File: NeuronPreviewFinal.doc
Version: February 23, 2015, 13:00
Deadline: March 2, 2015

Excitement about inhibitory presynaptic terminals

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Total text: 12808 characters (12000 – 14000)
Abstract 60 words (40)
References 15 (15)

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Based on extrapolation from excitatory synapses, it is often assumed that depletion of the releasable pool of synaptic vesicles is the main factor underlying depression at inhibitory synapses. Using subcellular patch-clamp recording from inhibitory presynaptic terminals, Kawaguchi and Sakaba show that at Purkinje cell–deep cerebellar nuclei neuron synapses, changes in presynaptic action potential waveform substantially contribute to synaptic depression.

Short-term synaptic depression is of critical importance for the processing of information in neuronal networks. Synaptic depression may, for example, underlie low-pass filtering, habituation, removal of redundant correlations, and dynamic input compression (Abbott and Regehr, 2004). Thus, it is important to completely understand the phenomenon of synaptic depression, including the underlying molecular and cellular mechanisms. In principle, several distinct mechanisms could contribute to depression, including failures in axonal action potential propagation, changes in presynaptic action potential waveform (Geiger and Jonas, 2000), inactivation of presynaptic Ca^{2+} channels (Xu and Wu, 2005), depletion of the releasable pool of synaptic vesicles (Dobrunz and Stevens, 1997; Schneggenburger et al., 1999), inhibition of release by presynaptic metabotropic receptors, and desensitization of postsynaptic ionotropic receptors (Fig. 1A). It is generally thought that depletion of the vesicle pool is the major factor underlying synaptic depression. However, this view is mainly based on the extensive analysis of various excitatory synapses (Dobrunz and Stevens, 1997; Schneggenburger et al., 1999). Whether it can be extrapolated to inhibitory synapses remains unclear.

Early experimental analysis of inhibitory synaptic transmission in hippocampus and cerebellum eliminated, one by one, several candidate mechanisms of synaptic depression. For example, depression at hippocampal basket cell–granule cell synapses (the main output synapse of fast-spiking, parvalbumin-expressing interneurons; Hu et al., 2014) and Purkinje cell–deep cerebellar nucleus (PC–DCN) synapses (the main output synapse of cerebellar Purkinje cells; Telgkamp et al., 2004) is not primarily generated by pool depletion, since changes in release probability via variation in extracellular Ca^{2+} concentration only have subtle effects on the extent of depression (Kraushaar and Jonas, 2000; Telgkamp et al., 2004). Failure of action potential propagation does not seem to contribute, since the axons of inhibitory neurons propagate action potentials highly reliably (Khaliq and Raman, 2005; Hu et al., 2014). Inactivation of presynaptic Ca^{2+} channels also cannot be the main mechanism, because inhibitory synapses typically rely on P/Q-type channels for the initiation of transmitter release (Hu et al., 2014), and these channels show only minimal inactivation or even facilitation during repetitive activation. Finally, desensitization of postsynaptic receptors cannot be involved, since depression primarily appears as a presynaptic change (Kraushaar and Jonas, 2000). These results illustrate the sometimes frustrating nature of a rigorous scientific approach. We falsify several

hypotheses underlying a phenomenon, until the remaining possibilities are almost impossible to test. Moving forward in this situation then requires new experimental strategies.

This is exactly the approach taken by Kawaguchi and Sakaba. To address the mechanisms of synaptic depression at GABAergic synapses, they approached the problem in the most direct possible way: by recording from inhibitory terminals. To perform these experiments, they focused on the PC–DCN synapse. This experimental system seems highly suitable, since the presynaptic terminals are large enough to enable subcellular patch-clamp recording (~3 µm; Kawaguchi and Sakaba, Fig. 1). Furthermore, this synapse shows robust depression of inhibitory postsynaptic currents during repetitive stimulation (Telgkamp et al., 2004). Using this system, they were able to perform simultaneous measurements of presynaptic voltage, presynaptic capacitance, and postsynaptic current. This almost sounds too nice to be true, but there is a price to pay: Currently, the experimental strategy used is primarily applicable to inhibitory terminals under culture conditions, although the authors offer a couple of additional experiments in acute slices.

Using this technique, Kawaguchi and Sakaba performed several “dream experiments“ at an inhibitory synapse. First, they measured stimulus-evoked capacitance changes in presynaptic terminals. As the specific capacitance of biological membranes is constant (~1 µF cm^{-2}), these measurements provide a quantitative assay of the number of vesicles fusing with the plasma membrane. The main advantage of capacitance measurements is that they provide a perfectly linear assay of exocytosis, in contrast to postsynaptic currents, which may exhibit nonlinearities because of saturation and desensitization of transmitter receptors. Using this approach, they found that the size of the releasable pool was surprisingly large, while the release probability per vesicle was small. Consequently, there is only minimal contribution of pool depletion to synaptic depression. Imaging experiments with synaptopHluorins further corroborated this conclusion.

Second, they directly measured the voltage changes in the presynaptic terminal preceding transmitter release, which were previously inaccessible to quantitative measurements. They showed that the action potential in the presynaptic terminal undergoes waveform changes during repetitive stimulation. These changes involve a reduction in peak amplitude and an increase in the half duration. Previous work at excitatory terminals showed that the relation between action potential waveform and presynaptic Ca^{2+} inflow can be complex. Reduction in action potential amplitude reduces the total presynaptic Ca^{2+} inflow, whereas action potential broadening enhances it (Bischofberger et al., 2002; Fig. 1B). In inhibitory terminals, the effects of amplitude reduction predominated, resulting in an attenuation of Ca^{2+} inflow and release probability. Hence, an activity-dependent reduction in the amplitude of the presynaptic action potential substantially contributed to synaptic depression. Interestingly, action potential waveform changes occurred specifically in

presynaptic terminals, and not in adjacent axons. Thus, the mechanism is implemented in a presynaptic terminal-specific manner.

Finally, they probed the mechanisms underlying the activity-dependent changes in presynaptic action potential amplitude. They found that the excitability of the inhibitory presynaptic terminals is low. The voltage-gated Na^+ channel density was small, whereas the K^+ channel density was large, resulting in a small Na^+-to-K^+ channel ratio. Pharmacological experiments further suggested that the presynaptic K^+ channels were primarily assembled from subunits of the Kv1 family (Kv1.1 and Kv1.3). As Kv1 channels have a low activation threshold, they may be ideally suited to regulate the amplitude of the presynaptic action potential. Taken together, these results suggest a novel mechanism underlying synaptic depression: An activity-dependent reduction in the amplitude of the presynaptic action potential, caused by a low excitability of the presynaptic terminal. Kawaguchi and Sakaba also performed additional experiments at inhibitory synapses in the hippocampus, suggesting that the new mechanism may be generalizable to at least some cortical inhibitory synapses. More work on inhibitory terminals of different GABAergic interneuron subtypes will be required to explore how far the generalization can go.

The results may trigger a *déjà vu*: The idea that changes in presynaptic action potential waveform may regulate synaptic strength was quite popular in the early scientific literature, especially for invertebrate synapses (Byrne and Kandel, 1996). Furthermore, dynamic changes in the presynaptic action potential waveform were previously documented at excitatory presynaptic terminals (Geiger and Jonas, 2000). However, in these cases activity-dependent broadening of the presynaptic action potential enhanced presynaptic Ca^{2+} inflow, and thereby facilitated transmitter release (Byrne and Kandel, 1996; Geiger and Jonas, 2000). In contrast, in inhibitory PC–DCN synapses, activity-dependent amplitude reduction of presynaptic action potential amplitude reduced presynaptic Ca^{2+} inflow, and therefore depressed transmitter release (Kawaguchi and Sakaba, this issue).

At inhibitory PC–DCN synapses, depression is prevented by a large vesicular pool, but re-introduced at a level upstream of the release machinery. What could be the functional advantage of such a weird design? As Kawaguchi and Sakaba speculate, such an organization may convey several advantages. First, it may facilitate reliable synaptic transmission, preventing pool depletion during high-frequency activity. This will be relevant under physiological conditions, because Purkinje cells fire high-frequency trains of action potentials *in vivo* in a wide range of behavioral conditions (Yang and Lisberger, 2014). Second, it may allow a flexible implementation of target cell-specific differences in synaptic dynamics, using different presynaptic Na^+-to-K^+ channel ratios at PC–DCN synapses versus, for example, synapses on vestibular nucleus neurons or other Purkinje cells (Kawaguchi and Sakaba, this issue). Third, the large releasable pool may provide room for the regulation of inhibitory synaptic strength by

neuromodulators or long-term plasticity, while synaptic depression would be maintained under all of these conditions (Hefft et al., 2002).

Short-term synaptic depression at excitatory synapses appears to serve several complex functions in neuronal networks (Abbott and Regehr, 2004). But what could be the function of depression at inhibitory synapses in the network? In hippocampal microcircuits, neuromodulators, such as oxytocin or cholecystokinin, depolarize fast-spiking, parvalbumin-expressing GABAergic interneurons. Under these conditions, short-term depression will ensure the reciprocity of changes in tonic and phasic inhibition: while tonic inhibition is enhanced, phasic inhibition would be reduced (Owen et al., 2013). Alterations in the temporal structure of inhibition will, in turn, enhance stimulus-evoked and reduce background activity in excitatory principal neurons. Thus, depression in inhibitory synapses may be critically important for setting the signal-to-noise ratio in neuronal networks (Owen et al., 2013).

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Figure legends

Figure 1. Short-term synaptic depression generated by changes in the presynaptic voltage waveform

A, Schematic illustration of the possible mechanisms of short-term depression at central synapses (boxes). Red, voltage-gated Na^+ channels (Na_V); blue, voltage-gated K^+ channels (K_V); magenta, voltage-gated Ca^{2+} channels (Ca_V); green, G-protein coupled receptors (GPCRs); gray, postsynaptic receptors (e.g. GluRs or $GABA_A$Rs). For details, see text. **B**, Efficient control of Ca^{2+} channel activation by changes in amplitude and duration of the presynaptic action potential. Top, amplitude reduction of the presynaptic action potential (AP, black) decreases both peak amplitude and integral of the Ca^{2+} current (I_{Ca}, magenta), reducing total Ca^{2+} inflow. Bottom, broadening of the presynaptic action potential slightly reduces the peak amplitude (due to reduction in driving force), but markedly increases the integral of the Ca^{2+} current, enhancing total Ca^{2+} inflow. Adopted from Bischofberger et al., 2002.