Big Minis from Hair Cells: Mechanism and Function

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What underlies the large variation in mEPSC amplitude in the auditory system? And is this variability important? In this issue of *Neuron*, Li et al. (2014) address the significance of large mEPSCs to auditory processing and Chapochnikov et al. (2014) describe a novel mechanism underlying them.

Neurotransmitter release from synapses is classically considered to be a stochastic process in which calcium driven into the synapse via voltage-gated calcium channels drives the fusion of neurotransmitter-containing vesicles. In the classical model, each vesicle acts independently to release neurotransmitter in a quantal all-or-none fashion, giving rise to a single miniature excitatory postsynaptic current (mEPSC). More recently, several studies on ribbon synapses of several cell types from different vertebrate preparations have demonstrated that many ribbon synapses exhibit large variations in the apparent mEPSC amplitude, presumed to arise from the release of multiple vesicles near simultaneously (Glowatzki and Fuchs, 2002; Singer et al., 2004; Keen and Hudspeth, 2006; Suryanarayanan and Slaughter, 2006; Li et al., 2009; Schnee et al., 2013). Ribbon synapses are hallmark features of graded nonspiking cells of the vertebrate auditory, vestibular, and visual systems. These synapses are demarcated by structures, synaptic ribbons, which tether vesicles at high densities near release sites on the plasma membrane. The role for the ribbon in synaptic transmission remains poorly understood. The mEPSC size in many of these preparations appears to be modulated by intracellular calcium with high intracellular calcium tending to favor larger mEPSCs (Singer et al., 2004; Keen and Hudspeth, 2006; Suryanarayanan and Slaughter, 2006; Li et al., 2009; Mehta et al., 2013). Of particular note, unlike in conventional synapses where action potentials can serve to synchronize the release of multiple vesicles (Tong and Jahr, 1994), these presumptive multivesicular events exist without synchronizing voltage signals and, in some cases, even persist after calcium channels are presumably closed (Singer et al., 2004) or when calcium spread from channels are severely restricted by fast calcium buffers (Li et al., 2009). Given the prevalence of these events in ribbon synapses, the large variability of mEPSCs may represent an important and possibly unique feature of ribbon synapses. Consistent with this idea, directed photodamage to the ribbon causes an acute reduction in mEPSC size in retinal bipolar cells, and photoreceptors of hibernating ground squirrels have reduced ribbon size compared to awake animals accompanied by a reduction in mEPSC amplitude (Mehta et al., 2013). Two articles in this issue of *Neuron* address two important aspects of mEPSCs in ribbon synapses: the mechanism giving rise to these events (Chapochnikov et al., 2014) and their physiological importance (Li et al., 2014).

Mechanisms Underlying the Variability in mEPSC Size

Models to explain the heterogeneity in mEPSC size at ribbon synapses have focused on mechanisms that lead to the near-simultaneous release of the contents of multiple vesicles, or "multivesicular release" (MVR). Specifically, variations of three models have been proposed to explain mEPSCs as a form of MVR (Figure 1). In one model, the ribbon or proteins associated with the ribbon facilitate the near-simultaneous fusion of multiple vesicles (Glowatzki and Fuchs, 2002; Singer et al., 2004). In a second model, calcium nanodomains near an open calcium channel drives release of multiple vesicles simultaneously (Jarsky et al., 2010; Graydon et al., 2011). In a third model, vesicles fuse to each other prior to fusing with the membrane (compound fusion) (Matthews and Sterling, 2008). Now, in this issue of *Neuron*, Chapochnikov et al. (2014) provide evidence supporting a new model. Specifically, the authors propose that changes in fusion pore properties give rise to mEPSC heterogeneity at the rat inner hair cell synapse.

Unlike the large mEPSCs described at some other ribbon synapses (Singer et al., 2004; Keen and Hudspeth, 2006; Li et al., 2009), the large mEPSCs from some hair cells, including rat inner hair cells, are predominantly multiphasic, exhibiting multiple rising phases that have been suggested to arise from multiple vesicles fusing with slightly staggered release times (Glowatzki and Fuchs, 2002; Schnee et al., 2013). To investigate the mechanisms underlying the mEPSC properties, Chapochnikov et al. (2014) took a closer look at the properties of the simple and multiphasic mEPSCs to determine whether the mEPSC properties had the expected properties of MVR. In a careful analysis of the properties of all mEPSCs, including both complex and simple mEPSCs, Chapochnikov et al. (2014) found that while the amplitudes of mEPSCs exhibited high variability and a skewed distribution, the charge of the mEPSCs showed a narrower and more symmetric Gaussian-like distribution, as one might expect if they were derived from single quanta (i.e., single vesicles).The multiphasic events persisted in the absence of extracellular calcium (although they were reduced in frequency), indicating that calcium entry through calcium channels is not required for these events. Moreover, simple events had nearly the same average total charge as the slower and more complex mEPSCs, suggesting that the same

amount of neurotransmitter is released during each type of event. To reconcile these results, Chapochnikov et al. (2014) propose that complex mEPSCs arise from multiple transient openings, or flickering, of a fusion pore of a single vesicle. This model has the appeal that there is significant precedent for flickering fusion pores in other systems (Lindau and Alvarez de Toledo, 2003) and, unlike the multivesicular models, can account for the invariance of the mEPSC charge between simple and multiphasic events. Interestingly, Chapochnikov et al. (2014) found that for multiphasic mEPSCs exhibiting more than two rising phases (presumed to be individual openings of a fusion pore),each rising phase was smaller than the one that preceded it, as expected from a vesicle being depleted of neurotransmitter with each opening of a fusion pore.

Will these results be the end of the multivesicular hypothesis for ribbon synapses? Most likely, no. While the property of large mEPSC variability may be shared among many ribbon synapses, many of the significant features appear to differ between preparations. For example, unlike rat inner hair cells, mEPSCs originating from hair cells of the amphibian papilla (Keen and Hudspeth, 2006; Li et al., 2009) and rodent retinal bipolar cells (Singer et al., 2004; Mehta et al., 2013) do not appear to be multiphasic and both the rise time and decay time kinetics are indistinguishable for small and large mEPSCs. Because of this, mEPSC charge shows a similar degree of variability as the mEPSC amplitude, unlike the rodent inner hair cells. Although such differences cannot definitively rule out a role for fusion pore modulation in these preparations, it does suggest that other mechanisms (perhaps multivesicular release) may be engaged at these other synapses. Future work will be necessary to determine whether a common model can be invoked or whether separate mechanisms have evolved in different systems.

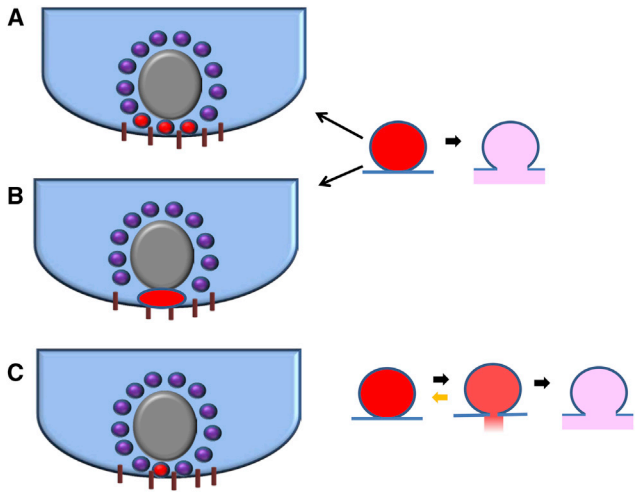

Figure 1. Models of mEPSC Heterogeneity from Ribbon Synapses
(A) Vesicles are induced to fuse simultaneously either by release site coordination (Singer et al., 2004; Glowatzki and Fuchs, 2002) or via a shared calcium nanodomain (Jarsky et al., 2010; Graydon et al., 2011).
(B) Vesicles fuse with one another prior to fusion with the plasma membrane (compound fusion) (Matthews and Sterling, 2008).
(C) Flickering fusion pores control the release of neurotransmitter from a single vesicle (Chapochnikov et al., 2014). Gray circles represent the ribbon. Small circles represent vesicles associated with ribbon, and red represents vesicles releasing neurotransmitter.

Relevance of mEPSC Size to Audition

While much debate has been centered on the mechanisms behind the variability in mEPSC size in ribbon synapses, a particularly central question about the phenomenon has remained largely unanswered: does the difference in mEPSC size matter? A paper by Li et al. (2014) addresses this fundamental question using the bullfrog amphibian papilla, a specialized auditory organ of the amphibian inner ear, which converts auditory sound pressure waves into afferent nerve fiber spikes. As in other auditory organs, spikes are phase locked to low-frequency sound pressure waves. This phase locking, which originates in the hair cell and is propagated throughout auditory circuits, is essential for many aspects of auditory processing (Trussell, 1999). Remarkably, the phase locking is largely independent of intensity, with low-amplitude sounds and high-amplitude sounds giving rise to neurotransmitter release at the same phase, running counter to the relationship between synaptic delay and membrane potentials. How such phase locking is precisely maintained remains incompletely understood.

Previous work from the von Gersdorff laboratory had shown that mEPSCs arising from hair cells of the amphibian papilla exhibit a tremendous amount of variability in their amplitudes and charge. Moreover, their previous work showed that the size and variability of the mEPSCs increase in a calcium-dependent manner (Li et al., 2009). In the present work, Li et al. (2014) find that most spontaneous mEPSPs fail to evoke action potentials in afferent fibers, with only the largest mEPSPs being sufficient to generate spiking. Hence, large mEPSPs, regardless of the underlying mechanism, are uniquely poised to drive spiking in afferent fibers. Li et al. (2014) go on to show that large and small mEPSCs are centered around the same preferred phase, but surprisingly the variability in the phase is much less for the large mEPSCs than for the small mEPSCs. Therefore, the auditory fiber improves its temporal precision by selecting only the large mEPSCs for generating spikes. Hence, the modulation of mEPSC size has critical importance to the precision of auditory processing. Why are large mESPCs better phase locked than small EPSCs? Li et al. (2014) propose that ribbons fully loaded with vesicles act as a diffusion barrier to trap calcium to sufficiently high calcium to drive release of multiple vesicles rapidly. By contrast, depleted ribbons would lose this diffusion barrier and calcium would rise more slowly, thus favoring longer synaptic delays of single vesicles. Further experimentation will be necessary to fully test this idea.

Together, Chapochnikov et al. (2014) and Li et al. (2014) have brought important new insight into the release properties of ribbon synapses, furthering our understanding of both the mechanism and importance of mEPSC size. However, the new work brings about intriguing new questions and leaves some important issues unanswered. How generalizable are the results to other ribbon synapses? What could be the role of large

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mEPSCs in retinal ribbon synapses, which presumably do not phase lock and often synapse on to nonspiking cells? What role does the ribbon structure and its molecular components play in regulating mEPSC size? Undoubtedly, the upcoming years will produce some more interesting insight into these problems.

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The Inside Track: Privileged Neural Communication through Axon-Carrying Dendrites

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Whether morphology tailors functional properties of pyramidal neurons is not completely understood. In this issue of *Neuron*, Thome et al. (2014) show that, in hippocampal pyramidal neurons, axons frequently originate from basal dendrites rather than the soma, constituting a "privileged" channel for synaptic inputs located in these axon-carrying dendrites.

Information processing occurs in the CNS on a variety of scales ranging from molecules to networks. At the cellular level, individual neurons integrate synaptic inputs in order to generate an action potential output. In pyramidal neurons—the most abundant principal neuron type in the mammalian cerebral cortex (Spruston, 2008)—the canonical flow of electrical signals follows this sequence: (1) integration of excitatory glutamatergic synaptic inputs in the dendrites, (2) active and passive propagation of the resulting depolarization first to the soma and then to the axon initial segment (AIS), and (3) action potential initiation at the AIS. Each of these steps of excitatory input processing is regulated by GABAergic inhibitory synaptic inputs, which are located in all three neuronal compartments (Klausberger and Somogyi, 2008).

Conserved across cortical regions and throughout the mammalian clade, the characteristic morphology of pyramidal neurons has been thought to support this dynamic polarization of the neuronal input-output transformation (Spruston, 2008). This canonical structure consists of a pyramidal-shaped soma, with a single branched apical dendrite emanating from its apex and multiple basal dendrites emanating from its base. The axon has also been thought to originate directly from the soma. While the exact functional significance of the separation of the dendritic input site into apical and basal domains remains unclear, the independent somatic origin of dendrites and the axon are understood to provide a structural basis for functional polarization and directional flow of information during neuronal input-output transformation: all synaptic signals from the dendrites must pass through the soma to reach the AIS, a highly specialized neuronal structure in the proximal axon that is enriched with voltage-gated Na^+ channels and functions as the site of output spike initiation (Rasband, 2010).

Despite many common structural traits, pyramidal neurons are not all identical.