg/kg) or saline solution (SAL) every other day between postnatal day (PN) 4 and PN10. At PN23 and PN30, Morris water maze performance was evaluated during 6 consecutive days. In the group of animals which started their tests at PN23, the VPA exposure impaired both, swimming speed and learning/memory performance. Interestingly, no differences were observed between VPA and control animals tested from PN30 to PN35. Our data suggests that the neurobehavioral deficits caused by VPA exposure during the brain growth spurt are transitory.

Keywords

valproic acid; fetal valproate syndrome; brain growth spurt; recovery; anticonvulsant

1. Introduction

Epilepsy is one of the most common neurologic disorders affecting 0.5 to 1% of pregnant women (Fairgrieve et al. 2000; Holmes et al. 2001; Tinker et al. 2010). The use of antiepileptic drugs, which typically must be continued throughout the pregnancy, increases the risk of malformations as well as of neurodevelopmental disorders in offspring (Meador et al. 2008; Tomson and Battino 2009). Among the vast number of anticonvulsants, valproate (depakene ©) has been recognized as one of the most teratogenic (Jentink et al. 2010; Ornoy 2009; Pennell 2008; Tomson et al. 2011). In addition to its anticonvulsant properties, VPA has also been used

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Conflict of interest statement

The authors report no conflicts of interest

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to treat other common neuropathies such as migraine headaches and bipolar disorder (Haddad et al. 2009; Vikelis and Rapoport 2010).

In humans, most of studies regarding the teratogenic effects of VPA focus on the exposure during the first trimester of gestation. During this period, the use of VPA has been associated with a cluster of facial abnormalities (e.g. cleft lip and palate, broad nasal base, and shallow philtrum), occasional major malformations (e.g. cardiovascular abnormalities, genitourinary defects, and limb defects) and neural tube defects (Alsdorf and Wyszynski 2005; Jentink et al. 2010; Kozma 2001). This constellation of physical features has been referred to as fetal valproate syndrome (Clayton-Smith and Donnai 1995; Kozma 2001). Children with valproate syndrome may also present cognitive problems including low verbal intelligence quotient, learning and memory deficits, and autistic spectrum disorder (Ornoy 2009; Vinten et al. 2005). While the effects of VPA during the first half of gestation are well known, recent studies have shown that exposure during late gestation may also contribute for the fetal anticonvulsant syndrome phenotype (Kim et al. 2011; Meador 2008; Pohl-Guimarães et al. 2011).

Relevant information about the teratogenic effects of VPA on brain function may come from studies using animal models. In rodents, the exposure to VPA during gestational period, which encompasses to the first two trimesters of human gestation (Clancy et al. 2007; Maier et al. 1999; Quinn 2005), promotes malformations and neurobehavioral deficits resembling autism that generally recapitulate the effects of VPA in humans (Ornoy 2009; Rouillet et al. 2010). In contrast, there are scant studies that focus on the effects of VPA during a period comparable to human late gestation which, in rats and mice, comprises the early postnatal life (Clancy et al. 2007; Maier et al. 1999; Quinn 2005). During the first 10 days of postnatal life, the brain rapidly increases in size and intense neurogenesis and synaptogenesis take place (Bandeira et al. 2009; Dobbing and Sands 1979; Patel 1983). Moreover, during this phase of “brain growth spurt” neuronal circuits are refined by pruning of connections and neuronal apoptosis (Olney et al. 2000). In fact, it is well known that alteration of these processes by external insults can disrupt the proper wiring of the brain and lead to numerous neurobehavioral problems (Medina 2011). The hippocampus is particularly vulnerable to teratogens during the brain growth spurt. For instance, exposure to VPA during this period can dramatically increase neuroapoptosis (Bittigau et al. 2003) and disrupt long-term potentiation (Zhang et al. 2003). Thus, VPA exposure during the brain growth spurt may have drastic effects on hippocampal development, which in turn may lead to impairments in hippocampal-dependent learning and memory. Here we use the Morris water maze to test whether exposure to VPA during the brain growth spurt impairs spatial learning of rats.

2. Methods

2.1. Animal Treatment

All experimental procedures were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee (IACUC) and were in compliance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health. Twenty pregnant Long-Evans dams (mid gestation) were purchased from Harlan Laboratories and offspring used as experimental subjects. Animals were maintained in a temperature- (approximately 22°C) and humidity- (approximately 50% relative) controlled room on a 12:12h light/dark cycle (lights on: 6:00, lights off: 18:00) with free access to food and water. The day of birth was considered PN0. Litters were not culled (mean litter size = 10.3 ± 0.7 pups) and the pups were left undisturbed until PN4, when pups within the same litter were randomly assigned to VPA or SAL treatment groups.

From PN4 to PN10, approximately ½ animals were injected with 200mg/kg of VPA (IP, in saline solution dissolved at 20mg/ml; n=49 pups) and the remaining with saline solution (n=60)

on alternate days. In order to minimize the risk of injury to internal organs, a 28-gauge needle was carefully inserted to just penetrate the abdominal wall and reach the peritoneal cavity. Leakage from the injection site was minimized by slowly withdrawing the needle from the abdominal cavity. At weaning (P21) males and females were separated and housed in groups of 2–3. At PN30, all rats were weighed. From the initial sample of 109 animals, only 39 (22 females and 17 males) rats treated with saline and 35 (21 females and 14 males) rats treated with VPA were used in this study. The remaining 35 animals were used in other studies (VPA: n=9; SAL: n=18) or died (VPA: n=5; SAL: n=3) during treatment. The whole sample (n =109) was used to estimate the mortality rate after VPA and SAL treatments.

Body-weight gain has been systematically used in developmental-toxicology studies as an indicator of general health status (Henck 2002). In addition, knowledge of body weight at the time of behavior testing is crucial to data interpretation because body-weight changes can confound behavioral results (Henck 2002). Therefore, the body weight data were evaluated from PN4 to PN10 (during exposure period) and at PN30 (during behavioral test period).

2.2. Morris Water Maze

To investigate the developmental outcomes of early VPA exposure, rats were tested in periods roughly equivalent to juvenile and early adolescence in humans. Animals were tested daily in the Morris Water maze during a period of 5 days starting at PN23 (SAL: 14 females and 9 males, VPA: 10 females and 7 males) and PN28 (SAL: 8 females and 8 males, VPA: 11 females and 7 males) for the infant and adolescent groups respectively. The Morris water maze consisted of a circular pool (180 cm diameter $\times$ 75 cm high) filled to a depth of 29 cm with water (22 ± 2 °C). The water was rendered opaque with white non-toxic tempera paint. Testing sessions took place between 13:00h and 16:00h. Each animal was given four trials a day. Each trial had a 60 sec ceiling, and the inter-trial interval ranged from 40 to 60 min. During the first five days of testing (training period), a circular escape platform (14 cm diameter), submerged 1.0 cm below the surface of the water, was located 38 cm away from the wall of the pool in the center of the northeast (NE) quadrant and remained there from the first to the fifth day of testing. On the sixth day (reversal session), the platform was placed in the center of the southwest (SW) quadrant. From the first to the fifth day of testing, the animal was introduced into the pool, facing the wall of the northwest (NW), southeast (SE), or southwest (SW) quadrants (quadrants pseudo-randomly assigned). On the sixth day of testing, the animal was introduced into the pool alternating between northwest (NW) and southeast (SE) quadrants. Once the animal reached the platform, it was allowed to remain on it for approximately 15 seconds. If the animal failed to reach the platform within 60 sec, the experimenter gently guided the animal through the water and placed it on the platform where it would remain for 15 sec. Performance on each trial was videotaped with a camera mounted directly above the pool and analyzed with image tracking software (Videomex-V, Columbus Instruments, Ohio). Latency to find platform, distance traveled to reach the platform, swimming speed (distance traveled/latency) and platform proximity were calculated for each trial. The platform proximity measure was obtained by sampling the position of the animal in the maze to provide a record of its distance from the escape platform in 1 s time-intervals. The percentage of time spent in each quadrant was also used to assess the performance in the maze.

2.3. Statistical analysis

Data are compiled as means and standard errors. To minimize the influence of litter effects, for all analyses, we considered the average of values from male and female rats of the same litter instead of using individual values (Wainwright 1998). Repeated measures analyses of variance (rANOVA) were performed for analyses of both the body weight from PN4 to PN10 and the Morris water maze data. For the body weight data, rANOVAs were carried out using “treatment” as between-subjects factor and “day” as repeated within-subject factor. The

analyses of Morris maze data were carried out using “treatment”, “age of testing” (rats tested from PN23 to PN28 or rats tested from PN30 to PN35) and “sex” as between-subjects factors and “trial” and “day” as repeated within subject measures. Appropriate lower-order ANOVAs were utilized whenever significant interactions of treatment with other factors were detected. In all cases, individual group differences were evaluated post-hoc by Fisher’s Protected Least Significant Difference (FPLSD). The analyses of body weight at PN30 was carried out by separate univariate ANOVAs using “treatment” (VPA or saline) and “sex” (male or female) as between-subjects factors. Significance was assumed at the level of $p < 0.05$. For the sake of simplicity, we will report results based only on the averaged univariate F tests. Whenever the sphericity assumption was violated, we used the Greenhouse-Geisser correction, which adjusts the degrees of freedom, in order to avoid Type I errors (Huynh and Feldt 1976).

3. Results

3.1. Survival rate and offspring growth

Survival rates of SAL and VPA groups were similar, 95.0% ($n = 57$) and 89.8% ($n = 44$), respectively and not statistically different (Fisher’s Exact Test, $P = 0.46$). Figure 1 shows offspring weights during SAL or VPA exposure (from PN4 to PN10). Note that for both groups, body weights increased significantly from PN4 to PN10 [$F(1.8, 53.5) = 931.4$, $P < 0.001$]. However, the VPA group presented a slower growth than the SAL group, as demonstrated by a significant treatment $\times$ age interaction [$F(1.8, 53.5) = 4.1$, $P < 0.05$] as well as a main effect of treatment [$F(1, 29) = 8.9$, $P < 0.01$].

At PN30, the average body weight of males (94.9 ± 1.9) was higher than in females (83.6 ± 1.6) [$F(1, 60) = 21.1$, $P < 0.001$]. However, there were no differences between the averaged body weight of SAL (90.8 ± 1.4) and VPA (87.7 ± 2.0) groups and there was no significant interaction between treatment and sex. Taken together, these results suggest that, during testing period, there were no differences between VPA and SAL groups regarding general health status.

3.2. Morris water maze

We first investigated whether VPA and SAL groups showed any differences in swimming speed since this could affect the outcome of latency and distance measures. Figure 2 illustrate that for both VPA and SAL groups, the swimming speed increased significantly over days [day effect: $F(4, 152) = 6.3$, $P < 0.001$]. The increase from the first to the second trial of the first day of testing was more accentuated than those observed afterwards [day trial interaction: $F(12, 456) = 5.9$, $P < 0.001$]. In general, the group of animals tested from PN30 to PN34 swam faster than the group tested from PN23 to PN27 [age effect: $F(1, 38) = 12.4$, $P < 0.01$]. The averaged swimming speed of the VPA group (25.8 ± 0.47 m/s) was significantly slower than that observed for the control (27.3 ± 0.4 m/s) [effect of treatment: $F(1, 38) = 5.6$, $P < 0.05$]. This effect was more pronounced in the group of females tested from PN23 to PN27 [trial age treatment sex interaction: $F(3, 114) = 2.7$, $P < 0.05$].

On the sixth day, when the platform was placed in the center of the SW quadrant, no differences in swimming speed were observed between groups with no effect of treatment (VPA: 28.2 ± 0.8 cm/s; SAL: 28.4 ± 0.7 cm/s), sex (female: 27.8 ± 0.8 cm/s; male: 28.7 ± 0.8 cm/s), age nor significant interactions involving these factors (figures 2E and 2F).

Considering that VPA and SAL groups showed differences in swimming speed and that such differences can directly affect, both latency and distance measures, these were not used to evaluate learning/memory performance. Therefore, learning/memory performance was assessed by the proximity to the platform and the percentage of time spent in each quadrant, variables that are not affected by swimming speed.

Figure 3A shows that the distance to the platform decreased significantly over days [day effect: $F(3,152) = 149.4$, $P < 0.001$] and trials [trial effect: $F(3,114) = 44.9$, $P < 0.001$] indicating that animals swim closer to the platform with training. This was less evident in the first day compared to subsequent ones [day trial interaction: $F(12,456) = 2.2$, $P < 0.05$]. The averaged platform proximity (all five testing days collapsed) for animals tested from PN23 to PN27 (57.2 ± 2.8 cm) was significantly greater than that observed for animals tested from PN30 to PN34 (46.3 ± 2.9 cm) [effect of age: $F(1,38) = 7.5$, $P < 0.01$]. As depicted in Figure 3A, the animals from the VPA PN23 group performed worse than those from other groups [day trial age treatment interaction: $F(12,456) = 2.1$, $P < 0.05$]. This effect was particularly evident from the first to the eighth trial (Figure 3B). No differences were observed between VPA PN30 and SAL PN30 as well as between males and females during training sessions.

On the sixth day (when platform was placed in the center of the SW quadrant), no differences were observed between groups regarding proximity to the NE platform. However, when the proximity to the SW platform was considered, animals from the VPA PN23 group performed worse than the other groups from the second to the fourth trial [trial treatment: $F(3,114) = 2.8$, $P < 0.05$; age treatment interaction: $F(1,38) = 5.0$, $P < 0.05$] (Figures 3C and 3D).

When we examined the percentage of time spent in the quadrant with the platform (NE quadrant) we found that, for all experimental groups, it significantly increased over days [day effect: $F(4,152) = 80.8$, $P < 0.001$] and trials [trial effect: $F(3,114) = 28.6$, $P < 0.001$]. This increase was less accentuated in the first day compared to subsequent ones [day trial interaction: $F(12,456) = 1.9$, $P < 0.05$]. As shown in the Figure 4A, the animals from the VPA PN23 group performed worse than the other groups [day trial age treatment interaction: $F(12,456) = 2.5$, $P < 0.01$]. In particular, the percentage of time spent in the quadrant NE of the VPA PN23 group did not change significantly along the first eight trials (Figure 4B). In contrast, for SAL PN23, SAL PN30 and VPA PN30 groups, the percentage of time spent in the quadrant NE significantly increased from the first to the eighth trial (Figure 4B). No differences were observed between males and females during training sessions.

On the sixth day, no differences in percentage of time spent both in the quadrant NE and in the quadrant SW (Figure 4C) were observed between groups.

The lower-order ANOVAs performed on percentage of time spent in each quadrant for each group separately confirmed that the VPA PN23 group performed worse during training sessions and on the sixth day (Figure 5). Note that the acquisition of a preference for the NE quadrant during training sessions is delayed in the VPA PN23 group compared to the other groups. Moreover, while all groups showed a clear preference for the NE quadrant on the first trial of the sixth day, VPA PN23 animals took longer to show a clear preference for the SW quadrant as compared to the other groups.

4. Discussion

Our data shows that VPA exposure during the brain growth spurt significantly affects the swimming speed and impairs learning and memory of animals tested during early postnatal developing stages (from PN23 to PN28). These findings are in accordance with studies that demonstrate poor motor performance and cognitive impairments in children exposed to VPA in utero (Koch et al. 1996; Meador et al. 2011; Ornoy 2009; Vinten et al. 2005) and in mice exposed to VPA (200 and 400 mg/kg) on PN14 and tested on PN22–26 (Wagner et al. 2006). While the mechanisms of action of VPA are not completely understood, there is a consensus that this anticonvulsant enhances GABAergic activity, suppresses NMDA and voltage-dependent sodium channels, and inhibits histone deacetylase (Johannessen and Johannessen 2003; Loscher 2002; Phiel et al. 2001). These multiple actions of VPA during the brain growth

spurt, even if transitory, may disturb the establishment of neuronal circuits leading to impairments in neuronal connectivity and plasticity (Pohl-Guimarães et al. 2011). Accordingly, the suppression of synaptic neurotransmission via blockade of NMDA receptors or activation of GABA receptors can trigger massive apoptotic neurodegeneration (Bittigau et al. 2003). Moreover, VPA actions on both NMDA receptors and voltage-dependent channels may affect the maturation of brain circuits by an overall reduction in neural activity (Maier et al. 1995). Finally, inhibition of histone deacetylase by VPA exposure may lead to increased demethylation of DNA, which in turn, can cause changes in gene expression that would affect, for instance, differentiation of neural progenitors (Shaked et al. 2008) and dendrite morphogenesis (Kim et al. 2009). Considering that, in rats, the brain growth spurt is a critical time for neurogenesis, dendritic arborization and synaptogenesis in the cerebellum and hippocampus (Gil-Mohapel et al. 2010; Guerri 1998), it is possible that damage to neuronal circuits in these areas account for the motor and learning/memory disturbances observed in the VPA group tested from PN23 to PN28.

The absence of differences between VPA and SAL groups in the sample of rats tested during early adolescence (from PN30 to PN35) is suggestive that some neurobehavioral deficits caused by VPA exposure during the brain growth spurt may be attenuated during subsequent development. It is important to notice that deficits in learning and memory, even transitory can still have a striking effect, especially in children at school age. In our study, deficits were observed in a juvenile period, but not during adolescence. While this reversibility may assure the lack of long term problems in our rodent model, the same may not hold true for humans. Deficits in learning and memory, even transitory can still have a striking effect, especially in children at school age. There is an extensive body of evidence on the importance of learning during early children development and how a disruption during this period can lead to poor outcomes (Barnett 1998; Baydar et al. 1993; Breslau et al. 2009).

The transitory deficits in learning and in memory may be associated with compensatory plastic changes, such as rearrangement of synaptic connections, changes in intrinsic physiological properties, or cross-modal regulation from other brain regions (Murphy and Corbett 2009; Plowman and Kleim 2010). Another possibility to be considered is whether the cell death due to VPA exposure was compensated at a later age by an increase in neurogenesis in the hippocampus. This could explain why no differences were observed between groups tested from PN30 to PN35. In fact, similar transitory effects have been reported in animal models of early alcohol exposure (Wozniak et al. 2004).

Considering that the Morris maze performance depends on complex motor skills and sophisticated sensory capabilities (such as visuospatial processing) it is possible that the slower growth observed in the VPA group have also contributed to a transitory deficit in learning and memory. Ontogenetic studies in Long-Evans rats demonstrated that, while pups tested from PN18 to PN19 did not improve their escape latencies over daily 10-trial sessions, the performance of animals tested from PN21 to PN23 improved dramatically (Rudy et al. 1987). Moreover, Long-Evans rats are not fully able to navigate using distal cues between PN21 and PN23 (Tonkiss et al. 1992) and albino rats do not develop mature swim posture and spatial navigation skills until approximately PN21-22 (Schapiro et al. 1970). Thus, it is reasonable to suppose that VPA could be delaying the development of such behavioral attributes. Interestingly, in ferrets, VPA exposure during the third trimester equivalent of human gestation postponed the time of natural eye opening in 4 to 5 days (Pohl-Guimarães et al. 2011). Mice exposed to a single injection of VPA (400 mg/kg sc) at P14 showed retardation in the maturation of behavioral response in inclined plane test (Wagner et al. 2006), while mice exposed to VPA in utero, presented delay in eye opening and nest seeking behavior that were accompanied by reduction in cortical brain-derived neurotrophic factor (BDNF) mRNA expression (Roullet et al. 2010).

5. Conclusion

Our data suggest that the learning memory deficits caused by VPA exposure during the brain growth spurt are transitory. However, it is important to note that childhood is a critical period of development and neurobehavioral deficits during this period, even if transitory, can markedly impair further developmental stages (Benton 2008; Bischof 2007; Innocenti 2007).

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Highlights

The rat brain is vulnerable to valproic acid during the growth spurt

Valproate exposure during the brain growth spurt impairs learning in juvenile rats

Deficits caused by valproate exposure during the brain growth spurt are transitory

Figure 1.

Mean body weight ($\pm$ SEM) of rats exposed to sodium valproate (VPA) or saline (SAL) from postnatal day (PN) 4 to PN10. Note that VPA exposure resulted in a significant body weight reduction. FPLSD: * $P < 0.05$, VPA vs. SAL.

Figure 2.

Mean ($\pm$ SEM) swimming speeds of females (A and B) and males (C and D) exposed to sodium valproate (VPA) or saline (SAL) from PN4 to PN10 and tested in the Morris water maze from PN23 to PN27 (PN23) or from PN30 to PN34 (PN30). In E and F, data represents averaged values from all trials of the females (E) and males (F) during reversal session. Note that during training session, the VPA-exposed animals were slower than SAL ones and such differences were more pronounced in females. During reversal session, no differences were observed between groups. FPLSD: *P < 0.05, **P < 0.01, VPA vs. SAL.

Figure 3.

Platform proximity during training sessions (A) for rats exposed to sodium valproate (VPA) or saline (SAL) from PN4 to PN10 and tested in the Morris water maze from PN23 to PN27 (PN23) or from PN30 to PN34 (PN30). (B) Percentage of reduction in platform proximity from the first to the eighth trial. (C) Platform proximity along trials on the sixth day (probe session). Note that the performance of the VPA PN23 group was worse than the other groups. During training and reversal sessions, the platform was located at NE and SW quadrants respectively. Values are means ($\pm$ SEM). FPLSD: * $P < 0.05$, ** $P < 0.01$ VPA PN23 vs. SAL PN23; # #.

Figure 4.

Percentage of time spent in the NE quadrant during training sessions (A) for rats exposed to sodium valproate (VPA) or saline (SAL) from PN4 to PN10 and tested in the Morris water maze from PN23 to PN27 (PN23) or from PN30 to PN34 (PN30). (B) Percentage of time spent in the quadrant with the platform in the first and the eighth trial. (C) Percentage of time spent in the quadrant with the platform on the sixth day (reversal session). During training and reversal sessions, the platform was located at NE and SW quadrants respectively. Values are means ($\pm$ SEM). Note that the VPA PN23 group performed worse than the SAL PN23 group from the first to the eighth trial. No differences were observed between VPA PN30 and SAL PN30. FPLSD: * $P < 0.05$, VPA PN23 vs. SAL PN23. Paired t-test ** $P < 0.01$, *** $P < 0.001$.

Figure 5.

Mean ($\pm$ SEM) of percentage of time spent in each quadrant for rats exposed to sodium valproate (VPA) or saline (SAL) from PN4 to PN10 and tested in the Morris water maze from PN23 to PN27 (PN23) or from PN30 to PN34 (PN30). Note that, during the training period (from the first to the fifth session), the VPA PN23 group presented a delayed preference for the NE quadrant (with the platform), of at least four trials compared to the other groups. On the sixth day (when the platform was placed in the SW quadrant), the VPA PN23 group took longer to show a clear preference for the SW quadrant. FPLSD: *P < 0.05: NE vs. all other quadrants; #P < 0.05: SW vs. all other quadrants.