Fast Ripples: What Do New Data about Gap Junctions and Disrupted Spike Firing Reveal about Underlying Mechanisms?

Gap Junctions on Hippocampal Mossy Fiber Axons Demonstrated by Thin-Section Electron Microscopy and Freeze Fracture Replica Immunogold Labeling. Hamzei-Sichani F, Kamasawa N, Janssen WG, Yasumura T, Davidson KG, Hof PR, Wearne SL, Stewart MG, Young SR, Whittington MA, Rash JE, Traub RD. *Proc Natl Acad Sci USA* 2007;104(30):12548–12553. Gap junctions have been postulated to exist between the axons of excitatory cortical neurons based on electrophysiological, modeling, and dye-coupling data. Here, we provide ultrastructural evidence for axoaxonic gap junctions in dentate granule cells. Using combined confocal laser scanning microscopy, thin-section transmission electron microscopy, and grid-mapped freeze–fracture replica immunogold labeling, 10 close appositions revealing axoaxonic gap junctions (30–70 nm in diameter) were found between pairs of mossy fiber axons (100–200 nm in diameter) in the stratum lucidum of the CA3b field of the rat ventral hippocampus, and one axonal gap junction (100 connexons) was found on a mossy fiber axon in the CA3c field of the rat dorsal hippocampus. Immunogold labeling with two sizes of gold beads revealed that connexin 36 was present in that axonal gap junction. These ultrastructural data support computer modeling and *in vitro* electrophysiological data suggesting that axoaxonic gap junctions play an important role in the generation of very fast (>70 Hz) network oscillations and in the hypersynchronous electrical activity of epilepsy.

Reduced Spike-Timing Reliability Correlates with the Emergence of Fast Ripples in the Rat Epileptic Hippocampus. Foffani G, Uzcategui YG, Gal B, Menendez de la Prida L. *Neuron* 2007;55(6):930–941. Ripples are sharp-wave-associated field oscillations (100–300 Hz) recorded in the hippocampus during behavioral immobility and slow-wave sleep. In epileptic rats and humans, a different and faster oscillation (200–600 Hz), termed fast ripples, has been described. However, the basic mechanisms are unknown. Here, we propose that fast ripples emerge from a disorganized ripple pattern caused by unreliable firing in the epileptic hippocampus. Enhanced synaptic activity is responsible for the irregular bursting of CA3 pyramidal cells due to large membrane potential fluctuations. Lower field interactions and a reduced spike-timing reliability concur with decreased spatial synchronization and the emergence of fast ripples. Reducing synaptically driven membrane potential fluctuations improves both spike-timing reliability and spatial synchronization and restores ripples in the epileptic hippocampus. Conversely, a lower spike-timing reliability, with reduced potassium currents, is associated with ripple shuffling in normal hippocampus. Therefore, fast ripples may reflect a pathological desynchronization of the normal ripple pattern.

COMMENTARY

Electrotonic coupling between pyramidal neurons is now amply documented, and the question is: "why does it occur?" Coupling could play an important role in brain oscillations as networks generate high-frequency oscillations, such as during rapid upstrokes (200 Hz ripples) (1) or when synaptic currents are completely blocked (2,3). In addition, a notably wide range of high-frequency oscillations are generated in relation to seizures; determining whether these oscillations are causally related to seizures and how they are generated may not merely be of academic interest, if they provide a signature of epilepsies, as several recent investigations suggest.

Using dye injections in axons of CA1–CA3 pyramidal neurons or granule cells, Traub and colleagues as well as Mercer et al. showed a diffusion of the injected dyes to adjacent neurons, presumably via gap junctions and indicated that high-frequency oscillations require gap junctions (4,5). In a more recent effort reviewed here, Hamzei-Sichani and colleagues used a multidisciplinary approach to assess the mechanisms underlying high-frequency oscillations, which included nothing less than thin-section transmission, confocal laser scanning microscope, electron microscopy immunogold labeling, and grid-mapped freeze–fracture replica! The investigators provide quite direct evidence for the existence of gap junctions. However, in purist electrophysiological terms, compelling evidence that gap junctions are functional requires pair recordings from the two connected elements and a demonstration that currents injected in one neuron, can generate a current in the other neuron. Anatomical observations may demonstrate that gap junctions are present but not that they are operative, as these experiments are not easily performed on axoaxonic connections.

In another heroic effort, Mercer and colleagues recorded over 1000 pairs of pyramidal neurons and showed that 19 out of the 1370 neuron pairs indeed were electrically coupled, such that a spike in one neuron generated a spikelet or an all-or-none spike in the other (5). The efficacy of the coupling index was high, suggesting that the currents will impact the other neuron, including by activating chemical synapses in target

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neurons. In addition, relying on spikelets and assuming that they are only generated by gap junctions, Mercer and coworkers found that over 70% of neurons may be coupled with a few other neurons—usually these couplings are spatially quite close. Therefore, gap junctions are more frequent than typically assumed, and they are axoaxonic, according to Traub et al., and somato-dendritic, according to Mercer et al. Traub and colleagues argue that the important feature is the propagation of spikes from axon to axon and that the soma and dendrites are not significant contributors in the equation. Clearly, the debate has not ended—it has just shifted from: "do gap junctions exist" to "where and how do they act"? Specific antagonists currently used (i.e., carbenoxalone and the antimalaria drug, mefloquine) are not as selective as often attributed in the literature, although this fact is not recognized or publicized. These observations suggest that gap junctions are functional but not necessarily that they are involved in any type of epilepsy.

More directly relevant to epilepsy is a recent study by Foffani et al. in which the mechanisms underlying the generation of fast ripples (<200–600 Hz) were investigated in an epileptic tissue. Fast ripples are thought to reflect a sort of hypersynchronization of pathological networks and to involve the intrinsic pathological feature or signature of epileptic networks in both human and experimental recordings (6,7). It is, therefore, of the utmost importance to determine their generating mechanisms and networks. Specifically, are fast ripples generated by the same networks that generate behaviorally relevant patterns? Is an additional inhibitory device that prevents the generation of fast activities above 200 Hz, somehow inhibited or malfunctioning? Or, are fast ripples generated by networks formed during the epileptogenic process and only present in pathological tissues because of cell loss and formation of aberrant excitatory synapses? In either case, there is an additional important question: do these patterns represent an increased fidelity of neuronal responses that tend to be over synchronized, as they become overexcited? Or, are they generated because of a reduced spike time reliability that leads to less stereotypic neuronal responses and, thus, more jitter and a larger window during which neurons can fire and generate responses?

In the study by Foffani et al., neurons were recorded in pilocarpine-treated animals in which fast ripples were generated *in vivo* but also were assessed in slices. The authors demonstrate that whereas naïve CA3 networks generate 200 Hz events in slices, in pilocarpine-treated animals, these same networks now generate 400 Hz or higher. Fast ripples emerge from a disorganized network associated with a fluctuation of spike responses and an unreliable firing of epileptic neurons, suggesting that reduced fidelity is an important component of epilepsy. This reduced fidelity is due to enhanced glutamatergic synaptic activity stimulated by epileptic neurons associated with lower field interactions. The initial suggested mechanism is a wide spread loss of neurons that reduces ephaptic and field interactions, leading in turn to reduced fidelity and seizures. In other terms, the central element is the initial cell loss produced by seizures. In this model, neurons do not fire at very high frequencies; rather, the crucial factor is that, in response to single stimuli, neurons trend toward having a jitter during spikes—it is this feature that promotes seizures. Although Foffani et al. have used a genuine model of temporal lobe epilepsy in which specific synapses are known to play central roles, the authors suggest that mossy fiber sprouting (now well established as occurring in epilepsy) plays neither a role in cell loss nor in the generation of further seizures. Their theory, however, cannot be readily reconciled with the repeated observations that reactive plasticity and, in particular, the sprouting of mossy fibers and the formation of aberrant synapses play a central role in temporal lobe epilepsy and are instrumental in how seizures beget seizures (8–12). Indeed, blocking mossy fiber synapses reduces seizures, and the mossy fiber kainate receptor-mediated EPSCs that synapse onto granule cells are present in epileptic, but not in naive granule cells (9,10). Therefore, seizures induce the formation of novel synapses that are both strongly excitatory and endowed with features that will heavily impact seizure generation.

The model proposed by Foffani and colleagues is akin to a statistical, nonevolving view of epileptic neurons, with the occurrence of seizures dependent upon the amount of cell loss. However, there is an ample body of observations to suggest that a wide range of conditions can lead to severe brain damage and cell loss, without necessarily being associated with seizures and fast ripples. Thus, a massive cell loss in CA1 (such as occurs with an ischemic insult) is not always associated with epilepsy, in spite of an increased excitability of CA3 pyramidal neurons following the destruction of their targets (13). Finally, a model relying on ephaptic interactions and cell loss as the nodal point does not readily take into account the exquisite differential voltage-gated and synapse-mediated currents of different neuronal populations that occur, notably, in the polarity of GABA actions produced by high-frequency oscillations and directly linked to epileptic tissues (14). To sum up, epilepsy is generated by continuously evolving networks endowed with unique features, and it is the dynamic evolution that must be deciphered to move toward a greater understanding of these networks.

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