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Cells That "Fire Together, Wire Together", but Do They Transcribe Together in Epilepsy?

Identification of Epilepsy-Associated Neuronal Subtypes and Gene Expression Underlying Epileptogenesis

Pfisterer U, Petukhov V, Demharter S, Meichsner J, Thompson JJ, Batiuk MY, Asenjo-Martinez A, Vasistha NA, Thakur A, Mikkelsen J, Adorjan I, Pinborg LH, Pers TH, von Engelhardt J, Kharchenko PV, Khodosevich K. *Nat Commun*. 2020;11(1):5038. doi:10.1038/s41467-020-18752-7 [published correction appears in *Nat Commun*. 2020;11(1):5988]

Epilepsy is one of the most common neurological disorders, yet its pathophysiology is poorly understood due to the high complexity of affected neuronal circuits. To identify dysfunctional neuronal subtypes underlying seizure activity in the human brain, we have performed single-nucleus transcriptomics analysis of >110 000 neuronal transcriptomes derived from temporal cortex samples of multiple temporal lobe epilepsy and nonepileptic subjects. We found that the largest transcriptomic changes occur in distinct neuronal subtypes from several families of principal neurons (L5-6_Fezf2 and L2-3_Cux2) and GABAergic interneurons (Sst and Pvalb), whereas other subtypes in the same families were less affected. Furthermore, the subtypes with the largest epilepsy-related transcriptomic changes may belong to the same circuit, since we observed coordinated transcriptomic shifts across these subtypes. Glutamate signaling exhibited one of the strongest dysregulations in epilepsy, highlighted by layer-wise transcriptional changes in multiple glutamate receptor genes and strong upregulation of genes coding for AMPA receptor auxiliary subunits. Overall, our data reveal a neuronal subtype-specific molecular phenotype of epilepsy.

Commentary

Seizure activity is a complicated biological process, wherein nearly every cell type has been implicated.[1-3] Neurons are obviously interesting because they drive electrical activity, but nonneuronal cells also play a critical role in brain function and are heavily implicated in the etiology of epilepsy. Gene expression studies to identify dysregulated gene sets in epilepsy have implicated dozens of novel genes relating to synaptic neurotransmission, metabolism, neuroinflammation, and so on.[4,5] However, these studies have largely relied on bulk RNA sequencing, which only captures changes in the pooled transcriptome of all the cells captured in the sample.[6] Thus, they've lacked the resolution to resolve changes in gene expression that may only appear in specific populations of cells—failing to account for the massive cellular heterogeneity that exists in brain tissue.[6]

This is a missed opportunity to explore the diversity within epilepsy-related transcriptomic changes. Are there neuronal subtypes that are more affected in epilepsy than others? What are the gene sets that are fundamentally dysregulated in epilepsy? Are these genes regulated in concert among neurons within a brain region or circuit? Pfisterer et al[7] addresses this gap in our knowledge by analyzing the single cell transcriptome of over 110 000 cortical neurons harvested from healthy and epileptic human brain tissue. Brain tissue was derived from temporal lobectomies of patients undergoing surgery for pharmacoresistant epilepsy or from control postmortem tissue. The authors performed single nucleus RNA sequencing (snRNA-seq), which allows transcriptomic analysis on a cell-by-cell basis, unlike traditional bulk RNA sequencing that takes a virtual average of all the cells. In this study, neurons were analyzed by selecting cells that express NeuN, a nuclear marker for neurons, for snRNAseq analysis. A hierarchical annotation strategy enabled them to identify 12 principal neuron and 23 GABAergic interneuron transcriptomic subtypes—a key advantage of their single cell approach. Previous works studying neuronal heterogeneity in the cortex have reported similar findings.[8,9]

Initial survey of the data shows a change in cellular composition in samples from patients with epilepsy. The authors note a particularly sharp decrease in L2/3 principal neurons as well as in a subfamily of fast-spiking GABAergic interneurons—although, there appears to be modest changes among most subtypes analyzed. Notably however, while the abundance of most neuronal subtypes is reduced in epilepsy, their data shows a slight increase in population size for a few principal and GABAergic interneuron populations. What could be driving this subtype-dependent change in cellular composition?

To determine the most severely epilepsy-affected neuron subtypes, the authors first ran a gene-expression correlation between neurons of healthy and epileptic data sets. Interestingly, they found a relatively small shift in the transcriptome of most neuronal subtypes. This analysis did reveal, however, a larger, more drastic shift in transcriptome among a few L5-6, L2-3 principal neuron and a few GABAergic interneuron populations. Together with the subtype-dependent changes in cellular composition, these reports support the notion of differential responses to epilepsy by neuronal subtypes. Thus, in order to better understand what processes may be underlying these differences, the authors used Gene Ontology (GO) term enrichment to assess the dysregulated biological processes fueled by the differentially expressed (DE) genes between healthy and epileptic neurons.

When the GO terms were clustered based on their enrichment pattern among the neuronal populations, they found strong correlation among diverse neuronal populations. Interestingly, while neurons in the same cortical layer generally varied together, within each layer there was still a great deal of heterogeneity at the level of sub-"families" of neurons. This suggests that sub-"families" of canonical cell types (eg, pyramidal neurons and parvalbumin-positive GABAergic interneurons) cluster together in a given layer. The authors argue that this represents circuit-specific changes in transcriptional regulation. This is an exciting and intriguing idea that has important implications for understanding the pathophysiology of epilepsy and its treatment. This idea remains to be validated with other approaches but is certainly consistent with the notion that subcircuit activity in the cortex is important to ictogenesis and seizure propagation. It also introduces the enticing possibility of coordinated transcriptional regulation assemblies of neurons involved in seizures.

To that end, Pfisterer et al uses an informed approach to focus the remainder of the study on GO terms relating to hyperexcitation of principal neurons and hypoinhibition of principal neurons by GABAergic interneurons. Through this guided approach, they describe complex dysregulation of expression of many genes relating to glutamate-mediated excitation and GABAergic inhibition. Notably, they report changes in expression that are subtype and layer-specific. Please see the study for details—there's a lot to consider!

Overall, Pfisterer et al presents a compelling story showing that heterogeneity exists within neuronal subtypes and their response in epilepsy. Not to be overlooked, the demonstration of genes being regulated in both cell-type-, layer-, and sub-"family"-specific manners is an exciting finding, especially coupled with the earlier reports showing some neuronal subtypes were more transcriptionally affected in epilepsy than others. However, as with any good study, it raises many questions. The authors chose to guide their investigation using known pathways related to seizure propagation, but one of the greatest advantages of a snRNAseq is its unbiased nature that allows for truly novel gene discovery. Are there any other distinct epilepsy-related gene signatures in the affected neurons? Perhaps relating to inflammatory state, metabolism, cytoskeleton, and so on. Also, what about other cell types in the brain? This study focused exclusively on neurons, but given the growing interest in glial cell diversity, it stands to reason that we could expect similar subtype-specificity. [10] Follow-up studies to investigate transcriptomic changes in epilepsy would serve well to include glial cells as well as neurons. Just as these authors identified DE gene sets that were highly correlated among neuronal subtypes, future studies could aim to identify common transcriptomic shifts that span across cell types.

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