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Chemical synaptic and gap junctional interactions between principal neurons: Partners in epileptogenesis

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ABSTRACT

Field potential signals, corresponding to electrographic seizures in cortical structures, often contain two components, which sometimes appear to be separable and other times to be superimposed. The first component consists of low-amplitude very fast oscillations (VFO, >70–80 Hz); the second component consists of larger amplitude transients, lasting tens to hundreds of ms, and variously called population spikes, EEG spikes, or bursts—terms chosen in part because of the cellular correlates of the field events. To first approximation, the two components arise because of distinctive types of cellular interactions: gap junctions for VFO (a model of which is reviewed in the following), and recurrent synaptic excitation and/or inhibition for the transients. With *in vitro* studies of epileptic human neocortical tissue, it is possible to elicit VFO alone, or VFO superimposed on a large transient, but not a large transient without the VFO. If such observations prove to be general, they would imply that gap junction-mediated interactions are the primary factor in epileptogenesis. It appears to be the case then, that in the setting of seizure initiation (but not necessarily under physiological conditions), the gain of gap junction-mediated circuits can actually be larger than the gain in excitatory synaptic circuits.

1. Introduction

A critical role for recurrent synaptic excitation, in the generation of synchronized bursts of action potentials (resembling interictal spikes and ictal burst complexes in epileptic patients), has been known for upwards of 30 years now. The role of excitatory synapses was inferred, for example, by the recording of "giant" EPSPs during epileptic bursts in *in vitro* rodent models (Johnston & Brown, 1981; Traub & Wong, 1982), and by the ability to shorten, or entirely block, population bursts using pharmacological antagonists of AMPA/kainate or NMDA types of glutamate receptors (Dingledine, Hynes, & King, 1986; Lee & Hablitz, 1989; reviewed in two previous monographs: Traub & Miles, 1991; Traub, Jefferys & Whittington, 1999).

Recurrent synaptic excitation, however, is not the whole story, either in human epilepsy or in experimental epilepsy models—even when one excludes the so-called field bursts in low-calcium media, wherein both excitatory and inhibitory chemical synapses are blocked (Jefferys & Haas, 1982; Taylor & Dudek, 1982). Epileptiform field potentials in the disinhibited hippocampal slice, in which glutamatergic neurotransmission is intact, have long been known to contain high-frequency components, sometimes up to several hundred Hz (Schwartzkroin & Prince, 1997; Wong & Traub, 1983; Fig. 1C illustrates this phenomenon for human electrocorticographic (ECoG) data). The existence of such high-frequency components, easily visible to the naked eye and reflecting synchronized collective activity in a neuronal population, is difficult to explain via glutamatergic interactions; and, recall that in these studies, fast GABAergic inhibition was blocked, resulting in a situation wherein phasic IPSPs cannot occur (unlike, for example, the case with hippocampal ripples in vivo Ylinen et al., 1995). The reason is that the time scale over which a single CA3 pyramidal neuron spike leads to a postsynaptic EPSP is several ms (see Fig. 2A); and, additionally, the giant EPSP, in any given pyramidal neuron during an epileptiform burst – resulting from inputs from all its presynaptic precursors – is large and smooth (see Fig. 3). As a consequence, a single EPSP alone would not allow the precise timing of a single presynaptic spike to measurably influence the timing of a postsynaptic spike: the effect of any one presynaptic spike is lost amidst the smoothed effects of the many other presynaptic spikes. Such considerations suggest that an alternative type of interaction between neurons might account for the high-frequency components of the epileptiform field potential—an interaction that should be extremely fast, and in

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which a single action potential in one cell can produce a temporally discrete and distinguishable effect in another cell, even during a collective synchronized burst. Gap junctions between principal neurons could, in principle, provide this sort of interaction. Indeed, we shall argue that not only is epilepsy far more complex than an imbalance between synaptic excitation and inhibition, but also that gap junctions between principal neurons are likely to play a crucial role, both before and during seizure activity.

Fig. 1 illustrates subdural grid ECoG data from a child who had a subcortical dysplasia and intractable seizures, with Fig. 1A and B showing the onset of an electrographic seizure, and Fig. 1C an interictal (i.e. between-seizure) event. From Fig. 1A and B, it is clear that in the epileptic brain, VFO can exist alone, not superimposed on burst discharges (as in Fig. 1C, and also occurred in seizure-associated burst discharges in this patient—see also Fig. 7); such an observation also suggests that VFO might not require significant glutamatergic neurotransmission. Furthermore, the common appearance of VFO just prior to a seizure (Bragin, Engel, Wilson, Fried, & Mathern, 1999; Fisher, Webber, Lesser, Arroyo, & Uematsu, 1992; Jacobs et al., 2008, 2009; Khosravani et al., 2008; Schevon et al., 2008; Traub et al., 2001; Worrell et al., 2004) suggests that distinctive tissue conditions might predispose the neural circuits to generate VFO and seizures, as part of a pathophysiology that encompasses both sorts of phenomenon together. [An example of such a possible tissue condition would be alkaline pH, which potentiates electrographic seizure activity *in vitro* (Traub et al., 2001, 2010), possibly by opening gap junctions.] Fig. 5 provides another example of VFO preceding an electrographic seizure, in a different patient.

VFO occurs under physiological conditions, as well as during epileptic events. For example, ∼200 Hz "ripples" occur superimposed on physiological sharp waves in the normal (non-epileptic) in vivo hippocampus, and in other limbic structures (Buzsáki, Horváth, Urioste, Hetke, & Wise, 1992; Chrobak & Buzsáki, 1996; Ylinen et al., 1995). Such combined VFO/transient events resemble, in form, interictal bursts with superimposed VFO, although not necessarily having the same amplitude (physiological events are smaller), and certainly not the same significance for predicting the occurrence of spontaneous seizures; additionally, VFO/transient events occur in epileptic human neocortex (Fig. 1C; Roopun et al., 2010) but not, to our knowledge, in normal neocortex. VFO per se can occur, however, in non-epileptic neocortex, although – at least in the anesthetized cat – the amplitude of VFO is significantly higher in epileptic cortex than non-epileptic cortex (Edwards et al., 2010; Grenier, Timofeev, & Steriade, 2001, 2003). Even so, the occurrence of VFO in normal brain suggests that the structural circuit substrate of VFO is not necessarily pathological, in and of itself.

A major advance in understanding ripple physiology came with the fortuitous discovery that ripples could occur in isolation, and not just superimposed on a sharp wave, at least *in vitro* (Draguhn, Traub, Schmitz, & Jefferys, 1998)—although sharp wave/ripples also can occur together *in vitro* (see below). The evidence that *in vitro* ripples are mediated by electrical coupling between pyramidal cells is compelling (Draguhn et al., 1998): (a) such ripples are enhanced in low-calcium media, that block chemical synapses (and probably also help to open gap junctions); (b) the ripples are enhanced by alkalinization of the medium, and suppressed by acidification, measures expected to open (respectively, close) gap junctions (Spray, Harris, & Bennett, 1981); (c) the ripples are suppressed by octanol, halothane and carbenoxolone (Juszczak & Swiergiel, 2009), all gap junction blockers (while, unfortunately, none are completely specific, the effectiveness of each of the three agents supports specificity). Additionally, ripples are associated with somatic spikelets, or fast prepotentials, which (in hippocampal neurons) can be evoked by antidromic stimulation, depend on gap junction coupling, and are conducted along axons (Schmitz et al., 2001). As of about 10 years ago, then, the existing data suggested a novel hypothesis, that pyramidal cells were electrically coupled between their axons, and that such coupling led to VFO. We shall consider later in this review how the generation might work, and we shall describe the tissue conditions that might favor VFO vis-à-vis synchronized burst discharges.

2. Properties of chemical synaptic vs. electrical coupling between pyramidal neurons

Fig. 2 illustrates chemical and electrical coupling between hippocampal pyramidal neurons. For chemical synapses between CA3 pyramidal cells, Miles and Wong (1986) found unitary EPSPs of 0.6–1.3 mV (of course with fluctuations) and time to peak of averaged events equal to 5–12 ms. As Fig. 2Aa indicates, a single presynaptic action potential would not generally cause firing of the postsynaptic pyramid, although this could happen at some connections from pyramidal cells to interneurons (Gulyás et al., 1993), and could happen rarely at pyramidal/pyramidal connections (probability about 0.05, Miles & Wong, 1986). In contrast to a single action potential, however, a *burst* of action potentials could evoke a somewhat delayed (11 ms) postsynaptic burst, although not with perfect reliability. Latencies to postsynaptic bursting could be up to 30 ms, and the failure rate for burst propagation ranged from 0.5 to 0.7 (Traub & Miles, 1991).

As Fig. 2B demonstrates, electrical coupling between pyramidal cells is (not surprisingly) quite rapid, with latencies 0.5 ms or less, when measured with an electrode in the soma of each neuron (the latency at the coupling site might be shorter). Furthermore, a single spike could, on occasion, evoke a spike in a coupled neuron (Fig. 2Bd, Mercer, Bannister, & Thomson, 2006). Such strong functional coupling – with a spike inducing a spike – is even more powerful than synaptic coupling wherein a full presynaptic burst is required to induce firing in the postsynaptic neuron. Unfortunately, in these examples the site of the coupling between the two neurons was not determined. If the coupling site were to be between axons, it is possible that the coupled axon actually fires a full spike in response to a "presynaptic" spike, but with the axonal spike conducting decrementally to the soma, where the electrode sits and sometimes records a spikelet, rather than a full spike. This issue is discussed in more detail in Schmitz et al. (2001).

The propagation of *bursts* of action potentials, from a neocortical pyramid to an electrically coupled pyramid, has recently been documented in a slice from a P32 rat (Wang, Barakat, & Zhou, 2010). The latency from the first full spike in one cell, to the first full spike in the coupled cell, was in the tens of ms; this latter spike was, however, preceded by a series of summating spikelets. Wang et al. (2010) also showed that the "postsynaptic" (i.e. across the gap junction) somatic response in a neuron to a "presynaptic" spike – that is, whether the response was a spikelet or a full action potential – was exquisitely sensitive to membrane potential, with 2 mV making a measurable difference.

The *density* of connections (between pyramidal cells) also appears to be quite different for chemical synapses vs. gap junctions. Thus, in the CA3 region *in vitro*, a pyramidal neuron was estimated (using dual intracellular recordings) to synapse onto roughly 2% of nearby neurons (MacVicar & Dudek, 1980; Miles & Wong, 1986). The CA3 region in a slice contains some thousands of pyramidal cells, so that (on average) a pyramidal cell should synaptically connect to dozens of others; and the connectivity in vivo is doubtless higher yet. In contrast, gap junctional connectivity between pyramidal cells, as estimated by dye coupling, appears to be much sparser (Church & Baimbridge, 1991). Dye coupling probability is enhanced by alkaline pH (Church & Baimbridge, 1991) and by low-calcium media (Perez-Velazquez, Valiante, & Carlen, 1994), but probably is not much over the percolation limit (i.e. wherein one cell couples to one other, on average), even in conditions optimal for coupling.