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Maximinis

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Llano and colleagues show that calcium released from presynaptic stores can drive simultaneous release of multiple vesicles at fast inhibitory synapses in the cerebellum.

Spontaneous synaptic release occurs at all chemical synapses, and is widely believed to be due to the fusion of individual synaptic vesicles. The currents are typically small, up to a few tens of picoamperes—thus, the usual nickname, miniature postsynaptic currents or mPSCs. Such small currents comfortably match the small size of vesicles. But not all mPSCs live up to their name. In the cerebellum, 20% of spontaneous inhibitory PSCs recorded in Purkinje cells are greater than 200 pA, with some as big as 1 nA, even in the presence of tetrodotoxin, cadmium and glutamate channel blockers. What could lead to such behavior? In this issue, Llano and colleagues[1] examine the basis for these large-amplitude mIPSCs (lamIPSCs, which they have also colloquially termed 'maximinis'), using electrophysiology, immunocytochemistry and two-photon calcium imaging. Taken together, their results demonstrate that calcium from internal stores in basket cell terminals can trigger the simultaneous release of multiple vesicles. This finding suggests that calcium stores could add to voltage-gated calcium channels in driving fast neurotransmitter release.

Calcium stores are involved in vesicular release in many systems, including neuroendocrine cells[2,3] and neurons[4]. Calcium is stored intracellularly in mitochondria and endoplasmic reticulum. The ligands ryanodine and IP_3 can trigger release of calcium from endoplasmic reticulum. Experimental manipulations of neuronal stores affect action-potential-evoked release, or the frequency of mPSCs, but manipulations of stores have not seemed to directly affect mPSC amplitudes[5,6].

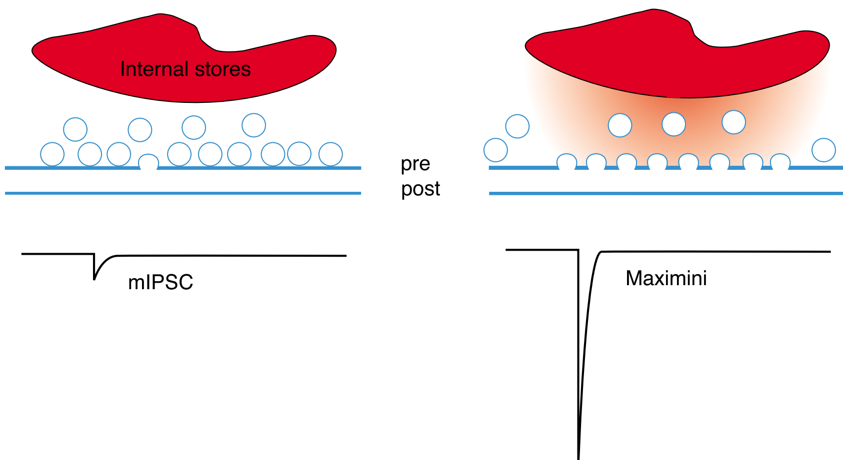
The system explored here by Llano and colleagues was exceptional, however, in that many spontaneous currents were of such large amplitude. Whereas most central synapses exhibit significant variance in mPSC sizes, none of the mechanisms proposed so far could easily explain spontaneous IPSCs as large as 1 nA. One plausible alternative is that these events are multivesicular, but the contribution of individual vesicles cannot be distinguished. The average single mIPSC seems to be 60 pA, which suggests that some mechanism synchronizes the release of as many as 15 vesicles. However, the mechanism could not involve sodium or calcium spikes, because lamIPSCs persist in tetrodotoxin, cadmium, and calcium-channel toxins. So what could supply the high concentrations of calcium usually required to elicit synchronous release?

The first clue that internal calcium stores could trigger lamIPSCs was the finding that manipulations affecting stores eliminated lamIPSCs. Starving the cells of external calcium led to a gradual rundown of average mIPSC amplitude and selective elimination of the large-amplitude events. In addition, they used ryanodine, which triggers release from ryanodine-sensitive stores at low concentrations, and blocks release at high concentrations. Using high concentrations of ryanodine, they found that the frequency of mIPSCs decreased, and half the events larger than 200 pA were eliminated. They then used immunocytochemistry to show that cerebellar basket cell presynaptic terminals, which supply these mIPSCs, do indeed contain ryanodine receptors. To do so, the authors developed a new antibody that recognized a highly conserved sequence in all known ryanodine receptor isoforms, which revealed a previously unrecognized population of ryanodine receptors in basket cell axons.

For the remaining piece of the puzzle, Llano and colleagues used two-photon microscopy of fluorescent calcium indicators to show that spontaneous calcium transients occur in basket cell terminals. These transients are reminiscent of calcium sparks in muscle, which are due to calcium-induced calcium release from internal stores[7]. In the basket cell axon, the transients spread up to 5 μm along the length of the axon, and may invade adjacent boutons. Just like the lamIPSCs, these calcium transients persisted in the presence of tetrodotoxin and ionotropic receptor blockers, and simultaneous recordings in the basket cell soma did not show any spontaneous calcium currents. In addition, when Llano and colleagues applied ryanodine at low concentrations, they saw a rise in frequency of spontaneous calcium transients, consistent with ryanodine triggering release from stores at low concentrations.

This study broadens our understanding of the potential involvement of calcium

Fig. 1. Proposed mechanism underlying large-amplitude mIPSCs at the basket cell to Purkinje cell synapse. Left, the familiar mechanism producing normal mIPSCs, in which a single vesicle randomly fuses with the presynaptic membrane, causing a small current in the postsynaptic cell. Right, the mechanism proposed by Llano and colleagues[1] to account for spontaneous large-amplitude mIPSCs. Calcium stores randomly release a high concentration of calcium, triggering the synchronous release of multiple vesicles.



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stores in the nervous system, where stores have usually been thought of as slower and modulatory. For example, glutamate release from parallel fibers activates metabotropic glutamate receptors on Purkinje cell dendritic spines, which in turn trigger a large postsynaptic release from IP3-sensitive calcium stores[8,9]. The time course of this release is on the order of hundreds of milliseconds. At some presynaptic terminals, calcium stores are involved in controlling resting and residual calcium due to posttetanic potentiation, which takes place over tens of seconds[10]. By contrast, the present study by Llano and colleagues suggests that calcium stores can synchronize release to within a few milliseconds (**Fig. 1**).

This ability to synchronize release on short time scales raises the possibility that stores contribute to fast neurotransmitter release, also called phasic release. Previously, the only source of the high concentrations of calcium that drive phasic release was believed to be voltage-gated calcium channels, which are tightly coupled to the synaptic release machinery[11]. Recently, calcium-permeable ionotropic receptors have been implicated as additional potential sources of calcium. For example, calcium influx through NMDA receptors alone can trigger GABA release at dendrodendritic synapses in the olfactory bulb[12,13]. The study by Llano and colleagues indicates that calcium stores can also supply sufficient calcium to drive fast multivesicular release. It is likely, therefore, that the stores lie very close to release sites (within a few hundred nanometers), and it will be interesting to examine this issue through studies of ultrastructure and of exogenous calcium chelators.

There are several interesting questions raised by this work. Mechanistically, how are stores being triggered to release calcium? How many release sites along the basket cell axon are recruited by calcium release from stores? Physiologically, what is the role of calcium stores in transmitter release during normal activity? Are the spontaneous release events themselves important, or do they reflect a process whose primary function is to amplify phasic release? If the latter, can the amplification, and thus synaptic strength, be modulated by activity or chemical messengers? Finally, do other synapses have calcium stores that can drive fast neurotransmitter release? Such large-amplitude mPSCs have not been commonly described in the nervous system, but because the antibody that Llano and colleagues introduced recognizes a previously unknown population of calcium stores, it remains to be seen whether these stores show up in other neuronal types.

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Dissecting the ins and outs of excitement: glutamate receptors on the move

Volker Haucke

AMPA, NMDA or insulin can cause endocytosis of AMPA receptors. Two papers now show that these stimuli act via distinct signaling pathways, some of which also induce LTD.

Ionotropic glutamate receptors (AMPARs) can move into and out of the postsynaptic membrane dynamically. Presumably such changes in the number of AMPARs at the membrane are important in activity-dependent synaptic plasticity, and thus the signaling pathways governing glutamate receptor trafficking have moved to center stage in molecular neurobiology. In this issue, Beattie *et al.*[1] and Lin *et al.*[2] begin to shed light on the mechanisms by which different effectors can induce AMPAR internalization. They find striking similarities between the signaling mechanisms governing AMPAR endocytosis and the induction of long-term depression (LTD). Although some of the results seem to be at odds with each other, these new studies provide a glimpse into an increasingly complex web of signaling networks at glutamatergic synapses.

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Because activity-dependent changes in synaptic efficacy correlate with changes in the surface levels of AMPARs, postsynaptic glutamate receptor trafficking between the plasma membrane and internal compartments is proposed to regulate the strength of excitatory synapses. Endocytotic internalization of most plasma membrane receptors involves clathrin and dynamin, which mediate selective transport from the plasma membrane to internal endosomal compartments. Over the past two years, it has become clear that clathrin-mediated endocytosis is also required for hippocampal long-term depression[3–6], with AMPARs being internalized rapidly into clathrin-coated vesicles[5]. Conversely, induction of long-term potentiation is accompanied by insertion of AMPARs from intracellular sites into the postsynaptic density[7,8], presumably by exocytotic membrane fusion. Thus, a picture emerges in which activity influences cycling of ionotropic glutamate receptors[9] in and out of the membrane.

So which signaling cascades control these processes, and thus synaptic responsiveness? Some answers to this intriguing question have now emerged from two elegant papers by Malenka[1], Sheng[2] and their colleagues. By using state-of-the-art immunofluorescence imaging techniques in live cultured hippocampal neurons labeled with antibodies, along with drugs that selectively stimulate distinct pathways of glutamate signaling, the authors provide a nascent picture of how activity may regulate synaptic strength at excitatory

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