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Effect of Multifactorial Genetic Liability to Exencephaly on the Teratogenic Effect of Valproic Acid in Mice

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ABSTRACT The present study shows that the multifactorial genetic liability to spontaneous exencephaly in the SELH/Bc mouse strain (10–20% of embryos) also confers an elevated risk of exencephaly induced by valproic acid. Treatment of pregnant dams (600 mg/kg sodium valproate in distilled water, i.p.) during the critical period on day 8 (D8) of gestation resulted in D14 exencephaly frequencies of 69% in SELH/Bc contrasted with 39% in each of the SWV/Bc and ICR/Bc strains. Analysis of these data under the assumptions of the threshold model indicated that the valproic acid-induced shift in mean liability was similar for all three strains, and therefore the effects of genotype and teratogen were additive, not synergistic. A similar exencephaly response pattern for the same three strains was observed previously with retinoic acid [Tom et al. (1991) Teratology *43:*27–40], a pattern that, combined with the data of Finnell et al. [(1988) Teratology *38:*313–320], argues that strain differences in exencephaly response are not due to strain differences in teratogen metabolism. SWV/Bc and ICR/Bc embryos differ in location of the Closure 2 initiation site of cranial neural tube closure [Juriloff et al. (1991) Teratology *44:*225–233], but the observation that they do not differ in risk of exencephaly produced by either valproic acid or retinoic acid contradicts the hypothesis that this particular morphological difference underlies strain differences in exencephaly risk. The high exencephaly response of SELH/Bc to two teratogens predicts that human conceptuses with a genetically determined elevated risk for neural tube defects could be easily tipped into high risk by mild teratogens. *Teratology 55:306–313, 1997.* © 1997 Wiley-Liss, Inc.

Risk of birth defects after exposure to a teratogen is determined in part by genotype. This principle is demonstrated by the differing responses of identically treated normal inbred strains of mice to a teratogen (e.g., Diwan, '74; Dagg et al., '66; Smithberg, '67; Biddle, '75; Biddle and Fraser, '76; Finnell et al., '86). The biological mechanisms that cause strain differences in teratogen response are not known. The possibilities include genetically determined differences in drug metabolism and genetically determined differences in embryology, such as timing of embryonic developmental processes or morphology of embryonic structures. In the absence of teratogens, some inbred strains have an elevated risk of spontaneous birth defects caused by multifactorial genetic mechanisms. A question that arises is whether a multifactorial genetic liability to a particular spontaneous birth defect will cause increased susceptibility to teratogen induction of the same defect. There are a few studies in which this has been explored (e.g., Ingalls et al., '53; Vekemans and Fraser, '79; Cassells et al., '87; Seller and Perkins-Cole, '87; Matsuda, '90), and the general pattern suggests that multifactorial liabilities to spontaneous birth defects do increase susceptibility to environmental teratogens that produce the same defect. However, it is premature to deduce any general law from the data available.

The anticonvulsant drug valproic acid (sodium valproate) is a teratogen that causes neural tube defects in both humans and mice. In humans it appears to cause elevated risk of spina bifida aperta but not of anencephaly (Robert and Guibaud, '82; Lindhout and Meinardi, '84; Lammer et al., '87; Lindhout et al., '92). The predominant neural tube defect induced by valproic acid in mice is exencephaly (Nau et al., '81; Kao et al., '81; Paulson et al., '85), a failure of closure of the cranial neural tube, equivalent to human anencephaly. However, a specific treatment regimen 1 day later in gestation can produce a low incidence (4–6%) of spina bifida aperta in mice (Ehlers et al., '92; Nau, '94). The effects of valproic acid (sodium valproate) at the cellular level during closure of the cranial neural tube in mice have been investigated by several approaches and the major abnormality appears to be loss of integrity of the basal lamina and apical surfaces of the neuroepithelium and a loss of organization of neuroepithelial cell shape, adhesion, and orientation (Turner et al., '90).

Various inbred strains of mice having no unusual incidence of exencephaly ("normal strains") differ markedly in susceptibility to induction of exencephaly by valproic acid (Finnell et al., '88; Finnell, '91), and the

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multiple strain-specific levels of susceptibility demonstrate the multifactorial genetic determination of susceptibility to valproate teratogenesis. Three normal strains, DBA/2J, LM/Bc, and SWV, share the same hierarchy of susceptibility to induced exencephaly for valproic acid and for hyperthermia (Finnell et al., '88), suggesting a common mechanism of liability to both teratogens. At least some of these strain differences are also expressed in cultured mouse embryos exposed to sodium valproate (Naruse et al., '88), indicating that the genetic difference is not solely due to differences in maternal metabolism. It has been suggested that valproic acid-induced teratogenesis may be mediated via an interaction with folate metabolism; the combination of folinic acid, vitamin B6, and vitamin B12 given around the time of teratogen exposure was observed to reduce the risk of exencephaly and other malformations in mice (Elmazar et al., '92). This suggests that metabolic differences in embryos could underlie strain differences in liability to valproic acid-induced exencephaly. In another approach, a morphological mechanism as the basis for strain differences in liabilities to induced exencephaly has been suggested, based on observed differences among strains in timing and location of sites initiating closure of the cranial neural tube (Juriloff et al., '91).

The SELH/Bc mouse strain has a high risk of spontaneous exencephaly (Macdonald et al., '89), which has been traced to the delayed elevation of the mesencephalic neural folds and absence of initiation of neural tube closure at the prosencephalon-mesencephalon junction in all embryos in this strain (Macdonald et al., '89; Gunn et al., '95). The majority of embryos successfully compensate for this developmental defect by extending fusion of the apposed neural folds caudally from the most rostral initiation site (Macdonald et al., '89; Juriloff, '94). The genetic cause of spontaneous exencephaly in SELH/Bc mice is multifactorial and involves 2–3 loci (Juriloff et al., '89; Gunn et al., '92).

The purposes of this study were 1) to test the prediction of the multifactorial threshold model that a strain with high genetic susceptibility to "spontaneous" exencephaly, SELH/Bc, will be at elevated risk for valproic acid-induced exencephaly, compared with genetically normal strains; and 2) to explore the effect of a previously observed normal strain difference, between SWV/Bc and ICR/Bc mice, in cranial neural tube closure morphology on liability to valproic acid-induced exencephaly.

MATERIALS AND METHODS

Mice

The strains used were chosen on the basis of our previous knowledge of their risk of spontaneous exencephaly, their differing morphologies of initiation of cranial neural tube closure, and, for one, its previous use in studies of teratogen-induced exencephaly in other laboratories.

The SELH/Bc strain was created in our laboratory with genetic selection to produce high frequencies of spontaneous exencephaly (Juriloff et al., '89). Under our standard conditions, about 10% of fetuses are exencephalic (Tom et al., '91). All embryos of the SELH/Bc strain have delayed elevation of the mesencephalic neural folds and lack initiation of cranial neural tube closure at the prosencephalic/mesencephalic boundary, Closure 2 (Macdonald et al., '89; Tom et al., '91).

The SWV/Bc strain is a normal genotype and has a low risk of spontaneous exencephaly, previously observed to be about 0.5% in our laboratory (Tom et al., '91). The initiation site of Closure 2 is rostrally offset in this strain (Juriloff et al., '91), compared with other normal strains. SWV/Bc embryos are known to be relatively susceptible to hyperthermia- or valproic acid-induced exencephaly, compared with other normal strains (Finnell et al., '88).

The ICR/Bc strain is a normal genotype historically related to SELH/Bc (Juriloff et al., '89) and of no known relationship to SWV/Bc (Juriloff et al., '91). The frequency of spontaneous exencephaly has been observed to be low, ranging between less than 0.2% (Juriloff et al., '89) and 1% (Tom et al., '91). The site of Closure 2 is similar to that of most normal strains studied (Juriloff et al., '91).

All mice originated from, and were maintained in, our animal unit in the Department of Medical Genetics at the University of British Columbia, under standard conditions described previously (Macdonald et al., '89). The light cycle was 6 AM to 6 PM PST. They were fed Purina Laboratory Rodent Diet (#5001) and acidified water (pH 3.1 by HCl) ad libitum. All females used in this study were nulliparous and 2–6 months of age when bred, with a mean age of 3 months within each strain.

Timed pregnancies were obtained by placing 1–4 females with singly caged males at 5 PM PST. Twelve to 15 males per strain were used. The females were checked for copulation plugs by 9 AM PST the following morning, and 9 AM was designated D0/9h of pregnancy, as ovulation in mice takes place around the midpoint of the dark cycle (Bronson et al., '66). Females with plugs were removed from the males' cages and housed together in groups of up to 4. Unmated females were left with the males until a plug was found; they were checked each day for plugs at 9 AM and 5 PM and the few with 5 PM plugs were discarded.

Valproic acid treatment

Within each strain, mated females were assigned sequentially to one of three treatment times: D8/8h, D8/12h, or D8/16h. Within a given treatment time females were assigned at random to valproic acid or distilled-water vehicle control groups. Subsequently, a second set of SWV/Bc and ICR/Bc was treated at D8/16h and D8/20h with valproic acid, to extend the critical period examined.

The dosage of valproic acid, route of administration, and treatment time paralleled the experiment by Finnell et al. ('88), which demonstrated strain differences among normal inbred strains of mice in susceptibility to valproic acid-induced neural tube defects. Sodium valproate (2-propylpentanoic acid sodium, Sigma P4543) was obtained from Sigma Chemical Co. (St. Louis, MO) and stored in desiccant at room temperature until use. For each treatment session, approximately 5 ml of a 0.06 g/ml solution of sodium valproate was prepared by dissolving a known weight of sodium valproate powder in freshly distilled water. The valproic acid solution and distilled-water vehicle control were prepared less than 1 h before treatment. Mated females were weighed at the time of treatment on D8 and were injected intraperitoneally (i.p.) with either 600 mg sodium valproate/kg body weight (in a volume of 100 µl/10 g of body weight) or an equivalent volume of distilled water. After treatment, females were housed by strain and treatment group in clean cages with fresh food and water.

All treated females were killed by carbon dioxide gas on D14 of gestation. The uterus was immediately removed, pinned to a layer of black wax in a Petri dish, and immersed in isotonic saline (0.85% NaCl). The embryos were exposed and examined under a dissection microscope, and major external malformations, particularly exencephaly, and moles (dead early postimplantation embryos) were recorded.

Data analysis

Two statistical approaches to data analysis were taken. First, the proportions of exencephalics among scoreable embryos and of moles among implantations, per treatment group (pooled litters), were used as the experimental units. These were compared by chi-square statistics (Sokal and Rohlf, '69). Second, when chi-square tests were significant, a statistically more conservative approach that removes the statistical impact of litter effects, if present, was applied as follows. The proportions of exencephalics among embryos per litter, and of moles among implantations per litter, were transformed to their Freeman-Tukey arcsine values (Mosteller and Youtz, '61), and mean litter arcsine values per group were compared by analysis of variance (ANOVA) statistics, either t-tests or the one-way ANOVA (Sokal and Rohlf, '69). The significance level for all statistical tests was $P < 0.05$. For significant F-tests comparing more than two groups, the Tukey test (Zar, '84) was applied to identify the groups that differed.

The significance of the observed increase in exencephaly frequency after valproic acid treatment was tested by comparisons between valproic acid-treated groups and vehicle-treated control groups at each treatment time within strains. To determine the statistical significance of the observed peak of the critical period (Dagg, '66) for exencephaly induction in each strain, comparisons were made within strains across groups differing in treatment time. To determine the statistical significance of the apparent strain differences in susceptibility to valproic acid-induced exencephaly, comparisons were made between strains at their respective treatment times that showed the highest exencephaly response. As the replicates for D8/16h did not differ by either statistical approach, they were pooled for this analysis. Further in this comparison of strains, data for each strain were corrected for the expected frequency of spontaneous exencephaly by use of Abbott's formula (Finney, '71) and the proportions of induced exencephalics compared by the chi-square approach.

Finally, the change in mean liability to exencephaly induced by valproic acid treatment at the peak of the critical period in each strain was calculated and compared. This approach (Fraser, '77; Finney, '71; Falconer, '81; Eales et al., '96) assumes that, within each strain, the liability to exencephaly of individual embryos is distributed around a mean value, with some embryos having more, and some less, liability than the average, and the distribution being normal or Gaussian. A threshold value on the liability scale denotes a value beyond which embryos are exencephalic. The effect of a teratogen, under this model, is to move the distribution of individuals on the liability scale, so that a greater proportion of the distribution lies beyond the threshold.

Using the known properties of the Gaussian distribution, the frequency of exencephalic embryos, an area under the curve, can be used to locate the distribution relative to the threshold on a scale of standard deviation units. The threshold can then be used as a biological fixed value, and the distributions and means of all strains compared on the standard deviation scale. These methods have been summarized by Falconer ('81).

In order to estimate a non-zero exencephaly frequency for the untreated normal strains we used the following. For ICR/Bc, there were two exencephalics in the pooled control sample of 225 embryos, a frequency of 0.9%. For SWV/Bc, in which we observed no exencephalics in the pooled control sample of 321 embryos, data from a set of 764 control SWV/Bc embryos collected in a previous study of exencephaly in our laboratory (Gunn et al., '92) were added to our present data, to give a total exencephaly frequency of 0.3% (3/1,085).

RESULTS

Maternal administration of valproic acid on D8 of gestation produced exencephaly in all three strains of mice (Table 1). In SELH/Bc mice, the frequency of exencephaly in valproic acid-treated embryos ranged between 42% and 69%, whereas in vehicle-treated embryos the range was 7–11%. Valproic acid treatment of the normal SWV/Bc strain caused exencephaly in 20–42% of embryos, in contrast with no exencephaly among 321 vehicle-treated control embryos. Similarly, 12–40% of valproic acid-treated ICR/Bc embryos were exencephalic, in contrast to 1% of 225 vehicle-treated controls. Within each strain and within each treatment

TABLE 1. Strain differenecs and critical periods of exencephaly induction by 600 mg/kg valproic acid given on D8 of gestation

Strain	Treatment	No. of litters	No. of litters affected	No. of embryos	Embryos with exencephaly		Mean litter arcsine (±SE)
					No.	%	
SELH/Bc	WT[1] D8/8h	12	7	132	10	7.6	17.1 (2.3)
	WT D8/12h	15	11	169	19	11.2	20.6 (2.2)
	WT D8/16h	13	7	165	11	6.7	15.5 (2.4)
	VA[2] D8/8h	12	12	134	57	42.5[a,b]	40.0[d] (4.3)
	VA D8/12h	17	17	169	117	69.2[a,b,c]	55.5[d,f,h] (3.6)
	VA D8/16h	12	12	135	80	59.2[a,b]	52.1[d] (4.3)
SWV/Bc	WT D8/8h	9	0	115	0	0	7.9 (0.2)
	WT D8/12h	8	0	94	0	0	8.3 (0.4)
	WT D8/16h	9	0	112	0	0	7.9 (0.1)
	VA D8/8h	8	6	95	19	20.0[a]	24.9[e] (4.7)
	VA D8/12h	8	8	103	31	30.1[a]	34.4[e] (3.7)
	VA D8/16h	8	8	95	33	34.7[a,c]	36.9[e,g] (2.5)
	VA D8/16h[3]	9	9	109	46	42.2[a,c]	40.5[e,g] (4.8)
	VA D8/20h[3]	8	8	92	23	25.0	31.1 (3.9)
ICR/Bc	WT D8/8h	8	1	77	1	1.3	10.8 (1.7)
	WT D8/12h	8	1	75	1	1.3	10.6 (1.3)
	WT D8/16h	8	0	73	0	0	9.2 (0.2)
	VA D8/8h	8	5	76	9	11.8[a]	20.4[e] (3.5)
	VA D8/12h	8	6	79	20	25.3[a]	28.8[e] (5.0)
	VA D8/16h	8	8	72	29	40.3[a,c]	40.3[e,g] (5.7)
	VA D8/16h[3]	9	9	83	31	37.3[a,c]	39.3[e,g] (3.8)
	VA D8/20h[3]	10	8	81	16	19.8	28.4 (3.7)

[1]WT = water-treated controls.
[2]VA = valproic acid-treated.
[3]A second set of D8/16h and D8/20h done to extend times tested.
[a,d]Significantly different ($P < 0.05$) from WT by chi-square test ([a]) or two-tailed t-test ([d]).
[b]Significantly different ($P < 0.001$) from SWV/Bc and ICR/Bc at same treatment time by chi-square test.
[c]Significantly higher ($P < 0.01$) than other treatment times within strain by chi-square test. Replicates did not differ significantly ($P > 0.25$) and were pooled.
[e]T-test comparison with WT not appropriate because there is no between litter exencephaly variance in the WT group.
[f]Significant effect of treatment time (ANOVA, $P < 0.05$); D8/12h significantly higher ($P < 0.05$) than D8/8h by Tukey test; all other pairs not significant.
[g]Borderline significant, or significant, effect of treatment time (for SWV, ANOVA, $P < 0.1$; for ICR, ANOVA, $P < 0.05$); pooled D8/16h significantly higher ($P < 0.05$) than D8/8h by Tukey test; all other pairs not significant.
[h]Comparing strains at the time of highest response to valproic acid, there are significant strain effects (ANOVA, $P < 0.001$). SELH differs significantly from SWV and from ICR (Tukey test, $P < 0.05$).

time, the exencephaly frequency in the valproic acid-treated group was significantly higher than in the control group (all $P < 0.05$ by chi-square test).

Valproic acid treatment did not increase embryonic mortality in any strain at any time of treatment (Table 2; all $P > 0.05$ by chi-square test).

The critical period for induction of exencephaly in each strain appeared to be relatively broad and to extend beyond the 8–12 h period tested (Fig. 1). A peak of responsiveness was observed within the range of times tested in each strain (Fig. 1). Within each strain there was a significant effect of time of treatment on exencephaly frequency (all $P < 0.05$ by chi-square test of heterogeneity and by ANOVA). For each of SWV/Bc and ICR/Bc, the replicate samples at D8/16h did not differ significantly (Table 1; both $P > 0.25$ by chi-square test) and the replicates were therefore pooled. The highest response of SELH/Bc (69% exencephalic) was observed after treatment at D8/12h, 4 h earlier than the highest response time for SWV/Bc (39%) and ICR/Bc (39%).

The peak exencephaly frequency in the SELH/Bc strain was significantly higher (Table 1) than that of either of the two normal strains, SWV/Bc and ICR/Bc, which did not differ from each other. After correction for the presence of spontaneous exencephaly by Abbott's formula (Finney, '71), which in effect removes from the sample individuals who would have had exencephaly spontaneously, the frequency of exencephaly induced by valproic acid was still significantly higher ($P < 0.001$) for SELH/Bc than for SWV/Bc and ICR/Bc.

There did not appear to be litter effects on occurrence of exencephaly. In all three strains, most litters contained exencephalic embryos after valproic acid treatment (Table 1), and the frequency of litters lacking exencephaly generally fits binomial expectations (Sokal and Rohlf, '69). For example, ICR/Bc treated with valproic acid on D8/8h at 12% exencephaly and average litter size of 9.5 would be predicted to have 70% of litters with at least one exencephalic embryo, and 5 of 8 such litters (63%) was observed, a good fit for a small number of litters.

TABLE 2. Embryonic mortality (moles) observed on D14 after 600 mg/kg valproic acid given on D8 of gestation

Strain	Treatment*	No. of litters	No. of litters with moles	No. of implants	Moles		Mean litter arcsine (±SE)
					No.	%	
SELH/Bc	WT[1] D8/8h	12	6	140	8	5.7	14.8 (2.0)
	WT D8/12h	15	8	179	10	5.6	15.0 (1.7)
	WT D8/16h	13	3	168	3	1.8	10.5 (1.5)
	VA[2] D8/8h	12	6	145	11	7.6	15.9 (2.3)
	VA D8/12h	17	7	178	9	5.0	15.3 (2.2)
	VA D8/16h	12	2	138	3	2.2	10.8 (1.6)
SWV/Bc	WT D8/8h	9	7	126	11	8.7	18.2 (2.3)
	WT D8/12h	8	7	107	13	12.1	22.0 (2.8)
	WT D8/16h	9	5	118	6	5.1	14.5 (2.2)
	VA D8/8h	8	7	104	9	8.6	19.0 (1.8)
	VA D8/12h	8	6	110	7	6.4	16.2 (1.8)
	VA D8/16h	8	7	107	12	11.2	21.3 (2.3)
	VA D8/16h[3]	9	7	124	15	12.1	20.4 (3.0)
	VA D8/20h[3]	8	5	103	11	10.7	18.6 (3.1)
ICR/Bc	WT D8/8h	8	7	92	15	16.3	24.5 (3.8)
	WT D8/12h	8	5	89	14	15.7	21.9 (4.2)
	WT D8/16h	8	6	84	11	13.1	21.7 (3.0)
	VA D8/8h	8	7	89	13	14.6	23.8 (2.6)
	VA D8/12h	8	5	90	11	12.2	20.4 (3.5)
	VA D8/16h	8	8	95	23	24.2	30.4 (2.8)
	VA D8/16h[3]	9	8	103	20	19.4	26.6 (3.4)
	VA D8/20h[3]	10	9	102	21	20.6	28.7 (3.5)

[1]WT = water-treated controls.
[2]VA = valproic acid-treated.
[3]A second set of D8/16h and D8/20h done to extend times tested.
*All differences are non-significant (WT vs. VA), $P < 0.05$ by chi-square test, within strain, within treatment time.

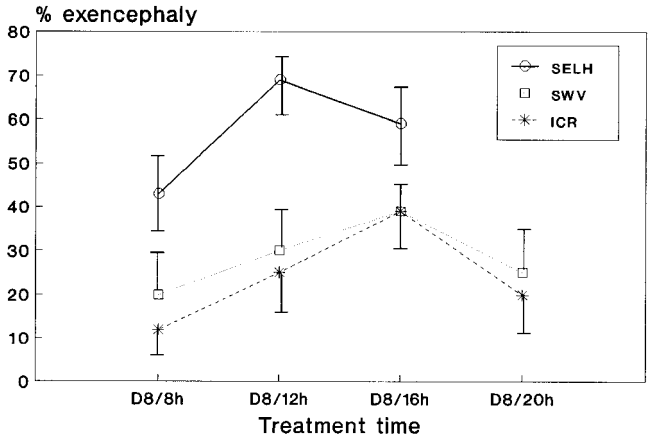

Fig. 1. Peaks of critical periods for exencephaly response to valproic acid treatment (600 mg/kg) in SELH/Bc, SWV/Bc, and ICR/Bc mouse strains. Data points for D8/16h for SWV/Bc and for ICR/Bc each represent pooled replicates. Bars represent 95% confidence intervals.

A few other, rarer, anomalies were seen in the valproic acid-treated embryos and were not seen in controls. They demonstrated some strain specificity. In particular, 23 SWV/Bc embryos had midfacial clefts (i.e., a complete midline gap between the two sides of the face, extending from the external surface to the oral cavity, and from the tip of the snout caudal to the level of the eyes) in combination with exencephaly. This trait was present after treatment at D8/12h (7%), D8/16h (5%), and D8/8h (5%). In contrast, only two ICR/Bc embryos had midfacial clefts with exencephaly (D8/12h). Additionally, two ICR/Bc embryos had midfacial clefts without exencephaly (D8/12h, D8/16h). No SELH/Bc embryos had midfacial clefts. One embryo with spina bifida and three with shrivelled tails were observed, all in SWV/Bc embryos after treatment on D8/20h.

DISCUSSION

Judged by the frequency of exencephaly at the peak of the critical period, SELH/Bc embryos are much more liable to valproic acid teratogenesis than are SWV/Bc or ICR/Bc. Even after the proportion of embryos expected to be spontaneously exencephalic is removed from the data by Abbott's formula (Finney, '71), the frequency of *induced* exencephaly was much higher in SELH/Bc (66%) than in SWV/Bc (39%) or ICR/Bc (38%). Examined in this way, the data support an interpretation that, relative to normal strains, the multifactorial genetic liability to exencephaly in SELH/Bc mice confers an increased liability to teratogen-induced exencephaly. Previously, SELH/Bc mice have also been demonstrated to have an increased liability to retinoic acid-induced exencephaly (Tom et al., '91).

The lack of difference in valproic acid-induced exencephaly frequency between the two genotypes of normal strain embryos, SWV/Bc and ICR/Bc, suggests that the previously observed difference in site of initiation of Closure 2 (normally at the prosencephalon/mesencephalon boundary) in these two strains (Juriloff et al., '91) has little or no impact on liability to teratogen-induced exencephaly. These two normal strains also did not

differ significantly in frequency of exencephaly induced by retinoic acid (Tom et al., '91).

The frequency of exencephaly induced by valproic acid in SWV/Bc embryos was similar to that observed by Finnell et al. ('88) after similar treatment. For example, after D8/h12 treatment, the frequency was 30% in the present study and 35% in the study by Finnell et al. ('88). It is interesting to note that SWV/Bc had the highest exencephaly response among the strains examined by Finnell et al. ('88) and that the large increase in liability in SELH/Bc compared with SWV/Bc as a representative normal strain would be even greater if SELH/Bc were compared with other normal strains, such as DBA/2J.

Our data support the previous observation (Finnell et al., '88), based on other strains, of shared hierarchies of relative liability to exencephaly induced by biochemically different teratogens. The previous work showed that the relative liability among DBA/2J, LM/Bc, and SWV/Bc was the same for hyperthermia-induced exencephaly and for valproic acid-induced exencephaly (Finnell et al., '88). In the present study, we note that the relative liability among ICR/Bc, SWV/Bc, and SELH/Bc is similar for retinoic acid-induced exencephaly and for valproic acid-induced exencephaly (SWV/Bc not different from ICR/Bc; both lower than SELH/Bc). These shared hierarchies across different teratogens would not be expected if strain differences in teratogen-induced exencephaly frequencies were due to differences in drug metabolism. In short, for strain differences in liability to teratogen-induced exencephaly, neither the hypothesis that it is due to difference in site of initiation of Closure 2, nor the hypothesis that it is due to difference in drug metabolism is supported by the data. The hypothesis that the common hierarchy of response by normal strains reflects strain differences in timing or robustness of mesencephalic neural fold elevation (e.g., genetically determined differences in cell proliferation) remains viable.

Although the difference in location of Closure 2 initiation site between ICR/Bc and SWV/Bc did not appear to influence exencephaly liability, we speculate that it did influence the risk of a midfacial cleft. This defect, which is a failure of closure of the most rostral region of the neural tube, was relatively common (5–7%) in SWV/Bc embryos and always combined with exencephaly. In contrast, it was rare in ICR/Bc (0.5%) and not correlated with presence of exencephaly, and it was not seen in any SELH/Bc embryos.

The greatly increased liability to teratogen-induced exencephaly in SELH/Bc embryos suggests that the various teratogens used interfere with the same developmental process as that weakened by the abnormal genotype, in this case, the elevation of the mesencephalic folds. Whereas from direct examination of the frequency of exencephaly one cannot infer whether the genetic and teratogenic effects interact or are simply additive (Fraser, '77; Biddle, '81), using the threshold model (below) one can make this distinction.

The threshold model (Falconer, '81; Fraser, '80) assumes that there is a quantitative trait in embryos that is strongly correlated with the risk of developing exencephaly. In this case, the quantitative variable might be the timing of mesencephalic neural fold elevation relative to other development in the embryo. For each genotype or strain there is an average value for this variable, and a symmetrical (Gaussian) distribution of individual values around this average, with those closest to the average being most common. There is, on this scale, a limiting value, a "threshold," beyond which normal development will not proceed. In this case, it might be a time in development after which the neural folds are unable to elevate. If the mean value for a genotype lies close to the threshold, part of the distribution of individual values will fall beyond the threshold, and those individuals will fail to develop normally; i.e., in this case, they will be exencephalic. The frequency of these individuals is the area under the distribution of values that falls beyond the threshold.

Using this model, it is possible to work backward from observed frequencies of exencephalics to deduce the location of the threshold relative to the mean (in units of standard deviation) for each strain (Tom et al., '91; Juriloff et al., '89; Falconer, '81; Eales et al., '96). As the threshold is a biological limit, it can be considered a constant, and the locations, relative to a constant threshold, of the various distributions can be deduced (Table 3). In this way, the shift of distributions caused by valproic acid treatment can be visualized (Fig. 2). It is clear from this approach that the SELH/Bc strain does not respond more to valproic acid than do the two normal strains. The change due to treatment is for all three strains approximately the same, indicating that the effects of genotype and valproic acid on the quantitative variable, perhaps timing of mesencephalic fold elevation, are additive (Fraser, '77; Biddle, '81). Alternative mechanisms that are not supported by the data include a synergistic mechanism whereby there would be specific interaction between the products of the liability genes and the teratogen's effects, as this would lead to a greater-than-additive response. Another possibility rejected by the data is the effect of liability genes and valproic acid at different times and locations in the embryo to produce exencephaly by completely different mechanisms (e.g., failure to close vs. reopening of the neural tube), as this would produce an obvious less-than-additive response under the threshold model.

Our study has indicated that, in mice, the presence of a multifactorial liability that causes elevated risk of "spontaneous" exencephaly also causes a greatly increased liability to valproic acid-induced exencephaly. This issue is important to human teratology, as it predicts that conceptuses receiving a genetically determined elevated risk for neural tube defects would be easily tipped into a very high risk of neural tube defects by teratogenic exposures that would cause low, albeit increased, risk in the general population.

TABLE 3. Threshold model calculations for valproic acid effect on mean exencephaly liability

Strain	Treatment	Exencephaly (%)	Location of mean, if T = 0 (SD units)[1]	Shift in mean due to valproic acid treatment (SD units)
SELH/Bc	Control[2]	8.6	−1.366	
	VA[4] D8/12h	69.2	+0.496	1.86
SWV/Bc	Control[3]	0.3	−2.770	
	VA D8/16h	38.7	−0.279	2.49
ICR/Bc	Control[2]	0.9	−2.366	
	VA D8/16h	38.7	−0.279	2.09

[1]Distance of the mean from the threshold in standard deviation units when the threshold is set at zero, determined from values given in Falconer ('81: Appendix Table A) for location of the threshold when the mean is set at zero. These two values are the same, but the sign is changed.
[2]Control = pooled water-treated groups: D8/8h, D8/12h, D8/16h.
[3]Control = pooled water-treated groups plus data from previous study (Gunn et al., '92).
[4]VA = valproic acid-treated.

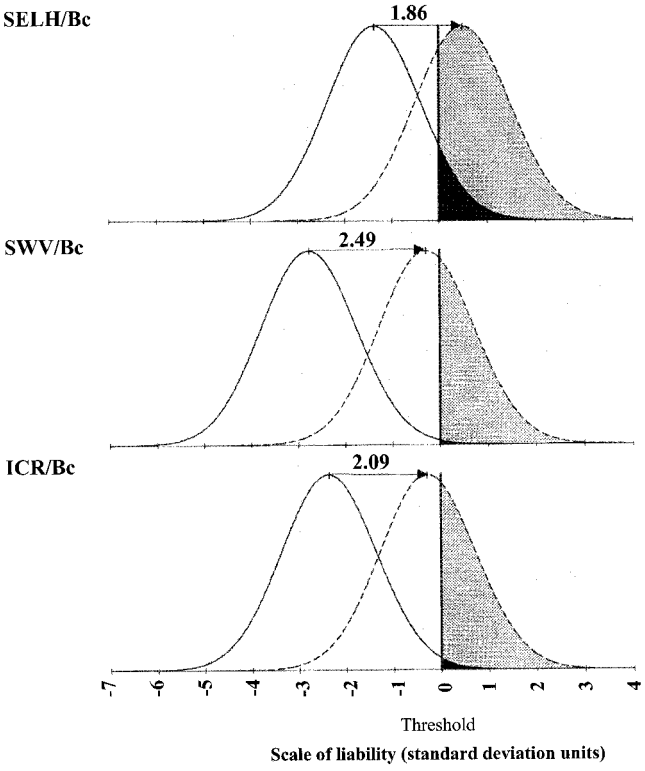

Fig. 2. Effect of valproic acid (600 mg/kg) on liability to exencephaly in SELH/Bc, SWV/Bc, and ICR/Bc mouse strains. Data from Table 1 are presented in terms of the multifactorial threshold model. Solid lines indicate water-treated control embryos; dashed lines indicate valproic acid-treated embryos. Calculations are presented in Table 3.

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