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One Ring to Bind Them: The Annulus of GABAergic Inhibitory Restraint Fades at Seizure Emergence

Extracellular Glutamate and GABA Transients at the Transition From Interictal Spiking to Seizures

Shimoda Y, Leite M, Graham RT, Marvin JS, Hasseman J, Kolb I, Looger LL, Magloire V, Kullmann DM. *Brain*. 2023: awad336. doi:10.1093/brain/awad336

Focal epilepsy is associated with intermittent brief population discharges (interictal spikes), which resemble sentinel spikes that often occur at the onset of seizures. Why interictal spikes self-terminate whilst seizures persist and propagate is incompletely understood. We used fluorescent glutamate and GABA sensors in an awake rodent model of neocortical seizures to resolve the spatiotemporal evolution of both neurotransmitters in the extracellular space. Interictal spikes were accompanied by brief glutamate transients which were maximal at the initiation site and rapidly propagated centrifugally. GABA transients lasted longer than glutamate transients and were maximal ~1.5 mm from the focus where they propagated centripetally. Prior to seizure initiation GABA transients were attenuated, whilst glutamate transients increased, consistent with a progressive failure of local inhibitory restraint. As seizures increased in frequency, there was a gradual increase in the spatial extent of spike-associated glutamate transients associated with interictal spikes. Neurotransmitter imaging thus reveals a progressive collapse of an annulus of feed-forward GABA release, allowing seizures to escape from local inhibitory restraint.

Commentary

In 1967, two young neurologists, David Prince and B. Joe Wilder, induced epileptiform activity by applying penicillin to exposed cortex of a cat and recorded unitary activity at the site of application and in the neighboring tissue.[1] What they noticed was some of the first evidence of surround inhibition in epileptogenic tissue. There was a prevalence of large depolarizing shifts at the "focus" and a predominance of inhibitory postsynaptic potentials in the penumbra. Since then, evidence across seizure models has amassed suggesting that several mechanisms of inhibitory signaling could be at play underlying ictogenesis and seizure propagation. One such mechanism is the breakdown of inhibitory restraint, where hypersynchronous activity at the ictal focus generates a feedforward inhibitory surround that in turn serves to restrain the excitatory activity.[2] Given the cortical cytoarchitecture, with its diversity of interneuron types, this inhibition occurs both locally through lateral inhibition and distally through long-range inhibitory cortico-cortical projections. Upon GABAergic failure, possibly due to synaptic fatigue or depolarization block, the excitatory activity overcomes this inhibition and, unchecked, can now propagate. Another inhibitory mechanism hypothesized is post inhibitory rebound, where GABAergic activity suppresses pyramidal neuron firing, but upon the cessation of this inhibition, the excitatory cells synchronously reactivate and self-perpetuate as a seizure. Changes in GABAergic interneuron activity may also serve other roles. It may prime the cortex to seize due to excessive potassium release from GABAergic neurons during interictal activity. Additionally, GABAergic transmission may become paradoxically excitatory following intracellular chloride buildup from excessive firing, shifting the GABA reversal potential.[3] Understanding the precise timing of inhibitory activity relative to excitatory activity during seizures in the intact nervous system can help to resolve the complexities in cellular mechanisms underlying seizure dynamics.

Until recently, *in vivo* seizure studies have been largely electrophysiologic, predominantly monitoring population level dynamics or sparse local recordings and thus often failing to capture the diversity of individual cell or microcircuit activity underlying epileptiform events. Intravital microscopy overcomes this limitation by enabling simultaneous high-resolution imaging of many individual cells and microdomains. This technique can be used to image voltage changes of specific cellular populations, changes in intra- and extracellular ion concentrations and even changes in protein concentration. While measuring ion or protein specific changes had been possible prior to the advent of optical measures, such as through ion specific electrodes, microfluidic recordings, fast scan cyclic voltammetry, or various electrochemical biocatalytic sensors, capturing dynamics with the spatiotemporal resolution

facilitated by imaging was prohibitively difficult. Over the last decade, optical studies have provided evidence of feedforward inhibitory neuron activation and the subsequent breakdown of this activity during ictal invasion.[4-7] However, the overwhelming focus of intravital microscopy has been on calcium imaging, in which transients serve as proxies for neural activity. With calcium being an imperfect reporter and voltage imaging still unable to meet the demands of long-term studies, Dimitri Kullmann and Vincent Magloire's team sought a more direct reporter of cortical activity during ictogenesis—neurotransmitter imaging.

In their recent paper[8] in *Brain* led by Yoshiteru Shimoda, the authors provide critical evidence of such a feedforward inhibitory surround and subsequent breakthrough. Using intravital 2-photon microscopy in awake head-fixed mice, the team studied excitatory and inhibitory spatiotemporal dynamics of interictal discharges and the interictal to ictal transition during seizures induced by intracortical injection of the neurotransmitter receptor modulators pilocarpine (cholinergic agonist) and picrotoxin (noncompetitive $GABA_A$ antagonist). Utilizing original and redshifted variants of the iGluSnFR and iGABASnFR genetically encoded neurotransmitter indicator families out of Janelia Research Campus, the authors devised a clever approach to multiplex the imaging of glutamate and GABA, leveraging 3 PMT detectors with partially overlapping bandpass filter sets, to mathematically unmix the recorded signals post hoc. To enhance the temporal resolution of their signal, the authors also employed a spiral line scan approach, which can achieve a 40 to 60 hz sampling frequency while still reflecting the 2 dimensionality of the recording field. The indicators were also targeted to extracellular domains to capture the neurotransmitter concentrations in the extracellular space. The authors recorded in cortical layer 2/3 ($\sim$100 μm below the pia) at various distances from the chemoconvulsant injection site, the purported ictal focus.

During interictal discharges, the authors observed increases in both glutamate and GABA, with the peak glutamate signal more proximal to the focus and the peak GABA signal occurring more distal. In addition to the spatial concentration gradients of the neurotransmitters being distinct with respect to the focus, their propagation patterns also differed, with glutamate expanding centrifugally away from the focus and GABA contracting centripetally toward the focus. The glutamate expansion occurred at a faster rate than the GABA contraction, with a persistence of GABA in the field suggestive of slower clearance. These results were consistent with the feedforward inhibitory surround hypothesis of the self-limiting nature of interictal discharges. Then at the interictal to ictal transition, the authors observed an increase in glutamate release and a corresponding decrease in GABA release, suggestive of a failure of inhibitory restraint. The authors followed this up by computationally modeling their findings to verify that the model they proposed for these dynamics was feasible.

Overall, this study provides convincing, direct visual evidence of an inhibitory surround and subsequent breakthrough of excitatory activity at the transition from interictal spiking to seizures, lending substantive support to the collapse of inhibitory restraint hypothesis. While it may be tempting to suggest this work reveals a broadly applicable mechanism by its use of 2 distinct pharmacologic models, it is important to note that epilepsy is a heterogenous disease studied at the bench by models limited by similar heterogeneity. These conclusions might only apply to focal onset seizures, or even intracortical chemoconvulsant models. Breakdown of inhibitory restraint also need not be the only mechanism at play, as evidence exists that different dynamics occur across the cortical layers and interneuron subtypes[6,9] and this study is limited to examination of glutamate and GABA released from the full variety of neuron subtypes that reside in or project to layer 2/3. This work also lends support to the notion that interictal discharges are self-limited epileptiform events that when left unrestrained progress to seizures. Indeed, the work demonstrates an inhibitory surround during both the interictal events and ictal events. However, this concept is not without controversy. It is a widely held contention that interictal spikes and seizures arise from similar underlying pathology, but whether a causal relationship exists between the events remains unresolved.[10]

Imaging has become a crucial modality in the investigation of basic mechanisms of epilepsy in the 21st century. This began with line scan calcium imaging coupled with electrophysiology in an *ex vivo* slice preparation, providing crucial evidence for the existence of a feedforward inhibitory signal during epileptiform activity.[11] However, this early method only captured pan neuronal activity, predominantly from neuropil, and was spatially limited to one dimension. Coming full circle, another technical advance is made here with spiral line scanning that captures 2-dimensional spread of transmitter levels *in vivo*, and now also coupled with a novel approach to multiplexed imaging of different neural substrates. And so it comes to pass that the posited ring of inhibition that binds seizure excitation is illuminated from the darkness.

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