Nanoscale Location Matters: Emerging Principles of Ca\textsuperscript{2+} Channel Organization at the Presynaptic Active Zone

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How does diversity in the organization of secretory machines determine properties of neurotransmitter release? In this issue of Neuron, Rebola et al. (2019) found that distinct nanoscale assemblies of Ca\textsuperscript{2+} channels and Munc13, not overall channel abundance, mediate differing release characteristics of two cerebellar synapses.

Atoms vibrate, clocks tick, and neurons fire. The brain is reminiscent of a modern computer, where many transistors work together to handle complex tasks using binary signals. Within individual neurons, most signals are indeed propagated digitally as action potentials. Signal transmission at synapses between neurons, however, is highly diverse and dynamic, dramatically increasing the complexity of brain signaling and its computational power.

Synaptic transmission is initiated by the opening of voltage-gated Ca\textsuperscript{2+} channels. The channels are strategically positioned in the presynaptic active zone where synaptic vesicles are released. Vesicle fusion is triggered by Ca\textsuperscript{2+}, and the probability of its occurrence is highly heterogenous between synapses and modulated during plasticity. Among the factors that control the vesicle fusion probability, the magnitude and spatial organization of Ca\textsuperscript{2+} signals take a central role (Eggermann et al., 2011). But many important factors, for example the numbers of Ca\textsuperscript{2+} channels within an active zone, their exact distribution, their spatial relationship to vesicle fusion sites and other secretory proteins, and how these parameters differ across synapses, remain uncertain.

In this issue of Neuron, Rebola et al. (2019) examined the nanoscale distribution and role of Ca\textsuperscript{2+} channels by comparing two distinct synapses in cerebellar slices: a “weaker” excitatory synapse and a “stronger” inhibitory synapse (Figure 1). The weaker synapse has a lower release probability and is formed by granule cells onto Purkinje cells. The stronger synapse is an inhibitory synapse with higher release probability, formed between stellate cells. Intriguingly, presynaptic Ca\textsuperscript{2+} entry, assessed as residual Ca\textsuperscript{2+} transients, did not positively correlate with release probability. The weaker synapses had ~3-fold higher Ca\textsuperscript{2+} entry than the stronger synapses.

How could it be that the lower amount of Ca\textsuperscript{2+} at the stronger synapses is more efficient in triggering release? The authors pursued a technical tour-de-force to answer this question. First, it is shown that the number of Ca\textsuperscript{2+} channels, but not their open probability, accounts for increased Ca\textsuperscript{2+} entry at the weaker synapses. Using functional and morphological experiments, the authors estimated that about ~100–200 channels are present within an active zone at the weaker synapses, but only tens of channels are so at the stronger synapses. One possible explanation for the differences in release probability is that the synapses with fewer Ca\textsuperscript{2+} channels are stronger because their channels are located closer to vesicular Ca\textsuperscript{2+} sensors, which would more efficiently activate these sensors. Indeed, the stronger synapses were resistant to EGTA, a slow Ca\textsuperscript{2+} chelator that only captures Ca\textsuperscript{2+} ions when they diffuse over long distances (Eggermann et al., 2011). Hence, at the stronger synapses the distance between the mouth of the Ca\textsuperscript{2+} channel and the Ca\textsuperscript{2+} sensor is too short for EGTA to capture the Ca\textsuperscript{2+} ions. In contrast, the weaker synapses with the large Ca\textsuperscript{2+} signals were highly sensitive to EGTA. Consequently, the lower amount of Ca\textsuperscript{2+} may be more efficient at triggering release because the Ca\textsuperscript{2+} channels are better positioned at the stronger synapses (Figure 1A).

The authors directly assessed Ca\textsuperscript{2+} channel localization at both synapses and complemented these studies with simulations of distributions of release sites and Ca\textsuperscript{2+} channels to test various models of the topographical organization. For the morphological experiments, the synapses were freeze-fractured, and then channel localization was directly assessed with nanometer precision by immunogold-labeling. Co-labeling by the same technique was used to determine the relative localization of Munc13, a protein that marks release sites at hippocampal synapses (Sakamoto et al., 2018). At the stronger synapses, Ca\textsuperscript{2+} channels were present in small clusters next to Munc13 assemblies, but the proteins were not intermixed. The weaker synapses were markedly different. While Munc13 was still present in small clusters, the Ca\textsuperscript{2+} channels were spread out over the presynaptic membrane but excluded from the areas where Munc13 was clustered.

The authors propose that two nanotopographical motifs account for the differing properties of these synapses. The stronger stellate cell inhibitory synapse uses a perimeter release model in which several Ca\textsuperscript{2+} channels are clustered within ~10 nm of each exocytotic site. Ca\textsuperscript{2+} channel opening leads to rapid and effective release triggering because the channels are efficiently included in the nano-assemblies that drive fusion. In contrast, the weaker...
granule cell excitatory synapse follows an exclusion zone model with an area of ~50 nm around release sites in which there are no Ca\textsuperscript{2+} channels, making release triggering less efficient. Outside of this zone, the channels are widespread.

The systematic side-by-side comparison is powerful as it provides the most direct evidence to date that differences in the molecular nanotopographies within an active zone exist, and it indicates that these motifs contribute to functional heterogeneity. The work provides compelling quantitative data to describe two specific cases of specialized synapses and other synapses have invented other motifs.

Do other proteins contribute to the heterogeneous release properties in granule and stellate cell boutons? The correlation between nanotopographies of Ca\textsuperscript{2+} channels and Munc13 with release at these synapses is striking, and the question arises whether additional mechanisms are at play. Two proteins that mediate heterogeneity in the hippocampus are RIM-BP and ELKS, integral members of the active zone. While mossy fiber boutons strongly depend on RIM-BP2, RIM-BPs are largely dispensable at other excitatory hippocampal synapses (Brockmann et al., 2019). Analogously, ELKS controls Ca\textsuperscript{2+} entry at inhibitory hippocampal synapses but mediates release-readiness independent of Ca\textsuperscript{2+} entry at excitatory synapses (Held et al., 2016). In these cases, causality for their diverse roles is indicated through loss-of-function approaches, but the nanotopographies of RIM-BP and ELKS are not well understood, and differences in protein function or organization could account for functional heterogeneity (Figure 1B).

Importantly, heterogeneity in the proteins of the core fusion machinery itself, for example in the Ca\textsuperscript{2+} sensors, could influence release properties as well.

Interestingly, functional diversity may even be controlled by Munc13 itself. While Munc13-1 marks release sites at hippocampal synapses (Sakamoto et al., 2018), experiments at the fly neuromuscular junction indicate that heterogeneity in Munc13 isoforms can generate release sites of distinct properties within a synapse (Böhme et al., 2016). Indeed, the weaker synapses in the present study (Rebola et al., 2019) may contain another Munc13 isoform, and its expression could contribute to the control of release. Taken together, several proteins likely contribute to functional heterogeneity and tune the influence of Ca\textsuperscript{2+} channel topographies on synaptic strength. Future studies should build on the presented work to further dissect the important question of presynaptic heterogeneity.

Finally, how do synapses accomplish the distinct Ca\textsuperscript{2+} channel organization? Currently, we do not know the molecular mechanisms that control the different Ca\textsuperscript{2+} channel nanotopographies, and a conundrum arises with respect to key candidates. Similar to Munc13 (Sakamoto et al., 2018), the active zone protein RIM marks release sites at hippocampal excitatory synapses and is aligned with postsynaptic receptor clusters (Tang et al., 2016). Importantly, RIM binds to both Ca\textsuperscript{2+} channels and Munc13, and these interactions control active zone levels of the respective proteins (Kaeser et al., 2011). This appears at odds with the separation of Munc13 clusters and Ca\textsuperscript{2+} channels, because their biochemistry, loss-of-function phenotypes, and positioning relative to postsynaptic receptor domains suggest that RIM, Munc13, and Ca\textsuperscript{2+} channels sit in the same protein complex. One potential solution could be that proteins such as RIM are present in different protein complexes that fulfill separate functions.
Consistent with this model, different fragments of RIM independently rescue its exocytotic functions via Munc13 or its scaffolding role for Ca\textsuperscript{2+} channels (Kaeser et al., 2011). This dichotomy establishes that RIM has separable functions, and these functions could be localized to distinct protein nano-assemblies within an active zone, at least in some cases. Recent technological advances for the high-resolution assessment of the composition and structure of protein complexes in situ will likely allow dissecting how individual protein assemblies within an active zone vary in composition, and how these exocytotic machines are aligned with postsynaptic receptor domains.

Ultimately, the ability of synapses to employ different topographical motifs of key molecular players offers neurons new ways to tune their release properties. It is likely that these assemblies do not only differ across synapse types, but also over time and as a function of past activity of a synapse. Future research should build on the striking findings by Rebola et al. (2019) to assess how the computational power of the brain is controlled and modulated by the molecular nano-assemblies that mediate synaptically triggered transmission.

REFERENCES


Decreasing Influence of Retinal Inputs on the Developing Visual Cortex

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Before vision matures, spontaneous retinal activity drives downstream visual targets. In this issue of Neuron, Gribizis et al. (2019) image activity simultaneously in connected mouse visual areas and demonstrate distinct developmental patterns of signal transformation in thalamocortical versus retinocollicular pathways.

During development, immature sensory circuits generate spontaneous neural activity, which propagates across nascent synaptic connections and plays important roles in setting up precise wiring patterns of adult circuits. A well-documented example is the spontaneous activity in the mammalian retina, termed “retinal waves.” Retinal waves are generated by distinct mechanisms in three developmental stages prior to the onset of vision (reviewed by Arroyo and Feller, 2016). In rodents, the initial retinal waves before birth (stage 1) are mediated by gap junctions. After birth, retinal waves subsequently rely on cholinergic signaling (stage 2) until around postnatal day 10 (P10). Between P10 and the onset of light response at around P12, cholinergic influence diminishes, and retinal waves are mediated by glutamate released from bipolar cells (stage 3). Stage 2 waves propagate to higher visual targets including the thalamocortical pathway and the superior colliculus (SC) (Ackman et al., 2012).