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Retrograde Signaling Causes Excitement

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Retrograde signaling is a powerful tool to shape synaptic transmission, typically inducing inhibition of transmitter release. A new study published in this issue of *Neuron* by Carta et al. (2014) now provides strong support for arachidonic acid as a potentiating retrograde messenger.

The recent two decades have seen a surge in research into mechanisms of retrograde synaptic signaling. In the wake of this development, a number of criteria have been proposed that need to be fulfilled for a molecule to be identified as a retrograde messenger (Regehr et al., 2009). First, both the postsynaptic production machinery and the presynaptic target of the messenger have to be present. Second, interference or blocking of either the postsynaptic production or the presynaptic target should lead to inhibition of the effect. And third, direct activation of the presynaptic target should mimic the effect of the retrograde messenger. All of these criteria have sufficiently and beautifully been met in the paper "Membrane Lipids Tune Synaptic Transmission by Direct Modulation of Presynaptic Potassium Channels" by Carta et al. (2014) in this issue of *Neuron*.

Carta et al. (2014) base their study on the finding that prolonged depolarization of postsynaptic CA3 pyramidal cells (either through direct depolarization steps or several types of more physiological synaptic input stimuli) leads to a transient potentiation of incoming mossy fiber (MF)-mediated excitatory postsynaptic currents (EPSCs) for about 10 min. Using electrophysiological, pharmacological, and uncaging tools, Carta et al. (2014) elegantly show that this depolarization-induced potentiation of excitation (DPE) is postsynaptically induced and dependent on a postsynaptic rise in Ca^{2+}, while it is clearly presynaptically expressed. Hence, there is a definite requirement for a retrograde signal to establish the presynaptic potentiation of MF synaptic currents. At great experimental lengths, Carta et al. (2014) investigate the nature of this retrograde messenger, meticulously excluding (1) conventional neurotransmitters and -modulators, (2) any messenger that is based on a Ca^{2+}-dependent vesicular release from CA3 pyramidal cells, and (3) also the "usual suspects" nitric oxide and endocannabinoids. They then successfully identify the membrane-derived lipid arachidonic acid (AA)—or one of its downstream metabolites—as the agent of the presynaptic potentiation of transmission: interference with the release or lipoxygenase-mediated metabolism of AA effectively inhibits DPE, while local photoactivation of caged AA results in potentiation of mossy fiber EPSCs comparable to DPE. Employing technically most challenging presynaptic patch-clamp recordings of mossy fiber boutons (Bischofberger et al., 2006), Carta et al. (2014) also elucidate the mechanism of DPE: they find that postsynaptically released arachidonic acid directly acts on presynaptic voltage-gated potassium channels by shifting the voltage dependence of the steady-state inactivation of K_v currents towards more negative values. As a result, the presynaptic action

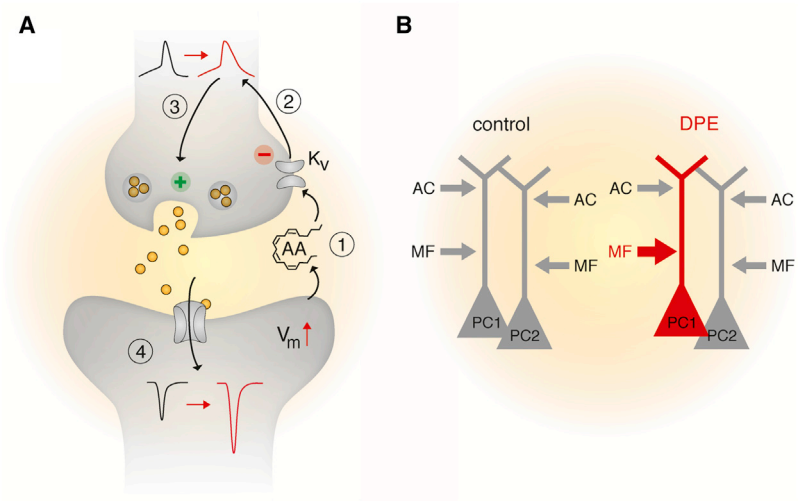

Figure 1. Schematic Diagrams Illustrating Depolarization-Induced Potentiation of Excitation
(A) Transsynaptic mechanism and (B) selective network effect of DPE on presynaptic mossy fiber input onto individual postsynaptic CA3 pyramidal cells.

potential waveform is broadened, probably leading to a larger influx of calcium upon invasion of the presynaptic terminal and consequentially an increased release of glutamate into the synaptic cleft (see Figure 1, steps 1–4). In a final set of experiments, Carta et al. (2014) shine light on an important functional consequence of the described phenomenon: DPE not only potentiates synaptic transmission over a time course of about 10 min, but also facilitates long-term plasticity at MF synapses by lowering the threshold of high-frequency stimulation required for the induction of LTP. To summarize, in this study Carta et al. (2014) provide comprehensive evidence for a retrograde signaling mechanism modulating synaptic transmission and plasticity at the hippocampal mossy fiber synapse.

Until now, the largest body of experimental evidence for retrograde signaling stem from the endocannabinoid system and cannabinoid receptors (Alger, 2002; Castillo et al., 2012; Kano et al., 2009; Wilson and Nicoll, 2002). The study by Carta et al. (2014) now adds another fascinating facet to the class of lipid-derived retrograde signals. The newly described AA-dependent form is peculiar in two ways. First, its presynaptic effects are receptor independent. Such direct modulation of the retrograde target has only been hypothesized for the gaseous NO signal before (Ahern et al., 2002), whereas all other examples involved an intermediate interaction with receptors (or enzymes in the case of NO). Using local photoactivation of caged AA (which the authors have specifically synthesized for this study), Carta et al. (2014) convincingly demonstrate the direct modulation of presynaptic potassium channels by the proposed retrograde messenger. These data nicely fit to previous reports describing enhanced inactivation of K_v channels induced by AA (Oliver et al., 2004). Second, and most importantly, this study marks a retrograde positive modulation of presynaptic transmitter release, thereby greatly expanding the repertoire of retrograde signaling mechanisms. Previously described retrograde signals typically exerted a negative modulation of presynaptic terminals, at least at excitatory connections, mostly via G protein-mediated inhibition of VDCCs (Regehr et al., 2009). In the current case, the retrograde signal leads to a modulation of presynaptic potassium channels, action potential broadening, and increased transmitter release in turn. Interestingly, in another study, it was recently proposed that postsynaptic activation of NMDA receptors and the resulting potassium efflux through these receptors could lead to a presynaptic depolarization, constituting another type of retrograde signal (Shih et al., 2013). This depolarization of the presynaptic terminal leads to increased calcium influx, most likely via action potential broadening and similarly results in increased presynaptic transmitter release. Taken together, both studies implicate the presynaptic action potential as a finely tunable player in the plastic regulation of transmitter release, expanding initial reports on activity-dependent broadening of action potentials (Geiger and Jonas, 2000).

Direct modulation of the broadly expressed voltage-gated ion channel K_v by the diffuse retrograde messenger arachidonic acid naturally raises the question of signal specificity. In this respect, Carta et al. (2014) elegantly demonstrate that DPE induced in a CA3 pyramidal neuron neither spreads to MF synaptic inputs on neighboring, nondepolarized CA3 pyramidal cells, nor does it affect more distally located associational-commissural (AC) synapses on the same pyramidal neuron. DPE does, however, occur in neighboring, previously unstimulated MF synapses on the same CA3 pyramidal cell. Thus, DPE combines postsynaptic cell specificity (only the activated neuron is affected) and presynaptic synapse-type specificity (see Figure 1B). The special microarchitecture of the mossy fiber synapse with its glove-like enwrapping of the postsynaptic thorny excrescence by the presynaptic bouton very likely plays an important role in the spatial limitation of the arachidonic acid signal. Functionally, DPE therefore transiently reinforces already active CA3 pyramidal neurons and selectively strengthens the information transmission between dentate gyrus granule cells and this particular postsynaptic neuron. In addition, postsynaptic depolarization might even lead to depolarization-induced suppression of excitation (DSE) via CBRs at AC terminals, which would further dissociate the relative input strengths of MF versus AC synapses. These events might endow the CA3 microcircuitry with important filtering properties during pattern completion or the formation of place fields (Marr, 1971; Neunuebel and Knierim, 2014).

Presynaptic action potential broadening through the retrograde messenger arachidonic acid lasted for several minutes as Carta et al. (2014) could evaluate in patch-clamp recordings of MF boutons: both uncaging of AA as well as theta-burst stimulation of the presynaptic terminal induced an increase in the AP half-width, suggesting a possible role

of this signaling mechanism during long-term modification of synaptic transmission. Adding to the physiological relevance of retrograde AA signaling on a cellular level, DPE also lowered the threshold for the induction of long-term potentiation. Yet again in contrast to cannabinoid signaling mediating long-term depression (eCB-LTD) (Chevaleyre and Castillo, 2003), AA participates in potentiating excitatory synaptic transmission also on longer timescales. Carta et al. (2014) propose that DPE most likely facilitates the induction process of LTP by allowing a larger calcium influx into the presynaptic terminal. However, it cannot completely be excluded that AA-induced broadening of the action potential waveform might also participate in the expression of LTP. Finally, the involvement of postsynaptic L-type VDCCS in establishing DPE, which in turn lowers the threshold for induction of LTP, might help to resolve the controversy regarding the induction locus of mossy fiber LTP (Nicoll and Schmitz, 2005) by suggesting that certain induction paradigms may have a postsynaptic component.

Carta et al. (2014) have presented an extraordinarily compelling and extensive set of experimental data, which introduces and elucidates a new type of retrograde signaling to modulate synaptic transmission and plasticity. These findings open up an exciting line-up of interesting questions for future studies. First, why is the retrograde signaling mechanism by AA seemingly specific for the mossy fiber synapse? Carta et al. (2014) describe that DPE is absent or at least silent at other connections, such as Schaffer collateral (CA3 to CA1) or AC synapses, and these synaptic pathways show a predominance of retrograde cannabinoid signaling. Is there a competition between the two retrograde pathways and cannabinoid receptors usually take the upper hand? Is the exact pattern of induction stimulus and/or the spatiotemporal calcium signaling profile in the postsynapse important for which pathway gets activated? The necessary enzymatic production machinery for AA as well as the appropriate target, namely the voltage-dependent potassium channels, are almost ubiquitously present. The absence of presynaptic CBRs at mossy fiber terminals perhaps "unmasks" the capability for AA signaling at this connection. Second, what mechanism terminates the AA signal at potassium channels? Is it the unbinding kinetics of AA in combination with diffusion or enzymatic degradation or uptake? In the same lines, is it possible to alter the temporal signaling profile of AA? Another interesting possibility that could be investigated is whether in addition to the retrograde signaling some kind of autocrine message is delivered at the same time to the neuron of its origin by AA, which would influence the shape of backpropagating action potentials in the dendrites of CA3 pyramidal neurons after the production of AA. Third, does the AA production machinery possess a coincidence detection capability? For example, are metabotropic receptor systems—in addition to calcium influx via VDDCs—able to trigger the enzymatic cascade for AA generation, which in turn enabled signal convergence at the postsynaptic side? Finally, it will be of pivotal interest for a better understanding of the complex microcircuitry in area CA3 to investigate whether a similar retrograde signal involving AA is present at synapses between mossy fibers and interneurons.

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