# Inherited Epilepsy: Spike-Wave and Focal Motor Seizures in the Mutant Mouse Tottering

Abstract. *Mice with the mutant gene tottering (*tg, *chromosome 8, autosomal recessive) show, in adolescence, abnormal bursts of bilaterally synchronous spike waves as revealed in electrocorticograms recorded over long periods. The spike waves are accompanied by behavioral "absence" attacks and intermittent focal motor seizures showing somatotopic progression. Cerebral metabolic activity during seizures was assayed by autoradiography of brain sections from mice injected intravenously with* $^{14}$*C-labeled 2-deoxyglucose. Metabolic activity was increased bilaterally in selected brainstem structures. Spontaneous electrocorticographic and clinical seizures of this general pattern were recognized hitherto only in humans.*

Defined, single-locus mutations in mice can be used to identify, with a high degree of precision, disturbances of brain development and function in various inherited diseases of the central nervous system (*1*). Although the hereditary transmission of certain forms of epilepsy has long been suspected in humans (*2*), a more detailed understanding of the biochemical and cellular pathophysiology of these seizure disorders awaits the development of suitable experimental models (*3*). In the present study we have analyzed in mice the neurological expression of the tottering mutation (gene symbol *tg* on chromosome 8). This mutation features a stereotyped, spontaneous behavioral seizure pattern and is transmitted in a simple recessive mode of inheritance.

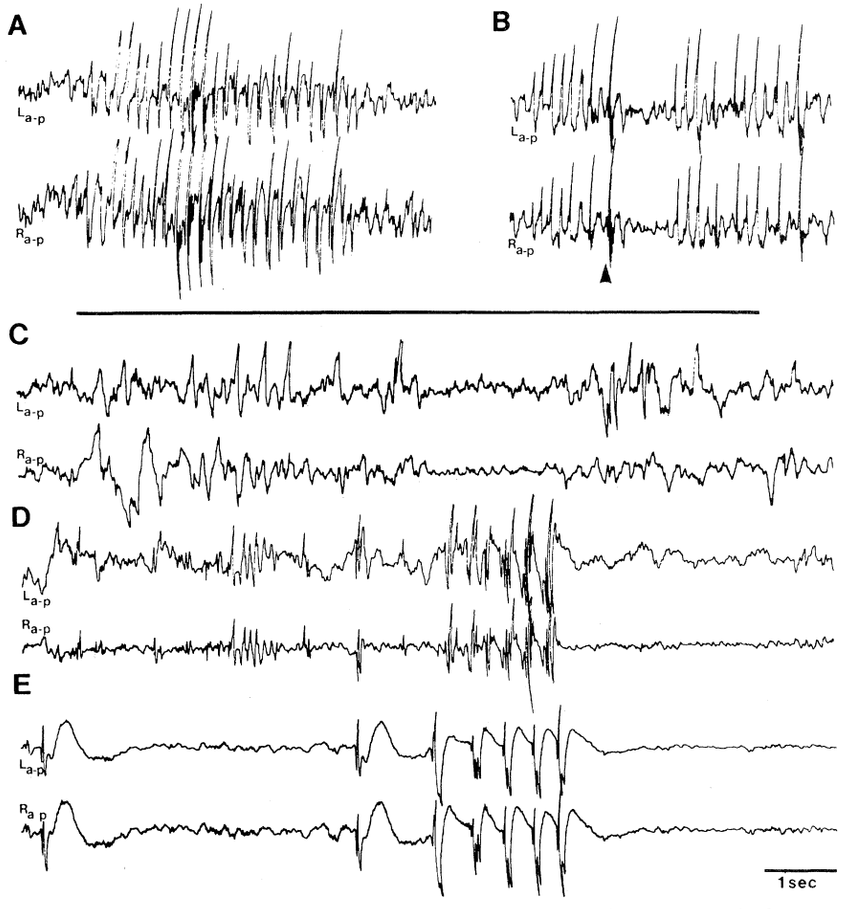


Fig. 1. Bipolar ECG recordings (left and right, anterior to posterior) from cerebral cortex of unanesthetized, unrestrained *tg/tg* mice. (A) Bilaterally synchronous, spike-wave bursts (six to seven per second) in a 32-day-old *tg/tg* mouse. The animal displayed sudden behavioral arrest and maintained a fixed, staring posture throughout the spike-wave discharge. Larger-amplitude spikes were accompanied by myoclonic head jerks. (B) Transient bilateral spike-burst suppression induced by startle (air puff, arrow) in a 12-week-old *tg/tg* mutant. The burst sequence demonstrates subharmonic spiking at a rate of three spikes per second superimposed on waves of six per second. (C and D) An ECG recording during stage 2 of spontaneous, partial tonic-clonic seizure in an adult *tg/tg* mouse. (C) Generalized and lateralized spike- and slow-wave dysrhythmias correspond with a behavioral sequence of bilateral and unilateral limb clonus. (D) Spiking at a rate of six spikes per second and a rare burst of spike waves, three per second, appear briefly during spontaneous partial motor seizure. (E) Polyspike-wave complexes during pentylenetetrazole-induced activation of the stereotyped motor seizure pattern. Calibration: (A to C) 100 $\mu$V; (D and E) 150 $\mu$V.

The *tg* mutant allele has been crossed into the C57BL/6J inbred strain where expression of the disorder is essentially the same as originally described on a different genetic background (*4*). Except for the behavioral seizures, the only visible neurological deficit in homozygous (*tg/tg*) mice is a wobbly gait that is first apparent at 3 to 4 weeks of age and is most prominent in the hindquarters. To study the neural substrate of the *tg* syndrome, we obtained electrocorticographic (ECG) recordings and autoradiograms of regional metabolic activity in the brain (*5*) prior to and during motor seizures.

We used adult (4 to 20 weeks old) control and affected mice. The mice were anesthetized with Avertin (tribromoethanol; 0.02 ml/g) and gold electrodes were implanted bilaterally at frontal and parietal sites in symmetrical skull holes drilled without injury to the underlying dura. The ECG recordings were obtained from unanesthetized, unrestrained animals over 1-month periods with a Grass model 6 electroencephalograph (linear frequency filtering 1 to 35).

The resting ECG in *tg/tg* mice displays abnormal bursts of bilaterally synchronous and symmetrical spike waves, six to seven per second, over the cerebral hemispheres (Fig. 1A). An apparently subharmonic spike burst, with three spikes per second, is sometimes present (Fig. 1B). These spontaneous bursts, 200 to 400 $\mu$V in amplitude, constitute an invariable characteristic of the *tg/tg* mutant in the waking state and were not seen in +/+ or +/*tg* control littermates. The abnormal bursts last from 0.3 to 10 seconds, occur hundreds of times per day, and can represent up to 10 percent of the resting ECG activity in an undisturbed adult mutant animal. Bilateral spike-wave paroxysms are always accompanied behaviorally by a sudden arrest of movement, a fixed staring posture, and often with twitching of the vibrissae or jaw. Single myoclonic jerks of a limb or the trunk or a sudden dropping of the head corresponded with larger-amplitude spike discharges (Fig. 1, A and B). Unilateral bursts were never observed. The spike bursts were not activated by any tested frequency of photostimulation (1 to 50 Hz) but could be blocked momentarily by single auditory, photic, or somatosensory stimuli (Fig.


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1B). The paroxysmal spike-wave abnormality is fully developed in the 4-week-old tottering animal and persists into adulthood (12 weeks after birth), although the wave component decreases substantially with age.

The motor seizures, typically seen within 1 to 3 days of positive identification of the homozygote on the basis of gait disturbance, occur one or more times per day without warning in animals up to 3 months old but may decline in frequency during the normal life-span of the mutant. The seizure pattern is remarkably stereotyped in all animals (*4*), allowing a division of the seizure sequence into three distinct stages.

The initial stage begins as a transient exacerbation of the ataxia with the hindlegs held stiffly against the trunk and is followed by asymmetrical dystonic posturing, flattening of the sacrum, and spastic paralysis of the hindlimbs which are adducted and dorsiflexed. The mouse is otherwise unaffected and can move voluntarily with the forelimbs. The second stage is heralded by the onset of focal tonic-clonic seizures of the extremities and shows a slow, unilateral somatotopic progression, affecting first only one hindleg with clonus while the forelimb is tonically flexed, then involving the forelimb alone. Over a period of 10 to 20 minutes, clonic seizures can alternate between the two sides, or may affect one forelimb and the contralateral hindlimb simultaneously. All limbs are affected at one time or another during the full temporal sequence. Squinting, adversive head movements, flattened pinnae, grinding of the jaws, and tonic elevation of an extremity are intermittently present during this stage.

The final stage involves unilateral or bilateral clonic movements of the forelimbs only. An entire seizure typically lasts 20 to 30 minutes, ends abruptly, and appears to be spontaneous since no sensory stimuli have been found to trigger the focal motor seizures (*6*). The animal remains apparently conscious throughout and shows no clinical signs of autonomic disturbance or postictal paralysis.

The abnormal patterns of ECG activity recorded during spontaneous motor seizures were less stereotyped than the spike-wave absence abnormality. In 12 serial recordings of seizure episodes in eight *tg/tg* mice, the most common pattern was low-voltage, desynchronized activity interspersed with long runs of generalized theta (4 to 7 Hz) waves occurring throughout all seizure stages. In 30 percent of the recordings, high-voltage generalized spikes, sharp waves, and slow waves appeared briefly during the alternating tonic-clonic movements of stage 2 (Fig. 1, C and D). In isolated instances in three animals, the paroxysmal discharges were intermittently lateralized during unilateral hindlimb clonus (Fig. 1C). The bursts of six spikes per second were primarily interictal with respect to the motor seizures, but were occasionally present during a quiescent period within the ictal episode (Fig. 1D). No postictal depression of ECG activity was present in any of the *tg/tg* seizure episodes.

The partial motor seizure pattern could be evoked in its entirety within 3 to 5 minutes after injection of subconvulsant doses of pentylenetetrazole (20 mg/kg, intraperitoneally) in *tg/tg* but not in proved +/*tg* or +/+ mice. Spike-wave discharges associated with myoclonic jerks were also intermittently present during drug-activated motor seizures (Fig. 1E).

To identify the neural pathways involved in the partial motor seizures, we injected $^{14}$C-labeled 2-deoxy-D-glucose intravenously into three *tg/tg* mice during and between spontaneous seizure episodes and three C57BL/6J-+/+ age-matched controls (*5*). Regional metabolic activity was then assayed qualitatively by autoradiography of serial brain sections.

Striking increases in 2-deoxyglucose uptake during seizures were consistently observed in bilateral thalamic nuclei (3+): nucleus centrum medianum, parafascicularis, and subparafascicularis; in extrapyramidal brainstem structures: red nucleus (4+) and zona incerta (3+). The reticular nuclei (3+), centralis oralis pon-

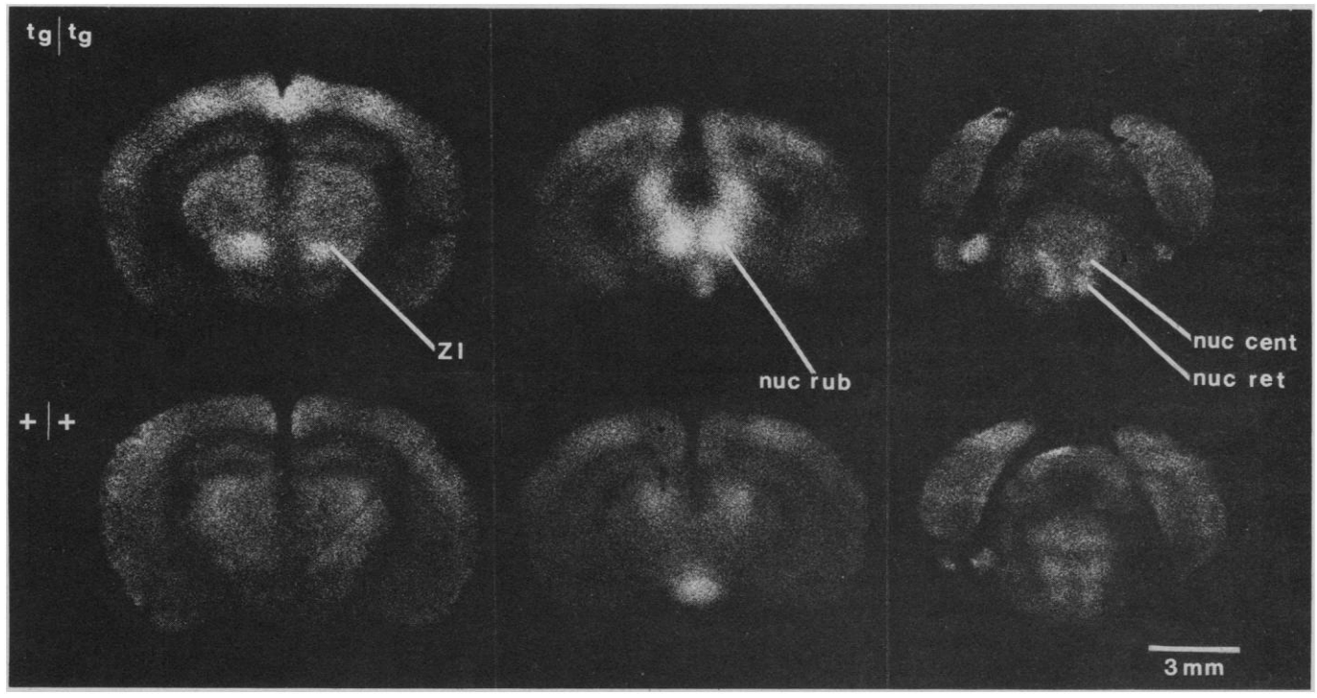


Fig. 2. Seizure-specific increases in regional cerebral metabolic activity revealed by autoradiograms of $^{14}$C-labeled 2-deoxyglucose uptake in *tg/tg* mutant during focal motor seizure (top row) and unrestrained wild-type control mouse (bottom row). The brainstem nuclei consistently activated during motor seizures were the zona incerta (*ZI*) and adjacent structures, red nucleus (*nuc rub*), and nucleus reticularis tegmenti pontis (*nuc ret*) and nucleus centralis oralis pontis (*nuc cent*). Sections from left to right correspond to coronal sections 321, 351, and 407 in Sidman *et al.* (*9*).

tis, and reticularis tegmenti pontis were also selectively activated (Fig. 2). Nuclear components in the small medulla and spinal cord were too close to the threshold of the method to allow reliable assessment. Bilateral decreases were noted in cerebellar deep nuclei and substantia nigra. Intense uptake (4+) was present in bilaterally symmetrical patches of frontal and cingulate cortex, ventrolateral and anterior dorsalis nuclei of the thalamus, and the striatum, but high levels of deoxyglucose uptake in these regions were also found in freely moving control mice. Low activity (1+) was observed in both mutant and control mice in hippocampus, entorhinal or temporal cortex, septal nuclei, amygdala, and hypothalamic nuclei.

No morphological expression of the *tg* mutation has yet been recognized. Preliminary neuropathological studies at the light microscopic level revealed no lesions in the adult *tg/tg* cerebrum, cerebellum, spinal cord, dorsal-root ganglia, or sciatic nerves. Although adult *tg* mutants weighed an average of 7 g less than normal adults, brain weights were equal to those of normal littermates. It is worth noting that other alleles at the *tg* genetic locus (leaner, $tg^{la}$, and rolling mouse Nagoya, $tg^{rol}$) have been reported to show loss of Purkinje neurons, predominantly in the anterior lobe of the cerebellar cortex (*7*).

The abnormal patterns of cerebral activation during behavioral seizures in the tottering mouse are of considerable theoretical and practical significance with reference to human epilepsy. The murine spike-wave seizure disorder described here resembles in many, but not all, respects the electroencephalographic, clinical, and developmental characteristics of the recurrent centrencephalic absence seizures of human childhood (*8*). Heredity has long been thought important in the etiology of "genuine" or "essential" seizure disorders in man, but the genetic components of these clinical syndromes have been difficult to isolate and interpret. Our data show that a spontaneous, paroxysmal electrocorticographic disorder of the centrencephalic type accompanied by behavioral arrest and partial motor seizures can, in principle, be inherited as a simple autosomal recessive trait in mammals.

The evidence suggests that a presumably single gene mutation at the *tg* locus in the mouse alters specific central nervous system pathways at critical stages of maturation. The *tg/tg* mutant provides an opportunity to identify the nature of the defect in neuronal organization, its biochemical and electrophysiological features, and the pharmacological sensitivity of naturally occurring, spontaneous paroxysmal discharges in the brain. This genetically defined model of spike-wave epilepsy provides a rational basis for a search to identify a gene product at the molecular level that might be used in the detection and prophylaxis of this neurological disease at preclinical stages.

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**References and Notes**

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3. Genetic variations in the triggering threshold for artificially induced seizures exist in several species [K. F. Killam, R. Naquet, J. Bert, *Epilepsia* **7**, 215 (1966); R. L. Collins and J. L. Fuller, *Science* **162**, 1137 (1968); R. D. Crawford, *J. Hered.* **61**, 185 (1970)]. Most sensory-precipitated seizures induce generalized convulsions, often lethal, and are rare (< 1 percent) as a form of epilepsy in man [H. Gastaut and C. A. Tassanari, *Epilepsia* **7**, 85 (1966)].
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5. Carbon-14 labeled 2-deoxy-D-glucose (New England Nuclear, 0.2 μCi per gram of body weight) was injected into the dorsal tail vein. After 45 minutes the animal was killed by cervical dislocation and the brain was excised and frozen at − 70°C in isopentane. Serial frozen sections (20 μm) were exposed to Kodak SB5 x-ray film and developed according to the method of C. Kennedy, M. Des Rosiers, M. Reivich, F. Sharp, and L. Sokoloff [*Science* **187**, 850 (1975)]. Qualitative analyses of nuclear regions identified on corresponding stained histological sections were compared by using grain density over white matter (1+) as the lower, and vestibular nuclei (4+) as the upper limits of isotope uptake.
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10. Supported by the William G. Lennox Trust (to J.L.N.) and research grant NS11237 (to R.L.S.) from the National Institutes of Health. We thank E. R. Grass of the Grass Instrument Company for the generous loan of an electroencephalograph.

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## Perception of Echo Phase Information in Bat Sonar

Abstract. *Echolocating bats (*Eptesicus fuscus*) can detect changes as small as 500 nanoseconds in the arrival time of sonar echoes when these changes appear as jitter or alternations in arrival time from one echo to the next. The psychophysical function relating the bat's performance to the magnitude of the jitter corresponds to the half-wave rectified cross-correlation function between the emitted sonar signals and the echoes. The bat perceives the phase or period structure of the sounds, which cover the 25- to 100-kilohertz frequency range, as these are represented in the auditory system after peripheral transformation. The acoustic image of a sonar target is apparently derived from time-domain or periodicity information processing by the nervous system.*

The biological sonar of bats in the suborder Microchiroptera is currently under intense scientific study as a relatively well-defined example of a biological communication system (*1, 2*). Behavioral observations have established that echolocation enables bats to detect, identify, and track prey; to avoid obstacles; and to navigate the near environment. Psychophysical experiments have shown that bats can perceive a variety of different target features by extracting information from echoes (*1–5*), although only a few of these studies have revealed anything directly about how sonar signals are processed in the brain (*2, 4, 5*). Many aspects of the auditory and neural basis of orientation by echolocation have been explored physiologically (*6*), and neurons within the bat's auditory system respond in ways relevant to target perception by sonar. I now report new psychophysical data from bats on the perception of changes in target distance (or range), data that provide conceptual order to many diverse observations on echolocation and that identify the form of the acoustic image of a target to a bat.

Two echolocating bats (*Eptesicus fuscus*) were trained with food as a reward to choose between a simulated stationary target represented by echoes arriving 3.087 msec after the sonar transmission, corresponding to a target range of about


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