

## NO CALM BEFORE THE STORM: REDUCED GABA INHIBITION PRECEDES SEIZURES IN TISH RATS

**GABAergic Synaptic Inhibition Is Reduced before Seizure Onset in a Genetic Model of Cortical Malformation** Trotter SA, Kapur J, Anzivino MJ, Lee KS. *J Neurosci* 2006;26(42):10756–1067. Malformations of the neocortex are a common cause of human epilepsy; however, the critical issue of how disturbances in cortical organization render neurons epileptogenic remains controversial. The present study addressed this issue by studying inhibitory structure and function before seizure onset in the telencephalic internal structural heterotopia (tish) rat, which is a genetic model of heightened seizure susceptibility associated with a prominent neocortical malformation. Both normally positioned (normotopic) and misplaced (heterotopic) pyramidal neurons in the tish neocortex exhibited lower resting membrane potentials and a tendency toward higher input resistance compared with pyramidal neurons from control brains. GABAergic synaptic transmission was attenuated in the tish cortex, characterized by significant reductions in the frequency of spontaneous IPSCs (sIPSCs) and miniature IPSCs recorded from pyramidal neurons. In addition, the amplitudes of sIPSCs were reduced in the tish neocortex, an effect that was more profound in the normotopic cells. Immunohistochemical assessment of presynaptic GABAergic terminals showed a reduction in terminals surrounding pyramidal cell somata in normotopic and heterotopic tish neocortex. The attenuation of inhibitory innervation was more prominent for normotopic neurons and was associated with a reduction in a subset of GABAergic interneurons expressing the calcium-binding protein parvalbumin. Together, these findings indicate that key facets of inhibitory GABAergic neurotransmission are disturbed before seizure onset in a brain predisposed to developing seizures. Such alterations represent a rational substrate for reduced seizure thresholds associated with certain cortical malformations.

### COMMENTARY

Disorders of cortical development comprise one of the most frequent causes of epilepsy. Such malformations include congenital errors in neuron proliferation, migration, and synaptogenesis. Numerous clinical syndromes of cortical malformation have been identified, and the genetic basis for some of these syndromes has been determined (1). Nevertheless, the mechanisms by which cortical malformations lead to epileptogenesis remain incompletely understood. Animal models have been used to study the mechanisms by which epilepsy can arise in dysplastic brain (2,3). Several animal models employ exogenous insults to the brain—such as freeze lesions, cranial irradiation, and prenatal exposure to toxins (e.g., methylazoxymethanol)—to produce disrupted cellular development and heightened excitability. While these models clearly provide mechanistic information about malformation-induced epileptogenesis, a naturally occurring or genetic malformation is more relevant clinically.

In contrast to lesion-induced models, the telencephalic internal structural heterotopia (tish) rat entails a genetic mutation that leads to both a specific cortical malformation (heterotopic band of unlaminated gray matter subjacent to a thinned but appropriately laminated normotopic cortex) and to the occurrence of spontaneous seizures at a certain age of development (about postnatal day 30 [P30]) (4). The seizures in tish rats appear to

originate in the normotopic cortex overlying the heterotopic band, rather than in the heterotopia itself (5). However, both normotopic and heterotopic cortex receive inputs from appropriate cortical and subcortical targets and send projections to other cortical regions (6); therefore, the heterotopic neurons also might create hyperexcitable circuits within neocortex. The tish model overrides some disadvantages of the lesion-induced variety and exhibits some features similar to the human syndrome of subcortical band heterotopia. However, human subcortical band heterotopia is usually caused by an X-linked mutation of the doublecortin gene *DCX*, whereas the gene mutation in the tish rat is autosomal recessive and has not yet been identified. Therefore, these two syndromes both produce heterotopic bands of abnormal neurons and spontaneous seizures but have different genetic bases.

Understanding of the pathophysiological basis of hyperexcitability and, hence, of seizure propensity has been based on the simple concept that seizures arise from increased excitation, decreased inhibition, or both. While this conceptualization now is considered to be oversimplified (e.g., overexpression of GABAergic inhibition sometimes causes enhanced excitation and seizures (7)), it remains a valuable construct for approaching epilepsy pathogenesis. In this regard, the paper by Trotter et al. examines possible mechanisms for cortical hyperexcitability in the tish mutant rat before the onset of spontaneous seizures by carefully dissecting potential alterations of GABAergic neurotransmission. Lee, Chen, Schottler, and colleagues have characterized the histological features and physiological aspects of the tish mutant rat in detail in previous reports (4–6). They now investigate some specific pathophysiological features that might lead to epileptogenesis in this model.

Electrophysiology (whole cell recordings) and biocytin staining of large layer V pyramidal cells and immunohistochemistry for interneurons were compared among normal cortex of Sprague-Dawley rats and normotopic and heterotopic cortex of tish rats of Sprague-Dawley background, all at P15. Membrane properties and firing patterns did not differ significantly among the groups. However, the frequency and amplitude of action-potential-dependent spontaneous inhibitory postsynaptic currents (sIPSCs) were reduced in tish normotopic cortex compared to control cortex, whereas action-potential-independent miniature IPSCs (mIPSCs) were similar in each group. These results suggest that, in tish brain, reduced sIPSCs are due to diminished multiquantal, multiterminal release events rather than to smaller quantal size. Increasing the release probability by exposure to low  $Mg^{2+}$ /high  $Ca^{2+}$  did not fully restore inhibition in the tish normotopic cortex, as assessed by sIPSC frequency and amplitude. Furthermore, calcium channels (N subtype) responsible for vesicle release from cortical interneurons are present and functional in tish brain, and there is no shift in calcium channel subtype (from N to P/Q), based on experiments with specific calcium channel blockers. Suspecting a presynaptic localization for the inhibition defect in tish brain, the investigators then examined the intensity and distribution of glutamate decarboxylase (GAD-65) immunoreactivity and found fewer GABAergic terminals innervating tish normotopic layer V neurons than cells from control cortex. In tish brains, there were fewer parvalbumin-containing interneurons, suggesting a decrease in this specific inhibitory interneuron subtype, as also seen in other malformation models (8). In tish heterotopic cortex, each of the above findings also occurred but less dramatically, consistent with the observation that normotopic cortex is the primary driver of seizures in tish rats.

The authors conclude that hyperexcitability in tish rat brain, at least in part, is due to a deficit in a specific subtype of interneuron that ordinarily provides dense innervation of neocortical pyramidal output neurons. The reduced inhibitory drive onto these principal neurons represents a plausible structural and neurochemical explanation for heightened seizure

predisposition in this model of cortical dysplasia. Other mechanisms, such as GABA<sub>A</sub> receptor subunit stoichiometry, alterations in GABA reuptake, and pathology of other interneuron subtypes, might also be involved. The key step now is to show directly that this reduced inhibition leads to seizures and to determine whether it is possible to modify epileptogenesis by enhancing GABA function in these mutants. It is also critical to learn how the calming effects of GABAergic inhibition change over time as the seizure (“electrical storm”) approaches and hits. This model provides an excellent substrate in which to pursue such questions and offers insights into epilepsy in human syndromes of cortical dysplasia.

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## References

1. Guerrini R, Filippi T. Neuronal migration disorders, genetics, and epileptogenesis. *J Child Neurol* 2005;20:287–299.
2. Baraban SC. Epileptogenesis in the dysplastic brain: a revival of familiar themes. *Epilepsy Curr* 2001;1:6–11.
3. Schwartzkroin PA, Roper SN, Wenzel HJ. Cortical dysplasia and epilepsy: animal models. In: Binder DK, Scharfman HE, eds. *Recent Advances in Epilepsy Research*. New York: Kluwer Academic/Plenum Publishers, 2004:145–174.
4. Lee KS, Schottler F, Collins JL, Lanzino G, Couture D, Rao A, Hiramatsu K, Goto Y, Hong SC, Caner H, Yamamoto H, Chen ZF, Bertram E, Berr S, Omary R, Scrabble H, Jackson T, Goble J, Eisenman L. A genetic animal model of human neocortical heterotopia associated with seizures. *J Neurosci* 1997;17:6236–6242.
5. Chen ZF, Schottler F, Bertram E, Gall CM, Anzivino MJ, Lee KS. Distribution and initiation of seizure activity in a rat brain with subcortical band heterotopia. *Epilepsia* 2000;41:493–501.
6. Schottler F, Couture D, Rao A, Kahn H, Lee KS. Subcortical connections of normotopic and heterotopic neurons in sensory and motor cortices of the tish mutant rat. *J Comp Neurol* 1998;395:29–42.
7. Cossart R, Bernard C, Ben-Ari Y. Multiple facets of GABAergic neurons and synapses: multiple fates of GABA signalling in epilepsies. *Trends Neurosci* 2005;28:108–115.
8. Roper SN, Eisenschenk S, King MA. Reduced density of parvalbumin- and calbindin D28-immunoreactive neurons in experimental cortical dysplasia. *Epilepsy Res* 1999;37:63–71.