

A New Neurobehavioral Model of Autism in Mice: Pre- and Postnatal Exposure to Sodium Valproate

George C. Wagner · Kenneth R. Reuhl ·
Michelle Cheh · Paulette McRae · Alycia K. Halladay

Published online: 12 April 2006
© Springer Science+Business Media, Inc. 2006

Abstract Autism symptoms, including impairments in language development, social interactions, and motor skills, have been difficult to model in rodents. Since children exposed in utero to sodium valproate (VPA) demonstrate behavioral and neuroanatomical abnormalities similar to those seen in autism, the neurodevelopmental effects of this antiepileptic agent were examined in mice following its pre- or postnatal administration. Exposed pups were evaluated in a battery of neurodevelopmental procedures designed to assess VPA-induced retardation (wherein a behavior fails to mature on schedule), regression (wherein a behavior does mature on time but then deteriorates), or intrusions (wherein normal behaviors are overshadowed by stereotypic or self-injurious behaviors). The resulting observations were interpreted in the context of this new strategy to model autism.

Keywords Animal model · Autism · Sodium valproate

G. C. Wagner · P. McRae
Department of Psychology, Rutgers University, New Brunswick,
New Jersey, USA

K. R. Reuhl · A. K. Halladay
Department of Pharmacology and Toxicology,
Rutgers University, New Brunswick, New Jersey, USA

M. Cheh
Department of Neuroscience, Rutgers University,
New Brunswick, New Jersey, USA

G. C. Wagner (✉) · K. R. Reuhl
Center for Childhood Neurotoxicology and Exposure
Assessment, Rutgers University, New Brunswick,
New Jersey 08854, USA
e-mail: gcwagner@rci.rutgers.edu

Autism is a developmental disorder characterized by impaired social interactions, impaired verbal and non-verbal communication, and the appearance of unusual stereotypic and sometimes self-injurious behaviors. The etiology of autism remains unknown but is thought to involve a genetic predisposition interacting with exposure to environmental neurotoxicants. One of the most striking features of autism is that cognitive and sensory/motor development may progress symptom free for several months to years but is then followed by a period of *retardation* (wherein some skills fail to develop or do so well behind schedule), or a period of *regression* (wherein some acquired skills are lost), or a period of *intrusion* (wherein acquired skills are overshadowed by the appearance of behaviors aberrant in form or frequency). The onset and duration of these symptoms may not coincide with time of injury. That is, following neurodevelopmental injury, behavioral deficits may fail to be observed until the time at which affected brain regions contribute to functional outcome. The maturation of different brain regions and cellular structures at the time of injury may explain recovery of function after damage or impairments specific to onset of testing (Goldman, 1971; Miller, Goldman, & Rosvold, 1973). Importantly, the period when clinical signs first begin to appear may represent a time when neurotoxic agents have already accumulated in brain to critical levels and/or the deleterious effects of earlier exposures may manifest through perturbation of normal ontogeny of neural pathways. Neurotoxicant exposure during critical periods may disrupt neurobehavioral development by altering neural migration, circuitry, and/or synaptogenesis of brain areas required for expression of these behaviors, resulting in behavioral retardation, regression, and/or intrusions.

Traditionally, animal models of autism have not been used to systematically examine retardation, regression and/

or intrusions but instead, tend to focus on a single aspect of neurobehavioral development. Likewise, evaluation of subjects in a battery of neurobehavioral tests may allow for detection of deviations from the normal ontogeny but traditionally, these functional test batteries have not been used to categorize patterns of toxicant-induced deficits into the categories of retardation, regression or intrusions. In the present studies, mice were exposed to sodium valproate (VPA) either pre- or postnatal and then evaluated in a series of neurodevelopmental tasks, which targeted cerebellar, striatal and hippocampal function during critical developmental periods. Based on their nature and timing, the VPA-induced deficits were characterized in this framework of retardation, regression or intrusions, thus reflecting the major developmental components of autism without attribution to specific symptoms of the disease. The treated mice are not considered autistic; rather, they show developmental deficits in a fashion parallel to those observed in autism.

Sodium valproate was chosen to test this model following reports of an association between autism and prenatal exposure to this teratogen. VPA was chosen for study based on several observations: First, VPA exposure at the critical time of neural tube closure produced changes in Purkinje cell number and cerebellar cell volume consistent with those seen in autistic children (Ingram, Peckharm, Tisdale, & Rodier, 2000; Sobaniec-Lotoweska, 2001). Furthermore, clinical studies of children exposed to VPA in utero have characterized a “fetal valproate syndrome” that show phenotypic similarities to autism and includes deficits in language and communication, stereotypic and hyperexcitable behavior, and global delays in behavioral development (Ardinger et al., 1988; Koch et al., 1996; Mawer, Clayton-Smith, Coyle, & Kini, 2002; Moore et al., 2000; Williams et al., 2001). Previous studies have also demonstrated impairment in cognitive, motor, attention and social development in rodents administered VPA in utero or during weaning (Chapman & Cutler, 1989; Voorhees, 1987; Wu & Wang, 2002). Accordingly, in the present studies, mice were exposed to VPA either in utero or postnatally. The exposure period of E13 was chosen as it corresponds to the final stages of Purkinje cell generation in the mouse (Inouye & Murakami, 1980). The early postnatal time point of day 14 was chosen on the basis of three observations: (a) cerebellar organization is essentially complete but hippocampal and striatal differentiation and migration are continuing (Bachevalier & Beauregard, 1993; Rice & Barone, 2000; Voorhees, 1986); (b) proliferation and migration are complete in the rat but synaptogenesis and gliogenesis continue beyond postnatal day 14 (Voorhees, 1986) and, (c) critical behaviors including mid-air righting and negative geotaxis, mature or first appear on this day in the BALB/c mouse. The behavioral tests

used for evaluation of the VPA-treated mice have been linked to brain regions known to be affected by VPA and/or show abnormal morphology in autism: cerebellum (mid-air righting, surface righting, negative geotaxis); striatum (motor activity, visible platform water maze) and hippocampus (passive avoidance, hidden platform water maze). Each of these behaviors consistently matures in control mice on a predictable day. Thus, the VPA-induced deficits observed in the present study could be characterized relative to the controls as retardations, regressions, or intrusions. It was concluded that this approach of categorizing neurobehavioral deficiencies according to this ontogenic timeline would be useful strategy for an animal model autism.

Methods

Prenatal Sodium Valproate (VPA) Administration

Male and female BALB/c mice (Taconic, Germantown, NY) were housed together in plastic cages with standard wood chip bedding and free access to food and water. All mice were maintained in an AAALAC-accredited facility under guidelines set forth by the National Institutes of Health. Lights were set on a 12 h on: 12 h off-cycle and temperature was maintained at 25°C. Females were checked before 10 AM for presence of a vaginal plug which was recorded as day 0 of embryonic development. Pregnant females were treated subcutaneously on embryonic days 12–17 (E12–17) with 200 mg/kg sodium valproate (VPA; 2-propylpentanoic acid sodium salt, Sigma, St. Louis MO) dissolved in saline and administered at a concentration of 0.1 ml/kg. Control females were treated with saline on days (E12–17). In a separate study, female breeding mice were treated with either saline or VPA 600 mg/kg s.c. on E13 only. Day of birth was recorded as day 0 and all pups were labeled for individual identification. Body weight was measured daily. Of the 22 VPA and 9 saline-treated dams treated on E13, a total of 9 VPA and 8 saline litters survived. This yielded a total of 31 VPA-exposed pups ($n = 14$ female and $n = 17$ male) and 39 saline pups ($n = 20$ female and $n = 19$ male).

Postnatal Sodium Valproate Administration

Untimed late-pregnant BALB/c female mice (Taconic, Germantown, NY) were housed individually in plastic cages in conditions identical to those above. Day of birth was recorded as day 0. Female pups were removed from the cage on day 5. Male mice were treated with either saline ($n = 45$) or VPA 200 mg/kg ($n = 25$) or VPA 400 mg/kg ($n = 17$) s.c. on postnatal day 14 of life. Behavioral testing

began on day 13 and continued on day 14 prior to the VPA/saline administration; thereafter behavioral testing continued through day 26 and again at 4 months of age.

Finally, since a high dose of sodium valproate produces sedation, an additional control group was used to determine if behavioral consequences 24 h after administration were due to residual drug effects. Mice were injected on day 14 with 50 mg/kg VPA ($n = 10$) or saline ($n = 10$) and behavioral testing conducted 3 h later. This dose was calculated based on previous pharmacokinetic studies in neonatal rats, guinea pigs and sheep showing the half-life to be about 7 h (Haberer & Pollack, 1994; Wong et al., 2000; Yu, Sugiyama, & Hanano, 1985). Therefore, 50 mg/kg was chosen as estimation of the concentration in the body if a dose of 400 mg/kg was administered 21 h earlier.

Behavioral Testing Procedures

Surface Righting

Each mouse was placed on its back and gently held with all four limbs extended outward at which time it was released. Time to right such that all four paws were touching the surface was recorded. A maximum score of 30 s was recorded when the mouse failed to right in that period. Mice were tested on days 5–9 of life.

Mid-air Righting

Ability to right in mid-air was assessed on days 13–19 of life by holding the mouse by the scruff of the neck ventral side up with all four paws extended upward 30 cm above a padded surface. Ability to right was scored positive if the mouse landed on all four paws. A score of two out of three successful mid-air righting attempts was recorded as ability to right on each day.

Hanging Wire Grip Strength

On postnatal days 13–19 of life, mice were placed on a grid wire surface (30 cm \times 18 cm divided into 1.2 cm grid squares). The plane was inverted and held 30 cm above a padded surface. Latency to fall was recorded with a maximum of 30 s for each trial.

Negative Geotaxis

Negative geotropism was tested on postnatal days 13–19 by placing the mouse on the same wire grid as used in the hanging wire test. Each mouse was placed facing downward along a 45° incline. Latency to turn 180° such that the head was facing upward along the incline was recorded with a maximum of 30 s for each trial.

Balance Beam

On days 20–26 of life, mice were placed mid-way down an elevated beam measuring 2 cm wide and 120 cm in length. With room lights-off, a 60 watt bulb illuminated one side of the beam while the other side led to an enclosed, darkened box measuring 15 cm³ and lined with bedding material from the animal's home cage. Latency to traverse the beam and enter the darkened box was recorded with a 60 s maximum for each trial.

Water Maze

The maze consisted of a circular tub measuring 71 cm in diameter and 29 cm in height. The tub was painted white on the interior and was filled 3/4 full with water maintained at 23–26°C and made opaque with white non-toxic latex paint. A starting point was determined randomly from one of four equally spaced quadrants. In the visible platform version of the water maze, a platform measuring 8 cm in diameter and painted black was placed in one quadrant of the maze and the water was only allowed to fill such that the platform sat 1.5 cm above the surface. In the hidden platform, an identical platform painted white sat 2 cm below the surface of the water. Animals received five trials each day and each animal was allowed a maximum of 60 s to reach the escape platform. The position of the hidden platform remained constant throughout the experiment and the room was illuminated and extramaze cues were present. If the animal did not reach the platform in 60 s, a score of 60 was recorded and the animal was gently guided to and placed on the platform. During the intertrial interval, all animals rested atop the platform until the next trial began.

Passive Avoidance

On postnatal day 21, mice were placed on one side of a Plexiglass shuttlebox 27 cm in length, 10.7 cm wide and 16.8 cm high. The floor was made of stainless steel bars with a 0.75 cm space between each. A 45-watt lightbulb illuminated the start side of the box and mice were allowed to enter the compartment on the opposite side, which was dark. Upon entering the darkened area, a 1 mA scrambled footshock was delivered on that side only. Animals were allowed to return to the start box or remain in the shocked compartment for a maximum of 10 s. The trial ended if the animal remained in the start box for 120 s. Escape responses (returning to the start box) also terminated the trial and ended the shock. Animals were tested on four trials per day from postnatal day 21 until reaching a criterion of three consecutive correct avoidances of the footshock.

Motor Activity

Habituation and motor activity were assessed on days 20–26, immediately prior to water maze and balance beam tests. The chamber consisted of a black 42 × 22 × 14 cm Plexiglass box. Six infrared sensors placed approximately 7 cm apart and 2.5 cm above the floor were used to measure activity over a 10 min period.

Self-injurious Behavior

At 11 weeks of age, male mice treated on postnatal day 14 with VPA 400 mg/kg s.c. ($n = 11$) or saline s.c. ($n = 12$) were administered d-amphetamine (Sigma, St. Louis, MO) at 22.5 mg/kg s.c. and observed in their home cage for incidence of self-injurious and stereotypic behaviors at 15, 30 and 60 min after injection. The presence of self-biting and oral dyskinesias was recorded as either “yes” or “no.” Stereotypic behavior was rated as either occurring over a large area of the cage (1), in bursts in the same area (2) or continuously in the same area (3). This rating scale was adapted from Kelley, Sevoir, and Iversen (1975).

Statistical Analysis

All behavioral analysis were performed using a repeated measures ANOVA including both group, day, and sex as main factors, with the exception of the mid-air righting response and self-injurious behavior, which was analyzed using Chi-Square and Fisher’s Exact Test.

Results

Prenatal Administration of 200 mg/kg, E12–E17

Mice born to dams treated on days E12–E17 with 200 mg/kg VPA showed minor deficits when tested for negative geotaxis on postnatal days 6–14. The ANOVA revealed a significant effect of day, where latency increased across days [$F(8,64) = 7.8, P < 0.0001$]. In addition, the VPA treatment resulted in a significantly longer latency on day 10 [$F(8,64) = 3.6, P = 0.002$] as compared to controls. Otherwise, there were no major effects of prenatal VPA treatment on any of the other behaviors in male pups given lower doses spread out across days.

Prenatal Treatment with 600 mg/kg VPA on E13

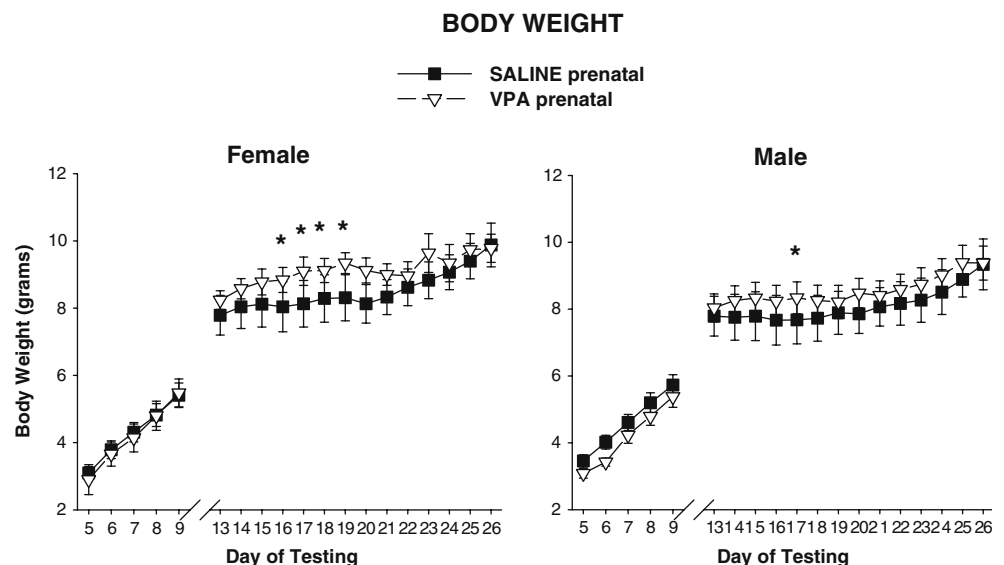
Body Weight

A two-factor repeated measures ANOVA of body weight data from postnatal days 5–26 revealed a significant increase in body weight [$F(18,612) = 222.4, P < 0.0001$]. In addition, there was a significant interaction of day and treatment with VPA-treated pups having higher body weights than saline-treated controls on days 18, 19, 21 and 21 [$F(18,612) = 2.2, P = 0.002$] (Fig. 1).

Surface Righting

Both male and female pups receiving VPA treatment in utero displayed longer latencies to surface right on P5 and P6 [$F(1,66) = 22.7, P < 0.0001$]. Both males and females, treated with saline or VPA, showed significant

Fig. 1 Changes in body weight in postnatal female (left) and male (right) BALB/c mice following administration of sodium valproate (VPA, 600 mg/kg s.c.) on embryonic day 13. *Indicates significantly different from saline-treated mice using Fisher’s PLSD, $P < 0.05$



improvements in this behavior over time, with VPA treatment resulting in a delay in the ability of this skill, independent of sex (Fig. 2).

Mid-air Righting

χ^2 analysis revealed a significant overall effect of VPA treatment on mid-air righting in males [$\chi^2(6) = 35.2, P < 0.0001$] and females [$\chi^2(6) = 23.2, P < 0.0001$]. Fisher’s Exact Test further revealed that female pups treated with VPA in utero were impaired in mid-air righting on P15 ($P = 0.0001$) and males were impaired on P14 ($P = 0.01$), P15 ($P = 0.004$) and P16 ($P < 0.0001$) (Fig. 3).

Hanging Wire

A two-factor repeated measures ANOVA revealed that both control and VPA-treated mice of both sexes showed a significant improvement in hanging wire grip strength over days tested [$F(6,288) = 36.2, P < 0.0001$]. There was also a significant effect of prenatal drug exposure [$F(1,48) = 20.8, P < 0.0001$] and of sex [$F(1,48) = 4.9, P = 0.03$] such that animals exposed to VPA gestationally showed a shorter latency to fall from a suspended wire, with males performing significantly better than females in the VPA treatment group only (Fig. 4).

Negative Geotaxis

A repeated-measures ANOVA revealed no significant effect of treatment, sex, or day of testing, on negative geotaxis. Pups exposed in utero to VPA did not show any deficits in acquiring this skill.

Balance Beam

There was no significant effect of sex or treatment on balance beam latency. However, there was a significant interaction of sex by day, with male VPA-treated pups being faster to cross the beam as compared to female VPA-treated pups on P24 and P26 [$F(6,228) = 2.9, P = 0.008$].

Water Maze

Preliminary investigation of the ontogeny of water maze performance in BALB/c mice revealed that, independent of what day training is initiated, a significant reduction in latency to escape to a hidden platform does not emerge until around P23. Analysis of escape latency was calculated based on the average of all five trials on days P20–26. On P23, saline-treated animals showed a significant improvement in ability to find the hidden platform [$F(6,264) = 8.4, P < 0.0001$]. This was not the case for VPA-treated pups, independent of sex [$F(1,44) = 6.3, P = 0.01$]. In fact, VPA-treated pups did not show a significant improvement across days. On days P23 and P25, both male and female pups previously exposed to VPA were significantly impaired in water maze performance; only males continued to be affected on P26 [$F(6,264) = 3.4, P = 0.003$] (Fig. 5).

Motor Activity

Each analysis consisted of a total activity count for the full 10-min period and an analysis of each 2 min bout, with both treatment and sex as independent variables. Between day analysis examining total activity counts for the 10 min period showed a significant increase in

Fig. 2 Impairments in the ability to right on a surface in postnatal female (left) and male (right) BALB/c mice following administration of sodium valproate (VPA, 600 mg/kg s.c.) on embryonic day 13. *Indicates significantly different from saline-treated mice using Fisher’s PLSD, $P < 0.05$

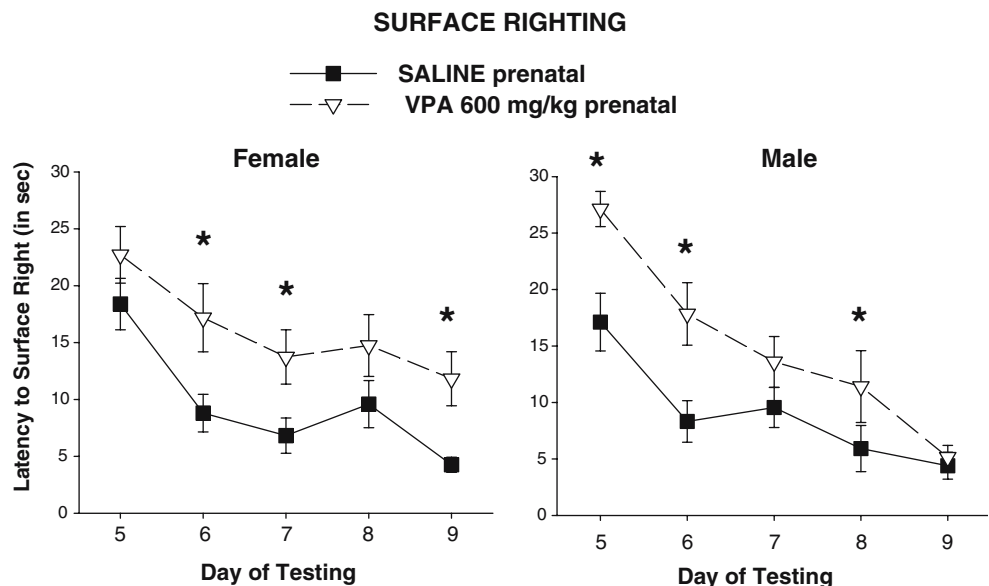
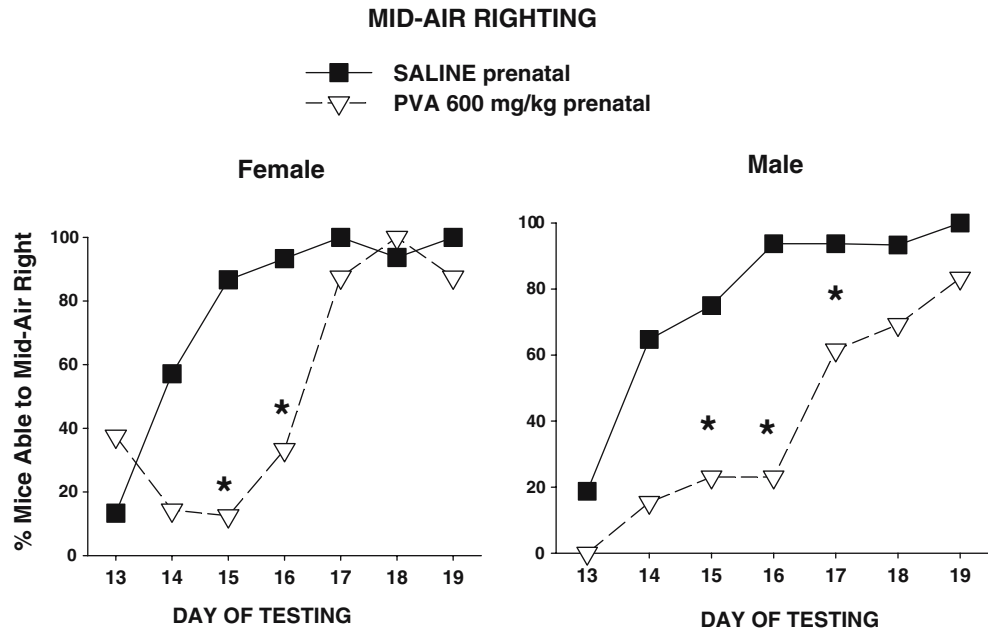


Fig. 3 Impairments in the ability to right in mid-air in postnatal female (left) and male (right) BALB/c mice following administration of sodium valproate (VPA, 600 mg/kg s.c.) on embryonic day 13. *Indicates significantly different from saline-treated mice comparing percentage of animals successful 2 out of 3 testing trials using Fisher's Exact Test, $P < 0.05$



activity across days [$F(6,204) = 7.8, P < 0.0001$] as well as a significant sex difference [$F(1,34) = 5.3, P = 0.02$], with females showing higher activity counts (Fig. 6). Within-days analysis on each specific 2 min bout revealed animals only showed within-days habituation on P23 (Fig. 7). On P22, VPA treatment resulted in consistently higher activity counts [$F(1,35) = 4, P = 0.05$] on bouts 2–4 (4–8 min) only, and a significant day by treatment interaction that approached significance ($P = 0.06$).

Treatment with VPA on P14

Body Weight

When expressed as a percent of saline-treated control, body weight analysis revealed a significant effect of both day [$F(6,198) = 15.9, P = 0.0001$] and an interaction of day and treatment [$F(6,198) = 15.8, P = 0.0001$] with no significant main effect of treatment. Treatment with 400 mg/kg VPA resulted in a significant decrease in body weight

Fig. 4 Grip strength ability measured using the hanging wire test on postnatal days 13–19 in female (left) and male (right) BALB/c mice administered sodium valproate (VPA, 600 mg/kg s.c.) on embryonic day 13. *Indicates significantly different from saline-treated mice using Fisher's PLSD, $P < 0.05$

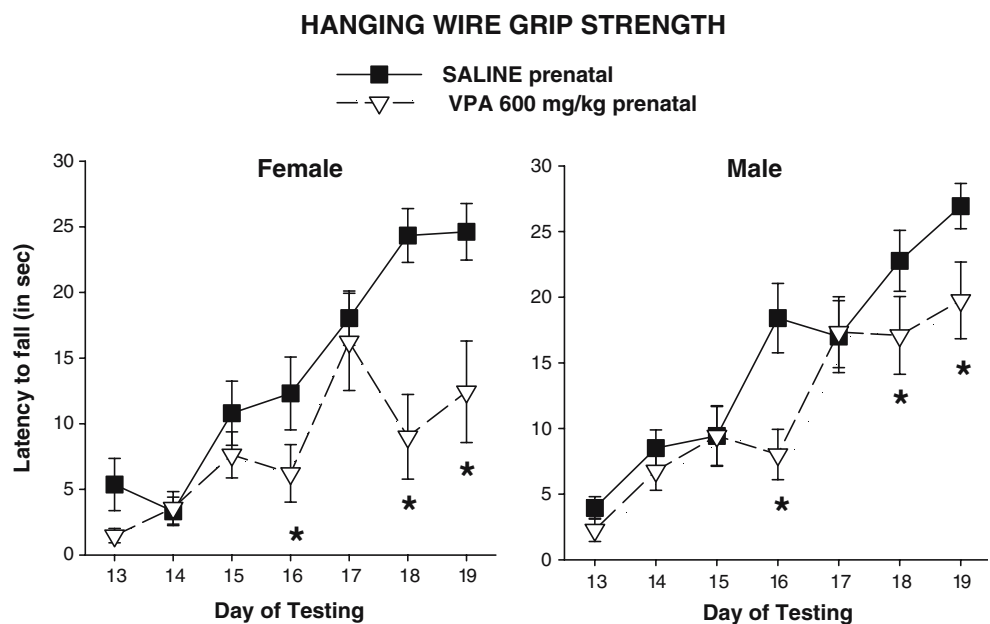
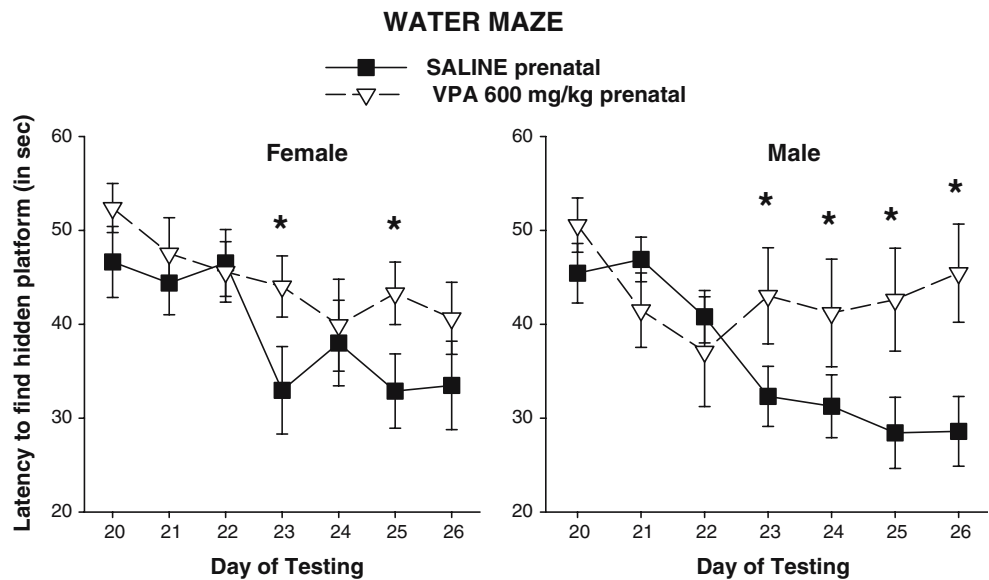


Fig. 5 Spatial learning ability measured in the water maze on postnatal days 20–26 in female (left) and male (right) BALB/c mice administered sodium valproate (VPA, 600 mg/kg s.c.) on embryonic day 13. *Indicates significantly different from saline-treated mice using Fisher's PLSD, $P < 0.05$



on postnatal days 16–18 (2–4 days following VPA administration). By day 22 and thereafter, no differences were observed between groups (Fig. 8).

Mid-air Righting

χ^2 Analysis revealed a significant overall effect of VPA treatment on mid-air righting for both the 400 mg/kg dose [$\chi^2(6) = 64.7, P < 0.0001$] and the 200 mg/kg dose [$\chi^2(6) = 66.2, P < 0.0001$]. Fisher's Exact Test further revealed that both the 200 mg/kg and 400 mg/kg dose produced significant ($P < 0.0001$) deficits in mid-air righting on P15 and P16 (24–48 h after treatment). Pups were able to mid-air right on P14 and this ability was lost following VPA treatment (Fig. 9).

Hanging Wire

A two-factor repeated measures ANOVA revealed a significant improvement in grip strength over days tested [$F(5,236) = 35.6, P < 0.0001$]. However, postnatal VPA treatment did not alter this behavior.

Negative Geotaxis

The effects of 400 mg/kg VPA on postnatal day 14 were analyzed using a repeated measures ANOVA. A significant decrease in latency to turn 180° was observed [$F(6,228) = 2.4, P = 0.03$], as well as a significant effect of drug treatment [$F(1,38) = 3.9, P = 0.05$]. Animals treated with VPA exhibited longer negative geotactic

Fig. 6 Horizontal Motor Activity on postnatal days 23–26 during a 10 min testing period. BALB/c mice were tested following administration of saline or sodium valproate (VPA, 200 mg/kg or 400 mg/kg s.c.) on postnatal day 14

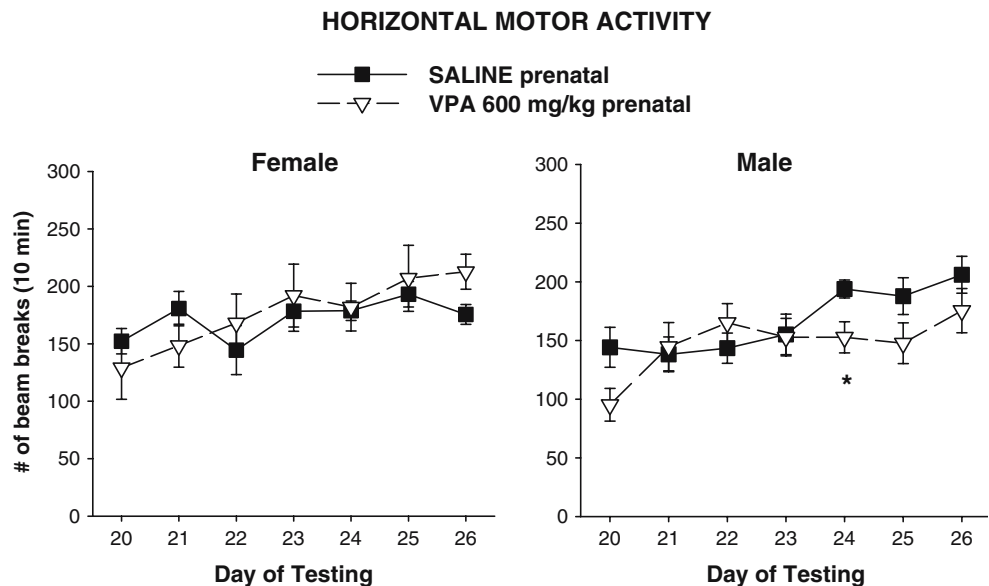
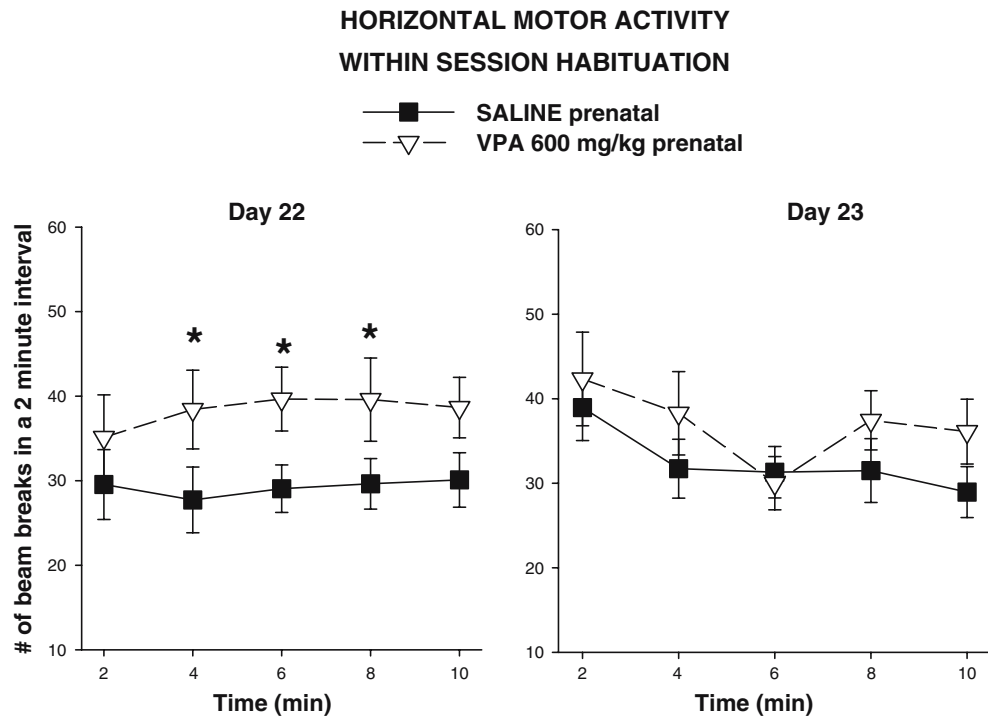


Fig. 7 Within-session habituation on postnatal days 22 and 23 in male BALB/c mice following administration of sodium valproate (VPA, 600 mg/kg s.c.) on embryonic day 13. The x-axis represents motor activity for each 2 min period across the 10 min trials on each day. *Indicates significantly different from saline-treated mice, using Fisher's PLSD, $P < 0.05$



latencies across days, an effect that was significant on P16 (Fig. 10).

[$F(3,93) = 5.7, P < 0.0001$]. This effect was unchanged by VPA treatment.

Balance Beam

Water Maze

All mice treated on P14 and tested on balance beam on P23–26 demonstrated shorter latencies across days

A repeated measures ANOVA was performed across days using the average of the five trials per day for saline

Fig. 8 Changes in body weight in postnatal male BALB/c mice following administration of sodium valproate (VPA, 400 mg/kg s.c.) on postnatal day 14. *Indicates significantly different from saline-treated mice using Fisher's PLSD, $P < 0.05$

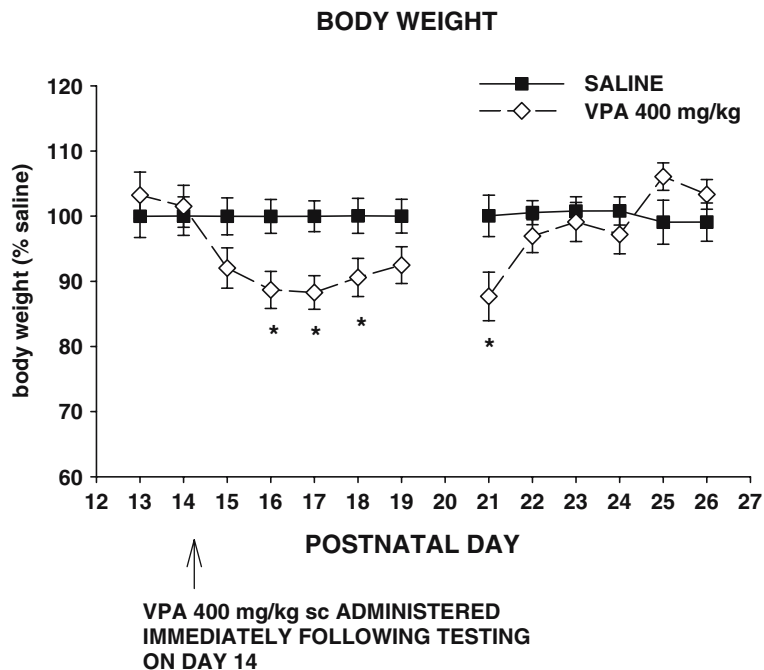
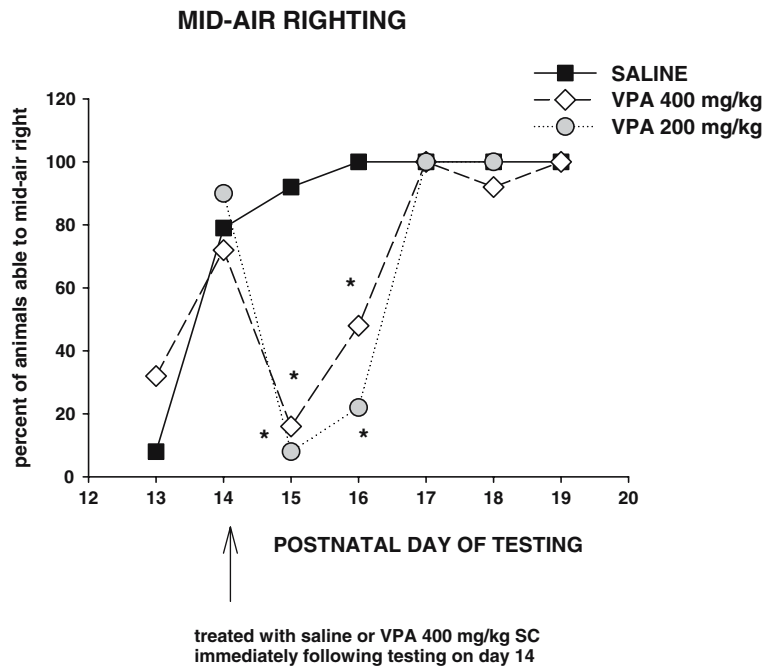


Fig. 9 Impairments in the ability to right in mid-air in postnatal male BALB/c mice following administration of sodium valproate (VPA, 200 mg/kg or 400 mg/kg s.c.) on postnatal day 14. *Indicates significantly different from saline-treated mice using Fisher’s Exact Test, $P < 0.05$



treatment as well as 400 mg/kg and 200 mg/kg VPA. Mice receiving 400 mg/kg VPA on P14 showed a longer latency to reach the visible platform [$F(2,38) = 3.9, P = 0.02$]. However, mice receiving 200 mg/kg VPA were not impaired, and were able to reach the platform in under 30 s (Fig. 11). When the platform was hidden, the saline-treated animals showed a significant improvement across days [$F(1,45) = 4.6, P = 0.004$]. This analysis also revealed a significant effect of treatment so that both VPA

doses produced a significant impairment in escape latency on all 4 days of testing [$F(1,45) = 15.9, P < 0.0001$] (Fig. 12).

Passive Avoidance

In lieu of water maze testing, separate groups of animals that received either saline or 400 mg/kg VPA on P14 underwent passive avoidance training on days P21–22.

Fig. 10 Ability of male BALB/c mice to display the negative geotactic response following administration of sodium valproate (VPA, 400 mg/kg s.c.) on postnatal day 14. *Indicates significantly different from saline-treated mice using Fisher’s PLSD, $P < 0.05$

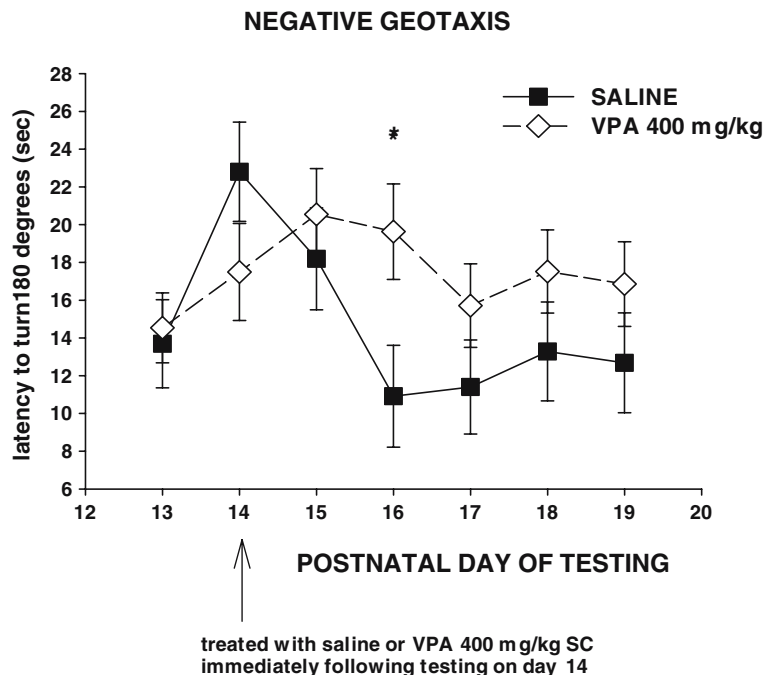
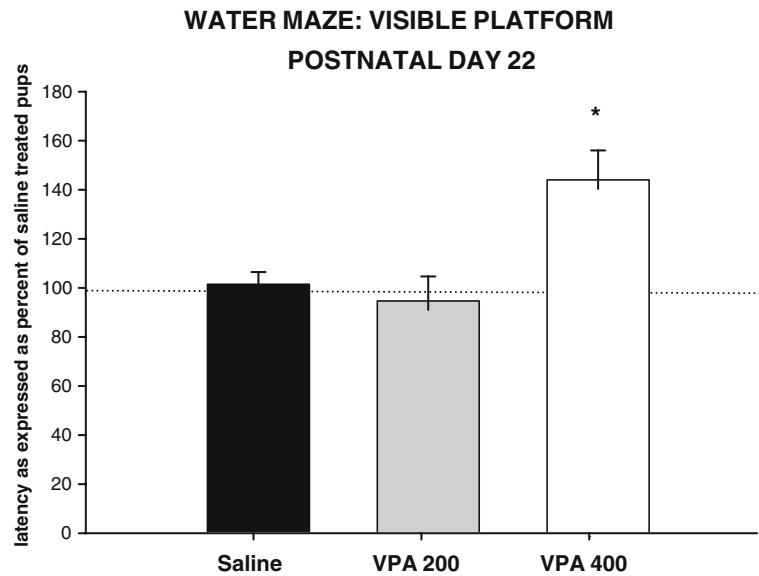


Fig. 11 Escape latency on a visible (cued) version of the water maze in animals treated with saline, 200 mg/kg or 400 mg/kg sodium valproate s.c. on postnatal day 14 and tested on postnatal day 22. Data is expressed as a percent of saline-treated control. *Indicates significantly higher than saline treated animals using Fisher's PLSD, $P < 0.05$



Both groups of animals reached criterion by day 22; while there was no difference in mean number of avoidances, both groups showed longer escape latencies (time spent to move into the “shocked” side of the chamber) on P22 compared to P21 [$F(1,16) = 5.2$, $P = 0.03$]. However, on P21, the VPA-treated animals exhibited a longer latency compared to controls [$F(1,16) = 8$, $P = 0.01$] (Fig. 13).

Motor Activity

Two separate replications of motor activity were made. Animals were tested on motor activity in an open field on either days P21–24 (saline or VPA 400 mg/kg only) or

days P23–26 (saline, VPA 200 mg/kg and VPA 400 mg/kg). Each analysis consisted of a total activity count for the full 10 min period and an analysis of each 2 min bout. For days P21–24, there was no significant difference between days on full 10 min motor activity counts. On the other hand, a significant increase in activity between days was observed between days P23 and P26 [$F(3,47) = 5.7$, $P = 0.001$], consistent with data obtained from prenatally exposed animals. On day P24, both groups showed a within-day habituation effect, reflected in significant decrease in activity on the third 2 min bout compared to the first 2 min bout (minutes 5–6 vs. minutes 1–2) [$F(4,60) = 5.7$, $P = 0.001$] (Fig. 14).

Fig. 12 Escape latency on a hidden platform (spatial learning) version of the water maze in animals treated with saline, 200 mg/kg or 400 mg/kg sodium valproate s.c. on postnatal day 14 and tested on postnatal day 23–26. *Indicates significantly higher than saline-treated animals using Fisher's PLSD, $P < 0.05$

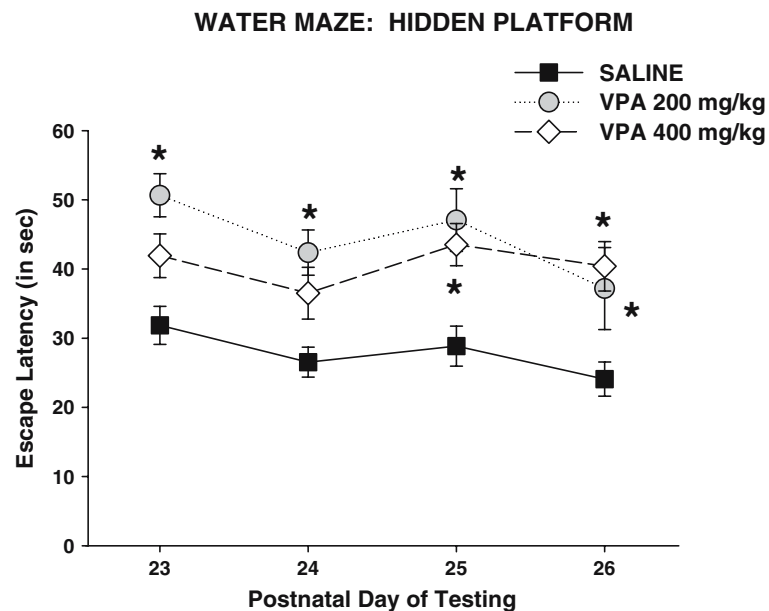
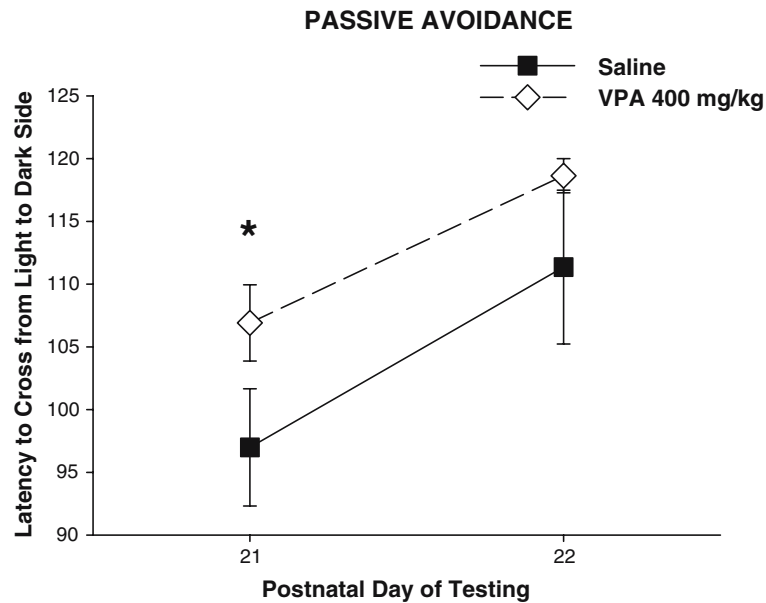


Fig. 13 Avoidance latency on a passive avoidance task in animals treated with saline or 400 mg/kg sodium valproate s.c. on postnatal day 14 and tested on postnatal days 21 and 22. *Indicates significantly higher than saline-treated animals using Fisher’s PLSD, $P < 0.05$



Acute VPA Administration in PND 14 Animals

VPA at 50 mg/kg s.c. did not produce any significant differences in ability to mid-air right, negative geotaxis, or grip strength compared to saline-treated pups when tested 3 h later (Fig. 15).

Self-injurious Behavior

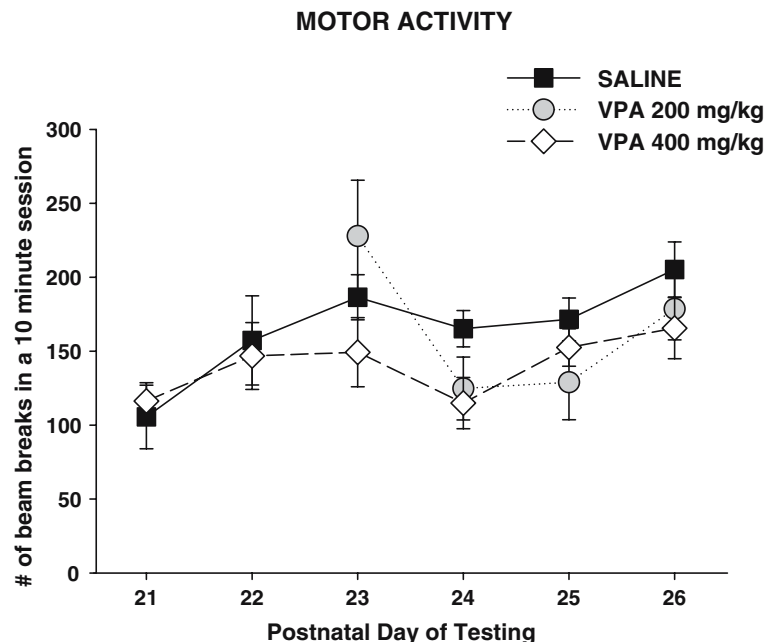
Following amphetamine administration (22.5 mg/kg s.c.), both groups exhibited self-injurious behavior (75% saline pretreated, 63% VPA pretreated), which included biting of

the forepaws and skin. This behavior peaked in frequency at 30 min postinjection. In addition, both saline and VPA-exposed animals tested at 11 weeks of life demonstrated high rates of oral dyskinesia/vacuous chewing (100% saline pretreated, 91% VPA pretreated), compared to saline treated controls (0%).

Discussion

The major observations in the present studies were that: (a) in utero exposure to VPA resulted in developmental

Fig. 14 Horizontal Motor Activity on postnatal days 20–26 during a 10 min testing period. BALB/c mice were tested following administration of sodium valproate (VPA, 600 mg/kg s.c.) on embryonic day 13. *Indicates significantly different from saline-treated mice using Fisher’s PLSD, $P < 0.05$



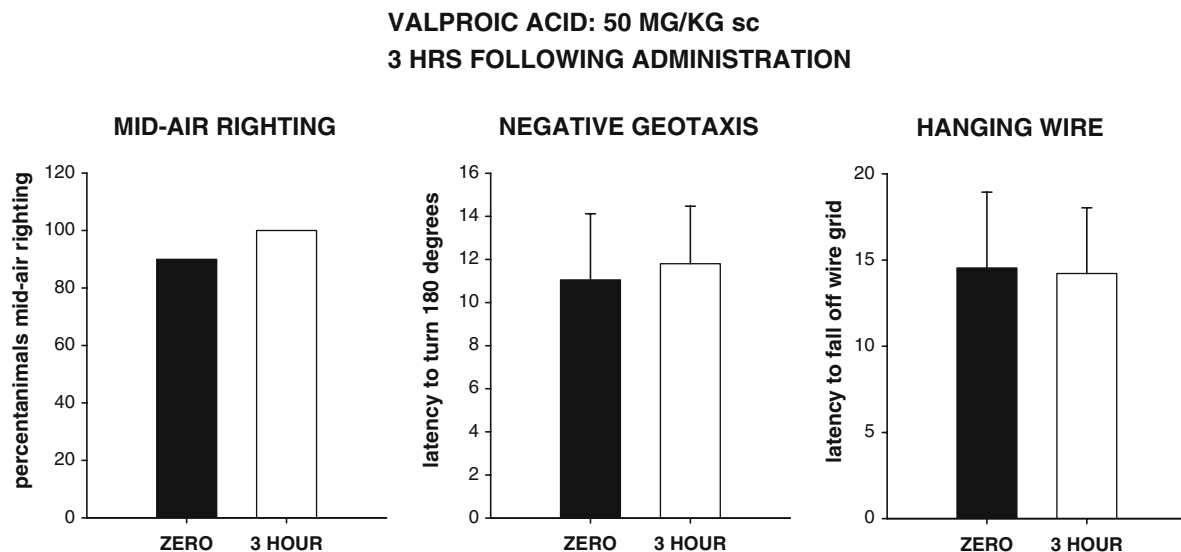


Fig. 15 Ability to right in mid-air (left), display negative geotaxis (middle) and hang from a suspended wire grid (right) prior to, and 3 h following, 50 mg/kg VPA s.c. in BALB/c mice on postnatal day 15

retardation, with delayed appearance in the maturation of surface and mid-air righting as well as negative geotaxis, grip strength, motor activity and water maze performance; (b) postnatal VPA administration caused similar retardation in the maturation of negative geotaxis and water maze performance; (c) regression of acquired skills (mid-air righting) was demonstrated following postnatal VPA exposure; and, (d) amphetamine challenge produced intrusive, self-injurious behavior in animals treated with both saline and VPA during early postnatal development. Collectively, these data indicate that a single exposure to VPA has long-lasting effects. Furthermore, the approach of assessing functional deficits following neurotoxicant exposure into broad categories of retardation, regression and intrusions may be of use for animal models of developmental disorders, particularly those thought to be associated with early developmental stressors or toxicant exposure.

The objective of these studies was not to directly attribute VPA-induced behavioral deficits to damage in particular brain regions. Rather, it was to use behavioral tasks known to involve hippocampal, striatal and cerebellar activity to ascertain if the VPA treatments disrupted the normal ontogeny of behavioral milestones. Prenatal exposure to VPA resulted in slower surface righting through day 8 of life as well as a delay in the appearance of mid-air righting. Likewise, mice exposed postnatally demonstrated a delayed negative geotactic response. In these cases, the behaviors did eventually develop to the levels exhibited by saline-treated pups. On the other hand, there were skills that failed to develop to control levels in the time period in which they were examined. This occurred with spatial

learning in the water maze and deficits in grip strength when animals had been exposed in utero. Finally, mice showed a regression in the mid-air righting response following postnatal exposure to VPA, though the ability to perform this response did eventually return.

The ability of subjects to perform the various tasks can, at least in part, be linked to the integrity of brain regions thought to be involved in autism. For example, animals with excitotoxic or electrolytic lesions of the hippocampus show impaired performance in their ability to swim to a hidden platform in the water maze task (Duva et al., 1997; Gallagher & Holland, 1992; Good & Honey, 1997; Morris, Schenk, Tweedie, & Jarrard, 1990; Packard & Teather, 1997). In contrast, when the water maze platform is visible, lesions of the striatum result in impairments to find the cued escape platform (Oliviera, Bueno, Pomarico, & Gugliano, 1997; Packard & Teather, 1997; Thullier, Lalonde, Mahler, Joyal, & Lestienne, 1996). Hippocampal lesions have also been shown to alter patterns of habituation in motor activity tasks (Galini, Weiss, Cassel, & Kelche, 1998; Wallace, Kaplan, & Werboff, 1977) as well as the ability to acquire or maintain a passive avoidance response (Kimble, Kirkby, & Stein, 1966; Sandi, Rose, & Patterson, 1992; Wincour, 1997). In the present studies, VPA-induced deficits in these tasks lead to the conclusion that there may be such damage following both the pre- and postnatal VPA treatments. Likewise, mid-air righting, surface righting and negative geotaxis have all been linked to cerebellar activity (Petrosini, Molinari, & Gremoli, 1990; Wolf, LaRegina, & Tolbert, 1996) and the deficits in the appearance and maintenance of these behaviors is consistent with previous reports that VPA causes cerebellar

damage in humans and other species. Finally, stimulant-induced stereotypic and appearance self-injurious behavior is dependent on changes in dopaminergic and serotonergic activity in the striatum (Allen & Davis, 1999; Halladay et al., 2003; Shishido, Watanabe, Kato, Horikoshi, & Niwa, 2000). The induction of self-injurious behavior following high doses of psychomotor stimulants such as amphetamine has been shown to mimic the behaviors seen in Lesch-Nyhan Syndrome and autism-like stereotyped behaviors (Jinnah, Gage, & Friedmann, 1990; Mueller, Saboda, Palmour, & Nyhan, 1982). While VPA-treated animals did not show increased behavioral sensitivity to amphetamine as adults; both groups demonstrated a high rate of self-injurious behavior (~69%) and stereotypy (100%) following amphetamine treatment. In addition, we have demonstrated that these intrusive behaviors do occur with increased sensitivity following early exposure to other neurotoxicants (Halladay, Wagner, Zhou, & Reuhl, 2004). It remains to be determined if repeated exposure to VPA, as opposed to the single injections used in the present study, will sensitize the mice to engage in stereotypic and self-injurious behavior.

Neuroanatomical studies performed on children with autism both pre- and postmortem report increased or decreased size cerebellum, along with changes in number of Purkinje cells (Bauman & Kemper, 1985; for review see Courchesne, 1997; Courchesne et al., 2001; Fatemi et al., 2002; Pierce & Courchesne, 2001; Sparks et al., 2002). Interestingly, high functioning children with autism do not show as severe alterations in cerebellar functioning (Goldberg, Landa, Lasker, Cooper, & Zee, 2000) and some autistic-like behaviors have been correlated with changes in cerebellar cell number (Pierce & Courchesne, 2001). In addition, these studies also demonstrate alterations hippocampal volume or hippocampal cell number in children with autism compared to controls and developmentally delayed children (Aylward et al., 1999; Bauman & Kemper, 1985; for review see Courchesne, 1997; Hebert et al., 2003; Sparks et al., 2002). Along these lines, children with autism show a specific pattern of memory disturbances such that associative type learning tasks are unimpaired while skills that require complex spatial organization, especially working memory, are compromised (Coldren & Halloran, 2003; Luna et al., 2002). Other studies have reported that malformation of, and decreased activity in the temporal lobe is associated with autism or autistic like symptoms, including impairments in facial processing (Sweeten, Posey, Shekhar, & McDougle, 2002; Pierce, Muller, Ambrose, Allen, & Courchesne, 2001). Finally, the basal ganglia have been reported to show structural changes (Kates et al., 1998; Sears et al., 1999) as well as functional impairments (Muller, Pierce, Ambrose, Allen, & Courchesne, 2001) in children with autism. Because the

symptom severity of autism is so broad, and because of a heterogeneous patient population under study, the magnitude and direction of the structural changes of these three brain areas has been disputed.

With respect to regression induced by the VPA, it was important to rule out the possibility that VPA may be exerting a protracted pharmacological effect 24 h after its administration. The half-life of VPA was estimated from the literature to be about 7 h in newborn rodents and sheep (Haberer & Pollack, 1994; Wong et al., 2000; Yu et al., 1985) and a dose approximating the calculated remaining level was administered to the pups prior to the behavioral testing. Since this dose was found not to cause any behavioral disruption, it was concluded that the loss of behavior was a true regression and not a pharmacological effect.

The goal of the present study was to evaluate a new strategy to model autism in mice, categorizing functional deficits as retardation, regression and intrusions. Sodium valproate was chosen for development of this model because of the similarity in symptoms exhibited by children exposed to VPA in utero and those diagnosed with autism. The conclusion is not that the treated mice are autistic but, rather, they show developmental deficits in an ontogenic fashion that parallels the clinical signs of autism. This strategy may prove useful for the assessment of the deleterious effects of early stressors including drugs, illness and environmental toxicants, on neurobehavioral development.

Acknowledgment This work was supported by: NS043981, ES05022, ES07148, ES11256, NJ Governor's Council on Autism, and Johnson & Johnson.

References

- Allen, S. M., & Davis, W. M. (1999). Relationship of dopamine to serotonin in the neonatal 6-OHDA rat model of Lesch-Nyhan syndrome. *Behavioral Pharmacology*, *10*, 467–474.
- Ardinger, H. H., Atkin, J. F., Blackston, D., Elsas, L. J., Clarren, S. K., Livingstone, S., Flannery, D. B., Pellock, J. M., Harrod, M. J., Lammer, E. J., Majewski, F., Schnizel, A., Toriello, H. V., & Hanson, J. W. (1988). Verification of the fetal valproate syndrome phenotype. *American Journal Medical Genetics*, *29*, 171–185.
- Aylward, E. H., Minshew, N. J., Goldstein, G., Honeycutt, N. A., Augustine, A. M., Yates, K. O., Barta, P. E., & Pearlson, G. D. (1999). MRI volumes of amygdale and hippocampus in nonmentally retarded autistic adolescents and adults. *Neurology*, *53*, 2145–2150.
- Bachevalier, J., & Beauregard, M. (1993). Maturation of medial temporal lobe memory functions in rodents, monkeys, and humans. *Hippocampus*, *3*, 191–202.
- Bauman, M., & Kemper, T. L. (1985). Histoanatomic observations of the brain in early infantile autism. *Neurology*, *35*, 866–874.
- Chapman, J. B., & Cutler, M. G. (1989). Effects of sodium valproate on development and social behaviour in the Mongolian gerbil. *Neurotoxicology & Teratology*, *11*, 193–198.

- Coldren, J. T., & Halloran, C. (2003). Spatial reversal as a measure of executive functioning in children with autism. *Journal of Genetic Psychology, 164*, 29–41.
- Courchesne, E. (1997). Brainstem, cerebellar and limbic neuroanatomical abnormalities in autism. *Current Opinion in Neurobiology, 7*, 269–278.
- Courchesne, E., Karns, C. M., Davis, H. R., Ziccardi, R., Carper, R., Tigue, Z., Chisum, H. J., Moses, P., Pierce, K., Lord, C., Lincoln, A. J., Pizzo, S., Schreibman, L., Haas, R. H., Akshoof, N. A., & Courchesne, R. Y. (2001). Unusual brain growth patterns in early life in patients with autistic disorder: An MRI study. *Neurology, 57*, 245–254.
- Duva, C. A., Floresco, S. B., Wunderlich, G. R., Lao, T. L., Pinel, J. P., & Phillips, A. G. (1997). Disruption of spatial but not object-recognition memory by neurotoxic lesions of the dorsal hippocampus in rats. *Behavioral Neuroscience, 111*, 1184–1196.
- Fatemi, S. H., Halt, A. R., Realmuto, G., Earle, J., Kist, D. A., Thuras, P., & Merz, A. (2002). Purkinje cell size is reduced in the cerebellum of patients with autism. *Cellular and Molecular Neurobiology, 22*, 171–175.
- Galini, R., Weiss, I., Cassel, J. C., & Kelche, C. (1998). Spatial memory, habituation and reactions to spatial and non-spatial changes in rats with selective lesions of the hippocampus, the entorhinal cortex or the subiculum. *Behavioral Brain Research, 96*, 1–12.
- Gallagher, M., & Holland, P. C. (1992). Preserved configural learning and spatial learning impairment in rats with hippocampal damage. *Hippocampus, 2*, 81–88.
- Goldberg, M. C., Landa, R., Lasker, A., Cooper, L., & Zee, D. S. (2000). Evidence of normal cerebellar control of the vestibulo-ocular reflex (VOR) in children with high-functioning autism. *Journal of Autism and Developmental Disorders, 30*, 519–524.
- Goldman, P. S. (1971). Functional development of the prefrontal cortex in early life and the problem of neuronal plasticity. *Experimental Neurology, 32*, 366–387.
- Good, M., & Honey, R. C. (1997). Dissociable effects of selective lesions to hippocampal subsystems on exploratory behavior, contextual learning and spatial learning. *Behavioral Neuroscience, 111*, 487–493.
- Haberer, L. J., & Pollack, G. M. (1994). Disposition and protein binding of valproic acid in the developing rat. *Drug Metabolism and Disposition, 22*, 113–119.
- Halladay, A. K., Kusnecov, A., Michna, L., Kita, T., Hara, C., & Wagner, G. C. (2003). Relationship between methamphetamine-induced dopamine release, hyperthermia, self-injurious behaviour and long-term dopamine depletion in BALB/c and C57BL/6 mice. *Pharmacology & Toxicology, 93*, 33–41.
- Halladay, A. K., Wagner, G. C., Zhou, R., & Reuhl, K. R. (2004). Neurodevelopmental consequences of MeHg in an animal model of autism. *Hawaii Neurotoxicology Conference*.
- Herbert, M. R., Ziegler, D. A., Deutsch, C. K., O'Brien, L. M., Lange, N., Bakardjiev, A., Hodgson, J., Adrien, K. T., Steele, S., Makris, N., Kennedy, D., Harris, G. J., & Caviness, V. S. (2003). Dissociations of cerebral cortex, subcortical and cerebral white matter volumes in autistic boys. *Brain, 126*, 1181–1192.
- Ingram, J. L., Peckharm, S. M., Tisdale, B., & Rodier, P. M. (2000). Prenatal exposure of rats to valproic acid reproduces the cerebellar anomalies associated with autism. *Neurotoxicology & Teratology, 22*, 319–324.
- Inouye, M., & Murakami, U. (1980). Temporal and spatial patterns of Purkinje cell formation in the mouse cerebellum. *Journal of Comparative Neurology, 194*(3), 499–503.
- Jinnah, H. A., Gage, F. H., & Friedmann, T. (1990). Animal models of Lesch-Nyhan Syndrome. *Brain Research Bulletin, 25*, 467–475.
- Kates, W. R., Mostofsky, S., Zimmerman, A. W., Mazzocco, M. M., Landa, R., Warsofsky, I. S., Kaufmann, W. E., & Reiss, A. L. (1998). Neuroanatomical and neurocognitive differences in a pair of monozygotic twins discordant for strictly defined autism. *Annals of Neurology, 43*, 782–791.
- Kelly, P. H., Sevoir, P. W., & Iversen, S. D. (1975). Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. *Brain Research, 94*, 507–522.
- Kimble, D. P., Kirkby, R. J., & Stein, D. G. (1966). Response perseveration interpretation of passive avoidance deficits in hippocampectomized rats. *Journal of Comparative and Physiological Psychology, 61*, 141–143.
- Koch, S., Jager-Roman, E., Losche, G., Nau, H., Rating, D., & Helge, H. (1996). Antiepileptic drug treatment in pregnancy: Drug side effects in the neonate and neurological outcome. *Acta Paediatrica, 84*, 739–746.
- Luna, B., Minshew, N. J., Garver, K. E., Lazar, N. A., Thulborn, K. R., Eddy, W. F., & Sweeney, J. A. (2002). Neocortical system abnormalities in autism. An fMRI study of spatial working memory. *Neurology, 59*, 834–840.
- Mawer, G., Clayton-Smith, J., Coyle, H., & Kini, U. (2002). Outcome of pregnancy in women attending an outpatient epilepsy clinic: Adverse features associated with higher doses of sodium valproate. *Seizure, 692*, 1–7.
- Miller, E. A., Goldman, P. S., & Rosvold, H. E. (1973). Delayed recovery of function following orbital prefrontal lesions in infant monkeys. *Science, 182*, 304–306.
- Moore, S. J., Turnpenny, P., Quinn, A., Glover, S., Lloyd, D. J., Montgomery, T., & Dean, J. C. S. (2000). A clinical study of 57 children with fetal anticonvulsant syndromes. *Journal of Medical Genetics, 37*, 489–497.
- Morris, R. G., Schenk, F., Tweedie, F., & Jarrard, L. E. (1990). Ibotenate lesions of hippocampus and/or subiculum: Dissociating components of allocentric spatial learning. *European Journal of Neuroscience, 2*, 1016–1028.
- Mueller, K., Saboda, S., Palmour, R., & Nyhan, W. L. (1982). Self-injurious behavior produced in rats by daily caffeine and continuous amphetamine. *Pharmacology, Biochemistry & Behavior, 17*, 613–617.
- Muller, R. A., Pierce, K., Ambrose, J. B., Allen, G., & Courchesne, E. (2001). Atypical patterns of cerebral motor activation in autism: A functional magnetic resonance study. *Biological Psychiatry, 49*, 665–676.
- Oliviera, M. G., Bueno, O. F., Pomarico, A. C., & Gugliano, E. B. (1997). Strategies used by hippocampal- and caudate-putamen-lesioned rats in a learning task. *Neurobiology of Learning & Memory, 68*, 32–41.
- Packard, M. G., & Teather, L. A. (1997). Double dissociation of hippocampal and dorsal-striatal memory systems by posttraining intracerebral injections of 2-amino-5-phosphonopentaonic acid. *Behavioral Neuroscience, 111*, 543–551.
- Petrosini, L., Molinari, M., & Gremoli, T. (1990). Hermicerebellectomy and motor behavior in rats. I. Development of motor function after neonatal lesion. *Experimental Brain Research, 82*, 472–482.
- Pierce, K., & Courchesne, E. (2001). Evidence for a cerebellar role in reduced exploration and stereotyped behavior in autism. *Biological Psychiatry, 49*, 655–664.
- Pierce, K., Muller, R. A., Ambrose, J., Allen, G., & Courchesne, E. (2001). Face processing occurs outside the fusiform 'face area' in autism: Evidence from functional MRI. *Brain, 124*, 2059–2073.
- Rice, D., & Barone, S. (2000). Critical periods of vulnerability for the developing nervous system: Evidence from human and animal models. *Environmental Health Perspectives, 108*, 511–533.

- Sandi, C., Rose, S. P., & Patterson, T. A. (1992). Unilateral hippocampal lesions prevent recall of a passive avoidance task in day-old chicks. *Neuroscience Letters*, *141*, 255–258.
- Sears, L. L., Vest, C., Mohamed, S., Bailey, J., Ranson, B. J., & Piven, J. (1999). An MRI study of the basal ganglia in autism. *Progress in Neuropsychopharmacology & Biological Psychiatry*, *23*, 613–624.
- Shishido, T., Watanabe, Y., Kato, K., Horikoshi, R., & Niwa, S. I. (2000). Effects of dopamine, NMDA, opiate, and serotonin-related agents on acute methamphetamine-induced self-injurious behavior in mice. *Pharmacology, Biochemistry & Behavior*, *66*, 579–583.
- Sobaniec-Lotoweska, M. E. (2001). Ultrastructure of purkinje cell perikara and their dendritic processes in the rat cerebellar cortex in experimental encephalopathy induced by chronic application of valproate. *International Journal Experimental Pathology*, *82*, 337–348.
- Sparks, B. F., Friedman, S. D., Shaw, D. W., Aylward, E. H., Echelard, D., Artru, A. A., Maravilla, K. R., Giedd, J. N., Munson, J., Dawson, G., & Dager, S. R. (2002). Brain structural abnormalities in young children with autism spectrum disorder. *Neurology*, *59*, 184–192.
- Sweeten, T. L., Posey, D. J., Shekhar, A., & McDougle, C. J. (2002). The amygdale and related structures in the pathophysiology of autism. *Pharmacology, Biochemistry & Behavior*, *71*, 449–455.
- Thullier, F., Lalonde, R., Mahler, P., Joyal, C. C., & Lestienne, F. (1996). Dorsal striatal lesions in rats. 2: Effects on spatial and non-spatial learning. *Archives of Physiological Biochemistry*, *104*, 307–312.
- Voorhees, C. V. (1986). *Handbook of behavioral teratology*. New York: Plenum Press.
- Voorhees, C. V. (1987). Behavioral teratogenicity of valproic acid: Selective effects on behavior after prenatal exposure to rats. *Psychopharmacology*, *92*, 173–179.
- Wallace, R. B., Kaplan, R., & Werboff, J. (1977). Hippocampus and behavioral maturation. *International Journal of Neuroscience*, *7*, 185–200.
- Williams, G., King, J., Cunningham, M., Stephan, M., Kerr, B., & Hersh, J. H. (2001). Fetal valproate syndrome and autism: Additional evidence of an association. *Developmental Medicine and Child Neurology*, *43*, 202–206.
- Winocur, G. (1997). Hippocampal lesions alter conditioning to conditional and contextual stimuli. *Behavioral Brain Research*, *88*, 219–229.
- Wolf, L. W., LaRegina, M. C., & Tolbert, D. L. (1996). A behavioral study of the development of hereditary cerebellar ataxia in the shaker rat mutant. *Behavioral Brain Research*, *75*, 67–81.
- Wu, Y., & Wang, L. (2002). The effects of antiepileptic drugs on spatial learning and hippocampal protein kinase C (in immature rats. *Brain Development*, *24*, 82–87.
- Wong, W., Kumar, S., Rurak, D. W., Kwan, E., Abbott, F. S., & Riggs, K. W. (2000). Ontogeny of valproic acid disposition and metabolism: A developmental study in postnatal lambs and adult sheep. *Drug Metabolism and Disposition*, *28*, 912–919.
- Yu, S. Y., Sugiyama, Y., & Hanano, M. (1985). Changes in pharmacokinetics of valproic acid in guinea pigs from birth to maturity. *Epilepsia*, *26*, 243–251.