

TOTTERING—A NEUROMUSCULAR MUTATION IN THE MOUSE

And Its Linkage With Oligosyndactylism

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IN April 1957, an abnormal, wobbly gait was observed in all three members of a five-month-old mated trio of littermate mice (one male, two females) of the DBA/2J strain in the Production Colony of the Roscoe B. Jackson Memorial Laboratory. The mice presumably appeared normal when weaned but had produced no offspring. They were turned over to the senior author for testing and subsequently were shown to be homozygous for a new recessive mutation, which was named tottering, symbol *tg*.

Genetics

The three original tottering mice were outcrossed to mice of the C57BL/10JGn strain. All proved fertile and produced normal F_1 offspring which, when intercrossed, produced F_2 offspring, most of which appeared normal when weaned at about four weeks of age. The F_2 were set aside and examined again at about six weeks of age, when some showed the wobbly gait of their grandparents. In this and subsequent crosses, classification was much more certain at six to eight weeks of age, although the abnormal gait could sometimes be recognized at four weeks. Therefore, nearly all litters were held until they were eight weeks old before final classification. Even so, as will be seen later, some mice were undoubtedly misclassified. The seizures which were later recognized as a regular manifestation of the new mutation occur at irregular intervals and were therefore not useful for routine classification.

Table I gives the numbers of normal and tottering offspring observed in the

F_2 and in crosses of other types. The results do not differ from expectation on the hypothesis that the tottering phenotype is determined by a recessive mutation at a single locus. There is a deficiency of *tg* in crosses 2 and 3 that is not significant in the separate crosses but is significant ($\chi^2 = 4.313$, $P < .05$) when the crosses are combined. The deficiency is probably due largely to lower viability of the mutant type but may also be due to misclassification of some *tg* mice as normal.

Tottering was tested for linkage with several markers and found to be very closely linked to Oligosyndactylism (*Os*)². Of 170 offspring of the cross $Os+ / +tg \times +tg / +tg$, 77 were recorded as *Os*+, 84 as *+tg*, 6 as *++*, and 3 as *Ostg* (the *tg* classification of 2 *++* and 2 *Ostg* was marked as questionable). The nine apparent crossovers all occurred in early litters (the first 40 mice classified) before the linkage had become obvious. Subsequently, all apparent crossovers were held until there was no doubt about the classification for tottering. Among 12 *Os* and six normal-footed mice which were doubtful when first classified, no true crossovers were found. It therefore seems probable that the nine recorded crossovers were the result of misclassification for *tg*. In later crosses of $Os+ / +tg \times Os+ / +tg$, which produced over 600 offspring, there were also no crossovers, but in this case less care was taken to classify correctly for *tg*. Some crossovers may have occurred and escaped detection; recombination between *Os* and *tg* is certainly rare, however, if it occurs at all.

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Os has been tested for linkage with markers in all known linkage groups except IV and XV^{1,3}. Possibly the *Os-tg* linkage represents a new linkage group, XVIII, but other tests must be performed before this can be stated with certainty.

Description

The severity and age of onset of symptoms in *tgtg* mice varies greatly on different genetic backgrounds. The mice on which the following description is based were from a line (called *Ostg*) formed by crossing offspring of the *Os-tg* linkage cross with the *Os* stock and intercrossing offspring of the type *Os+/+tg*. Homozygous tottering mice in this stock are easily recognized at weaning and usually by two and one-half weeks of age. The onset of symptoms thus seems to be considerably earlier than in the linkage cross or in the DBA × C57BL F₂. The seizures described below were not commonly observed in the linkage cross or in the F₂ and there is no record of their frequency or age of onset in those mice, but the tottering gait developed two to four weeks later than in the derived *Ostg* stock.

Homozygous tottering mice in the *Ostg* stock show signs of neurological disease at two weeks of age or a few days later. The first indication, not always detectable, is a more than normal toeing-out of the hind feet. A day or so later the gait becomes abnormal. The trunk is held closer to the ground than normal and the mouse may lean to one side when walking. Occasionally it falls over. An unmistakable sign at this stage is the development of intermittent focal seizures. These consist of a flattening of the trunk in the sacral area, spastic abduction and extension of one hind limb for a few seconds, recurring

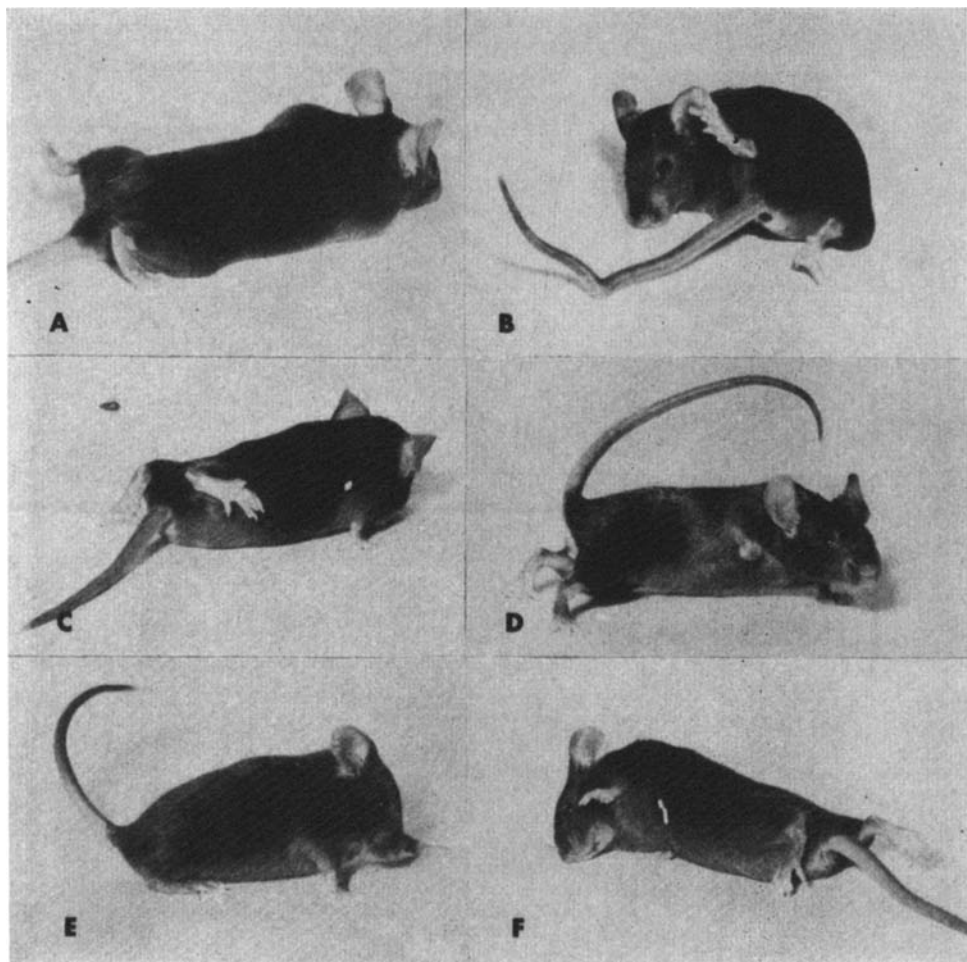
in the same pattern every few minutes (Figure 12A).

During the following week the seizures become more complicated, but the intervals between seizures become longer. By three weeks of age the complete seizure pattern has developed and persists essentially unchanged for the rest of the animal's life, although in some mice the seizures become less frequent with age. A seizure usually is heralded by a brief period of increased running with abrupt changes of direction, resembling "agitated" behavior. This is followed for a minute or so by decreased or virtually absent motor activity, usually accompanied by increased rate and depth of respirations. The mouse may urinate. Then a hind limb is raised and may be held against the trunk or may rotate outward (abduct) and pedal in the air a few times (Figure 12B). Or the limb may rise and drop suddenly to the tabletop. While a hind limb pedals, a fore limb may be lifted and flexed at wrist, elbow, and fingers.

The seizure then spreads. A typical sequence is for one or both hind limbs to abduct severely at the hip and knee, extend at the ankle and paw the air, while the back is held stiffly flexed so that the perineum is pressed against the table (Figure 12C). Next the hind limbs become less involved and a fore limb lifts and paws gently or flexes under the trunk (Figure 12D). A strong tonic flexion of the neck sustained for about 10 seconds (Figure 12E) may then occur. Subsequently, the tail may dorsiflex (just beginning in Figure 12E), muscles about the nose, cheek, or eyelid may quiver (left side of mouth pulled back in Figure 12F), an eye may blink several times, or the lower jaw may work briefly. In these later phases the mouse

TABLE I. Results of crosses with tottering mice

Cross	Parents		Offspring			χ^2
	♀	♂	Normal	Tottering	Total	
1	<i>tg+</i>	<i>tg+</i>	124	45	169	0.239
2	<i>tgtg</i>	<i>tg+</i>	48	35	83	2.036
3	<i>tg+</i>	<i>tgtg</i>	64	48	112	2.286
4	<i>tgtg</i>	<i>tgtg</i>	—	48	48	



STAGES OF SEIZURES IN TOTTERING MICE

Figure 12

A—Flattened trunk and spastic extension of the left hind limb. *B*—Left hind limb pedalling. *C*—Marked abduction at right hip and knee, extension at ankle, and depression of perineum and abdomen against table. *D*—Flexing of the right fore limb. *E*—Tonic flexion of the neck and beginning dorsiflexion of tail. *F*—Simultaneous involvement of all limbs, trunk, neck, and face in the same patterns as recorded for individual body parts in the focal seizures.

may perform well-coordinated face-washing movements with the fore paws or may walk about intermittently. While walking, the animal sometimes shows sudden momentary releases of motor tone throughout the body. A seizure can be interrupted at any stage by lifting the mouse by the tail, although it usually resumes, sometimes at an earlier stage, upon the mouse's release.

It should be emphasized that a seizure

almost always has a focal onset and the pattern is not uniform from attack to attack, even in the same animal. An attack may begin with quivering of an ear, repeated blinking of an eye, or other signs. Some seizures abort without showing any progression, while occasional ones progress to involve all body parts simultaneously in the patterns described above for the individual parts (Figure 12*F*). A representative seizure,

documented below, shows the pace of events:

- 4:25 p.m. Left hind limb abducts at hip and flexes at knee. Mouse urinates.
- 4:26 p.m. While mouse is walking, right hind limb rotates outward and abducts at hip. Back flexes ventrally and both hind limbs abduct and paw the air.
- 4:27 p.m. Fore limbs paw air.
- 4:28 p.m. Tonic flexion of neck. Brief face-washing movements with fore paws.
- 4:29 p.m. Tail lifts slowly and is held stiff and arched.
- 4:30 p.m. Mouse rises slowly upon hind limbs and one fore limb, then tips over backward.
- 4:32 p.m. Beginning to recover.
- 4:33 p.m. Recovered and walking about cage.
- 4:44 p.m. Face washing. Over next five minutes mouse runs about cage and appears confused, then appears weak and stops running.
- 4:50 p.m. Urinates. Hind limbs begin to paw air.
- 4:55 p.m. Unconscious, convulsion involving all limbs, neck, trunk, and tail.
- 4:58 p.m. Lifts head, opens eyes, moves left front foot.
- 4:59 p.m. Lies motionless, with abdomen touching floor of cage and hind limbs splayed outward.
- 5:09 p.m. Lifts head, drags body forward by use of fore limbs. Hind limbs move but are still abducted.
- 5:15 p.m. Hind limbs now active, but trunk held close to floor. Plows along through shavings in cage and frequently topples to one or the other side.

Between seizures, a tottering mouse can be recognized by its wobbly gait. In the *Ostg* stock the gait is usually abnormal by two and one-half or three weeks of age. In an adult mouse with

fully developed symptoms, the hindquarters and tail wobble from side to side and at times tip over momentarily. The tail is often held arched upward and forward over the back, a sign useful in quickly detecting affected mice. The head does not move laterally like the hindquarters, but bobs and tosses up and down, sometimes severely enough to be suggestive of an intension tremor. A mouse may walk with the hind legs partially extended, the trunk tottering, and the perineum and lower jaw rubbing the ground.

The wobble of the hindquarters prevents the mouse from balancing well on its hind limbs. Turning is awkward; when at the edge of the table, the animal backs away by extending both hind limbs laterally and forward. Frequently it executes several complete circles in one direction while moving backward. If the mouse is lifted by the tail and a pencil placed between the hind limbs, it fails to clasp the pencil with feet and toes as a normal mouse does. Instead, the legs are held stiffly extended, either laterally or crossed below the pencil. The mouse frequently misjudges distances with the fore limbs and may make several passes at an object before reaching it.

Homozygous tottering mice swim poorly. A normal mouse holds itself horizontally with the upper part of head and trunk out of the water, paddles rapidly with all limbs, and skims along the surface of the water. Affected mice usually hold themselves vertically with only the mouth, nose, and eyes above water. Paddling is intermittent, the trunk tilts from side to side, and little distance is covered. By the end of a minute they have sunk one or more times.

A number of normal neurological findings were recorded. Tottering mice are alert and active. Pupil response to light is brisk; hearing is present. When dropped, the mouse lands on its feet. When oscillated on a raised platform, the tail swings back and forth appropriately in the direction of tilt. The mouse responds normally to blunt, strong pres-

sure, a pinch, or a light touch with a camel's hair brush on ears, all limbs, and tail.

Despite the neurological signs, including seizures virtually every day and probably several times a day, the life span is relatively normal. Body weights of heterozygous and homozygous animals are comparable through the first two weeks of life, but from about two months of age and usually earlier, the tottering mice are consistently lighter, averaging about 70 to 80 percent of the weight of heterozygous littermates.

Homozygous affected animals of both sexes sometimes mate and give viable litters but are not reliable breeders. Seizures have been noted just after parturition. One female was observed in a seizure involving all four limbs. Simultaneously she was attempting to gather her newborn litter and managed, after several attempts, to pick up one baby in her mouth and turn the forward part of her own trunk toward the nest she had made. At this point the baby slipped from her mouth, her jaws underwent several clonic contractions, and her

tongue protruded far out for about one second.

No observations have been made on the effect of pregnancy on the clinical signs.

A preliminary survey of tissue sections has not revealed pathological changes in the central nervous system.

Summary

A new neuromuscular defect in the mouse has been shown to be due to a recessive mutation. The mutation, called tottering, symbol *tg*, is very closely linked to Oligosyndactylism, *Os*. Homozygous tottering mice are characterized by: (1) intermittent seizures which may begin as early as two weeks of age and continue throughout life; and (2) a wobbly gait (affecting particularly the hindquarters) which may appear before four weeks of age but in some stocks is not detectable until eight weeks or more, and which continues throughout life. Tottering mice of both sexes are often fertile.

Literature Cited

1. FALCONER, D. S. Private communication. *Mouse News Letter* 14:13. 1956.
2. GRÜNEBERG, H. Genetical studies on the skeleton of the mouse. XVIII. Three genes for syndactylism. *Jour. Genet.* 54:113-145. 1956.
3. ST. AMAND, W. and M. B. CUPP. Private communication. *Mouse News Letter* 19:38. 1958.



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FOR the past few years the *Journal of Heredity*, along with many other non-profit journals, has found itself in a financial crisis in the face of a continuing rise in publication costs. Each year has ended with a larger and large debt to the printer. Last July, page, illustration, and reprint charges to the author went into effect in order to off-set some of the

cost of publication. These revenues will not begin to be effective for several months to come. In the meantime the National Science Foundation has awarded us a grant of \$10,810 to help maintain continued publication until we are more able to meet our obligations from our own resources.

—B.C.L.