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A Tisket a Tasket: Chandelier ≠ Basket (Cell Bouton Density in Sclerotic DG)

GABA bouton subpopulations in the human dentate gyrus are differentially altered in mesial temporal lobe epilepsy

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Medically intractable temporal lobe epilepsy is a devastating disease, for which surgical removal of the seizure-onset zone is the only known cure. Multiple studies have found evidence of abnormal dentate gyrus network circuitry in human mesial temporal lobe epilepsy (MTLE). Principal neurons within the dentate gyrus gate entorhinal input into the hippocampus provide a critical step in information processing. Crucial to that role are GABA-expressing neurons, particularly parvalbumin (PV)-expressing basket cells (PVBCs) and chandelier cells (PVChCs), which provide strong, temporally coordinated inhibitory signals. Alterations in PVBC and PVChC boutons have been described in epilepsy, but the value of these studies has been limited due to methodological hurdles associated with studying human tissue. We developed a multilabel immunofluorescence confocal microscopy and a custom segmentation algorithm to quantitatively assess PVBC and PVChC bouton densities and to infer relative synaptic protein content in the human dentate gyrus. Using en bloc specimens from MTLE subjects with and without hippocampal sclerosis, paired with nonepileptic controls, we demonstrate the utility of this approach for detecting cell-type specific synaptic alterations. Specifically, we found increased density of PVBC boutons, while PVChC boutons decreased significantly in the dentate granule cell layer of subjects with hippocampal sclerosis compared with matched controls. In contrast, bouton densities for either PV-positive cell type did not differ between epileptic subjects without sclerosis and matched controls. These results may explain conflicting findings from previous studies that have reported both preserved and decreased PV bouton densities and establish a new standard for quantitative assessment of interneuron boutons in epilepsy.

Keywords
MTLE, bouton, GABA, interneuron, basket cell, axo-axonic, chandelier

Commentary

In mesial temporal lobe epilepsy (MTLE), the classic trisynaptic feedforward loop of the hippocampus malfunctions in a way that leads to sustained high levels of activity (ie, seizures). In the "dentate gate" model, the dentate gyrus (DG) in a healthy brain has intrinsic properties and inhibitory circuitry that lead to very sparse firing,[1,2] preventing most cortical input from reaching pyramidal cells of the hippocampus. In MTLE, it is hypothesized that some aspect of the gate breaks down, permitting abnormally high levels of cortical activity to propagate to the hippocampus and initiate a seizure.[3,4] The excess of spiking activity that composes a seizure makes particularly attractive the hypothesis that a loss of inhibition in the DG underlies MTLE. However, the experimental evidence for preferential loss of GABA synapses onto dentate granule cells in epileptic tissue is complicated and sometimes confusing. For example, there is an apparent loss of interneurons in and around the granule cell layer,[5] but the density of parvalbumin (PV+) boutons onto granule cells is preserved.[6]

There are at least 2 issues that complicate the measurement of changes to inhibition onto DG granule cells. First, a loss of interneurons does not necessarily correspond to a loss of inhibitory synapse density. For example, following pilocarpine-induced status epilepticus (a rodent model of MTLE that leads to spontaneous seizures), Thind et al[7] observed an initial loss of glutamic acid decarboxylase positive (GAD+) interneurons, accompanied by a loss in GABA synapses in the DG, in the first week post-SE. This was followed by a large and significant increase in GABA synapse count during the chronic epilepsy phase, suggesting that the surviving interneurons have compensatory synaptogenesis. The second primary factor complicating interpretation of GABA synapse changes in MTLE is the

diversity in the interneuron population.[8,9] Simply looking at the distribution of all GABAergic boutons (eg, by staining tissue for vesicular GABA transporter, vGAT, or GAD isoforms) or boutons of one interneuron subtype (eg, somatostatin, parvalbumin, cholecystokinin) gives an incomplete picture of MTLE-associated changes. The statistical composition of GABAergic boutons may shift among the interneuron subtypes, without changing the global GABA bouton density.

In the highlighted paper, Alhourani et al[10] addressed both of these problems using a sophisticated multilabel immunofluorescence platform to analyze resected tissue from patients with intractable MTLE (with and without hippocampal sclerosis). Synaptic boutons were multiply labeled with antibodies for vGAT, PV, GAD65, and GAD67 and automatically segmented and coregistered. Together, this enabled simultaneous quantification in the DG of GABA boutons from multiple interneuron subtypes. Consistent with previous findings, vGAT+ boutons were positive for GAD65, GAD67, or both; 20% of vGAT+ boutons were PV+, none of which were positive for GAD65 only.

The authors compared GABA bouton density statistics in MTLE tissue with that from age-matched postmortem controls. In MTLE, there was an overall decrease in vGAT+/GAD+ boutons, but when normalized to the number of surviving granule cells, the GAD+ boutons/cell was actually elevated in sclerotic tissue (owing to a large decrease in total granule cell count). The PV+ boutons were separated into putative basket cells (BCs) and chandelier cells (ChCs) based on GAD isoform content (GAD67 and GAD65, or GAD67-only, respectively). Interestingly, in tissue from sclerotic hippocampi, the number of BC boutons per granule cell *increased* by more than 200%, while the ChC bouton density *decreased* by 75%. This type of discrepancy may give some insight into why it has been so difficult to identify epilepsy-associated changes in the inhibitory circuitry of the DG.

Finally, the authors infer the density of the labeled antigens based on the intensity of fluorescent labels and find that GAD and vGAT levels are elevated in MTLE tissue across all interneuron types, suggesting that the labeled boutons are functional and perhaps potentiated. Although many controls were performed by the authors in prior studies, quantifying protein content in this way remains an imperfect measure. Immunofluorescent intensity can vary for a number of reasons, some of which may be related to the tissue being sclerotic (but not related to protein concentration). For example, the spatial inhomogeneity of sclerotic tissue may lead antibodies to variably penetrate the sample or fluorescent light to variably scatter out of the tissue. Technologies for single-cell quantitative proteomic measurements are only recently emerging, and future studies may look to validate findings using mass cytometry.[11]

Given that BCs are prominent perisomatic inhibitory cells of the DG and ChCs provide (debatably, and perhaps state-dependent) excitatory input onto the axon initial segment of granule cells,[12] both the increase in BC bouton density and the decrease in ChC bouton density could be seen as compensatory changes to a "hyperexcitable" brain. However, increases in BC bouton density in adult tissue could mean that the number of GABA bouton output per BC has increased. This could have paradoxical functional implications in terms of the nature of GABAegic inhibition provided to granule cells. An increase in the number of outputs per BC would suggest that there is a higher degree of synchronous inhibition to the DG (ie, all outputs from one cell would release GABA synchronously). This effect could be even more dramatic if, similar to the findings of Thind et al,[7] the BC synaptogenesis was accompanied by a loss of BCs. The highlighted study did not quantify cell bodies by interneuron subtype to provide a measure of GABA boutons/interneuron; doing so could more directly test this hypothesis.

Rich histological data sets such as the one summarized here will undoubtedly inform the nature of epileptogenic changes in human tissue, which will be critical in developing novel treatments for MTLE. For example, the findings in the highlighted study might help explain why drugs that target $GABA_A$ receptors, such as benzodiazepines, have such variable results in patients: A global modulation of $GABA_A$ may not be the best treatment for heterogeneous changes in GABA circuitry. At a basic neuroscience level, this study further highlights the importance of annotating connectomic data, currently being collected using high-throughput electron microscopy, with inormation about protein expression.

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