Postnatal Development of Brain and Behavior of Shaker Short-Tail Mice

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Lyons, J.P., and Wahlsten, D. Postnatal development of brain and behavior of shaker short-tail mice. Behavior Genetics, 1988, 18, 35-53.

Abstract:
Effects of the shaker short-tail gene (an allele at the dreher locus) on behavior and brain morphology were studied. A total of 20 separate litters was tested, one each day from birth to 20 days and one every other day from 22 to 30 days. Within a litter, all homozygous mutant mice (dr^{sst}/ dr^{sst}) and one normal sibling (+ / -) were examined. A larger sample of mutant mice and their siblings from seven litters was observed 10 days after birth. The homozygous recessive genotype (dr^{sst}/dr^{sst}) resulted in retardation of the overall growth of the body and brain, but genetic effects on the tail and cerebellum, which were highly variable and uncorrelated, could not be attributed solely to a general retardation of development. Performance on a standard battery of reflex tests was also severely retarded in mutant mice, especially the righting reflex and cliff aversion response. When frequencies of 36 behavior patterns during 5 min in a simple environment were compared, immature behavior patterns such as pivoting disappeared from the repertoire of mutant mice but normal patterns of locomotion were never achieved. Instead of walking in a straight path, the animals often circled or walked while swaying from side to side. Older mutants were almost always active. When the four paws were motionless on the ground, the head was usually shaking or tossing. Only grooming behavior appeared to be relatively normal. The degree of defect in motor behaviors was positively correlated with the degree of abnormality in the cerebellum, which in some cases was almost absent.
KEY WORDS: dreher; brain weight; cerebellum; reflex ontogeny; developmental rate; behavioral repertoire.

Article:

INTRODUCTION

Animals with neurological mutations provide unique opportunities for the study of genetic effects on the development of brain and behavior, but they also pose special problems. A mutation often impairs development severely, leads to unusual characteristics that are rarely seen in laboratory strains and, in some cases, gives rise to extraordinary variability in morphology and behavior (e.g., Morse, 1979; Sidman *et al.*, 1965). However, a large majority of research with neurological mutants has been reported by neurologists and neuroanatomists using somewhat imprecise descriptive methods that cannot answer certain questions posed by deleterious mutations.

We sought to overcome these problems in a study of a spontaneous, single-locus autosomal recessive mutation which occurred in our breeding population of BALB/c mice in 1980 (Wahlsten *et al.*, 1983). The gene, which is an allele at the "dreher" *(dr)* locus on chromosome 1 (Washburn and Eicher, 1986), was named "shaker short-tail" because of a strong resemblance to the extinct "shaker short" *(st)* gene discovered by Dunn (1934). It was impossible to ascertain whether our mutation was a recurrence of *st*, partly because Dunn used colorful, but vague, terms for behaviors, such as "chaotic circus movements".

Several problems were addressed in this research. First, mice homozygous for dr^{sst} appear to be very different from their siblings on almost every measure of body, brain, and behavior. They clearly do not grow as rapidly as normal mice, and because of this general developmental delay, they appear inferior to their siblings on

virtually every characteristic which normally changes rapidly over the neonatal period. Hence, it is important to distinguish between deficits in the overall rate of growth and deficits which are specific to a certain tissue, organ, or behavior. This problem was addressed by measuring normal littermates of the mutant mice at a wide range of ages and fitting curves to their data to describe the general progress of development. In order to avoid problems created by repeated testing of the same mouse and to allow brain—behavior correlations, each animal was given behavioral tests only once and then processed for histology. The equations were later applied to a larger sample of mice at a single age to determine the degree of growth retardation for various measures of the mutant mice.

Second, in order to quantify the degree of retardation of behavioral development, standardized tests of behaviors of neonatal mice must be used. The batteries of reflex tests published by Fox (1965a) and Williams and Scott (1954) had previously been incorporated into a time scale of postnatal reflex ontogeny (Wahlsten, 1974), which made it possible to quantify effects on developmental rate of heredity (Wahlsten, 1975), maternal environment (Wainwright, 1980), and protein malnutrition (Wainwright and Russell, 1983). This same battery was employed to measure the overall retardation of reflex ontogeny and to detect reflexes which were most impaired by dr^{sst}.

Third, shaker short-tail homozygotes exhibit highly unusual postures and sequences of behaviors which are never seen in normal siblings and are not revealed by numerical scores on a battery of standard reflex tests which are relatively insensitive to the specific kinetic pattern of movement. This problem was addressed by observing the behavioral repertoire in a simple environment and devising new categories of behavior to describe unique patterns in addition to those exhibited by normal mice.

The first experiment examined changes from the day of birth to weaning in several gross measures of body and brain size as well as behavior. A second study was carried out to obtain larger samples of the brains of mutant mice and their siblings at a single age and to examine reflex ontogeny in greater detail.

METHODS

Experiment 1

Animals. The parent mice mated to produce offspring for this study were all hybrid mice descended from a BALB/cCF carrier of dr^{sst} crossed with either a C57BL/6J inbred mouse or a B6D2F3/J hybrid mouse descended from a cross of the inbred strains C57BL/6J and DBA/2J. Care was taken to ensure that litters with different genetic backgrounds were distributed uniformly across ages of testing. Litters were obtained from 40 hybrid females mated with 16 different hybrid males. Matings involved one male placed with one, two, or three females in a 29 × 18 × 13-cm opaque plastic mouse cage containing Beta-Chip bedding. After a successful mating was detected by the presence of a vaginal plug, the female was isolated in a fresh cage and allowed to give birth and rear her litter until the age of testing. All mice were maintained at 22 ± 1°C with a 12- h light:12-h dark cycle and given free access to Master MLM rodent food and tap water.

Assignment to Test Age. Among the 48 litters alive at birth (Day 0), 16 contained no mutant mice and therefore were not included in the study. The 32 other litters were assigned randomly to a test age with the restriction that litters from a particular genetic mating type were not concentrated around a particular age. In six cases all the mutants in a litter died before the assigned age of testing. When this occurred, the next litter born was assigned to that test age, in order to reduce the bias produced by testing only the most viable mutants at the oldest ages and a large proportion of severely affected mutants at the younger ages. Eventually 26 litters were tested, one each day from Day 0 to Day 20 and one every other day from Day 22 to Day 30.

Procedure. On the day of birth each mouse in the litter was examined, weighed, and marked with a felt pen for later identification. Marks were maintained with hair dye in mice older than 9 days. On the assigned test day all mice were again weighed, and then all mutants and one normal control mouse ($+/dr^{sst}$ or + / +) were subjected to the testing procedure. The control mouse was generally near the median body weight for normal mice in the litter. If there was only one mutant mouse in the litter, a control mouse was chosen that had the same sex and

coat color as the mutant, if one was available. One normal control at each age was considered sufficient to establish an adequate curve for normal development because changes during the age range under study were known from previous research (Wahlsten, 1974) to be very large and consistent, and effects of the mutation were also generally large.

The testing procedure for each mouse consisted of behavioral assessment followed by perfusion. At all ages, each mouse was first observed for 5 min in an opaque 29 × 18 × 13-cm plastic mouse cage containing fresh Beta-Chip hardwood bedding. The light level in the case was approximately 500 lux from overhead flourescent room lights. After being placed in the middle of the cage with its four paws on the bedding, the mouse was observed by an experimenter (J.P.L.), who recorded verbal codes for each distinct behavior onto a cassette tape. The duration of each occasion of a distinct behavior was later determined from measurement of the tapes, and then the total time spent performing each behavior was obtained and converted to the percentage total time. At ages 8 to 20 days, each mouse was then given a battery of 14 reflex tests (see Table IV) modified from the tests used by Fox (1965a) and described in detail previously (Wahlsten, 1974, 1975; Wainwright and Russell, 1983). Behavior on each test was assigned a score ranging from 0 to 1.0 to indicate the degree of maturity of the response. Criteria for fractional scores on each test are available from the authors upon request.

Behavior Categories. A few preweaning mice were observed in the simple test situation prior to the formal study in order to develop categories suitable for the final study. The mutant mice were especially challenging because of their bizarre activities which required novel categories. The list of behavior categories was developed from descriptions given in several previous studies (van Abeelen and Kalkhoven, 1970; Wainwright, 1980; Williams and Scott, 1954), modifying and adding categories to fit our mice and test environment. Brief names and descriptions of behaviors are listed in the Appendix. Only those which appeared in more than five mice in the study are described.

Histology. Behavioral testing was followed by anesthesia with chloroform vapor, measurement of tail length, and then intracardiac perfusion with 0.9% NaC1 followed by 10% buffered neutral formalin. The brain was extracted from the skull at least 2 days later, and after at least 1 further week in the fixative the brain was weighed to the nearest milligram. Each brain was saturated in 10% buffered sucrose, embedded in 10% sucrose dissolved in 18% gelatin, and stored in 10% sucrose in 10% buffered formalin until sectioning. Serial sagittal sections were cut on a freezing microtome at 25 μm and every tenth section was stained with cresyl violet for Nissl substance. The volume of the cerebellum was determined by tracing the outline of each section, measuring its area with an Apple graphics tablet, multiplying by 0.25 mm, and then summing over all sections. The thickness of the external granular layer (EGL) of the cerebellum of normal mice was determined from 10 separate measurements in the midsagittal section as described previously (Wahlsten, 1974). A similar method was used for the EGL of the mutants, except that fewer measures were taken because certain lobes of the cerebellum were always missing and measures were sometimes taken from unusual locations near the mid-plane because of a contorted foliation pattern. Certain mutant mice had no EGL score because they had no cerebellum present near the mid-sagittal plane.

Statistical Analysis. Data for the normal mice were used to fit equations for each of the six variables shown in Fig. 1 using the least-squares criterion. For the four variables where scores were evidently approaching an upper limit by Day 30, logistic growth curves were used. For the others, hierarchical quadratic regression was used and the quadratic term was included only if it was statistically significant ($\alpha = 0.05$). Hierarchical quadratic regression was also used to estimate tail length, brain weight, and cerebellum volume from body weight.

Experiment 2

Animals. Hybrid mice which were known carriers of dr^{sst} were maintained and mated as in Expt 1. Ten females mated to two males yielded 10 litters with 100 live offspring, 24 of which were dr^{sst}/dr^{sst}. Six of the mutants died within 2 days after birth, but the other 18 all survived until perfusion on Day 10. Three of the litters contained no mutant mice and therefore were not included in the study.

Procedure. A modified battery of 11 reflex tests was given to 18 mutant mice and their 48 normal siblings at 6, 8, and 10 days after birth. The last three tests used in Expt 1 (eyes open, visual placing, auditory startle) were omitted because normal mice rarely score above 0 at Day 10. On Day 10, each mutant was perfused with formalin. Within each litter, one normal mouse was perfused for each mutant. An effort was made to include the same numbers of each sex within a litter, but this was not always possible because of the composition of the litter. The 18 control mice included 9 females, whereas the 18 mutants included 6 females. This difference in sex composition of the samples was of little consequence because mice were tested at Day 10, a time when sex differences in normal mice were very small. In litters with three or four mutants, it was necessary to perfuse almost the entire litter, which meant many normal controls were not near the median body weight for the litter. Histological methods and measurements of all variables were done as in Expt 1.

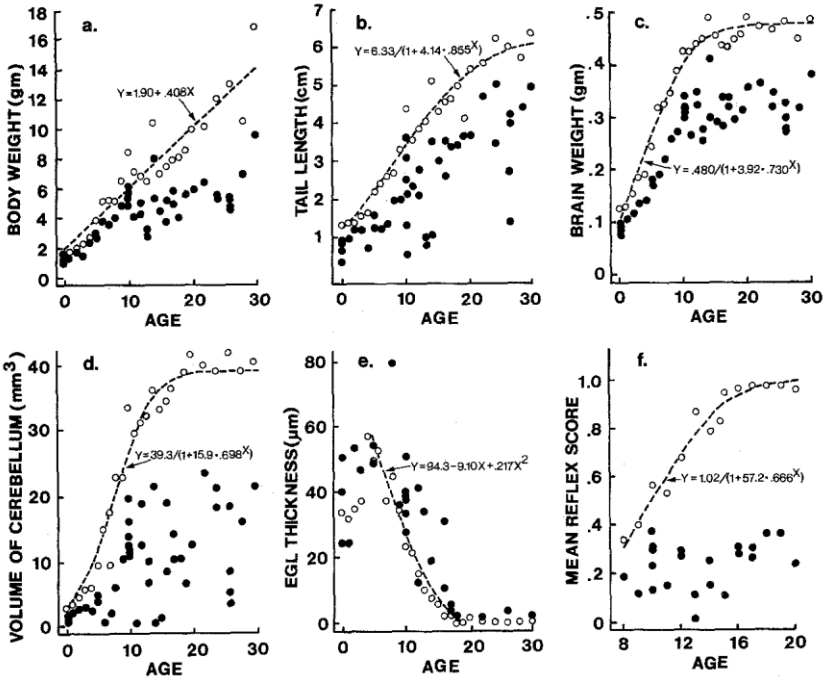

Fig. 1. Individual scores for mutant mice (filled circles) and normal siblings (open circles) at several ages for (a) body weight, (b) tail length, (c) brain weight, (d) volume of the cerebellum, (e) thickness of the external granular layer (EGL) of the cerebellum, and (f) the mean of 14 reflex tests. Curves of the best fit for the data on normal siblings are indicated by a dashed line and their corresponding equations relating expected phenotypic value (Y) to age (X).

Statistical Analysis. Because individual mutant and control mice were not strictly matched, means of the groups were compared using *t* tests on independent groups. The principal virtue of the choice of controls was that it ensured similar distributions of genotypes at loci other than dr^{sst} as well as similar gestation lengths and other maternal variables. When within-group variances were significantly different, group means were compared using an approximation of *t* with separate variance estimates and adjusted degrees of freedom (Hays, 1981). The use of equal sample sizes for mutants and controls simplified the analysis and minimized the problem of unequal variances for certain measures. Deviations of tail lengths, brain weights, and cerebellar volumes from values expected on the basis of body weights were obtained using the regression equations derived from normal mice in Expt 1. Calculation of correlations between phenotypes was problematic in two cases. The body weight:brain weight correlation tends to be elevated because the brain is part of the body, and the brain weight : cerebellar volume correlation is similarly inflated. Consequently, for the purpose of calculating Pearson correlations, a corrected "body" weight score was obtained by subtracting the brain weight from the body weight of each mouse. The "brain" weight was corrected by subtracting the weight of the cerebellum from the brain weight. The weight of the cerebellum was estimated by multi-plying the cerebellar volume of each mouse by 1.05 g/cm^3, an estimate of the density of formalin-fixed brains of 10-day-old mice obtained in our laboratory using Archimedes' principle.

RESULTS

Experiment 1

Behavioral Repertoire. Some of the 36 behavior patterns were seen very rarely and therefore were of little interest in a first study of behavior of dr^{sst}/dr^{sst} mice. For example, the side walk occurred in only three of 54 mutant mice and never in normal mice. In two mutants at 13 and 19 days it occupied little of the 5-min period, but one mutant on Day 26 spent 64% of the time on its side doing the side walk, which indicated a severe deficiency of the righting reflex. Results are presented in Table I only for behaviors exhibited by more than 5 of the 80 mutant and normal mice during the 5-min test and averaging at least 5% of the period on at least 1 day. Data are combined for 5-day intervals in the form of the average percentage time spent doing the specific behavior.

Normal mice had difficulty maintaining an upright posture only within the first 5 days. Immature behavior patterns, such as weak rest and pivot, disappeared and were replaced by more mature patterns at 2 weeks from birth. From 10 to 19 days, normal mice spent considerable time resting, whereas from 20 days onward, they actively explored the cage and exhibited many behaviors seen in adult mice. Among mutant mice, righting behaviors were observed at all ages, and animals were rarely at rest at any age after Day 14. Except for grooming, behaviors common among normal mice from Day 20 to Day 30 were very rare or totally absent in mutant mice. The total percentages of the 5-min period occupied by the behaviors listed in Table I indicate that they comprised almost the entire repertoire of normal and mutant mice.

Table I. Percentages of the 5-min Period Spent Performing Behaviors[a]

	Age range (days)				
	0–4	5–9	10–14	15–19	20–30
Normal mice					
(*N*)	(5)	(5)	(5)	(5)	(6)
Side righting	6	0	0	0	0
Leaning rest	10	0	0	0	0
Weak rest	70	57	0	0	0
Mature rest	0	29	68	51	10
Pivot	10	7	2	0	0
Walk	0	1	16	18	28
Sniff	0	0	10	18	23
Stand	0	0	0	8	11
Rear	0	0	0	2	10
Groom	0	0	2	2	12
Total	95.5	94.0	99.0	97.5	95.4
Mutant mice					
(*N*)	(8)	(6)	(13)	(7)	(10)
Weak rest	20	6	1	1	0
Mature rest	0	0	7	2	2
Groom	0	0	1	2	7
Front righting	4	5	4	0	0
Flail	12	3	6	8	5
Roll	0	0	0	7	6
Leaning rest	7	32	12	4	0
Back rest	12	12	7	2	0
Side rest	37	17	23	7	0
Trunk rest	0	10	9	1	0
Circle	0	0	0	37	38
Sway walk	0	0	0	12	23
Head shake	0	1	4	6	3
Head toss	0	0	1	5	6
Back extension	0	2	14	3	0
Total	92.2	88.3	89.4	95.5	89.6

[a] An entry of zero indicates less than 0.51% of the period.

Many of the behaviors were rare or absent among normal mice but frequent among mutant mice. The less active front righting disappeared and the more active roll appeared at about 2 weeks after birth. Righting behaviors were highly variable among older mutant mice. For the 10 mice tested from Day 20 to Day 30, 2 remained on their feet at all times and never had to right themselves, and 4 spent less than 5% of the time righting or attempting to right themselves, but 4 others spent 16.4, 19.3, 62.2, and 80.6% of the time, respectively, engaged in righting behaviors. These data indicate extreme variability in the extent of impaired behavior among

surviving mutant mice. For those mutants which did acquire a competent righting response, motor behavior was still far from normal; most showed incessant circle or sway walk behaviors interspersed with head toss movements. Head shake and/or head toss behaviors were noted in every mutant mouse after Day 15.

Certain patterns of behavior in mutants suggested seizures, particularly back extension, front extension, paw grasp, and tumble. Bouts of these activities were generally quite brief, but three mice on Day 10 spent more than 25% of the time in the back extension posture. After 15 days, seizure-like activities were seen in only 6 of 16 mice and were very brief, which may reflect a higher mortality among seizure-prone mice.

The grooming behavior of dr^{sst}/dr^{sst} mice was similar to the normal pattern in frequency and topography of motion of the forelimbs with respect to the body, but it was often performed while the mutants were lying on their backs or sides. When a mutant mouse in an upright posture began to groom, it usually tipped over but continued grooming for a few seconds.

Morphological and Reflex Development. At birth, the 258 normal mice had a mean body weight of 1.44 g, which was significantly greater than the average weight of 1.33 g for the 91 mutant mice ($t = 5.85$; df = 347; $P < 0.001$), but the standard deviations were similar for the two groups (0.154 for normals, 0.166 for mutants) and many mutants were within the normal range.

The scores on each of six variables and curves of best fit for the normal mice are presented in Fig. 1. The phase of reduction in EGL thickness could be fit precisely using the quadratic curve for only Days 5 to 20. Otherwise, the curves described the change in each measure from Day 0 to Day 30 with great precision. The proportion of variance in each variable accounted for by the curve of best fit shown in Fig. 1 was at least 0.98 in every instance.

It is apparent from Fig. 1 that at birth several of the mutant mice were quite close to the values expected for normal mice on all five variables measured. Body weight and brain weight increased for the first 10 days at a rate close to normal, although in every case the mutant mouse was smaller than its matched control, which indicates a small difference in median size even at the earliest phase of postnatal growth. By 2 weeks after birth, body and whole brain growth was clearly retarded in all surviving mutant mice. The tails of some of the mutants grew rapidly but the ones with almost no tail at birth remained very short, which gave rise to extreme variability in tail length as exemplified by the six littermates tested at Day 10. The cerebellum grew substantially in several animals but remained very small in several others, and variability in the cerebellar volume was extremely large after 10 days. Despite the impaired growth of the cerebellum as a whole, the granule cells migrated away from the EGL on schedule for those mice with enough cerebellum present at mid-plane to allow a reasonable measure of EGL thickness. The generally greater thickness of the EGL for mutants was probably an artifact of the bizzare foliation patterns, which made it difficult to obtain a sagittal section that was perpendicular to the axis of a lobe; any section cut obliquely to the axis would necessarily make the EGL appear thicker at that point than it truly was. Reflex ontogeny was severely impaired, to the extent that for mutants the linear change in the mean reflex score across the ages studied was not significant and most mutants performed below the level expected for a normal 8-day-old mouse. Inspection of scores on individual tests revealed that a few mutants did acquire a competent righting reflex after Day 16 and a forelimb grasp reflex after Day 11. No mutant mouse showed any sign of an auditory startle response at any age, which suggests that dr^{sst}/dr^{sst} mice are deaf, as were Dunn's *st/st* mice studied by Bonnevie (1936).

Growth Retardation. Inspection of the data for the older mutant mice (Fig. 1) revealed obvious correlations of brain and cerebellum size with body weight. Equations regressing tail length, brain weight, and cerebellar volume on body weight were derived for the 21 normal mice from Day 0 to Day 20, an age range where normal scores spanned almost all scores for mutant mice, but were not yet asymptotic for normal brain and cerebellum size, and where sex differences were very small. These equations were then used to predict the value for a mutant mouse, given its body weight. The deviation between the predicted score and the observed score indicated the degree of effect of the mutant genotype on the particular phenotype in addition to its effect on

overall size of the mouse. Summary statistics for the deviation scores of two age groups with similar numbers of mutant mice are given in Table II along with one-tailed *t* tests comparing the mean deviation from predicted values to an expected deviation of O. In every case the mean difference between the scores of mutant mice and the values predicted on the basis of body weight was much smaller than the mean difference between mutants and their normal controls, which indicates that substantial portions of the deficits produced by the dr^{sst}/dr^{sst} genotype were produced by growth retardation. For ex-ample, the brains of mutants older than 10 days averaged 144 mg smaller than controls, but they were only 19 mg less than expected on the basis of the body weights of the mutants. Nevertheless, the deviations from predicted values were statistically significant in all but one instance, which shows that the dr^{sst}/dr^{sst} genotype did have deleterious effects in addition to effects on overall growth rates.

Table II. Mean Deviations of Mutant Mice in Two Age Ranges from Values for Normal Control Mice and Values Expected from Regression Equations on the Basis of Body Weight

	Days 0 to 10 (N = 20)			Days 11 to 30 (N = 24)		
Variable	Difference from controls	Difference from expected	t	Difference from controls	Difference from expected	t
Tail length (cm)	−0.80	−0.57	−4.09**	−1.85	0.18	0.82
Brain weight (mg)	−56.0	−27.0	−4.31**	−144.0	−19.0	−2.33*
Cerebellar volume (mm^3)	−6.7	−4.7	−4.43**	−25.0	−8.1	−6.09**

* Deviation from expected value of 0 significant, $P < 0.05$, one tailed.
** $P < 0.01$.

Experiment 2

Physical Measures at Day 10. Means and standard deviations for mutant and control mice are given in Table III along with tests of significance. Mutants had significantly lower averages on every measure. In fact, no mutant mouse scored higher than the lowest control mouse for any variable. The means of control mice were reasonably close to the values expected at Day 10 on the basis of the curves given in Fig. 1, but the deviations of control mice from the values predicted from their body weights were significantly different from zero for tail length and cerebellar volume. That is, the control mice in Expt 2 had slightly shorter tails and somewhat smaller cerebellar volumes for their body weights than expected on the basis of data for control mice in Expt 1. Because of this, the deviation scores of mutant mice were compared to those of their siblings, not to an expected value of zero. In all three cases, deviations for mutants were significantly greater (more negative) than those for their normal siblings, which replicates the finding in Expt 1 that deficits produced by the dr^{sst}/dr^{sst} genotype entailed more than just growth retardation of the whole mouse. Again, growth retardation was an important part of the story, because differences between deviation scores of mutants and those of controls were substantially smaller than differences between their raw scores.

Table III. Mean Values at 10 Days After Birth

	Controls (N = 18)		Mutants (N = 18)	
Variable	Mean	SD	Mean	SD
Body weight (g)	6.85	0.52	4.77*	0.66
Tail length (cm)	3.50	0.23	1.87*	0.87
Brain weight (mg)	415.0	17.0	305.0*	35.0
Cerebellar volume (mm^3)	23.1	2.6	8.0*	4.7
Difference from prediction				
Tail length (cm)	−0.27**	0.27	−0.85*	0.82
Brain weight (mg)	−4.0	13.0	−18.0*	20.0
Cerebellar volume (mm^3)	−4.7**	2.7	−9.5*	3.6

* Significantly less than control mean, $P < 0.01$, one tailed.
** Significantly less than 0.0, $P < 0.01$, two tailed.

Reflex Development. Analysis of the averages of scores on 11 reflex tests on each day for mutants and controls indicated a highly significant interaction between genetic group and test day (F = 38.8; df = 2,68; $P < 0.0001$), which meant that control mice improved their reflex performances much more rapidly than did mutant mice. According to the simple main effect for days, the mutant mice did improve significantly over days (F = 9.3; df = 2,68; $P < 0.01$) (means of 0.17, 0.21, and 0.25 on days 6, 8, and 10), although their change was modest in

comparison with the large change for the controls (means of 0.36, 0.49, and 0.71). By Day 10, the mutants still had not achieved the average level of performance shown by controls on Day 6.

Comparisons of performances on individual tests averaged over the 3 days revealed that dr^{sst}/dr^{sst} mice were impaired on all 11 tests but to varying degrees (Table IV). Group differences were highly significant on every test (all $P < 0.001$). Deficits for the mutants were especially severe for the righting reflex and cliff aversion tests, whereas they were least pronounced for the level-screen grasp test. On the remaining tests, differences between mutant and control mice were intermediate and the rank orders of tests by average scores for the mutants and controls were almost identical.

Table IV. Mean Scores for Specific Reflex Tests Averaged over Days 6, 8, and 10 for 18 Control and 18 Mutant Mice

Reflex test	Controls		Mutants		
	Mean	SD	Mean	SD	*t*
Righting reflex	0.88	0.08	0.24	0.13	17.3*
Cliff aversion	0.83	0.10	0.17	0.25	10.2*
Forelimb graps	0.82	0.09	0.57	0.10	8.3*
Hindlimb grasp	0.47	0.09	0.21	0.12	7.2*
Vibrissa placing	0.32	0.09	0.04	0.03	12.2*
Level screen grasp	0.38	0.07	0.27	0.09	4.1*
Vertical screen cling	0.70	0.10	0.38	0.17	6.7*
Screen climb	0.32	0.13	0.02	0.05	9.2*
Pole grasp	0.44	0.06	0.26	0.08	7.4*
Forelimb stick grasp	0.39	0.09	0.11	0.09	9.5*
Hindlimb stick grasp	0.16	0.10	0.04	0.07	4.5*

* $P < 0.01$, one tailed.

Retardation of Development. It is apparent that mutant mice had lower values on all measures at Day 10 than their normal siblings and that the difference was partly a manifestation of retarded development. However, it is not at all clear from Table III whether there are substantial differences in the degree of retardation of the various phenotypes. To compare development of phenotypes measured on different scales such as grams and centimeters, it is helpful to transform them to a common scale of developmental age measured in days, as was done in previous studies (Wahlsten, 1974, 1975; Wainwright and Russell, 1983). Using the equations of best fit from Expt 1 (Fig. 1), it is possible to estimate the developmental age by substituting the phenotypic value for a mouse into the equation and solving for days. For example, a mouse with a brain weight of 0.25 g would be assigned a developmental age of 4.6 days, which is the age at which a group of normal control mice would achieve a mean brain weight of 0.25 g. Average developmental ages derived from five different measures are listed in Table V for the normal siblings and mutant mice. The controls were close to 10 days on three measures, as would be expected, whereas their body sizes were more advanced and their cerebellar volumes less advanced compared to the sample of normal mice observed in Expt 1. The variabilities of developmental age were quite similar for all five measures of the control mice. The mutant mice were 4 to 5 days behind controls on every measure except tail length, which was severely retarded for most mutants and much more variable in comparison to controls. Cerebellar volume was no more retarded on average than was body weight, but its variability was much greater than for controls. Thus, the dr^{sst}/dr^{sst} genotype caused moderate retardation of development for several gross measures of body size, brain size, and behavior, but it severely impaired growth of the tail and cerebellum for some, but not all, mutant mice.

Table V. Developmental Ages in Days Estimated from Each of Five Variables Measured at Day 10 for 18 Control and 18 Mutant Mice

Variable	Controls		Mutants		Group difference	Ratio of variances
	Mean	SD	Mean	SD		
Body weight	12.14	1.29	7.03	1.63	5.14*	1.61
Tail length	10.41	0.94	3.80	3.36	9.61*	12.91*
Brain weight	10.37	1.04	6.15	1.06	4.22*	1.05
Cerebellar volume	8.71	0.79	3.50	2.25	5.21*	8.20*
Reflex score	10.39	0.64	6.29	1.32	4.10*	4.21*

* $P < 0.01$, one tailed.

Correlations Between Phenotypes. For a mutation with widespread adverse effects on development and variable expressivity, it is important to know whether deficits on the various phenotypes are highly correlated or, perhaps, are independent. Are the mutant mice with the shortest tails also the ones with smallest cerebellar volumes? To answer this question, Pearson correlations were computed for several measures, including corrected values of "body" and "brain" weights and average reflex score on 11 tests over 3 test days. All correlations were positive and most were significant for both the control and the mutant mice. A noteworthy exception was the correlation of 0.205 between tail length and cerebellar volume for the mutant mice, which was not significant ($P > 0.05$). Cerebellar volumes of mutants were highly correlated ($r = 0.847$) with their corrected brain weights, which shows that the effects of the dr^{sst}/dr^{sst} genotype on the nervous system are not restricted to the cerebellum. Cerebellar volume was also significantly correlated with average reflex score for mutants ($r = 0.630$; $P < 0.001$) but not for controls ($r = 0.364$; $P > 0.05$). It is likely that all correlations for the control mice were reduced somewhat because of restricted range.

DISCUSSION

The results of these two experiments comprise the first systematic reports of the effects of the "shaker short-tail" gene on growth and development. The general retardation of growth by drsst/drsst must be considered in future research because differences between mutants and siblings of the same chronological age will always be large and probable trivial. It would be more informative to compare mice equated for the overall degree of morphological maturity, as has been done for prenatal fiber tract ontogeny (Wahlsten, 1981; Wainwright and Deeks, 1984). Retardation probable has several causes, including normal nutrition, because dr^{sst}/dr^{sst} mice are less able to compete with normal siblings in the nest for good suckling locations. If the amount of food consumed is indeed a problem, then body size should suffer relatively more than brain size (Nagy, 1979). If this is true, then the deficits in brain size given in Tables II and III probably underestimate the size of the detriment. It should be possible to change the rearing conditions experimentally and modify the consequences of the drsst/drsst genotype, as has been done with the "staggerer" mutation by Guastavino (1984a,b).

Neonatal and preweaning shaker short-tail mice have serious problems of motor coordination, and these problems seem to intrude into almost every waking activity. In the first week after birth, they frequently rest, but instead of the normal pattern of rest on the ventral abdomen, they often rest on the back, shoulder, or side. Most of the unusual postures seen in this period seem to be derived from a poorly developed or absent righting reflex; the mice struggle to right themselves and periodically rest in whatever position they happen to be. Immature behavior patterns such as weak rest and pivot disappear from the repertoire, albeit a little behind schedule in comparison to normal siblings, and most of them acquire a reasonable proficiency at righting. Once these animals are able to locomote in an upright posture, they are almost always active and rarely rest, but their motor patterns are unusual. Although some dr^{sst}/dr^{sst} mice exhibit what appears to be normal grooming, this behavior is also unusual in that the mouse often falls over while rubbing its face. It is likely that abnormalities of the motor patterns of grooming would be revealed by more detailed observation and sophisticated analysis of the limb motion topographies (Fentress and Stilwell, 1973).

The postnatal development of several other neurological mutations has been studied systematically, but direct comparisons with dr^{sst} are difficult because so many details of methodology vary between studies. Cripps and Nash (1983) examined reflex ontogeny of "pigtail-rotator" *(pr)* mice and found that *pr/pr* mice achieved full responses on several items in the Fox (1965a) test battery about 2 days later than normal sib-lings. van Abeelen and Kalkhoven (1970) applied the Fox (1965a) reflex tests to "Nijmegen waltzer" *(nv)* mice and found no consistent difference from controls in rate of ontogeny, although *nv/nv* mice expressed certain responses significantly earlier and others later than controls. Myers (1970) applied the Fox (1965a) tests to "reeler" *(rl)* mice and found no consistent differences prior to Day 12. He noted extreme behavioral variation among *rl*/*rl* mice and chose to test only the most active ones. Fox (1965b) himself examined reflex ontogeny of mice with nine different neurological mutations but presented the data in a manner that precluded accurate estimates of developmental delay. None of these investigators obtained brain weights, and all used repeated testing of the same mice over several days. It appears that the dr^{sst}/dr^{sst} genotype has much more deleterious effects on postnatal development than the mutations mentioned above.

The dr^{sst}/dr^{sst} genotype is remarkable for the highly variable defects which accompany it, in both brain anatomy, cerebellum size in particular, and behavioral repertoire. A few animals never gained a competent righting reflex prior to weaning, and these also had exceptionally small cerebellar volumes. This great variability may prove useful for future studies of relations between brain structure and behavior. The low correlation between tail length and cerebellar volume of dr^{sst}/dr^{sst} mice suggests variation in expression of the gene within the same mouse. Correlations between phenotypes of dr^{sst}/dr^{sst} mice tend to be large because of substantial individual differences in the rate of overall development. That is, phenotypes tend to be correlated not so much because the gene has direct physiological effects on both of them but because all parts of the system tend to be smaller in developmentally younger mice. Furthermore, increased variability among mutant mice makes a phenotypic correlation more visible, if one exists. However, both tail length and cerebellar volume show extreme variability but are nevertheless uncorrelated. Evidently the shaker short-tail gene can influence events at opposite ends of the neutral tube independently.

APPENDIX: CATEGORIES OF BEHAVIOR

Items with an asterisk(*) were seen only in mutant mice.

Several behaviors occurred when mice were attempting to right them-selves after falling over.

1. Front righting: The head and torso are raised with the support of one or both forelimbs; the trunk is raised and then thrust ventrally.
2. Side righting: One forelimb is extended from the shoulder to grasp the substrate, and the trunk is thrust to one side.
3. Fall: The mouse falls from an upright position onto one side of the body.
4. Flail (*): All limbs move in an uncoordinated pattern while the mouse is on its back.
5. Roll (*): The mouse briefly gains an upright position but continues turning and rolls onto its back again.

Several postures occurred while a mouse was at rest and not moving its limbs.

6. Weak rest: The abdomen is on the bedding, with the limbs not supporting the body.
7. Mature rest: All four limbs are under and supporting the body.
8. Sway rest: The body is stationary but the head and shoulders move from side to side with the support of the forelimbs.
9. Leaning rest (*): One shoulder and the head are on the bedding while the hindlimbs are splayed and on the bedding.
10. Trunk rest (*): The forelimbs support the body but the hindlimbs are splayed and the trunk rests on the bedding.
11. Side rest (*): The entire length of one side of the body rests on the bedding.
12. Back rest (*): The entire length of the back rests on the bedding; the limbs are motionless.

Several behaviors resulted in the mouse moving from one portion of the cage to another and therefore were termed exploratory.

13. Pivot: The forelimbs propel the body in a circular motion while the hindlimbs are stationary.
14. Walk: All four limbs are under the body and moving in a coordinated manner.
15. Circle: The mouse walks rapidly in a tight circular path.
16. Sway walk (*): The body lurches from side to side while walking.
17. Stand: The hindlimbs are on the bedding with the forelimbs against the side of the cage.
18. Rear: The hindlimbs are on the bedding with the forelimbs not touching any object.

There were also behaviors involving motion of part of the mouse.

19. Sniff: The body is stationary and erect but the whiskers are moving rapidly.
20. Dig: The mouse pushes or scrapes the bedding material with both forelimbs or both hindlimbs while the body is erect.
21. Groom: The mouse rubs or scratches the body with one or two paws.

Finally, there were several patterns seen only in the dr^{sst}/dr^{sst} mice.

22. Head shake (*): The mouse repetitively moves the head horizontally.
23. Head toss (*): Acute retroflexion of the head occurs.

24. Back extension (*): The mouse is on its back with the limbs extended and rigid; the back and neck are arched.

Other behaviors which were very infrequent were trunk rock, body grasp, air push, side walk, run, jump, crawl, drag, head bend, front ex-tension, paw grasp, and tumble. Descriptions of these are available from the authors.

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