Epilepsy Currents
2019, Vol. 19(2) 124-125

DOI: 10.1177/1535759719835658
journals.sagepub.com/home/epi
SAGE

Seizing Sequencing Data to Consider Cell and Circuit Complexity

Classes and Continua of Hippocampal CA1 Inhibitory Neurons Revealed by Single-Cell Transcriptomics

Harris K, Hochgerner H, Skene NG, et al. *PLoS Biol*. 2018;16(6):e2006387. doi:10.1371/journal.pbio.2006387.

Understanding any brain circuit will require a categorization of its constituent neurons. In hippocampal area CA1, at least 23 classes of GABAergic neurons have been proposed to date. However, this list may be incomplete; additionally, it is unclear whether discrete classes are sufficient to describe the diversity of cortical inhibitory neurons or whether continuous modes of variability are also required. We studied the transcriptomes of 3663 CA1 inhibitory cells, revealing 10 major GABAergic groups that divided into 49 fine-scale clusters. All previously described and several novel cell classes were identified, with 3 previously described classes unexpectedly found to be identical. A division into discrete classes, however, was not sufficient to describe the diversity of these cells, as continuous variation also occurred between and within classes. Latent factor analysis revealed that a single continuous variable could predict the expression levels of several genes, which correlated similarly with it across multiple cell types. Analysis of the genes correlating with this variable suggested it reflects a range from metabolically highly active faster-spiking cells that proximally target pyramidal cells to slower-spiking cells targeting distal dendrites or interneurons. These results elucidate the complexity of inhibitory neurons in one of the simplest cortical structures and show that characterizing these cells require continuous modes of variation as well as discrete cell classes.

Commentary

Neural circuits are comprised of distinct cell populations, each an important cog in the neural machine. Knowledge of these distinct cell populations is crucial to understand how that circuit functions, and, in turn, how changes in these cell populations and the circuit (like those that take place in epilepsy) affect its function. Inhibitory neurons play important roles in circuit function and dysfunction and have been the subject of considerable study regarding seizure generation, propagation, and cessation. Temporal lobe epilepsy is often accompanied by the loss of interneurons,[1] the loss of interneurons is sufficient to induce spontaneous recurring seizures,[2] the replacement of lost interneurons reduces seizures and comorbidities,[3] and on-demand optogenetic activation of interneurons can stop seizures.[4] However, the role of interneurons in seizures and epilepsy is complex, in part reflecting the complexity of interneurons themselves.

Interneurons are typically characterized by their firing properties, gene expression, morphology, and synaptic partners. These properties determine and reflect their unique roles in the circuit. Recently, Harris et al took advantage of advances in single-cell RNA sequencing to take both a broad (profiling over 3000 cells) and deep (performing on average over 100 000 reads per cell) look at inhibitory neurons in CA1 of the mouse hippocampus, classifying cells based on their gene expression profiles. These profiles were then compared to the literature to assign tentative labels to each cluster. In total, Harris et al report 49 distinct inhibitory cell clusters. Consistent with classical classification schemes,[5] they found that no single genetic marker was sufficient for defining a cell class. Although this is not surprising, it highlights an obvious limitation of many prior studies (including some of our own work[4,6]), which investigate inhibitory neurons through a single molecular marker. For example, there are disparate reports of the effect of optogenetically stimulating parvalbumin (PV) cells on seizures.[7] Although there are many important considerations regarding the different results of these studies (including cell location, seizure focus location, seizure model, and status of the chloride reversal potential), an additional compounding factor is that "PV cells" are a heterogeneous population of cells.

Moreover, Harris et al found that even within more specifically defined cell classes (eg, "PV axo-axonic") there were multiple distinct clusters. Again, this drives home findings of others—consider, for example, work by Varga et al, where they were able to break down each of the 3 major PV cell classes into subclasses based on their distinct firing characteristics during high frequency oscillations.[8] Varga et al further found

that these subclasses were also well predicted by the cell's dendritic structure or somatic location.[8]

Of course, which cell type a cell belongs to cannot entirely determine its gene expression profile; many genes are indicative of not only cell type but also, for example, activity level. In this vein, Harris et al report that there is variability in gene expression somewhat orthogonal to cluster identity: this variability may correspond to differing activity levels and action potential kinetics, as well as the postsynaptic somatodendritic target location. To some extent, this is a reminder that gene expression is not fixed, and there are therefore limitations to using gene expression alone to define cell types. It also reflects an opportunity to use this method in future studies to further examine changes in gene expression that occur with seizures and in epilepsy.[9] For example, research by Wu and colleagues used a modified protocol to examine activity dependent changes in gene expression using single-cell sequencing, including changes in the amygdala following acute seizure activity.[9] Although there are caveats and hurdles to using single-cell sequencing to look at changes in epilepsy, the benefits include the breadth of cells and genes which can be examined simultaneously; further research can expand on the work of Wu et al and Harris et al to study cell-type specific changes in gene expression in epilepsy and to determine, with greater precision, the types of cells lost in epileptic tissue.

The data provided by Harris et al are an excellent reference and starting point. Future studies can further confirm and update the cell-type labels and clustering provided in their study (eg, through patch-seq[10]), examine additional regions within the hippocampal formation, and extend the work to encompass changes in epilepsy. Additionally, and particularly noteworthy, there is more to be done to appreciate the full diversity of clusters in CA1, even in healthy tissue. Specifically, Harris et al found that the number of identified cell clusters linearly increased with the number of cells that were sequenced and that their study of over 3000 cells remained on this linear trajectory (ie, the saturation point has not been found yet). Making this point even more striking and salient is the fact that especially rare cell types or cell types particularly sensitive to the isolation methods would have been missed. All together, these findings strongly suggest that we have been underestimating the number of meaningfully distinguishable cell populations and that we do not yet fully comprehend the extent of inhibitory neuron diversity. Further underscoring this message—despite the prior extensive characterization of interneurons in CA1, there are a number of clusters that Harris et al identified but were unable to pair to previously characterized cell populations. Additional work is needed to characterize these novel cell clusters and determine their role in the circuitry.

Interneurons are critical to healthy hippocampal functioning, and a proper understanding of their role in seizures and epilepsy is complicated by their sheer diversity. This work by Harris et al provides a reference point to further extend our knowledge, both through additional studies using similar techniques, and by applying the knowledge already gleaned. For example, the information from the deep transcriptomic analysis could be utilized (especially in combination with recent advances in genetic targeting techniques such as INTRSECT[11]) to develop methods to label, manipulate, and study more specific cell populations—so that we can stop examining what effect activation of "PV cells" has on seizures, and start determining the role of (more) specific and homogenous neuronal populations. It is time to seize single-cell sequencing data, appreciate the extent of neuronal diversity, and profit from a deeper understanding of neuronal circuits in health and disease.

By Zoé Christenson Wick and Esther Krook-Magnuson

References

1. Klein P, Dingledine R, Aronica E, et al. Commonalities in epileptogenic processes from different acute brain insults: do they translate? *Epilepsia*. 2018;59(1):37-66.
2. Spampanato J, Dudek FE. Targeted interneuron ablation in the mouse hippocampus can cause spontaneous recurrent seizures. *eNeuro*. 2017;4(4).
3. Hunt RF, Girskis KM, Rubenstein JL, Alvarez-Buylla A, Baraban SC. GABA progenitors grafted into the adult epileptic brain control seizures and abnormal behavior. *Nat Neurosci*. 2013;16(6): 692-697.
4. Krook-Magnuson E, Armstrong C, Oijala M, Soltesz I. On-demand optogenetic control of spontaneous seizures in temporal lobe epilepsy. *Nat Commun*. 2013;4:1376.
5. Freund TF, Buzsáki G. Interneurons of the hippocampus. *Hippocampus*. 1996;6(4):347-470.
6. Christenson Wick Z, Leintz CH, Xamonthiene C, Huang BH, Krook-Magnuson E. Axonal sprouting in commissurally projecting parvalbumin-expressing interneurons. *J Neurosci Res*. 2017; 95(12):2336-2344.
7. Maguire JL. Implicating interneurons: optogenetic studies suggest that interneurons are guilty of contributing to epileptiform activity. *Epilepsy Curr*. 2015;15(4):213-216.
8. Varga C, Oijala M, Lish J, et al. Functional fission of parvalbumin interneuron classes during fast network events. *Elife*. 2014; 3(e04006).
9. Wu YE, Pan L, Zuo Y, Li X, Hong W. Detecting activated cell populations using single-cell RNA-Seq. *Neuron*. 2017;96(2): 313-329.e6.
10. Cadwell CR, Palasantza A, Jiang X, et al. Electrophysiological, transcriptomic and morphologic profiling of single neurons using Patch-seq. *Nat Biotechnol*. 2016;34(2):199-203.
11. Fenno LE, Mattis J, Ramakrishnan C, et al. Targeting cells with single vectors using multiple-feature Boolean logic. *Nat Methods*. 2014;11(7):763-772.