

# THALAMIC RETICULAR NEURONS ARE UNEXCITED BY NEW STARGAZER SEIZURE MECHANISM

**Loss of Inhibitory Neuron AMPA Receptors Contributes to Ataxia and Epilepsy in Stargazer Mice.** Menez K, Nicoll RA. *J Neurosci.* 2008;28(42):10599–10603. *Stargazer* mice are characterized by ataxia and seizures, which resemble the human disorder absence epilepsy. Stargazin, the protein mutated in *stargazer* mice, promotes the expression and function of neuronal AMPA receptors (AMPA). However, it is unclear how decreased expression of excitatory AMPARs generates *stargazer* seizures, given that seizures often result from increased neuronal excitability. Additionally, although *stargazer* ataxia has been attributed to loss of AMPARs from cerebellar granule cells, other cerebellar neurons have not been examined. To examine the role of AMPAR dysfunction in these behavioral phenotypes, electrophysiological recordings were used to probe AMPAR regulation in relevant brain regions. We found that both cerebellar Purkinje cells and inhibitory thalamic reticular nucleus neurons have strongly reduced synaptic AMPAR function in *stargazer* mice. Together, our data suggest that impaired AMPAR regulation in multiple neuron populations may contribute to the behavioral phenotypes of absence seizures and ataxia seen in *stargazer* mice and imply that an understanding of human genetic disorders will require knowledge of both the genes that are mutated as well as their precise cellular expression pattern.

## COMMENTARY

A dominant and straightforward concept in epilepsy research is that seizures result from an imbalance between excitation and inhibition in neuronal networks. Within this framework, seizures are thought to arise from excessive excitation or disrupted inhibition. The theory makes intuitive sense to clinicians and scientists who see major motor manifestations of generalized seizures in patients and EEG traces that register synchronized discharges of excitatory principal neurons of

the cortex. Sensibilities remain unchallenged by treatment efficacy, given that pharmacotherapy for different types of epilepsy is aimed at reducing excitation (e.g., by blocking glutamate receptors or voltage-gated ion channels that tend to depolarize neurons) or enhancing inhibition (e.g., by facilitating GABAergic synaptic transmission). However, what is the explanation for epilepsy in a mouse whose known genetic defect predicts a *loss* of excitation?

The *stargazer* mouse, which arose as a spontaneous mutant in an inbred colony at the Jackson Laboratory, has both ataxia and epilepsy, with spike-wave seizures resembling those of absence epilepsy in humans (1). An early study reported increased excitability of layer V pyramidal neurons in *stargazer*

mice, with spontaneous giant depolarizing EPSPs generating bursts of action potentials, and reduced afterburst hyperpolarization (2). Increased excitability was associated with a threefold enhancement of a hyperpolarization-activated, cesium-sensitive inward current, suggesting that epilepsy in stargazer mice could result from a defect in the cortex that lowered the threshold for aberrant thalamocortical spike-wave oscillations (2). Thus, at first, the stargazer mouse seemed to be a simple case of cortical hyperexcitability leading to epilepsy.

Within a year, understanding the mechanisms of epilepsy in stargazer mice became more complicated. First, Letts and colleagues mapped the stargazer locus to a small interval on mouse chromosome 15 (3); and then, the gene that is disrupted by the stargazer mutation, *Cacng2*, was identified (4). *Cacng2* encodes a 36-kD transmembrane protein, stargazin, that is homologous to the  $\gamma$  subunit of voltage-gated calcium ( $\text{Ca}^{2+}$ ) channel found in skeletal muscle, which prompted not only its nomenclature but also the primary efforts to characterize its function as a  $\text{Ca}^{2+}$ -channel subunit. Indeed, stargazin was noted to increase steady-state inactivation of P/Q-type  $\text{Ca}^{2+}$  channels when expressed in oocytes, consistent with a potential role as a  $\text{Ca}^{2+}$  channel subunit. Physiological investigation of stargazer mice revealed potentiated low-voltage-activated  $\text{Ca}^{2+}$  currents and enhanced intrinsic membrane excitability in thalamocortical relay neurons (5). Thus, potential mechanisms of absence epilepsy in stargazer mice now included hyperexcitability of thalamocortical relay neurons, causing abnormal thalamocortical synchronization and absence epilepsy (5). While a bit more complicated, the general theme of imbalance between excitation and inhibition was intact.

In parallel to the focus on  $\text{Ca}^{2+}$ -channel modification, several investigators simultaneously, but independently, discovered an explanation for the ataxia of the stargazer mouse—cerebellar granule cells from stargazin and the allelic mutant, waggler, were essentially deafferented, largely lacking AMPA-receptor currents (6,7). Building upon these observations, a collaborative effort enabled the discovery that stargazin (and several other related proteins) was an auxiliary subunit of the predominant excitatory neurotransmitter receptor in the brain, the AMPA receptor (8,9). Absence of stargazin in cerebellar granule cells led to the inability of AMPA receptors to traffic to the surface membrane or synapses, likely explaining the ataxia of stargazer mice (8). It now was considered possible that a defect in AMPA receptors elsewhere in the brain might underlie the absence epilepsy in stargazer animals. However, how do these new data fit within the framework of the theory of an imbalance between excitation and inhibition in epilepsy? How might loss of an AMPA-receptor auxiliary subunit, which is expected to reduce AMPA-receptor-mediated excitation, lead to absence epilepsy?

Before answering these questions, it is germane to review mechanisms of absence epilepsy. Absence seizures are thought to arise from abnormalities that cause hypersynchronization in the thalamic circuitry; the seizures depend upon intrathalamic connections between excitatory thalamocortical relay neurons and inhibitory perigeniculate (PG) and reticular thalamic (RT) neurons (10). Briefly, thalamocortical and PG/RT neurons are reciprocally connected, leading to recurrent network activity; the oscillatory activity requires: 1) PG/RT excitation by thalamocortical neurons (glutamatergic); 2) thalamocortical neuron inhibition by PG/RT (GABAergic) neurons; and 3) postinhibitory bursting in thalamocortical neurons (dependent upon T-type  $\text{Ca}^{2+}$  channels). As opposed to normal oscillations (sleep spindles), spike-wave discharges in absence seizures involve a much larger proportion of the thalamic circuit (11). In addition, corticothalamic pathways are involved in controlling thalamic synchronization and contribute to generation of spike-wave seizures, wherein cortical projections directly activate PG/RT neurons, leading to feed-forward inhibition onto thalamocortical cells (12). In summary, absence epilepsy depends upon inhibition and excitation within the thalamus; it also is influenced by reciprocal connections between the thalamus and cortex.

The current paper by Menuz and Nicoll highlights the mystery of epilepsy in stargazer mice—the seeming contradiction between the predicted loss of AMPA-receptor excitation and the common theme of excess excitation in epilepsy. Utilizing physiological recordings to directly explore AMPA-receptor function in the principal neurons of the thalamus, they found that AMPA-receptor function is downregulated in inhibitory RT neurons, but not in excitatory thalamocortical relay neurons. Thus, in two simple experiments, the investigators demonstrated that loss of excitatory AMPA receptors, when it occurs in inhibitory neurons, might lead to an excitation/inhibition imbalance in the thalamic neurons critical for synchrony. The selective loss of AMPA-receptor function in RT neurons compared with thalamocortical neurons likely is due to compensation by other stargazin-like molecules expressed in thalamocortical neurons (9), although experimental data for this potential mechanism are not provided in the paper. Furthermore, although the data correlate loss of RT neuron AMPA-receptor function with absence epilepsy in the stargazer mice, the authors do not show reduced inhibitory RT neuron output or, ultimately, causation. Other experiments presented in the paper demonstrate reduced AMPA receptors in inhibitory Purkinje cells (which, in addition to loss of AMPA receptors from excitatory granule cells (8), also could contribute to ataxia), and there must certainly be a large inventory of cell types selectively affected by loss of stargazin. Whether other cells yet to be studied, such as cortical interneurons, similarly might contribute to

epilepsy in the stargazer mice is unknown; it also is unknown whether or not the proepileptic changes, previously described in thalamocortical (5) and cortical neurons (2), result from changes in AMPA-receptor function.

In summary, Menuz and Nicoll show that a neuronal population critical for thalamocortical synchrony is altered in stargazer mice in a way that comports with conventional assumptions about the balance between excitation and inhibition in epilepsy. These authors also illustrate beautifully how genetic mutations may be localized, affecting specific neuron populations. However, despite advances in understanding physiological effects of the stargazer mutation, the link between the loss of thalamic RT neuron AMPA receptors and absence seizures remains correlative and offers one of several mechanisms that may contribute to epilepsy in the stargazer mouse.

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