On the Brink: A New Synaptic Vesicle Release Model at the Calyx of Held

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How vesicle calcium sensors interact with calcium channels at synapses affects neurotransmitter release dynamics. In this issue of *Neuron*, Nakamura et al. (2015) propose that synaptic vesicles are tightly coupled around the perimeter of a voltage-gated calcium channel cluster.

Synaptic transmission occurs when neurotransmitter-filled, membrane-bound synaptic vesicles fuse with the presynaptic membrane, releasing their contents into the synaptic cleft and activating specific receptors on the postsynaptic membrane. The fusion process is triggered by calcium influx in the presynaptic terminal induced by action potential-mediated depolarization of voltage-gated calcium channels (VGCCs). The speed and precision of this process relies heavily on the vesicle's fusion machinery, proteins which overcome a large energy barrier to catalyze the fusion of two membranes, sensing an influx of calcium. Therefore, how synaptic vesicles are coupled to voltage-gated calcium channels (VGCCs) at sites of neurotransmitter release is a topic of great interest in the field of synaptic transmission.

In attempts to understand the mechanisms of fast, calcium-evoked release at synapses, several models of calcium sensor-VGCC coupling have been proposed. One type of synapse at which this relationship has been extensively studied is the calyx of Held in the auditory brainstem (Eggermann et al., 2012), a large synapse with multiple release sites capable of high-frequency neurotransmission. At the calyx, models of the calcium sensor-calcium source relationship are constrained based on experimentally determined parameters, such as synaptic delay, vesicle release probability (Pv), and sensitivity of release to exogenous calcium chelators. However, one missing link in the synaptic vesicle-VGCC coupling debate has been a description of VGCC topography at the release sites in presynaptic active zones (AZs). In this issue of *Neuron*, Nakamura et al. (2015) propose a new model for calcium sensor VGCC coupling using quantitative ultrastructural imaging to constrain VGCC topography. In combination with experimentally defined parameters, the authors use this model to simulate neurotransmitter release characteristics observed in electrophysiological recordings.

Until recently, the topography of VGCCs at central synapses has eluded electron microscopists. As calcium channel antibodies improved, immunoelectron microscopy (immuno-EM) studies revealed that VGCCs are clustered at hippocampal (Holderith et al., 2012) and cerebellar (Indriati et al., 2013) synapses. Using immunolabeling of detergent-digested freeze-fracture replicas (SDS-FRL), Nakamura et al. (2015) find that at the calyx of Held VGCCs also cluster, presumably in the AZs. With age, these VGCC clusters increase in area, but not channel density, similar to what was observed at synapses onto Purkinje neurons using the same methods (Indriati et al., 2013). Comparing the topography of VGCCs between the ages of postnatal day 7 (P7) and P14 in the calyx of Held provides a framework in which the ultrastructural data can be applied to functional, age-related changes in synaptic transmission, which have been previously characterized (Taschenberger and von Gersdorff, 2000).

With the VGCC topography in hand, Nakamura and colleagues went on to determine the functional distance by which the calcium sensor of synaptic vesicles and the calcium channels are coupled (Nakamura et al., 2015). A technique that is used to determine coupling is assessing the degree of release inhibition by exogenous calcium buffers, such as EGTA (for review, see Eggermann et al., 2012). The calyx of Held is particularly amenable to this technique, as the large size of the calyx allows for access by a presynaptic patch pipette, while the postsynaptic response can be simultaneously monitored by patch clamp. Therefore, Nakamura et al. (2015) determined the coupling distance between the calcium sensor and VGCCs by assessing the inhibition of release by 10 mM EGTA. Though similar experiments to these have been performed (Borst and Sakmann, 1996; Meinrenken et al., 2002), the innovation provided in the current manuscript is a dialysis of the presynaptic calyx through the patch pipette, allowing for precise control of EGTA concentrations during baseline and release-inhibited conditions.

Traditionally, an effect on release of the slow calcium buffer EGTA has been associated with longer coupling distances, in the range of >100 nm (Eggermann et al., 2012). However, in simulations of release using ultrastructural information about VGCC cluster size and channel number, Nakamura and colleagues found that the inhibition by EGTA observed could only be reproduced by coupling the calcium sensor in close proximity to the edge of the VGCC cluster (Nakamura et al., 2015). This finding led the authors to propose a new release model, the release perimeter model (Figure 1). In this model, even though release is inhibited by EGTA, they propose that the calcium sensor is closely coupled to the edge of the VGCC cluster at a perimeter coupling distance of ~30 nm at P7 to ~20 nm at P14, significantly closer than calcium sensor-calcium source distances previously estimated (Borst and Sakmann,

1996; Meinrenken et al., 2002). Thus, perhaps the EGTA sensitivity could reflect inhibition of a calcium sensor physically coupled tightly to the VGCC cluster but functionally distant from the actual site of calcium influx. Interestingly, the estimated coupling distance is similar to that previously reported at older calyces (>P18; Wang et al., 2009), which was proposed based on a lack of inhibition of release by EGTA at more mature release sites, a measurement more commonly associated with a tight coupling relationship (Eggermann et al., 2012).

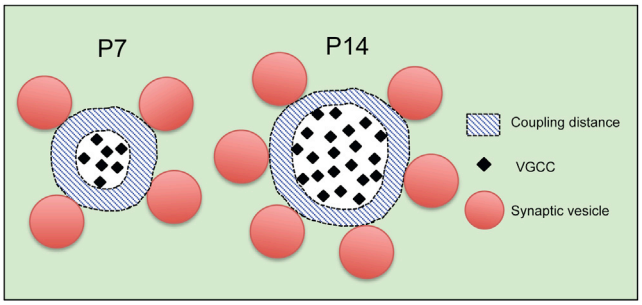

Figure 1. The Perimeter Release Model at Two Developmental Stages of the Calyx of Held

This illustration depicts the relationship between voltage-gated calcium channels (VGCCs; black diamonds) and synaptic vesicles according to the perimeter release model proposed by Nakamura et al. (2015) at the calyx of Held synapse from a postnatal day 7 (P7) and P14 animal. Ultrastructural analysis revealed an increase in the diameter of VGCC clusters with age, and the combination of this information with functional data predicted a decrease in the coupling distance between the synaptic vesicle calcium sensor and the VGCC cluster. The number of vesicles coupled to the cluster is speculative. Images are drawn approximately to scale with synaptic vesicles (50 nm diameter).

How does the perimeter release model compare with previously proposed coupling mechanisms for the calcium sensor and VGCCs at the calyx? Another model that also includes a clustered VGCC topography, as was observed by Nakamura et al. (2015), is the clustered VGCC-random vesicle placement model (random placement model; Meinrenken et al., 2002). In this model, VGCCs are placed in clusters, while vesicles are placed randomly throughout the AZ, which results in a variable coupling distance between the calcium sensor and calcium source from ~30 nm to ~300 nm, with an average distance of ~100 nm (Meinrenken et al., 2002). As the distance of the calcium sensor to the calcium source affects the release probability of a vesicle, one experimentally observed phenomenon that is well explained by the random placement model is the heterogeneity of vesicular release probability observed at the calyx (Meinrenken et al., 2002). The perimeter model can also account for some heterogeneity of release probability, albeit by different means. In the perimeter model, Pv is greatly affected by the number of VGCCs found in the cluster. At P14, the number of VGCC subtype CaV2.1 per cluster ranged from 3 to 73 as estimated by SDS-FRL (Nakamura et al., 2015). Thus, perhaps variability in VGCC number within a cluster accounts for heterogeneity of vesicles coupled along the perimeter. On the other hand, a second source of Pv heterogeneity could come from where along the perimeter the vesicle is docked relative to where in the cluster the calcium influx occurs. These two possibilities could provide for two different results in Pv heterogeneity: one in which heterogeneity occurs between clusters and one in which heterogeneity occurs within clusters. Therefore, experimentally determining the source of Pv heterogeneity will further elucidate the model of VGCC-calcium sensor coupling.

The proposal of the perimeter coupling model leads to the question: what is the mechanism determining the vesicle-VGCC distance? Perhaps one intriguing possibility is the calcium sensor protein itself, synaptotagmin. Previous work suggests that beyond the calcium-sensing function, synaptotagmin uses a different region to couple the vesicle to the calcium channel (Young and Neher, 2009). Another obvious candidate to serve as the link between synaptic vesicles and calcium channels is Rim. This AZ protein has been shown to interact with synaptic vesicles through the Rab3/27 proteins and VGCCs either directly or through Rim-binding protein (Südhof, 2013). However, evidence from genetic elimination of two Rim isoforms, Rim1 and 2, from the calyx revealed a role for this protein in VGCC clustering and release probability, but no role in vesicle-VGCC coupling (Han et al., 2014). Nevertheless, an AZ protein may be responsible for the coupling. At the *Drosophila* neuromuscular junction, the AZ protein, Bruchpilot, affects both VGCC clustering and tethering of vesicles around the cluster (Matkovic et al., 2013). One factor that the coupling mechanism should not change is the intrinsic release probability of the vesicle itself, as both fast and slowly releasing vesicles evoked by action potential are released equally well with calcium uncaging, indicating no relationship between the molecular composition of the release machinery and vesicle position (Wadel et al., 2007).

Is the perimeter release model specific to release sites at the calyx, or is this a general mechanism used for assuring fast and precise calcium-induced release at many synapses? Because other synapse types also have VGCC clustering (Holderith et al., 2012; Indriati et al., 2013), it is possible that calcium sensor may couple to the cluster in a perimeter fashion. However, unlike at the calyx release sites, the cerebellar parallel fiber synapses, which exhibit VGCC clustering (Indriati et al., 2013) and a short coupling distances, show no inhibition of release by EGTA (Schmidt et al., 2013). Perhaps the structure of the bouton-type parallel fiber synapse assures a tighter coupling of the calcium sensor to calcium influx even at the center of the VGCC cluster. At the mossy fiber bouton, experimental evidence, including a strong inhibition of release by EGTA, suggests that this synapse displays loose calcium sensor-VGCC coupling (Vyleta and Jonas, 2014). Nakamura et al. (2015) suggest that the perimeter release model predicts a similar coupling distance at this synapse as reported. Though this suggests that different synapses may employ similar release-coupling mechanisms, the necessity of constraining the parameters of the model as much as possible for a given synapse, including ultrastructural topography, remains.

One major piece of evidence for the model that is still missing is the

topography of the synaptic vesicle to VGCC cluster relationship. As of yet, the coupling distance between vesicles and the calcium source must be functionally determined. A major breakthrough in ultrastructural analysis could combine the resolution of vesicle placement through tomography of synapses fixed by high-pressure freezing (Imig et al., 2014) with immunogold labeling techniques. This could address the open question of whether VGCC perimeter size itself determines the number of vesicles that can be coupled within close proximity; i.e., does a larger VGCC cluster lead to more readily releasable vesicles (Figure 1)? At hippocampal synapses, Holderith et al. (2012) found that both the number of docked vesicles and VGCC cluster size correlated with AZ size. Additionally, in the calyx, the readily releasable pool of vesicles was determined to increase approximately 2.5- to 3-fold with age (P7–P14; Taschenberger and von Gersdorff, 2000). These could be hints that the available perimeter affects the number of release-ready vesicles. Nevertheless, structural information of the synaptic vesicle-VGCC relationship will provide insight into neurotransmitter release mechanisms. Hopefully, with the rapid development of high-resolution imaging techniques, determining the physical distance between docked synaptic vesicles and calcium channels within a synapse is on the horizon.

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CA2: It's About Time—and Episodes

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In this issue of Neuron, Mankin et al. (2015) show that CA2, an oft-neglected hippocampal subregion, has place representations that change from one episode to the next, even as the spatial environment does not. This finding may help explain how time is encoded in episodic memories.

We form memories of what happens to us by organizing all components of each episode in space and time. Much of this process takes place in the hippocampus, and it has been long known that lesions of this structure impair episodic memory in humans and other animals. The hippocampal code for space is expressed by place cells, neurons that activate as the subject traverses a specific spatial location. Place cells provide the brain with useful information for self-localization and navigation, but can also be seen as scaffolding for episodic memories: items found at one place, or occurrences taking place there, may be represented in the hippocampus by modulations in the activity of place cells tied to that location, in a phenomenon known as rate remapping (Leutgeb et al., 2005).

Thus, the hippocampus has the daunting task of combining sensory information of all modalities with a spatial metric, probably supported by self-motion signals. It accomplishes this feat with a very complex wiring pattern, involving the interplay of multiple substructures. In the traditional view, metric information and sensory inputs flow into the hippocampus, respectively, from the medial (where the eminently spatial responses of grid cells are measured) and the lateral entorhinal cortex. Within the hippocampus,