1999

The Behavior of Preweanling Pups Exposed to Valproate *In-Utero*

Wayne Briner
University of Nebraska at Kearney, Briner@platte.unk.edu

1999. *Transactions of the Nebraska Academy of Sciences*, 25: 77–81

THE BEHAVIOR OF PREWEANLING PUPS EXPOSED TO VALPROATE *IN-UTERO*

Wayne Briner

Department of Psychology
University of Nebraska at Kearney
Kearney, Nebraska 68849
E-mail: Briner@platte.unk.edu

ABSTRACT

Valproate is a known human and animal teratogen that produces neural-tube defects. Valproate is also known to produce fetal death and a variety of fetal deformities. The present study considered the role of valproate as a behavioral teratogen in rats. Pregnant rats were administered saline or valproate, or simply handled at 10 days gestation and the offspring tested for a variety of behaviors and physical measures. Valproate-exposed animals demonstrated lags in the emergence of swimming behavior, negative geotaxis and weight gain. This evidence points to valproate being a behavioral teratogen producing delays in neuromuscular development.

† † †

Neural-tube defects (NTDS), such as spina-bifida (SB), are one of the most prevalent forms of congenital defects, accounting for perhaps 15 percent of perinatal deaths. Estimates indicate 0.4 to 1.0 of every 1000 American births have some form of SB. In the United States some 1500 babies are born with myelomeningocele, a clinically important form of SB, each year.

The consequences of SB are not limited to the external physical deformities and disability. Members of the SB population are often labeled as distractible, restless and inattentive and are beginning to be diagnosed and treated for attention deficit disorder (Anderson and Spain 1977, Horn et al. 1985). The early sensorimotor precursors of intelligence (object permanence, development of schemas, object relations in space) in infants with SB are delayed or reduced, compared to their nonhandicapped peers, indicating a developmental lag (Morrow and Wachs 1992). Humans with SB usually have accompanying hydrocephalus and/or Arnold-Chiari Malformation (ACM) where the contents of the posterior fossa (medulla, pons, cerebellum) are displaced toward the foramen magnum.

The anticonvulsant valproic acid (VA) and its salts (valproic acid sodium/sodium valproate; brand names Depakene, Depakote) have been well established as human teratogens, with an incidence of SB at 5 times greater than usual for mothers taking this drug (for review see Vorhees 1986). Valproic acid is a widely used anticonvulsant and represents a substantial risk to the epileptic mother needing such medication. Valproic acid is of additional interest because of the development of an animal model of VA-induced SB (Ehlers et al. 1992a, b), making it a useful tool to investigate the physiologic processes involved in the formation of NTDS. Valproic acid also appears to be a behavioral teratogen in humans. Valproic acid has been linked to developmental delays, impaired cognitive and social functioning, mental retardation, and speech delay in humans (Ardinger et al. 1988, Christianson et al. 1994).

The animal model of VA-induced SB is of further interest because rat pups treated with VA develop SB and demonstrate a malformation similar to ACM in humans (Briner and Lieske 1995). Valproate-exposed animals also exhibit behavioral changes such as poorer maze performance, altered open-field, and hole board activity (Vorhees 1987a).

It is clear that VA is an important human and animal teratogen in both the physical and behavioral spheres. We have undertaken this study to assess the effects of prenatal VA exposure on the emerging neuromuscular development of young rats.

MATERIALS AND METHODS

Animals

Nulliparous female Long-Evans rats originally derived from Charles River stock were used. The rats were maintained on an ad-lib diet of Purina lab chow

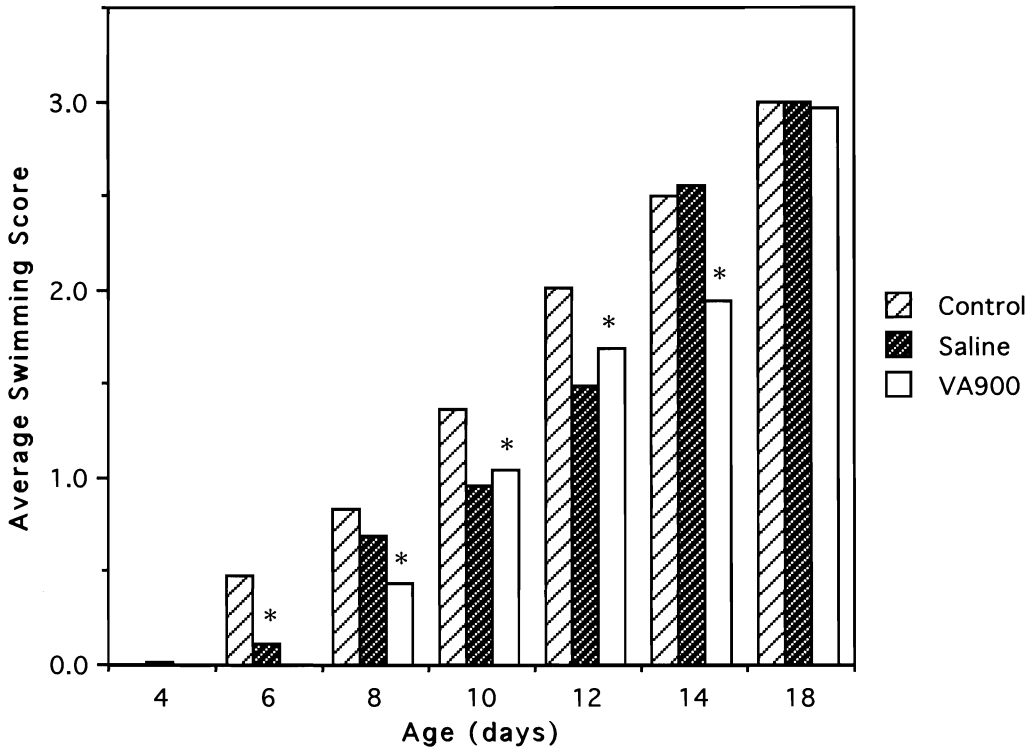

Figure 1. Development of swimming behavior across groups and over time. The VA group significantly lags the control group from 6 to 14 days of age. Asterisks (*) indicate a statistically significant lag for the VA group.

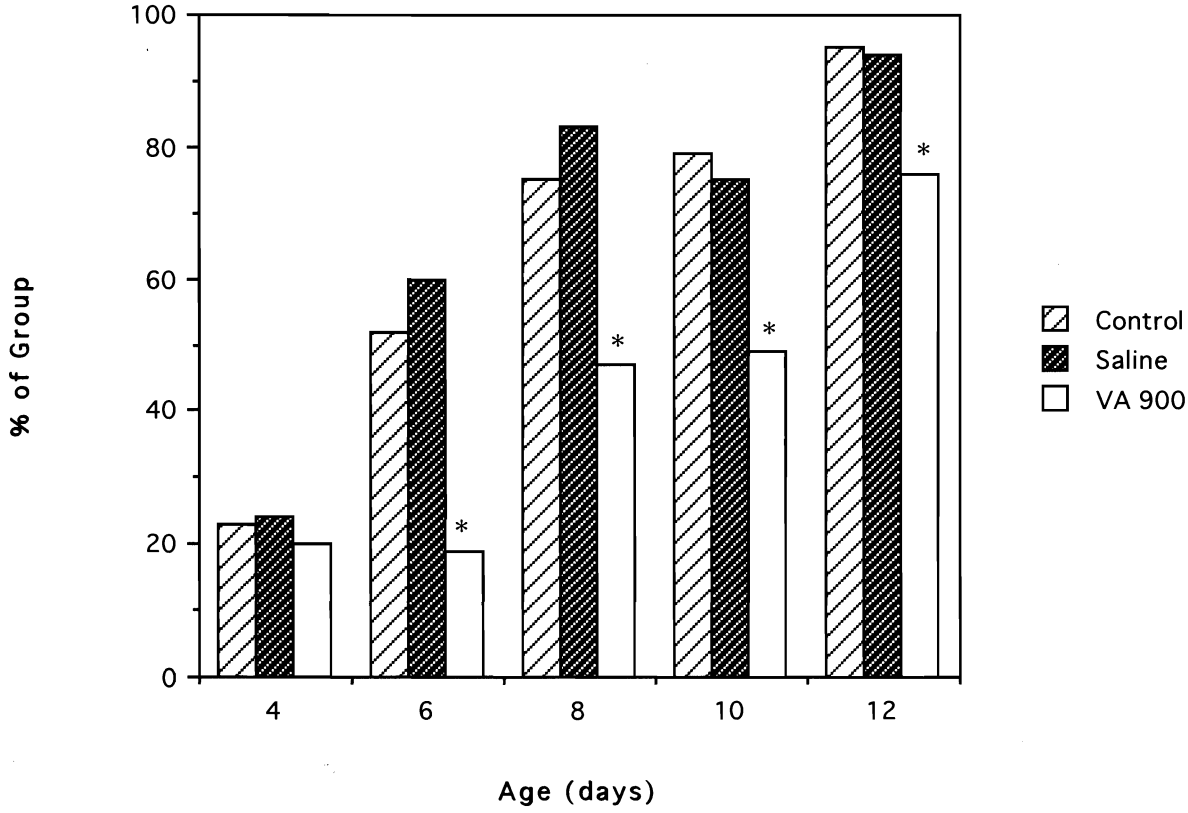

Figure 2. Negative geotaxis development for each group across ages. The VA exposed group significantly lags behind the control and saline treated groups as indicated by an asterisk (*).

and water and a 12-12 hour light-dark cycle. The females were placed with males for 24 hours and the presence of a copulatory plug noted, indicating day 0 of gestation. After we detected a copulatory plug, the females were removed from mating and housed separately. Just before birth of the pups, the females were placed in cages with wood-chip bedding and remained there with the pups for the duration of the experiment. At four days of age the pups were individually identified by toe clipping. All animals were maintained in accordance with federal guidelines and AALAC principles, as well as standards established by the Animal Welfare Act. This study was approved by the Institutional Animal Care and Use Committee of the University of Nebraska at Kearney.

Drugs and exposure

At 10 days gestation the females were injected with either physiological saline or VA sodium (Sigma Chemical, St. Louis) 900mg/kg, subcutaneously as an aqueous solution over the hindquarters. This dosage was selected because it is teratogenic yet has minimal impact on litter size (Briner and Lieske 1995). Control females were simply handled and not injected. Pregnancy was otherwise allowed to proceed normally and the date of birth of the pups was noted.

Weight

Animals were weighed every day of testing on an electronic scale to the nearest 0.1 g.

Swimming

Pups were tested beginning at 4 days and tested every other day until 12 days of age and also at 14 and 18 days. All tests were performed in the same order for each litter. The experimenter was blind to the treatment condition of the pups. From a height of six inches the pups were dropped into warm water and observed for several seconds. Animals were scored in a standard fashion (Schapiro et al. 1970, Vorhees et al. 1979) using the following criteria: unable to keep nose out of water = 0; keep nose out of water but not to top of ears = 1; keep nose and top of ears out of water = 2; keep nose, top of head and ears at least half-way out of water = 3.

Righting

The pups were placed on their backs on a flat hard table surface and allowed 30 seconds to right themselves. This was scored as pass/fail. The animals were not tested for righting at 14 and 18 days of age because they universally perform this task.

Negative geotaxis

The pups were placed head down on a 15° incline and allowed 60 sec to orient head up and remain head up for at least 10 sec. This was scored as pass/fail. The animals were not tested for negative geotaxis at 14 and 18 days of age because they universally perform this task.

Analysis

Righting and negative geotaxis were analyzed with Chi-square. Weight and swimming were analyzed with ANOVA with the Scheffé correction for follow-up analyses.

RESULTS

General observations

The numbers of litters and animals examined were as follows: eight litters of control animals for a total of 91 pups (mean litter size 11.4); seven litters of saline treated animals for a total of 76 pups (mean litter size 10.9); and 12 litters of VA-treated animals for a total of 97 pups (mean litter size 8.1); these differences were not statistically significant ($F(2, 24) = 2.14$, p = NS). None of the three groups of rat pups had any obvious deformities, and all seemed generally healthy and otherwise normal, except as indicated below. All groups had normal eye and ear opening as well as normal emergence of walking.

Weight

Weight of the pups was generally consistent until 14 and 18 days of age when the VA group demonstrated a slight lag behind that of both the control and saline group (Day 14 weights: 24.5, 25.4 and 22.4 g; Day 18 weights: 30.4, 31.8, 28.9 g for control, saline, and VA groups respectively). The differences on days 14 and 18 were statistically significant ($F_{(2, 225)} = 7.42$, $p < .001$ and $F_{(2, 220)} = 4.72$, $p < .001$, respectively).

Swimming

For the sake of clarity, the swimming scores were averaged for each group for each day of testing. The VA group consistently lagged behind the other two groups in terms of swimming development. At 4 days of age there was no significant difference ($F_{(2, 240)} = 1.21$, p = NS) between the groups (all the groups exhibiting little development of swimming behavior) and at 18 days of age there was no significant difference($F_{(2, 196)} = 2.00$, p = NS) (all groups had developed to nearly the same extent). However, for each of the days between, the VA-treated group lagged (6 days of age $F_{(2, 237)} = 27.40$, $p < .05$); 8 days of age $F_{(2, 225)} = 8.05$, $p < .05$; 10 days of age $F_{(2, 204)} = 9.83$, $p < .05$; 12 days of age $F_{(2, 218)} = 11.14$, $p < .05$; 14 days of age $F_{(2, 209)} = 17.83$, $p < .05$). Specifically, they were the last to advance to a score of 1 (the ability to keep the nose out of the water), and the last to develop a score of 3 (ability to keep nose and head out of water past the middle of the ear). The results are graphically displayed in Fig. 1.

Righting

There was no difference in the development of surface righting for the three groups at any age.

Negative geotaxis.

While the VA group performed as well as the other two groups at 4 days of age ($\chi^2_{(2)} = .56$, p = NS) they lagged behind the other groups at 6 ($\chi^2_{(2)} = 33.23$, $p < .001$), 8 ($\chi^2_{(2)} = 27.82$, $p < .001$), 10 ($\chi^2_{(2)} = 18.47$, $p < .001$), and ($\chi^2_{(2)} = 16.75$, $p < .001$) 12 days of age (Fig. 2).

DISCUSSION

The developmental toxicity of VA has been documented in previous studies (Binkerd et al. 1988, Vorhees 1987b) and the behavioral impacts have been described in humans and rats (Ardinger et al. 1988, Christianson et al. 1994, Vorhees 1987a). This study again demonstrates that VA is a teratogen that affects emerging behaviors in developing rat pups. The findings of this study point to an overall lag in neuromuscular coordination as a consequence of VA exposure. Swimming behavior is considered to be a good index of neuromuscular development and integration because of the number of neurological and muscular systems that need to be coordinated to bring about effective swimming (Kallman 1994). Similarly, negative geotaxis is a measure of neuromuscular integration that depends on information from the inner ear being coordinated with muscular activity to orient the animal head up (Kallman 1994).

The observation of normal development of surface righting, walking, ear and eye opening for the VA group, while other behaviors were negatively affected, argues that the deficits induced by VA are not global, but rather relatively specific. Previous work in our laboratory demonstrated that VA induces a form of the Arnold-Chiari malformation (Briner and Lieske 1995) that affects the brainstem and cerebellar region. This would be consistent with the findings presented here. The cerebellum and brainstem are required to coordinate complex sensory input required for tasks such as swimming and vestibular responses, and their alteration would presumably be reflected in development. Other behaviors, such as surface righting and walking, may be more dependent on motor programs (Grillner and Wallen 1985) and therefore less susceptible to moderate anatomic disruptions.

The differences in weight seen at 14 and 18 days of age are most probably due to valproate's effect on a system not examined in this study. In any event, the differences are small and do not appear to directly impact the animals' performance on tests.

Overall, the results of this study point to VA being a behavioral teratogen, at least as expressed in the behavior of preweanling rats. Valproate produces deficits in neuromuscular development and coordination that fade as the animal develops. This corresponds well to data from humans where the spina bifida population often experiences developmental lags but typically "catch up" to mainstream groups, although other permanent deficits are often seen.

ACKNOWLEDGMENTS

This research was supported by the Research Services Council of the University of Nebraska at Kearney. The author wishes to thank Drs. Joe Benz and Kevin Byrd for reviewing the manuscript.

LITERATURE CITED

Anderson, E. M., and B. Spain. 1977. *The Child with Spina Bifida*. London, Oxford University Press: 352 pp.

Ardinger, H., J. Atkin, R. Blackston, L. Elsas, S. Clarre, S. Livingston, D. Flannery, J. Pellock, M. Harrod, E. Lammer, F. Majewski, A. Schinzel, H. Toriello, and J. Hanson. 1988. Verification of the fetal valproate syndrome phenotype. *American Journal of Medical Genetics* 29: 171–185.

Binkerd, P., J. Rowland, , H. Nau, and A. Hendricks. 1987. Evaluation of valproic acid (VPA) developmental toxicity and pharmacokinetics in Sprague-Dawley Rats. *Fundamental Applied Toxicology* 11: 485–493.

Briner, W., and R. Lieske. 1995. Arnold-Chiari-like malformation associated with a valproate induced model of spina bifida in the rat. *Teratology* 52: 306–311.

Christianson, A., N. Chesler, and J. Kromber. 1994. Fetal valproate syndrome: clinical and neuro-developmental features in two sibling pairs. *Developmental Medicine and Child Neurology* 36: 357–369.

Ehlers, K., H. Sturge, H-J. Merker, and H. Nau. 1992a. Spina-bifida aperta induced by valproic acid and by all-trans-retinoic acid in the mouse: Distinct differences in morphology and periods of sensitivity. *Teratology* 46: 117–130.

———, ———, ———, and ———. 1992b. Valproic acid-induced spina-bifida: A mouse model. *Teratology* 45: 145–154.

Grillner, S., and P. Wallen. 1985. Central pattern generators for locomotion, with special reference to vertebrates. *Annual Review of Neuroscience* 8: 233–61.

Horn, D., E. Lorch, R. Lorch, and B. Culatta. 1985. Distractibility and vocabulary deficits in children with spina bifida and hydrocephalus. *Developmental Medicine and Child Neurology* 27: 713–720.

Kallman, M. 1994. Assessment of motoric effects. *In*:

G. Harry (ed.), *Developmental Neurotoxicology*. Ann Arbor, CRC Press: 103–122.

Morrow, J. D., and T. D. Wachs. 1992. Infants with myelomeningocele: Visual recognition memory and sensorimotor abilities. *Developmental Medicine and Child Neurology* 34: 488–498.

Schapiro, S., M. Salas, and K. Vukovich. 1970. Hormonal effects on ontogeny of swimming ability in the rat: assessment of central nervous system development. *Science* 168: 147–150.

Vorhees, C. 1986. Behavioral teratology of anticonvulsant and antianxiety medication. *In*: P. Riley and C. Vorhees (eds), *Handbook of Behavioral Teratology*. New York, Plenum Press: 211–241.

———. 1987a. Behavioral teratogenicity of valproic acid: selective effects on behavior after prenatal exposure in rats. *Psychopharmacology* 92: 173–179.

———. 1987b. Teratogenicity and developmental toxicity of valproic acid in rats. *Teratology* 35: 195–202.

———, C., R. Brunner, and R. Butcher. 1979. Psychotropic drugs as behavioral teratogens. *Science* 205: 1220–1225.