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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 (200 mg/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.

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

In humans, most of the 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 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; Roullet 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

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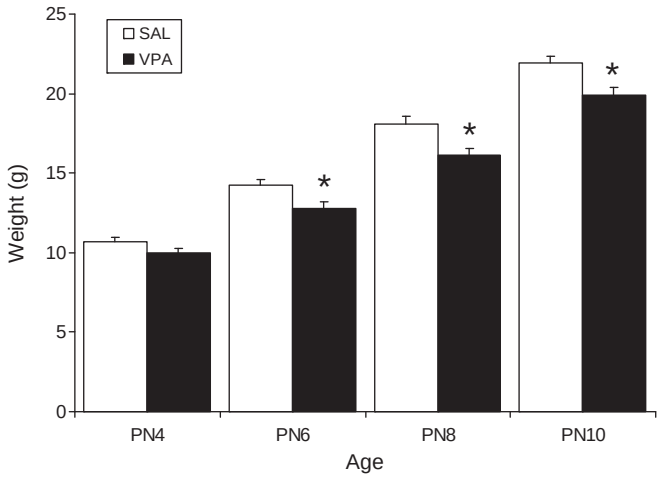

Fig. 1. Mean body weight (±SEM) of rats exposed to sodium valproate (VPA) or saline (SAL) from postnatal day (PN) 4 to PN10. Note that VPA exposure resulted in a significant body weight reduction. FPLSD: *P<0.05, VPA vs. SAL.

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 12:12 h 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 1/2 animals were injected with 200 mg/kg of VPA (IP, in saline solution dissolved at 20 mg/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 × 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 13:00 h and 16:00 h. Each animal was given four trials a day. Each trial had a 60 s 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 s. If the animal failed to reach the platform within 60 s, the experimenter gently guided the animal through the water and placed it on the platform where it would remain for 15 s. 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 GreenhouseGeisser