Oh, What a Tangled Web Epilepsy Genes May Weave

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Aberrant Neuronal Connectivity in the Cortex Drives Generation of Seizures in Rat Absence Epilepsy

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Absence epilepsy belongs to genetic epilepsies and is characterized by recurrent generalized seizures that are concomitant with alterations of consciousness and associated with cognitive comorbidities. Little is known about the mechanisms leading to occurrence of epileptic seizures (i.e., epileptogenesis) and, in particular, it remains an open question whether neuronal hypersynchronization, a key feature in seizure initiation, could result from aberrant structural connectivity within neuronal networks endowing them with epileptic properties. In the present study, we addressed this question using a genetic model of absence epilepsy in the rat where seizures initiate in the whisker primary somatosensory cortex. We hypothesized that alterations in structural connectivity of neuronal networks within wS1 contribute to pathological neuronal synchronization responsible for seizures. First, we used rabies virus-mediated retrograde synaptic tracing and evidenced that cortical neurons located in both upper- and deep-layers of whisker primary somatosensory cortex displayed aberrant and significantly increased connectivity the genetic model of absence epilepsy, as highlighted by a higher number of presynaptic partners. Next, we showed at the functional level that disrupting these aberrant whisker primary somatosensory cortex neuronal networks with synchrotron X-ray-mediated cortical microtransections drastically decreased both whisker primary somatosensory cortex neuron synchronization and seizure power, as revealed by in vivo local field potential recordings with multichannel probes. Taken together, our data provide for the first time strong evidence that increased structural connectivity patterns of cortical neurons represent critical pathological substrates for increased neuronal synchronization and generation of absence seizures.

Commentary

Childhood absence epilepsy (AE) is an idiopathic generalized epilepsy (IGE) syndrome that results from a genetic etiology.[1] Over the last decade, numerous genetic variations have been associated with AE including highly penetrant rare variants as well as common variants of small effect (presumably with cumulative polygenic risk).[2] Given the heterogenous genetic causes of absence seizures, it is not surprising that multiple alterations of the absence seizure circuit can produce seizures. For example, some genetic and pharmacological models of AE are associated with an increase in extra synaptic $GABA_A$ receptor currents in the thalamocortical neurons of the ventral posteromedial nucleus.[3] In contrast, another genetic model of AE is associated with a selective reduction in glutamatergic synaptic transmission from cortical layer VI to the reticular nucleus due to reduced postsynaptic GluA4-containing postsynaptic AMPA receptors.[4] Although AE is not associated with structural changes that are visible on clinical MRI exams,[1] quantification of interneuron density within certain cortical layers revealed microstructural changes in interneuron density in three genetic forms of AE.[5,6]

Here, Studor et al tested if a genetic model of AE is associated with a different type of microstructural change, an altered number of intracortical synaptic projections within the whisker-receptive somatosensory cortex (wS1) of the Genetic Absence Epilepsy Rat of Strasbourg (GAERS), a well-characterized AE model associated with a homozygous missense mutation in the Cav3.2 T-type calcium channel. They focused on wS1 given a wealth of prior studies demonstrating its importance in the absence seizure circuit.[7,8]

The investigators used an elegant, virus-mediated approach to trace microstructural connectivity. First, the upper or deep layers of wS1 were stereotactically injected with a lentivirus that encodes for the avian tumor virus receptor A (TVA) as well a

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green fluorescent reporter. The lentivirus can infect cortical neurons while the TVA protein they express allows for subsequent infection by a rabies virus. Two weeks later, they injected the same cortical layers with a modified rabies virus encoding a red fluorescent protein. The rabies virus only infects those neurons previously infected with lentivirus ("starter neurons") expressing TVA. The rabies virus spread from the starter neuron to the first order presynaptic neurons that project to the starter neuron and fluoresce red, but not green.

For starter neurons in the upper layers of wS1 (layer 2/3), GAERS rats had significantly more presynaptic partners than nonepileptic controls and the increased presynaptic partners occurred both within these upper layers as well as in layers L5/6. For starter neurons in the lower wS1, GAERS rats had increased presynaptic partners than nonepileptic controls localized in layer 5/6, but not in the upper layers. The increased microstructural connectivity to both layer 2/3 and 5/6 starter neurons was predominantly from close anterior/posterior sections. Costaining the sections for GABAergic interneurons revealed that in both layer 2/3 and layer 5/6 GAERS rats had increased GABAergic presynaptic partners originating from the lower layers.

The team next used an innovative method to causally manipulate the increased microstructural connectivity in GAERS rats. This technique is conceptually similar to multiple subpial transections, an epilepsy surgery that uses a surgical wire to disrupt horizontal, but not vertical connections within an eloquent epileptogenic gyrus thereby disrupting intracortical microstructural connectivity while sparing function.[9] However, instead of mechanically disrupting the horizontal connections with a surgical wire, they made eleven unilateral 50 μm wide synchrotron-generated X-ray microbeams spaced 400 μm apart. Immunohistochemical analysis demonstrated that the microbeams reduced neuron staining within discrete narrow vertical columns without disrupting the microvasculature and producing no discernable microglial activation and only minimal gliosis.

The X-ray microbeam transections were well tolerated; they did not alter performance on locomotion, or sensorimotor behavior tests and did not produce a significant decrease in somatosensory evoked potential amplitudes. Measurement of microstructural connectivity demonstrated that X-ray microbeam transections significantly reduced microstructural connectivity in layers 2/3 and 5/6. Physiological studies were then performed to determine the effects of disrupting microstructural connectivity on the synchrony on the epileptiform discharges. First, cortical surface local field potential recordings in freely moving GAERS revealed that the transections did not change the incidence of the epileptiform discharges, but did substantially reduce their spectral power, a finding consistent with a reduction in neuronal synchrony. Next, local field potentials obtained with a transcortical 16 channel probe in sedated rats were obtained to localize reduction of epileptiform spectral power found substantial decreases of current flow in layers 5/6. Interestingly, there was also an increase in spike-associated current flow in layers 2/3 and 4, a result indicating that the lateral microstructural connectivity is not as important for synchrony in these layers and, possibly, that transecting GABAergic projections increases the epileptiform-activity in these layers. Finally, analysis of the multiunit action potentials from the transcortical recordings was also consistent with disrupted synchrony. In the non-transected rats, action potential firing probability at the time of an epileptiform spike fit single Gaussian distributions in all cortical layers, but firing probability fit much less-well to single Gaussian distributions in the transected rats. Moreover, the latency from the peak action potential firing to the epileptiform spike was significantly prolonged in the transected GAERS in layer 5/6.

The importance of this study is the demonstration, for the first time, of increased cortical microstructural connectivity in a genetic model of AE. Moreover, the increased connectivity was directly associated with hypersynchrony of the epileptiform discharges. Therefore, IGE syndromes should not be considered as disorders that only result from pathological changes in intrinsic cell excitability or synaptic strength.

Although the authors were careful to test the effects of synchrotron microbeam transection on tissue damage, inflammation, and gliosis, one may question if off-target effects of irradiation contributed to the decreased synchrony. Now, with evidence that GABAergic microconnectivity produce the epileptiform synchrony, experiments that selectively target GABAergic neurons in specific cortical layers with chemogenetic or optogenetic proteins will be able to test the effects of reversibly modulating structural connectivity in synchrony without mechanical disruptions.

It should be emphasized that this study identified increased cortical connectivity in one genetic model of AE and it may not be present in all AE genotypes. GAERS rats have a higher density of parvalbumin-positive interneurons in wSI, a factor likely related to the increased microconnectivity found in this study.[6] In contrast, the wSI parvalbumin-positive interneuron density in Wag/Rij rats, another well-studied genetic AE model, is lower than that of GAERS and nonepileptic controls[6] and thus Wag/Rij rats may have reduced connectivity than GAERS. Future studies need to determine if increased connectivity is present in different AE genotypes. Moreover, if cortical transections are studied as a therapeutic option in refractory human AE, the presence of increased microstructural connectivity would need to be identified in individual patients.

Seizure pathophysiology is often described as resulting from neuronal hyperexcitability and hypersynchrony and thus it was surprising that this study found that disrupting neuronal synchrony did not lower frequency of the epileptiform discharges. However, synchrony may shape the clinical expression of the seizures. In this paper, the authors did not test the behavioral consequences of reduced ictal synchrony. However, previous EEG-fMRI studies in human AE patients demonstrated that the degree of impaired consciousness in absence seizures correlated to the amplitude of the EEG and fMRI responses, i.e., measurements of synchrony.[10] Future experiments testing the effects of reduced synchrony on behavior are necessary to determine if detangling the web of cortical connections has a therapeutic role in clinical treatment of intractable AE.

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