Inhibitory Neurons Cut a New Path in Epilepsy Development

A Reorganized GABAergic Circuit in a Model of Epilepsy: Evidence from Optogenetic Labeling and Stimulation of Somatostatin Interneurons.


Axonal sprouting of excitatory neurons is frequently observed in temporal lobe epilepsy, but the extent to which inhibitory interneurons undergo similar axonal reorganization remains unclear. The goal of this study was to determine whether somatostatin (SOM)-expressing neurons in stratum (s.) oriens of the hippocampus exhibit axonal sprouting beyond their normal territory and innervate granule cells of the dentate gyrus in a pilocarpine model of epilepsy. To obtain selective labeling of SOM-expressing neurons in s. oriens, a Cre recombinase-dependent construct for channelrhodopsin2 fused to enhanced yellow fluorescent protein (ChR2-eYFP) was virally delivered to this region in SOM-Cre mice. In control mice, labeled axons were restricted primarily to s. lacunosum-moleculare. However, in pilocarpine-treated animals, a rich plexus of ChR2-eYFP-labeled fibers and boutons extended into the dentate molecular layer. Electron microscopy with immunogold labeling demonstrated labeled axon terminals that formed symmetric synapses on dendritic profiles in this region, consistent with innervation of granule cells. Patterned illumination of ChR2-labeled fibers in s. lacunosum-moleculare of CA1 and the dentate molecular layer elicited GABAergic inhibitory responses in dentate granule cells in pilocarpine-treated mice but not in controls. Similar optical stimulation in the dentate hilus evoked no significant responses in granule cells of either group of mice. These findings indicate that under pathological conditions, SOM/GABAergic neurons can undergo substantial axonal reorganization beyond their normal territory and establish aberrant synaptic connections. Such reorganized circuitry could contribute to functional deficits in inhibition in epilepsy, despite the presence of numerous GABAergic terminals in the region.

Commentary

Simplistically, an excitatory–inhibitory imbalance in key brain areas supports an increased propensity for seizure generation. From a structural reorganization viewpoint, that concept translates to a loss of inhibition due to cell loss and altered responses to GABA, accompanied by an increase in excitation mediated by axon sprouting and synaptic reorganization among principal neurons. Excitatory axon sprouting and synaptic reorganization (exemplified by mossy fiber sprouting in the dentate gyrus) have been widely reported in animal models of acquired temporal lobe epilepsy (TLE) and in TLE patients. Concurrently, altered GABAergic inhibition and loss of select groups of GABAergic neurons in the hippocampal formation is also associated with epilepsy development. Reorganization of inhibitory synaptic circuits in epilepsy, however, has been less well characterized than for excitatory connections. Somatostatin-immunoreactive (SOM) GABAergic interneurons in the hilus represent one group of inhibitory interneurons that is particularly vulnerable to cell death associated with induction of TLE. These neurons normally innervate the distal dendrites of dentate gyrus granule cells, providing important recurrent inhibition. The loss of a significant portion of this critical class of cells during epileptogenesis may contribute to reduced seizure threshold. However, afferent synaptic drive to surviving hilar SOM neurons is reorganized and increased in animal models of TLE (1, 2), and the axons of hilar SOM neurons sprout and form new connections with granule cells, possibly reestablishing a portion of the normal inhibitory circuitry (3).

After noting that SOM immunolabeling in the dentate gyrus increased progressively after pilocarpine treatment, status epilepticus, and accompanying loss of SOM neurons in the hilus, Peng and colleagues investigated axon reorganization of SOM neurons originating in CA1 stratum oriens and found that their axons arborized aberrantly in the molecular layer of the dentate gyrus. These neurons are relatively spared in epilepsy development, and their axons do not normally invade the dentate gyrus. Using sophisticated labeling methods targeted to the stratum oriens in SOM-Cre transgenic mice, axon terminals of stratum oriens SOM neurons in pilocarpine-treated, but not control mice, were found to form symmetric (inhibitory) synaptic contacts with granule cell dendrites and dendritic spines. Normally, hilar SOM neurons make synapses on granule cell dendrites, near terminations of excitatory perforant path axons. The hilar SOM neurons are depleted in this model, but newly formed synapses from stratum oriens SOM neurons were often located near asymmetrical (excitatory) synapses on...
dendritic spines, hinting that the new synapses took the place of those lost when the hilar cells were killed. Unlike the axon reorganization observed in surviving hilar SOM cells—which occurs in the same domain as the normal projection—the axonal reorganization of stratum oriens SOM cells extended beyond their normal termination regions, forming an entirely new structural connection.

Functional connectivity of sprouted axons with granule cells was also established. The viral construct used to identify SOM neurons expressed channelrhodopsin, a cation channel that gets inserted into the membrane of the infected neuron, allowing for optogenetic activation of transfected SOM cells selectively. Electrophysiological recordings in pilocarpine-treated mice revealed optogenetically activated inhibitory postsynaptic currents (IPSCs) in granule cells after stimulation of stratum oriens SOM neurons, consistent with the formation of symmetric, inhibitory synapses by the sprouted axons. Identification of functional synaptic reorganization in local inhibitory circuitry during epileptogenesis has suffered from difficulties in specifically identifying and activating newly formed connections. Peng and colleagues’ use of optogenetic technology overcame this barrier and defined a new, functional inhibitory synaptic connection that forms during the process of epileptogenesis.

Reduced GABA cell density is often associated with increased network excitability. Coupled with axon sprouting and synaptic reorganization among principal neurons, decreased inhibition due to cell death has been incriminated as a contributor to the development of spontaneous seizures. Positive seizure control outcomes in patients treated medically to enhance surviving GABA connections are consistent with the critical nature of intact GABAergic circuitry in regulating brain activity. Recent reports suggesting that replacing specific types of GABA cells that are “lost” during epileptogenesis might abrogate seizure activity in mice (4), consistent with the hypothesis that diminished endogenous inhibition contributes to the expression of seizures. Emerging evidence also suggests that surviving hilar SOM neurons are morphologically and functionally altered in a manner that implies an endogenous attempt by the system to restabilize functional inhibition in key circuits of seizure initiation and propagation. These features of reorganized, surviving inhibitory circuitry imply that “normal” inhibitory circuits are somehow being augmented. Provocative as it sounds, one implication of reorganized inhibitory circuitry is that it serves a restorative function, operating to rebalance inhibitory tone.

However, the functional implications of the re-innervation of granule cells from stratum oriens are difficult to appreciate. The new connections seem unlikely to be effectively activated by the same stimuli that trigger recurrent inhibition from hilar SOM neurons (e.g., granule cell activity), so normal inhibitory feedback is probably not established by the new circuit. Perhaps the additional GABA being released is sufficient to maintain a level of synaptic inhibition adequate to suppress some forms of excessive activity, even if the functional feedback duties of the circuit are altered or not intact. Conversely, it was proposed long ago that sprouted GABAergic axons might help to synchronize neural activity (5), and certain types of developmental- and state-dependent cognitive behaviors may be mediated by cortical circuits that rely on this mechanism (6, 7). Inhibitory neurons with enhanced incoming and outgoing connectivity may act as “hubs” to promote hyperexcitability under certain conditions (8). Excessive inhibitory input to distal dendrites may even be more likely to result in paradoxi
cal depolarization (9). The behavioral effect on seizures of optogenetically stimulating this new connection in vivo was not tested, but outcomes of that experimental manipulation might address this issue in the future.

While the concept of synaptic imbalance underpinning TLE seems intuitive and even attractive, a more pragmatic view indicates that epileptogenesis resulting in TLE is probably more complex than a simple change in the balance of inhibition and excitation. The findings of Peng and colleagues demonstrating the formation of entirely new inhibitory connections in the hippocampal formation highlights the variety and complexity of epilepsy-related synaptic reorganization in the temporal lobe, and they also underscore the need to conceptualize TLE development and treatment in a way that takes this complexity into account.

by Bret N. Smith, PhD

References

5. Babb TL, Pretorius JK, Kupfer WR, Crandall PH. Glutamate decarbox
A, Gozlak H, Esclapez M, Bernard C. Hub GABA neurons mediate gamma-frequency oscillations at ictal-like event onset in the imma

by Bret N. Smith, PhD

References

5. Babb TL, Pretorius JK, Kupfer WR, Crandall PH. Glutamate decarbox
A, Gozlak H, Esclapez M, Bernard C. Hub GABA neurons mediate gamma-frequency oscillations at ictal-like event onset in the imma
American Epilepsy Society
Epilepsy Currents Journal
Disclosure of Potential Conflicts of Interest

Instructions
The purpose of this form is to provide readers of your manuscript with information about your other interests that could influence how they receive and understand your work. Each author should submit a separate form and is responsible for the accuracy and completeness of the submitted information. The form is in four parts.

1. Identifying information.
   Enter your full name. If you are NOT the main contributing author, please check the box “no” and enter the name of the main contributing author in the space that appears. Provide the requested manuscript information.

2. The work under consideration for publication.
   This section asks for information about the work that you have submitted for publication. The time frame for this reporting is that of the work itself, from the initial conception and planning to the present. The requested information is about resources that you received, either directly or indirectly (via your institution), to enable you to complete the work. Checking “No” means that you did the work without receiving any financial support from any third party – that is, the work was supported by funds from the same institution that pays your salary and that institution did not receive third-party funds with which to pay you. If you or your institution received funds from a third party to support the work, such as a government granting agency, charitable foundation or commercial sponsor, check “Yes”. Then complete the appropriate boxes to indicate the type of support and whether the payment went to you, or to your institution, or both.

3. Relevant financial activities outside the submitted work.
   This section asks about your financial relationships with entities in the bio-medical arena that could be perceived to influence, or that give the appearance of potentially influencing, what you wrote in the submitted work. For example, if your article is about testing an epidermal growth factor receptor (DGFR) antagonist in lung cancer, you should report all associations with entities pursuing diagnostic or therapeutic strategies in cancer in general, not just in the area of EGFR or lung cancer.

   Report all sources of revenue paid (or promised to be paid) directly to you or your institution on your behalf over the 36 months prior to submission of the work. This should include all monies from sources with relevance to the submitted work, not just monies from the entity that sponsored the research. Please note that your interactions with the work’s sponsor that are outside the submitted work should also be listed here. If there is any question, it is usually better to disclose a relationship than not to do so.

   For grants you have received for work outside the submitted work, you should disclose support ONLY from entities that could be perceived to be affected financially by the published work, such as drug companies, or foundations supported by entities that could be perceived to have a financial stake in the outcome. Public funding sources, such as government agencies, charitable foundations or academic institutions, need not be disclosed. For example, if a government agency sponsored a study in which you have been involved and drugs were provided by a pharmaceutical company, you need only list the pharmaceutical company.

4. Other relationships
   Use this section to report other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work.
Section #1 Identifying Information

1. Today's Date: 01/02/2014

2. First Name Bret Last Name Smith Degree PhD

3. Are you the Main Assigned Author? ☑ Yes ☐ No

If no, enter your name as co-author:

4. Manuscript/Article Title: Inhibitory Neurons Cut a New Path in Epilepsy Development

5. Journal Issue you are submitting for: 14.4

Section #2 The Work Under Consideration for Publication

Did you or your institution at any time receive payment or services from a third party for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc.)?

Complete each row by checking “No” or providing the requested information. If you have more than one relationship just add rows to this table.

<table>
<thead>
<tr>
<th>Type</th>
<th>No</th>
<th>Money Paid to You</th>
<th>Money to Your Institution*</th>
<th>Name of Entity</th>
<th>Comments**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Grant</td>
<td>☑</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Consulting fee or honorarium</td>
<td>☑</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Support for travel to meetings for the study or other purposes</td>
<td>☑</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Fees for participating in review activities such as data monitoring boards, statistical analysis, end point committees, and the like</td>
<td>☑</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Payment for writing or reviewing the manuscript</td>
<td>☑</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Provision of writing assistance, medicines, equipment, or administrative support.</td>
<td>☑</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Other</td>
<td>☑</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* This means money that your institution received for your efforts on this study.

** Use this section to provide any needed explanation.
Section #3 Relevant financial activities outside the submitted work.  
Place a check in the appropriate boxes in the table to indicate whether you have financial relationships (regardless of amount of compensation) with entities as described in the instructions. Use one line for each entity; add as many lines as you need by clicking the “Add” box. You should report relationships that were present during the 36 months prior to submission.

Complete each row by checking “No” or providing the requested information. If you have more than one relationship just add rows to this table.

<table>
<thead>
<tr>
<th>Type of relationship (in alphabetical order)</th>
<th>No</th>
<th>Money Paid to You</th>
<th>Money to Your Institution*</th>
<th>Name of Entity</th>
<th>Comments**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Board membership</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Consultancy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Employment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Expert testimony</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Grants/grants pending</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Payment for lectures including service on speakers bureaus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Payment for manuscript preparation.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Patents (planned, pending or issued)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. Royalties</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. Payment for development of educational presentations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. Stock(stock options)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12. Travel/accommodations/meeting expenses unrelated to activities listed.**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13. Other (err on the side of full disclosure)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* This means money that your institution received for your efforts.
** For example, if you report a consultancy above there is no need to report travel related to that consultancy on this line.

Section #4 Other relationships
Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?

☑ No other relationships/conditions/circumstances that present a potential conflict of interest.
☐ Yes, the following relationships/conditions/circumstances are present:

Thank you for your assistance.

Epilepsy Currents Editorial Board