

# Interneurons, Spike Timing, and Perception

# Minireview

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**Rhythmic  $\gamma$  oscillations at 30–70 Hz in cortical and hippocampal slices depend on a maintained excitation and on interactions between interneurons and pyramidal cells. These interactions include gap-junctional connections between inhibitory cells and fast excitatory and inhibitory chemical synapses. Spike timing with precision in the range of several ms may be assured by biphasic signaling mechanisms operating at these different connections. Such temporal precision may be important in cognitive processing.**

Can clapping help us understand consciousness? Maybe it can. Studies in applause physics have examined the delightful social self-organization that occurs when an audience claps at first randomly and then synchronously to express their pleasure after a performance (Neda et al., 2000). The mechanisms involved are reminiscent of those for a synchronous inhibitory neuron activity which may underlie EEG rhythms associated with activated cortical states. Cortical oscillations at  $\gamma$  frequencies of 30–70 Hz are associated with distinct behaviors including perception, attention, and sensorimotor coordination. They have been suggested to link neurons engaged in different aspects of a cognitive task. Recently, attention has focused on the possibility that electrical coupling between inhibitory cells plays a crucial role in generating such synchronous activity.

Both synchronized clapping and interneuron synchrony need several ingredients. First, an excitatory drive—both the audience and the interneurons must be excited. Second, interactions—between neighbors in the audience or between connected interneurons. Third, the population must be homogenous. If clappers persist in applauding at different rhythms or if interneuron properties are too different, synchronous activity does not emerge. We will consider each of these elements in turn.

## ***The Ingredients of Interneuron-Based Synchronies***

***Excitatory Drive.*** Data on the role of  $\gamma$  oscillations in perception originate in work on behaving animals. In contrast, information on the mechanisms that might underlie such activity derives mostly from slice models. In hippocampal or cortical slices, multiple stimuli can initiate  $\gamma$  rhythmicity. The excitatory drive used in models of  $\gamma$  includes tetanic stimulation of afferent pathways, and activation of muscarinic or kainate receptors or metabo-

tropic receptors for glutamate. Each stimulus, just like an excellent performance, causes a sustained neuronal excitation. These diverse agents may mimic in vitro the glutamatergic excitation and the activity in specific brainstem cholinergic nuclei associated with the emergence of  $\gamma$  oscillations in vivo.

***Interactions.*** Interneurons play a key role in the genesis of  $\gamma$  oscillations (Bragin et al., 1995; Traub et al., 1999). While a persistent excitatory drive is needed, inhibitory cell interactions with other interneurons are crucial to sculpt the rhythm. These interactions are constrained by anatomical connectivity. The characteristic axonal ramifications of two distinct types of inhibitory cells help us understand their involvement in the generation of rhythmic oscillations. Most cortical and hippocampal inhibitory cells contact several hundreds of pyramidal cells, as well as several tens of inhibitory cells, so forming ideal cheerleaders for synchronous clapping. In fact, they act as inverse cheerleaders generating a periodic synaptic inhibition which dictates when pyramidal cells do not fire. The second group of interneurons talk only to each other in a sort of cheerleaders cabal to propagate a coherent anti-clapping message. For instance, a group of interneurons containing the calcium binding protein calretinin form inhibitory synapses exclusively with other interneurons (Gulyas et al., 1996). Modeling work has shown how the superposition of inhibitory synaptic events generated within such a network of spontaneously active inhibitory elements induces synchrony (Traub et al., 1999).

If inhibitory interactions within clusters of interneurons suffice to generate autonomous rhythms, now it seems that excitatory interactions between the same cells act to sharpen up the synchrony. The excitation is mediated by gap junctions formed between interneurons. Gap junctions transmit electrical signals directly so that an action potential in one neuron induces, with minimal delay, a smaller spikelet in a coupled cell. This rapid communication underlies the involvement of gap junctions in many synchronous processes in the brain.

The molecular basis for gap-junctional coupling consists of the expression of proteins of the connexin family at coordinated membrane sites in connected cells. Connexin 36 (Cx36) is the major neuronal connexin. The deletion of this protein in two different knockout mice suggests that it contributes to  $\gamma$  frequency oscillations. In both animals, gap-junctional coupling between interneurons was largely absent. A 30–70 Hz synchrony initiated by activating kainate or muscarinic receptors was maintained in hippocampal slices but at reduced power (Hormuzdi et al., 2001). In neocortical slices, high-frequency oscillations induced by activating metabotropic receptors for glutamate were reduced in both duration and spatial extent (Deans et al., 2001). These findings suggest perhaps that the relative roles of Cx36-dependent electrotonic interactions in  $\gamma$  synchrony differ subtly in the cortex and hippocampus. Indeed in some slice models of  $\gamma$  activity, a rapid synaptic excitation of inhibitory cells mediated via glutamate receptors

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is needed, while oscillations in other models persist when chemical excitatory transmission is suppressed.

**Homogeneity or Diversity?** As synchronous applause emerges only when clappers clap at similar rates, so interneuron synchrony is facilitated when inhibitory cells have uniform properties. However, recent work from multiple laboratories shows that interneurons are anything but uniform. Can interneuron diversity be reconciled with synchronized clapping?

Interneurons possess different synaptic targets, are excited by different afferent systems, express different receptors, and discharge with different patterns. Dividing them into classes according to these differences helps us think about the roles that distinct interneurons might fulfill in cortical or hippocampal circuits. The problem is that classifications based on different criteria may not be consistent with each other (Parra et al., 1998), so subtypes of interneurons proliferate.

Further data on interneuron diversity has emerged from studies on the variability in expression of two molecules involved in models of  $\gamma$  oscillations. First, metabotropic receptors for glutamate (mGluRs). A study on a spatially defined subset of hippocampal inhibitory cells that first were thought to express just one type of mGluR revealed considerable diversity (van Hooft et al., 2000). Single cell RT-PCR techniques showed that many cells contained mRNA coding for either, and sometimes both, of two functionally similar mGluRs. However, mGluR receptor activation did not excite all of these cells and the axons of excited interneurons contacted diverse regions of pyramidal cell dendrites. The second molecular family is that of the connexin proteins, the substrate of gap-junctional communication. As for the mGluRs, different connexin molecules expressed in different cells might do a functionally similar job. Evidence was obtained by single cell PCR for the presence in interneurons of mRNA coding for three distinct connexins, Cx26, Cx32, and Cx36. Most, but not all, cells of four distinct groups of interneurons expressed Cx36, while this connexin was coexpressed with Cx26 or with Cx32 in two of the groups (Venance et al., 2000).

Physiological data on gap-junctional coupling between cortical interneurons strongly supports the notion that groups of inhibitory cells with similar firing patterns form functional entities (Beierlein et al., 2000; Szabadics et al., 2001). In dual records, gap junctions were frequently observed between pairs of cortical fast spiking cells (FS), pairs of low threshold spiking (LTS) cells, or pairs of regular spiking (RS) cells. In contrast, electrical connections between cells from these different groups were rarely detected. This raises two questions. What cell biological mechanisms underlie this specificity? And might the different groups of interconnected inhibitory cells underlie distinct oscillations? FS cells provide a perisomatic inhibition to cortical pyramidal cells and so presumably influence their discharges most strongly. In contrast, RS cells form chemical inhibitory synapses exclusively with pyramidal cell dendrites. In principle, these different groups of coupled inhibitory cells might selectively deliver distinct rhythmic inhibitory signals to somatic or dendritic regions of pyramidal cell membrane.

So how does interneuron diversity influence inhibitory synchronies? The question of how much diversity is permitted before cells must be put in different groups

remains difficult. For example, consider two similar interneurons, one expressing mGluR1 and the other mGluR5 receptors. Should we put them into different groups, or might this molecular diversity instead underlie a robust unity of function? Interneuron diversity might also be a way to assure a robust solution to the clapping problem, although simulation studies suggest that too much variability can disrupt inhibitory rhythms. The tendency of gap-junctional coupling to homogenize the electrical properties of connected interneurons may also be important in enhancing rhythmicity.

#### **Spike Timing in Inhibitory Circuits**

But how does it really work? Can the details of synaptic interactions in circuits underlying  $\gamma$  oscillations really help understand how the rhythm emerges? Synchrony depends on the simultaneous discharge of action potentials. So, perhaps it is useful to examine the temporal precision for action potential transmission between pairs of cortical neurons connected in distinct ways (Figure 1). The transduction of synaptic signals into action potentials, EPSP-spike coupling, involves both spatial and temporal filtering due to cellular geometry and intrinsic currents of the postsynaptic cell. Recent work shows how different biphasic signaling mechanisms control the precision of spike generation at three of the connections involved in the generation of  $\gamma$  oscillations.

**Interactions between Inhibitory Cells.** Dual records show inhibitory cells may be connected in three ways—by chemical or electrical junctions, or by both. In the first case, the kinetics of GABAergic inhibitory currents at synapses between interneurons have a fast decay time course of 2 or 3 ms (Bartos et al., 2001). This time constant is important in setting the frequency of inhibitory cell population oscillations. At purely electrical junctions between inhibitory cells, potential changes are transmitted bi-directionally with a pronounced low-pass frequency filtering. This filtering implies that slow potential changes, such as spike pre-potentials and after-hyperpolarizations, are more effectively transmitted than rapid events like action potentials (Figure 1A). Pre-junctional spikes induce spikelets of amplitude 0.5–2 mV in coupled cells, and such depolarizations can initiate firing at latencies as short as 1–2 ms. However, interaction of gap-junctional potentials with inward currents can extend the time window for postsynaptic spike initiation up to 10–20 ms (Mann-Metzer and Yarom, 1999; Tamas et al., 2000).

The third possible coupling between two inhibitory cells comprises both a gap junction and an inhibitory chemical synapse (Figure 1A). Such sites may be located close to each other and to the postsynaptic soma so reducing both dendritic filtering and propagation times. The resulting postsynaptic potentials interact. A presynaptic action potential generates a biphasic event where chemical synaptic inhibition curtails the spikelet due to gap-junctional coupling (Tamas et al., 2000). This biphasic signaling enforces a spike timing within a window of 1–2 ms, considerably shorter than that obtained by gap-junctional coupling alone (Figure 1A).

**Synaptic Excitation of Inhibitory Cells.** Biphasic signaling mechanisms also ensure that EPSPs which excite inhibitory cells generate action potentials rapidly and precisely. Excitatory currents have fast kinetics with overall duration less than about 5 ms due to a selective

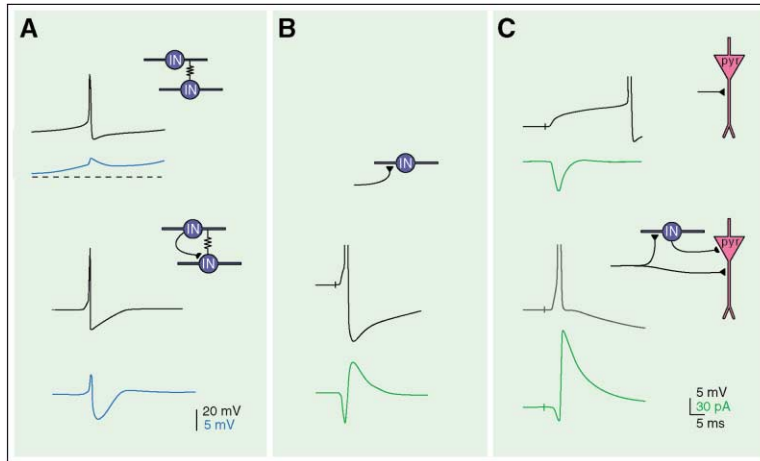


Figure 1. Biphasic Signaling Assures Precise Spike Timing in Three Interactions

(A) Gap-junctional coupling transmits subthreshold potential changes as well as spikelets at connections between interneurons. At subthreshold potentials, intrinsic currents carry on the depolarization after the spikelet. When an interneuron pair is also coupled by a chemical inhibitory synapse, the IPSP speeds up the repolarization after the spikelet. The resulting biphasic signal enhances the precision of spike transmission.

(B) Signaling is rapid at excitatory synapses made with interneurons. A biphasic sequence of inward and outward currents (green trace) is induced by an EPSP-shaped test pulse. Intrinsic postsynaptic currents close to threshold potential help to assure precise EPSP-spike coupling in interneurons.

(C) EPSP-spike coupling is less precise in pyramidal cells. EPSP-like test pulses are much less effective in activating outward currents near threshold (green trace). But in a circuit context, the rapid recruitment of synaptic inhibition produces a biphasic signal restricting spike generation to a window of duration of several milliseconds.

Traces were provided by authors from data sets the results of which were published in: Mann-Metzer and Yarom (1999); Fricker and Miles (2000); Galarreta and Hestrin (2001); and Pouille and Scanziani (2001).

expression of glutamate receptor subunits. In some inhibitory cells, dendritically located sodium channels boost distal EPSPs and may elicit local dendritic action potentials (Martina et al., 2000). Furthermore, voltage-gated outward currents are activated by small synaptic depolarizations near to firing threshold (Fricker and Miles, 2000). These currents add an active repolarization to the fast kinetics of glutamate receptor deactivation and prevent the generation of delayed action potentials. In this case then, the biphasic signal consists of a rapid EPSC assisted by intrinsic inward currents and then cut short by an intrinsic outward current (Figure 1B). This restrains spikes initiated by the synaptic excitation of interneurons to a narrow time window of 2–4 ms duration.

**Excitation of Pyramidal Cells.** While EPSPs induce inhibitory cell firing with high temporal precision, EPSP-spike coupling in pyramidal cells is much less precise. The hyperpolarizing component of the biphasic cellular signal seems to be absent—small EPSPs at subthreshold potentials initiate cellular potassium currents much less efficiently. Predominantly inward currents favor EPSP amplification, prolong EPSP decay, and initiate action potentials with poor temporal precision (Fricker and Miles, 2000). Action potentials may arise from plateau potentials with latencies that vary over 10 s of ms, resulting in an integrate-and-fire mode of function (Figure 1C).

Pyramidal cells do however receive biphasic signals when afferent EPSPs recruit GABAergic interneurons. Perhaps this sequence of an EPSP followed by an IPSP is the original biphasic signal. In a new twist, Pouille and Scanziani (2001) recently showed how somatic inhibition exerts a temporally precise control on the summation of afferent EPSPs. With fast kinetics, excitatory depolarizations are rapidly curtailed, leaving a time window of about 2 ms during which EPSPs can sum to induce pyramidal cell firing (Figure 1C).

So can the details of spike timing at single connections help understand the genesis of  $\gamma$  rhythms? During population oscillations, signals are transmitted not just between pairs of cells but rather in parallel between

many connected elements. Are such interactions just a larger version of those between two cells or does  $\gamma$  rhythmicity depend on emergent properties? For gap-junctional signaling, more should be better. The summed gap-junctional potential due to several interneurons discharging should induce more precise firing than that due to just one coupled cell. For pyramidal cells at low levels of network activity, the precision of EPSP-spike encoding is poor. However, at higher levels of network activity, when interneurons are recruited, the resulting somatic inhibition can impose a temporal precision in the millisecond range on spike initiation in pyramidal cells.

#### **Gamma Oscillations and Information Coding**

The slice models show how synchrony at  $\gamma$  frequencies might be generated. In vivo, oscillations in a similar range of frequencies occur in multiple cortical areas, induced by sensory stimuli with variable latency, but also spontaneously during states of expectancy or attention. Spike synchrony at  $\gamma$  frequencies is not thought to carry information. Rather, some authors suggest, it provides a temporal signal that binds together in transient assemblies cells involved in different aspects of a cognitive task.

Evidence of a role for spontaneous  $\gamma$  oscillations emerges from multi-unit records in conscious monkeys (Fries et al., 2001). Repeated presentations of the same visual stimulus initiated discharges in visual cortex with large latency fluctuations—on the order of  $50 \pm 30$  ms. Latency variations were correlated with the frequency and the trajectory of the macroscopic cortical field potential. Spontaneous oscillations at  $\gamma$  frequencies were associated with shorter latencies and enhanced coherence of multi-unit responses when compared to lower frequency field oscillations. By enhancing the temporal coherence of population responses, spontaneous  $\gamma$  oscillations essentially accelerate perception in a top-down modulation that could be specific to states like attention or expectancy. Recent EEG studies also showed that spatially extensive  $\gamma$  oscillations were induced in human subjects by both phases of a task involving the recognition of a face followed by a motor

reaction (Rodriguez et al., 1999). Interestingly, increases in phase synchrony in the  $\gamma$  band during the two parts of the task were separated by a period of negative synchrony. Perhaps the cellular ensemble activated in face recognition must be actively undone before formation of the ensemble corresponding to the motor reaction. The cellular processes involved in transitions between  $\gamma$  oscillations involving distinct cells in the same population remain to be elucidated.

Gamma synchrony might do more than just tag cells involved in distinct aspects of a cognitive task. It may also facilitate the detection of ensemble activities in downstream cells. So, it is argued, neurons are more likely to fire when excitatory synaptic inputs are coincident, as during  $\gamma$  synchrony, than when they are temporally dispersed. The *in vitro* data suggest that pyramidal cells can function as coincidence detectors with a 2–3 ms precision in high-throughput conditions when sequential EPSP-IPSP signals are generated. However, interneurons seem to be better detectors than pyramidal cells. Intrinsic postsynaptic currents already enhance the precision of EPSP-spike coupling in interneurons. This precision is reinforced for clusters of inhibitory cells connected by both electrical and chemical inhibitory junctions (Galarreta and Hestrin, 2001). The probability that such interneurons will discharge is elevated when EPSPs are coincident since the afferent events sum with excitatory gap coupling potentials. In contrast, delayed EPSPs coincide with the GABAergic IPSP and are much less likely to initiate firing. The relevance of this capacity to detect coincident firing in processes of perception awaits experimental testing.

### Conclusion

So, is this audience excited? Do we clap in synchronous appreciation of an excellent performance or is more work needed? More work, probably. More work especially to confront the slice data on possible mechanisms for  $\gamma$  oscillations, with the reality of rhythms generated by animals engaged in cognitive tasks. Knockout mice exist for molecules supposed to be crucial in  $\gamma$  synchrony, including muscarinic, kainate, and mGlu receptors as well the Cx gap junction channels. Do they have specific defects in perception? If not, might the diversity of mGluR or Cx expression in interneurons be responsible? We must find a way to knockout the function rather than the molecule.

More work, too, is needed on how different inhibitory cells participate in cognition. Extracellular spikes from interneurons are recognizable, yet little is known of their activity, and less still of their role, in cognitive tasks. Are they simply elements that generate a population clock sans content or might they have a real coding function?

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