



“Maestro” Hub Neurons Orchestrate the Immature GABA Network Symphony

Hub GABA Neurons Mediate Gamma-Frequency Oscillations at Ictal-Like Event Onset in the Immature Hippocampus.

Quilichini PP, Le Van Quyen M, Ivanov A, Turner DA, Carabona A, Gozlan H, Esclapez M, Bernard C. *Neuron* 2012;74:57-64.

Gamma-frequency oscillations (GFOs, >40 Hz) are a general network signature at seizure onset at all stages of development, with possible deleterious consequences in the immature brain. At early developmental stages, the simultaneous occurrence of GFOs in different brain regions suggests the existence of a long-ranging synchronizing mechanism at seizure onset. Here, we show that hippocamposeptal (HS) neurons, which are GABA long-range projection neurons, are mandatory to drive the firing of hippocampal interneurons in a high-frequency regime at the onset of epileptiform discharges in the intact, immature septohippocampal formation. The synchronized firing of interneurons in turn produces GFOs, which are abolished after the elimination of a small number of HS neurons. Because they provide the necessary fast conduit for pacing large neuronal populations and display intra- and extrahippocampal long-range projections, HS neurons appear to belong to the class of hub cells that play a crucial role in the synchronization of developing networks.

Commentary

Coordinated activation of neural networks is associated with most complex brain function in adults, and has been implicated in the maturation of neural circuits postnatally. Production of orchestrated neural population activity involves changes in cellular excitability, superimposed on synaptic and extrasynaptic communication characteristics that, in turn, require specific network architecture. In particular, gamma frequency oscillations (GFOs) involve relatively high frequency activity (> 40 Hz), transiently generated by repetitive and synchronized activity of relatively large groups of neurons, resulting in a fluctuating period of increased and decreased excitability in target neuron populations (1, 2). These GFOs can also be coordinated across multiple networks almost simultaneously, providing a substrate for synchronization of activity across brain regions. Interestingly, GFOs often precede seizures and ictal-like activity and may, therefore, be an electrographic hallmark of chronic epilepsy. Synaptic interactions between excitatory glutamatergic and inhibitory GABAergic neurons can contribute to generation of GFOs—and seizures—in the adult brain. Experimental data indicate that GFOs in cortical areas, including the hippocampus, can also be generated and coordinated across regions by interconnected GABAergic neurons in the immature brain; these data are well-supported by theoretical models (1). Coordinated activation of neuronal assemblies, such as that revealed by population oscillations

(i.e., GFOs), has been proposed to involve superconnected nodes (i.e., “hubs”) of local networks, in which a few so-called hub neurons link nodes via long-range connections (3). Both within and between networks, GABAergic neurons appear to fulfill this role (2). The work by Quilichini and Le Van Quyen et al. aims to better understand the mechanisms of GFO generation, including the potential involvement of hub cells that might trigger GFO generation, as well as the relationship of GFOs to ictal-like activity in immature hippocampal networks, where GABA neurons appear to be the predominant substrate of network function.

The authors investigated oscillations in immature mice and rats at an age when pyramidal cells are immature, ion cotransporter balance has not been established as it is in adults, and in which GABA tends to be excitatory. They used an *in vitro* septohippocampal preparation that allowed for study of local excitability within discrete regions of the hippocampus, as well as communication between distinct cell assemblies. Using recording conditions designed to evoke epileptiform activity in the preparation, the authors recorded activity of neuron populations as well as synaptic input to individual neurons within a population. These strategies allowed recordings from multiple hippocampal regions and also an assessment of the nature and timing of synaptic inputs to cells within each region. In low-Mg²⁺ recording conditions, ictal-like events of several seconds duration were generated in all principal cell populations, which were preceded by transient GFOs that were phase-locked across the various areas of the septohippocampal preparation. This suggested a high degree of synchronization within and *between* populations across the hippocampus and septum. The authors then determined the



firing pattern of different neuron classes, focusing on the CA1 hippocampal area during GFOs. Interestingly, the activity of principal neurons (i.e., pyramidal cells) did not increase during GFOs but increased only after GFO development. However, GABAergic interneurons fired at high frequency and in phase with the oscillatory activity during the GFOs, highlighting their contribution to the oscillations.

Unlike local interneurons, GABAergic hippocampal-septal (i.e., H-S) cells, which have long-range axonal projections through the CA1 and septum and are known to contact other GABA neurons exclusively during development, dramatically increased their firing activity just prior to GFO development. Indeed, the increased firing in these cells was progressively recruited, and the increased H-S cell activity, in turn, recruited locally-projecting interneurons via increases in depolarizing GABAergic synaptic input to the interneurons. The synchronized activity of interneurons, which contact pyramidal cells, could result in increased GABAergic synaptic frequency in the pyramids after GFO onset. Thus, H-S cells signaled the synchronization of interneurons during the GFO, which eventually may activate principal neurons via depolarizing GABAergic synaptic inputs. Because of their ability to drive the activation of GFOs, GABAergic H-S cells assume the role of conductor, signaling to local GABAergic network hubs to increase activity that initiates orchestrated function.

Whether H-S cell recruitment was necessary for GFO development or was simply closely associated with these synchronized events remained a key question. In addition to their apparent involvement in cognitive processes and other coordinated cortical output, GFOs precede ictal-like activity. Thus, H-S cell involvement in GFO initiation may also be relevant to generation of ictal-like events, at least in vitro. Investigation of this question was aided by the transgenic “GIN” mouse, in which the somatostatinergic subset of GABAergic neurons expresses green fluorescent protein (GFP) (4). Early postnatally in this mouse, most H-S cells were found to be GFP-labeled, and most GFP-labeled cells were H-S cells. Selectively eliminating labeled H-S cells one at a time from the preparation using focused fluorescence illumination resulted in the elimination of GFOs in CA1. This occurred after just a few H-S cells were killed, but not when other GABA neuron types were removed from the circuit. However, a causative relationship of GFOs to ictal-like events was not supported: Although the GFOs disappeared after selective elimination of H-S cells, the ictal-like events remained.

The finding that a relatively few H-S cells in the septohippocampal formation act as so-called “hub” neurons, triggering coordinated oscillatory activity in relatively large assemblies of GABAergic interneurons in the immature brain may have im-

portant implications for development of epilepsy in immature hippocampal circuits. In the immature brain, seizures beget seizures in a manner that requires GABAergic network-driven oscillations (5). Whether similar mechanisms exist in the adult remains uncertain since GFO generation in adults involves somewhat different mechanisms, including engagement of glutamatergic circuits. Thus, seizure susceptibility in the immature brain is relatively high (6), but basic epileptogenic mechanisms are different than in adults, at least in terms of neurotransmitter involvement.

Although GFOs are *associated* with seizure-like activity in the immature preparation used, the finding that ictal-like activity continues without their presence makes uncertain the GFO relationship to epileptiform events, seizures, or epilepsy, as noted by the authors. Even though they may not cause seizures, GFOs appear to signal an impending activity state change that is driven by activity in just a few exquisitely well-positioned inhibitory neurons. Since GFO generation is associated with large-scale cognitive activity, it is intriguing to hypothesize that their development coincident with seizures in the immature brain may be more closely related to epileptogenesis itself or to comorbidities of hippocampal function, rather than to acute seizure generation (ictogenesis) per se.

by Bret N. Smith, PhD

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