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Effect of early postnatal exposure to valproate on neurobehavioral development and regional BDNF expression in two strains of mice

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ABSTRACT

Valproate has been used for over 30 years as a first-line treatment for epilepsy. In recent years, prenatal exposure to valproate has been associated with teratogenic effects, limiting its use in women that are pregnant or of childbearing age. However, despite its potential detrimental effects on development, valproate continues to be prescribed at high rates in pediatric populations in some countries. Animal models allow us to test hypotheses regarding the potential effects of postnatal valproate exposure on neurobehavioral development, as well as identify potential mechanisms mediating observed effects. Here, we tested the effect of early postnatal (P4–P11) valproate exposure (100 mg/kg and 200 mg/kg) on motor and affective development in two strains of mice, SVE129 and C57Bl/6N. We also assessed the effect of early valproate exposure on regional BDNF protein levels, a potential target of valproate, and mediator of neurodevelopmental outcomes. We found that early life valproate exposure led to significant motor impairments in both SVE129 and C57Bl/6N mice. Both lines of mice showed significant delays in weight gain, as well as impairments in the righting reflex (P7–8), wire hang (P17), open field (P12 and P21), and rotarod (P25 and P45) tasks. Interestingly, some of the early locomotor effects were strain- and dose-dependent. We observed no effects of valproate on early markers of anxiety-like behavior. Importantly, early life valproate exposure had significant effects on regional BDNF expression, leading to a near 50% decrease in BDNF levels in the cerebellum of both strains of mice, while not impacting hippocampal BDNF protein levels. These observations indicate that postnatal exposure to valproate may have significant, and region-specific effects, on neural and behavioral development, with specific consequences for cerebellar development and motor function.

1. Introduction

Epilepsy is the most common neurological condition in pediatric populations, affecting 10.5 million or 1% of children under the age of 15, constituting ~25% of individuals with epilepsy [1,2]. While a number of treatments have been developed for pediatric seizures, valproate is still heavily utilized in many pediatric populations around the world, with prescribing rates as high as 50% in Jordan [3], 40% in Singapore [4], 25% in India [5], and 22% in the US [6]. While valproate is known to have teratogenic effects on fetal development, the impact of pediatric exposure on neurobehavioral development has remained less clear [7]. In humans, few effects of drug exposure (between the ages of 6 and 15 years old) have been identified on measures of cognitive functioning [8,9], and some studies have actually identified possible improvements in cognitive functioning and IQ scores in individuals with epilepsy on valproate [10]. However, these studies contradict previous work that failed to identify beneficial effects of valproate on cognitive outcomes and identified potential negative effects on motor performance and visuospatial functioning [11] as well as high rates of co-morbid pathology [6].

In animal models, the majority of work related to valproate exposure has focused on the teratogenic effects of prenatal exposure and its possible contribution to the development of ASD-like phenotypes [12,13]. Study of the effects of postnatal exposure to valproate has been limited, with a great deal of variation in the dosing regimen as well as the timing and duration of drug exposure. For example, work in mice (BALB/c) found that postnatal exposure (P14) to a single dose of valproate (200 or 400 mg/kg) led to transient impairments in midair righting reflex and negative geotaxis, effects that rapidly resolved, and found no effect of drug treatment on general locomotor activity [14,15]. Interestingly, using a near-identical paradigm of drug exposure in C57BL/6 mice, other groups have found more limited effects of valproate on these same measures [16]. Long Evans rats exposed during the early postnatal period (P6–P12) to valproate (150 mg/kg) were found to have sensory-motor gating abnormalities and impairments in fine motor performance [17], while Sprague Dawley rats exposed from P6–P18 to 150 mg/kg of valproate showed deficits in the development of social play [18]. Relevant to neural development, P4–P18 rats

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exposed to 75-200 mg/kg of valproate were found to have a significant decrease in brain weight [19] and rats exposed to three days of 50 to 400 mg/kg of valproate between the ages of P3 and P30 were found to have increased apoptotic cell death throughout the brain, suggesting widespread loss of cells [20–22]. Together, these results provide compelling support for more in-depth studies of the effects of valproate on postnatal brain development.

The mechanisms underlying the potential adverse effects of postnatal exposure to valproate remain unknown. Recent evidence suggests that valproate may impact expression of key genes that support neural development, such as brain derived neurotrophic factor (BDNF) [21,23,24], potentially through epigenetic effects on the timing or level of expression [25]. Brain derived neurotrophic factor is a critical signaling molecule that is highly expressed in the brain and supports such basic processes as cell proliferation, migration, survival, and growth [26–28]. In addition, BDNF expression is regionally selective, and changes dynamically over development [29]. Studies of valproate-induced changes in BDNF expression during the prenatal and postnatal period in rodents have also been limited. Rats receiving high doses of valproate (600 mg/kg) during the prenatal period (E8) were shown to have elevated expression of BDNF in the neural tube [23]. Similarly, *in vitro* cortical neuronal cultures from postnatal rat showed elevations in *bdnf* mRNA levels following valproate treatment [24]. Rats receiving valproate during the postnatal period have been found to show reduced *bdnf* gene expression within 24 h of treatment [21,30], however, these studies all report only transcript levels, and are based upon whole brain lysates. Whether these observations translate into changes in BDNF protein, or if the effects of valproate on BDNF levels are regionally restricted at these points in development remain open questions.

Here, we sought to test the behavioral consequences of early postnatal exposure to valproate on motor and affective outcomes, and to compare and contrast results from two independent strains of mice. In addition, we tested the effect of early postnatal valproate treatment on BDNF protein levels by ELISA, across strains of mice and doses, and in two separate brain regions, the cerebellum and the hippocampus. Based upon these results, we hope to better understand the effects of postnatal exposure to valproate on brain and behavioral development, identify possible mechanism driving observed outcomes, and test for possible regional differences in susceptibility to drug exposure.

2. Methods

2.1. Animals

Male and female C57Bl/6N as well as SVE129 founder mice were obtained from Charles River labs and maintained as separate breeding lines. All mice in the current study were bred in house from approximately 10 breeding pairs (5 for each line), weaned and sex segregated at P21, and were housed under a 12-h light/dark cycle with food and water *ad libitum*. Testing occurred prior to sexual maturation, and we did not observe any apparent sex differences on behavioral outcomes. Thus, data from both males and females were merged. All handling, injections, and behavioral tests were done between the hours of 9 a.m. and 12 p.m., during the lights on phase of the diurnal cycle. All testing was approved by the Brown University internal animal care and use committee (IACUC) and was consistent with the guide for the care and use of laboratory animals.

2.2. Drug delivery

To eliminate potential effects of differences in maternal care on behavioral outcomes, mice from all treatment groups were included in each litter (e.g. each litter included saline, Valp100, and/or Valp200 treated mice). Specifically, pups within litter were color coded by daily marking of the tail with lab marker (red, black, or blue). Within each litter, one group of pups received saline injection and the remaining animals received treatment with valproate. Color associated with saline treatment was counterbalanced between litters. Valproate was prepared by dissolving valproic acid (10 mg or 20 mg; Sigma Aldrich) in sterile saline (1 mL). Drug delivery began on postnatal day 4, with daily injections occurring through postnatal day 11. Mice received one of two doses, 100 mg/kg (Valp100) or 200 mg/kg (Valp200) valproate. Injections were administered subcutaneously, under the nape of the neck, once daily from postnatal day 4 (P4) to P11 between the hours of 9 a.m. and 12 p.m.

2.3. Righting reflex

To measure basic motor coordination during early postnatal development, we tested mice on the righting reflex on postnatal days 7 and 8. Testing occurred in the a.m. prior to the administration of saline or valproate for that day, to insure that effects were not the result of acute effects of drug exposure or stress associated with injection. Briefly, mice were placed onto their backs on a flat dry surface and the latency to right themselves (as defined as all four paws on the ground) was measured over six consecutive trials. The surface was thoroughly cleaned with 70% ethanol between animals. Behavior of the mice was video recorded and latency to right was assessed from video recordings by an observer blind to the treatment condition. If a mouse failed to right, it was given a time of 90 s (maximum trial duration) indicating the failure to right. The average latency to right for each animal was calculated and mean latency to right was compared across groups within each mouse strain. For SVE129 mice, we tested $n = 8$ saline and $n = 4$ Valp200. For C57Bl/6N mice, we tested $n = 16$ saline, $n = 3$ Valp100, and $n = 7$ Valp200.

2.4. Small open field

To measure the effect of early life valproate treatment on early locomotor behavior, mice were tested with the small open field test at postnatal day 12. Briefly, mice were placed into a small open field ($12 \times 6 \times 5$ in.) under low light (~5–10 lx) and allowed to freely explore for a period of 5 min. Behavior of the mice was digitally recorded and locomotor activity was assessed with the aid of digital tracking software (Noldus Ethovision XT 8.5). Total distance traveled was measured for each mouse. The boxes were thoroughly cleaned with 70% ethanol between subjects. For SVE129 mice, we tested $n = 18$ saline and $n = 6$ Valp200. For C57Bl/6N mice, we tested $n = 42$ saline, $n = 14$ Valp100, and $n = 16$ Valp200.

2.5. Large open field

To test the effect of early life valproate on locomotor activity and anxiety-like behavior at the time of weaning (P21), mice were tested in the large open field. Briefly, mice were placed into a large empty box ($24 \times 20 \times 12$ in.) under low light (~5–10 lx) and allowed to freely explore for 10 min. Behavior of the mice was digitally recorded and tracked with the aid of digital tracking software (Noldus Ethovision XT 8.5). Boxes were thoroughly cleaned with 70% ethanol between animals. To test for drug effects on locomotion, we tracked the total distance traveled during the trial. To assess anxiety-like behavior, the arena was digitally divided into 12 equal zones and the two center zones were defined as the center area. The percent entries as well as percent time spent in the center zones was used as an index of anxiety-like behavior. For SVE129 mice, we tested $n = 13$ saline and $n = 6$ Valp200. For C57Bl/6N mice, we tested $n = 48$ saline, $n = 14$ Valp100, and $n = 15$ Valp200.

2.6. Wire hang

To measure both strength and stamina of mice receiving early valproate treatment, we employed the wire hang test at postnatal day 17 (P17). Using a standard cage top (Allentown Cages), mice were