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Neuronal Superhubs: Elite Networks that Rule Synchrony

Maximally selective single-cell target for circuit control in epilepsy models

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Neurological and psychiatric disorders are associated with pathological neural dynamics. The fundamental connectivity patterns of cell–cell communication networks that enable pathological dynamics to emerge remain unknown. Here, we studied epileptic circuits using a newly developed computational pipeline that leveraged single-cell calcium imaging of larval zebrafish and chronically epileptic mice, biologically constrained effective connectivity modeling, and higher-order motif-focused network analysis. We uncovered a novel functional cell type that preferentially emerged in the preseizure state, the superhub, and was unusually richly connected to the rest of the network through feedforward motifs, critically enhancing downstream excitation. Perturbation simulations indicated that disconnecting superhubs was significantly more effective in stabilizing epileptic circuits than disconnecting hub cells that were defined traditionally by connection count. In the dentate gyrus of chronically epileptic mice, superhubs were predominately modeled adult-born granule cells. Collectively, these results predict a new maximally selective and minimally invasive cellular target for seizure control.

Commentary

Hub cells have long been identified as well-connected neurons, identifiable early in the immature brain, that play a critical role in orchestrating local and long-range activity for assemblies of neurons associated with specialized circuits. The earliest hub cells in immature circuits were identified as GABAergic neurons[1] shown to be critical in guiding both early circuit formation and maturation. The concept of superhub neurons then are network titans connected to many such hub cells in a feedforward motif that can control and drive brain synchrony, mirroring the "superhub" phenomenon reported in the financial world[2] where powerful networks of a few elite individuals impact all of our lives. Clinically, neuronal superhubs have been described in the claustrum of an epileptic patient where stimulation lead to electroencephalogram (EEG) signal synchrony within frontal-parietal networks and reversible loss of consciousness,[3] supporting their role in driving network synchrony.

Here, the authors[4] report the identification and connectivity profile of superhub neurons during the "preseizure" phase in two animal models using in-vivo two-photon Ca^{2+} imaging of neuronal activity using computational modeling to allow for predictions of seizure origins and paths of synchrony. It is well known that even through EEG is the gold standard for epilepsy diagnosis, it has poor spatial resolution. Therefore, the ability to conduct large-scale Ca^{2+} in-vivo imaging in zebrafish larvae that are both tiny and translucent allows the ability to capture neuronal activity in the form of transients of fluorescent Ca^{2+} signals for their entire brain comprising a few thousand neurons with the spatial resolution of individual neurons in real time. For this larval fish model, they used a chemoconvulsant (pentylenetetrazole)–induced seizure protocol to capture Ca^{2+} imaging during baseline, preseizure, seizure, and postseizure phases. When analyzing activity motifs from the captured dataset, two subsets of neurons were defined as incoming hub (IH) neurons and outgoing hub (OH) neurons. A subset of the OH neurons identified to be especially associated with other feedforward motif neurons were subclassified as "superhub" neurons. The presence of superhub neuronal activity in the preseizure recording phase in a model of induced seizures indicates that these neuronal networks are present and functional in "normal" immature brains, supporting similar observations related to the pioneer GABAergic superhub cells that were also shown to remain functional into adulthood.[5] This indicates that superhub neurons and their circuit assemblies are not a product of pathogenic brain plasticity or epileptogenesis following brain trauma or insults but rather circuit titans that are critical to brain function and likely maturation from an early stage. Computational simulations further illustrated that OH with the highest feedforward motif conductance had the most influence over network dynamics and displayed increased activity preceding and in between high calcium events compared to the traditional outgoing hub population of neurons. Disconnecting superhubs robustly stabilized the preseizure networks. The role of these

OH superhub circuits was then tested in a mouse model of TLE with chronic spontaneous seizures, the intrahippocampal kainate model. Two-photon calcium imaging of DG granule cells was captured for 600–800 neurons/mouse, in 3- to 5-min windows. Reproducing these experimental calcium data using applied FORCE optimization[6] showed that feedforward motif conductance of individual outgoing hubs was significantly higher in the modeled chronically epileptic dentate network than in the modeled control network, likely representing the classic imbalance of inhibition and excitation described for the DG gate.[7] Disconnecting all superhubs simultaneously in the modeled chronically epileptic dentate network significantly reduced the outgoing hub perturbation, as measured by the percentage change in global signal variance.

An interesting question arises from the concept of the existence of superhub circuits in the brain both in health and disease: who or what inhibits or modulates these titans? What role does the superhub GABAergic titans play early in the preseizure phase? The superhub motifs were activated preseizure both in the zebrafish larvae and the DG of the epileptic mouse. In the acute PTZ model, given the known mechanism of action of PTZ, GABAergic antagonism likely played a dominant role in the activation of the superhub excitatory neurons. In the spontaneously seizing intrahippocampal kainate mouse model, the imbalance of inhibition vs. excitation were both likely at play. Adding to the complexity of extrapolating Ca^{2+} imaging data to understanding circuit recruitment are the imaging results from the zebrafish larvae showing the IH were consistently localized to telencephalon and mesencephalon. In contrast, OH were consistently localized to diencephalon. Does that mean the seizures would not propagate to the telencephalon in the larvae? This may be because in the telencephalon of an immature brain, the OH are not yet well "superhubbed." Additionally the baseline and preseizure networks showed similar macroscale spatial organization of both incoming and outgoing hubs which may not allow for its use as an early predictive marker for seizure onset probability.

For a while now, epilepsy research has focused on the uncertainty regarding whether there is heterogeneous firing during seizures or synchronous neuronal firing in the seizure onset zones. Several research groups have investigated this question specifically in models of temporal lobe epilepsy. In the hippocampus, there are strong excitatory connections laterally within the septotemporally organized lamella along with inhibitory translamellar connections. Recent work testing this hypothesis, for evoked seizures using small microelectrode arrays, showed that the firing pattern was correlated along the lamellar, but not the septotemporal axis[8] and were markedly reduced by antiseizure medications. These results underscore that superhub synchrony at least as relevant to seizure onset foci in the hippocampus may also depend on anatomic connectivity and plasticity. Additionally, destabilization of epilepsy networks as a factor underlying the unpredictability of episodic seizures in patients has been shown to be modulated by multiday rhythms.[9] Within the circadian cycle, seizures are more likely to arise from NREM sleep[10] where superhub circuits may also play a role in synchronizing cortical slow-wave rhythms. For translational applicability, it remains unclear how superhub circuits may contribute to multidien rhythms and circadian seizure susceptibility in epileptic brains.

All epilepsy research is translational, and the authors highlight this by citing the success of closed-loop stimulation techniques in curbing seizures in preclinical models of spontaneously seizing rodents. These methods are already actively applied in the clinic in the form of Responsive Neurostimulation Systems (RNS) like NeuroPace Inc. as a therapeutic option to control seizures. RNS technology is a valuable alternative to removing or lesioning eloquent brain structures when they are the epileptic foci. However, the therapeutic response to RNS is often slow, variable, and defies prognostication based on clinical factors. Elegant work by Chang et al[11] using years-long intracranial neural recordings collected during RNS therapy has found that patients with the greatest therapeutic benefit underwent progressive, frequency-dependent reorganization of interictal functional connectivity. The extent of this reorganization scaled directly with seizure reduction within the first year, likely revealing altered network plasticity as a mechanism underlying successful RNS. Given the important physiological role that superhubs play in brain function, it would be of interest to understand whether any of the RNS-related antiseizure plasticity involved the superhub excitatory or inhibitory circuits. A better understanding of specific molecular biomarkers to identify these titans is needed. The possibility of specifically targeting superhub neurons to help curb drug refractory seizures during the "preseizure" phase could then be achieved using next generation of RNS guided by optogenetics.

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