Shaker short-tail, a spontaneous neurological mutant in the mouse

By: Douglas Wahlsten, Jill P. Lyons, and Walter Zagaja

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Abstract:

A spontaneous mutation that occurred in the Inbred strain BALB/cCF In 1980 proved to be inherited as a single autosomal recessive gene with complete penetrance. Homozygous recessive animals have a short or blunt tall, an irregular pattern of foliation of the cerebellum, delayed development of the righting reflex, and a wide variety of other peculiar behaviors. The effects of this mutation strongly resemble those of the shaker-short (st) gene reported by Dunn in 1934 that is now extinct. The gene designation, sst, is proposed for the new shaker short-tall mutation.

Article:

A large number of mutations known to affect the nervous system of mice has proven useful in research on mechanisms of brain development and function[7,10], and among these, defects of the structure of the cerebellum have been especially informative[8,9]. The present report describes a new recessive neurological mutation that radically alters the pattern of foliation of the cerebellum and blunts or shortens the tail. The mutation occurred spontaneously in one of 18 separate substrains of BALB/c mice maintained by full-sib mating at the University of Waterloo, and it was first detected on October 20,1980, when one male and one female in a litter of six were observed to have no righting reflex at two weeks after birth. Crossing experiments involving mice descended from the parents of these two defective animals revealed that the mutation is inherited as a single autosomal recessive gene with complete penetrance but variable expressivity. Study of the behavior, external morphology, and brain anatomy of several dozen afflicted animals indicated a novel phenotype associated with this mutation.

Materials and Methods

Laboratory mice of the species *Mus domesticus*[5.6] used in this study were all bred and reared in the authors' laboratory using methods described in detail in a previous report[12]. The mutation originally occurred in the strain BALB/cCF that had been inbred at Waterloo for seven generations after mice were procured from Carworth Farms, It is estimated that this substrain of BALB/c had been maintained by full-sib matings for at least 100 generations prior to 1980. Presumed carriers of the mutation were crossed with mice of various genotypes in order to determine the mode of inheritance. Some matings were with noncarrier BALB/cCF mice, and others were with B6D2F_3/J mice, the F_3 cross derived from B6D2F_1/J parents obtained from the Jackson Laboratory. Carriers also were mated with C57BL/6J mice, and the offspring were then crossed with known carriers to determine which of them were carriers. These F_1 carriers were then backcrossed to C57BL/6J, and offspring were in turn mated with known carriers. Because the mode of inheritance of the gene proved to be the same regardless of genotype with which crosses were made, data on inheritance are pooled across strain of noncarrier for presentation here.

In the early phases of the study mice in each litter were tested for the righting reflex at 10 days after birth, More careful observation of newborn mice detected anomalies of the tail and skull that made it possible to distinguish affected animals prior to the age at which the righting reflex normally appears. Consequently, in later phases of the study each mouse was examined on the day of birth for physical abnormalities as well as later in life for behavioral abnormalities. Later behavioral testing involved a standardized battery of 14 reflex tests[11] designed

to assess many aspects of sensory and motor function, as well as observation of response repertoire during exploration of a clean cage.

Defective animals were usually sacrificed at 10 days of age or later in order to examine their brains. They were anesthetized with pentobarbital sodium and then perfused intracardially with saline followed by 4 percent neutral buffered formaldehyde. Brains extracted from the skull were examined for external abnormalities and most were then sectioned at 25 µm with a freezing microtome in either the coronal or sagittal plane. Sections were stained with either metachromatic thionin, cresyl violet, or Sudan black B. A few brains were embedded in glycol methacrylate plastic, sectioned at 2 µm with a Sorvall JB4A microtome and stained with toluidine blue O. Because many of the affected mice were per-fused only when their condition had clearly begun to deteriorate, the observations of their brains can reliably show gross structural defects but are not suitable for analysis of small quantitative differences.

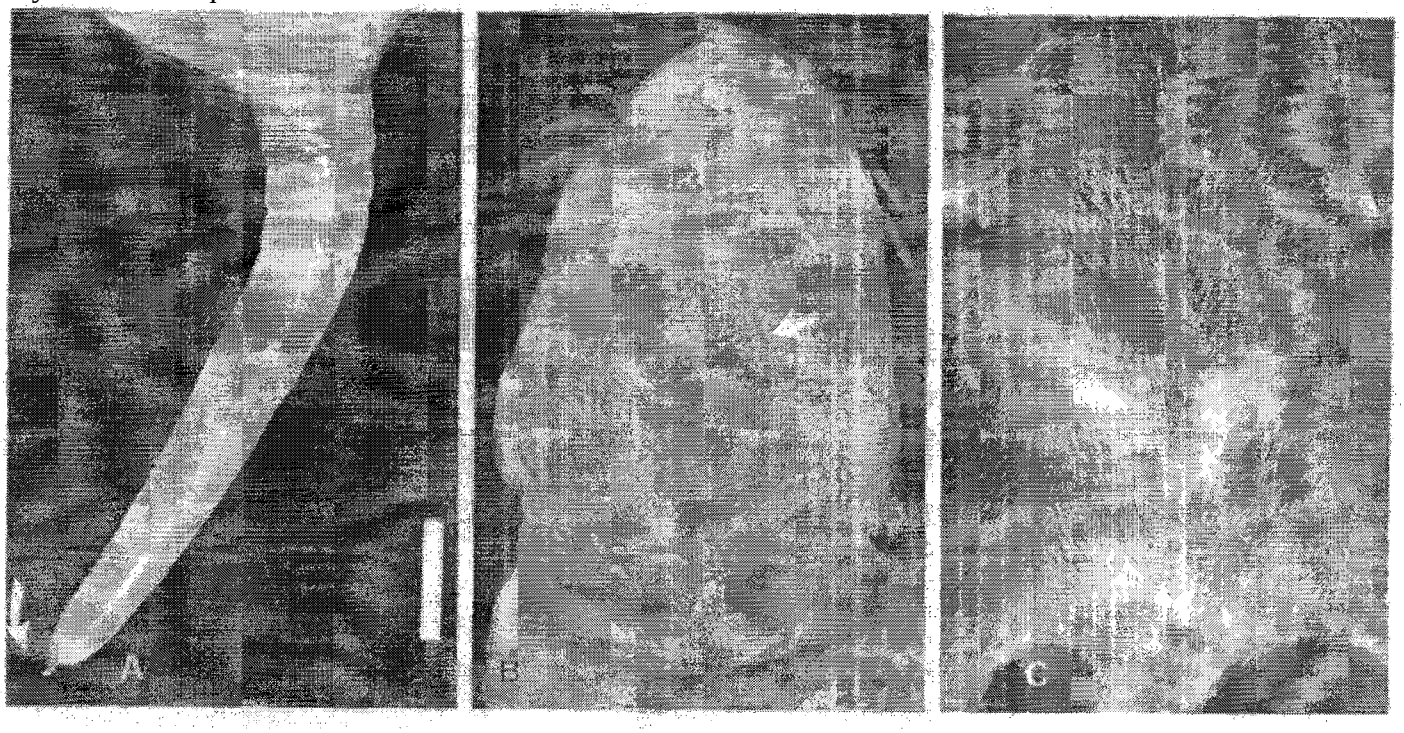

FIGURE 1 Abnormalities of external appearance in *sst sst* mice. *A*—blunt tail in a one-day-old mouse with a small filament (arrow) at the tip (bar represents 0.2 mm). *B*—dorsal bleb (arrow) with a small scab in a one-day-old littermate of the mouse in *A*; this animal had just died. (Same magnification as in *A*.) *C*—patches of white hair on the ventral abdomen of a 13-day-old mouse with a black agouti coat. (Magnification is 0.6 × that of *A*.)

Results

Definition of the phenotype

Among the 1072 neonatal mice examined in this study, there were 151 with features typical of the phenotype associated with this mutation. There were 97 abnormal animals observed closely on the day of birth that were recognized by the presence of either; a) a blunt tail with a small filament at the distal tip (Figure 1A) or an obviously short tail; or b) a small blood bleb at the junction of the parietal and interparietal plates of the skull at the dorsal midline (Figure 1B) As indicated in Table I, a defect of the tail was evident in all but one animal (it had a bleb), whereas a bleb was not always seen. In every animal with either or both of these anomalies at birth that survived until 10 days of age, and was perfused, the righting reflex and cerebellum were clearly abnormal. Likewise, every animal that showed an abnormal righting reflex and was then perfused also had an abnormal cerebellum. Thus, the components of the phenotype, which are virtually always present when rigorously assessed, are a defective tail and/or a bleb at birth, with an abnormal righting reflex and cerebellum later in development. Because of the relatively high mortality (Figure 2), especially during the first two days after birth, many mice found to be abnormal at birth were never subjected to reflex testing, and some mice given reflex tests died before they could be perfused. Those with a bleb at birth were much less likely to survive beyond one week of age than those with only the tail defect evident, almost all of whom survived at least one week. A few of the 63 pigmented mice had a peculiar absence of pigmentation in a patch of fur on the ventral abdomen (Figure 1C).

Table I. Number of mutant mice assessed (*N*) and percent with each symptom (%S)

Symptom	*N*	%S
Tail blunt or short	97	99
Bleb under skull	97	74
White patch in coat	63	26
Poor righting reflex	55	100
Abnormal cerebellum	36	100

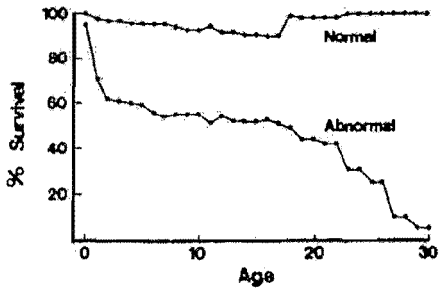

FIGURE 2 Percent survival of 96 liveborn *sst sst* mutant mice and 259 of their normal littermates from the day of birth to 30 days. The few instances where survival seems to increase slightly occurred because several animals were occasionally sacrificed for histology or dropped from the study after behavioral testing at one age, thereby reducing the sample size of the remaining mice.

Inheritance

For the purpose of exploring the mode of inheritance of the mutation, a carrier was defined as a mouse that produced a litter containing one or more animals with the abnormal phenotype, and a noncarrier was a mouse from a substrain of BALB/cCF or a non-BALB strain that had never produced an animal with the abnormal phenotype. The F_1 hybrids listed in Table II were offspring of a cross between a carrier and a noncarrier. No F_1 hybrid animal showed the abnormal phenotype, thus indicating recessive inheritance. Because there was no significant difference between the number of affected males and females, and each sex could transmit the mutation with approximately the same probability, data for sexes are pooled in Table I. Inheritance was evidently autosomal. Observed numbers of defective animals in three kinds of crosses were compared to expectations derived from the hypothesis of single-locus Mendelian inheritance, and in two instances there was no significant discrepancy. However, for crosses between a carrier and a sibling of a mutant. two-thirds of which should have been carriers, there were significantly fewer mice with the abnormal phenotype than expected. This result probably occurred because many defective animals in the first series of observations died before they were 10 days old. Among 147 offspring from this type of cross in the first series there were 9.5 percent defective animals, whereas among 303 offspring in the second series observed on the day of birth 14.2 percent were found to be abnormal, All of these results are consistent with the hypothesis that the mutation involves a single autosomal recessive gene with complete penetrance, one that has clear effects in the homozygous state regardless of whether it is on an inbred BA LB/cCF or a hybrid genetic background. This conclusion can only be made tentatively because it amounts to acceptance of the truth of the null hypothesis. Demonstration of linkage- with a known locus will strengthen this hypothesis.

FIGURE 3 Resting posture of a 31-day-old *sst sst* mouse showing the typical splaying of the hind limbs and squinting of the eyes.

Behavior

One consistent behavioral abnormality is that there is no righting reflex or a very poorly executed righting response at 10 days after birth, a time when nonaffected mice always right themselves quickly. Some of the

affected mice do eventually develop a righting response by 16 days of age, but others never are able to right themselves and instead show a futile flaying of the limbs and flexing of the spine. Those that can right themselves usually display a wide splay of the hind limbs when they try to sit (Figure 3), and they usually fall over when they try to groom or pick up a piece of food. Locomotion in awake and aroused animals is incessant and chaotic. Some animals spend long periods of time circling rapidly, and others show frequent head-tossing and a lurching gait. During exploration of the cage the mice seem unable to remain near any one object for long or focus attention on any particular feature of the environment. Eating hard lab chow blocks poses great difficulty and often results in death from starvation when the mother stops nursing during the fourth week after birth (Figure 2). Only two mice given a diet of wet mash in a cup on the cage floor have been able to survive more than two months after birth, The animals have effective suckling behavior in the nest and sometimes have body weight within the normal range prior to weaning, but those in larger litters with many normal siblings do not compete well for good nursing positions and usually deteriorate. Olfactory, tactile, and visual sensory functions seem to be present in most animals that survive to three weeks after birth, but auditory function is clearly impaired. No affected animals have ever shown an auditory startle response, which means they are probably deaf or nearly so. A systematic extensive study of behavioral development is currently in progress and will be reported later in detail.

Table II. Tests of single-locus Mendelian inheritance

Cross	Total offspring	Mutants obs. N	obs. %	exp. %	χ^2
Carrier × non-carrier	120	0	0	0	—
Carrier × carrier	324	76	23.5	25.0	0.3
Carrier × sib of mutant	450	57	12.7	16.7	5.0*
Carrier × F_1 hybrid	178	18	10.1	12.5	0.7

* $P = 0.025$

Brain

Whole brain weights of abnormal animals are always less than those of normal litter-mates of the same age, which primarily reflects severe deficiency in size of the cerebellum. The cerebellum is always reduced in volume and is sometimes only a small fraction of the normal size. In brains with a moderate sized cerebellum there is an obvious abnormality in the pattern of foliation both in the number of lobes and the contorted configuration of the fissures. Even the superior and inferior colliculi are sometimes misshapen. Sagittal sections of the cerebellum show the bizarre foliation pattern and reduced size very clearly (Figure 4A-C). Despite these most unusual structural arrangements, development of the cerebellum is relatively normal in many respects. The external granular layer forms and then dissipates as the granule cells migrate to their correct locations beneath the Purkinje cells, and the positions of major cell types seem to be normal, provided a section is examined where a lobe happens to be in the proper plane of sectioning. Major fiber tracts show intense myelin staining with Sudan black B four weeks after birth. In one animal, which was perfused 107 days after birth, there was no apparent degeneration of any cerebellar cell type or myelin sheaths.

Abnormalities of the brain are not confined to the cerebellum. For example, in the dorsal portion of the hippocam pus the dentate gyrus is often absent, although the cells of the hilt's are in place and the regio inferior and regio superior have reasonably normal shapes (Figure 54 and B), The corpus callosum is usually quite thin near the mid-sagittal plane Various peculiar features of the cytoarchitectonics of the brain are under investigation.

Other organs have not yet been examined, but in view of defects of the brain, tail, and ventral pigmentation, it would not be at all surprising to find abnormalities of the autonomic nervous system and Schwann cells. The

array of abnormalities observed so far is strongly suggestive of a defect in the forniation of the neural tube.

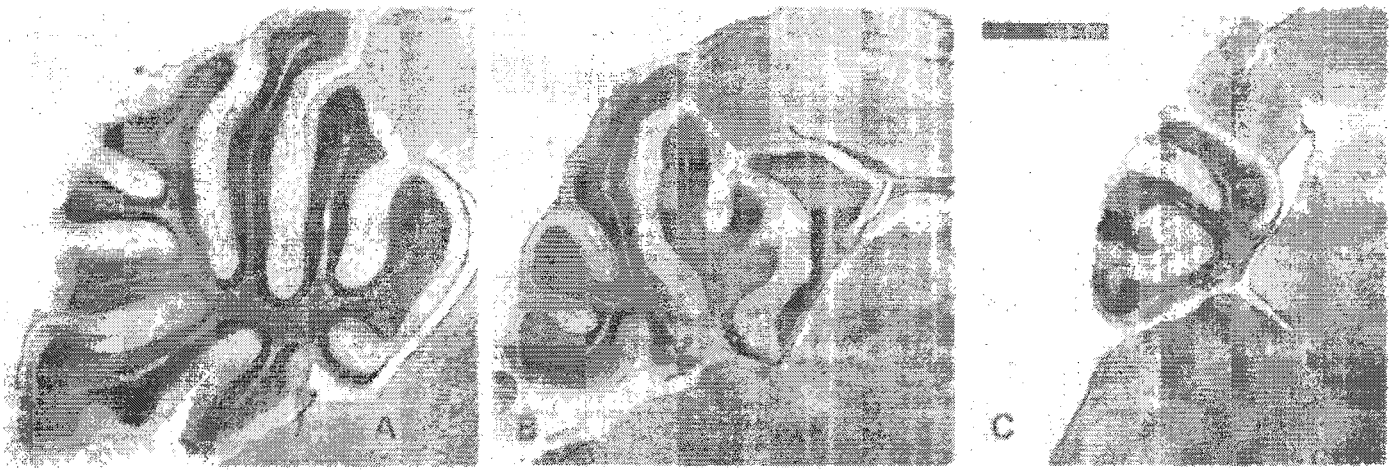

FIGURE 4 Mid-sagittal sections of the cerebellum stained with metachromatic thionin and photographed with the same magnification. *A*—a normal 20-day-old mouse showing the typical pattern of foliation. *B*—a 20-day-old *sst* *sst* littermate of the mouse in *A* having slightly reduced size but very unusual pattern of foliation of the cerebellum. *C*—a 29-day-old *sst sst* mouse with a very small cerebellum lacking several lobes (bar represents 1.0 mm).

Discussion

Results of breeding experiments provide sufficient evidence to make a tentative claim that a single-locus mutation has been discovered. The distinct phenotype associated with this mutation makes it likely to be a new locus rather than an allele at a locus already known to affect the cerebellum and motor coordination, although definitive tests of allelism have not yet been done. The association of short tail with the peculiar defect of foliation of the cerebellum is apparently unique among existing neurological mutations of the mouse.

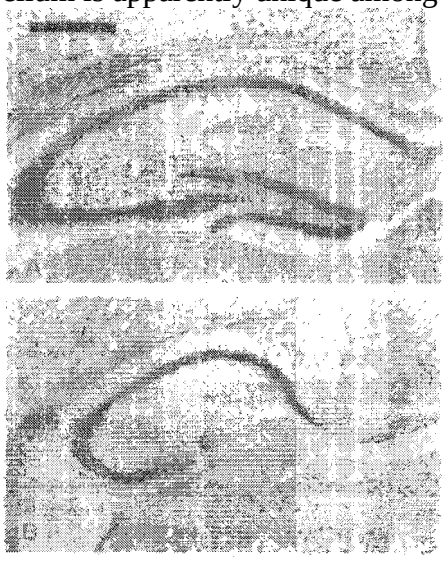

FIGURE 5 Coronal sections of the dorsal hippocampus stained with metachromatic thionin and photographed with the same magnification. *A*—a normal 20-day-old mouse having the typical arrowhead-shaped dentate gyrus (bar represents 0.5 mm). *B*—a 31-day-old *sst sst* mouse having no dentate gyrus present in the dorsal hippocampus.

The mutation bears a striking resemblance to the recessive shaker-short (st) gene[4] reported by Dunn[4] in 1934, but that is now extinct. Dunn described mice with a very short tail or a tail that was"... three-fourths of the length of the normal tail and usually ends in a slender filament „ ." as well as "...at birth a marked lesion near the median point of the parieto-occipital suture." Five-day-old mice showed "severe disturbances in equilibration" and older animals showed "erratic circus movements, more chaotic than those seen in the waltzing mouse ..." and "... ataxia of the head." Bonnevie[1] investigated the embryology of *stst* mice and traced the origins of the "small blebs on top of the skull" and defects of the inner ear leading to deafness[2]. In their catalog of neurological mutants, Sidman et al.[10] remarked that further analysis of development of homozygous recessive mice "must await a recurrence of the mutation."

There is good reason to believe that the mutation detected in our laboratory in 1980 is indeed a recurrence of *st*, as judged by the similarity of phenotypes and mode of inheritance, but proof of this is not possible because no

linkage of *st* with other loci was ever established. We therefore propose that the present mutation be symbolized *sst*, shaker short-tail, to emphasize the similarity to Dunn's mutation.

A recessive mutation occurred at the Jackson Laboratory in 1978 that produced .a phenotype very similar to our defective mice. Carriers provided by H. 0. Sweet have now been crossed with carriers of our mutation, and three litters have yielded seven pups with the characteristic phenotype out of 24 liveborn mice, indicating allelism of the two mutations. Sweet proposes the designation sst^J for the Jackson allele at this locus (Mouse News Letter, 1983, No. 69).

There is a possibility that the *sst* mutation may serve as a model for a rare human disorder known as the Dandy-Walker syndrome in which there is a ventriculocele of the fourth ventricle and greatly reduced size of the cerebellum[3], and that Caviness describes as sometimes being "ulegyric."

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