Journal Club

Editor's Note: These short, critical reviews of recent papers in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

Spike-Timing-Dependent Plasticity Induces Presynaptic Changes at Immature Hippocampal Mossy Fiber Synapses

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Review of Sivakumaran et al. (http://www.jneurosci.org/cgi/content/full/29/8/2637)

Activity-dependent modulations of synaptic strength play a crucial role in the structural and functional refinement of neuronal connections (Goda and Davis, 2003), which are thought to be the cellular basis of information storage and memory formation. Long-lasting modifications in the efficacy of synaptic transmission can be induced by coincident presynaptic and postsynaptic activity in a form of plasticity known as spike-timing-dependent plasticity (STDP) (Caporale and Dan, 2008). In this form of plasticity, the precise timing and the order of presynaptic and postsynaptic action potentials determine the magnitude and the direction of the change in synaptic strength.

STDP can occur at both glutamatergic and GABAergic synapses, with slightly different induction rules. At glutamatergic synapses, when a postsynaptic action potential follows a presynaptic spike within a window of tens of milliseconds, long-term potentiation (LTP) usually occurs, whereas the reverse order results in depression (LTD). The induction rules for STDP at GABAergic synapses appear to be more variable (Caporale and Dan, 2008). The current hypothesis suggests that the direction of plasticity depends on the peak amplitude of the postsynaptic intracellular calcium concentrations: high calcium levels lead to LTP, whereas low to moderate levels lead to LTD (Caporale and Dan, 2008).

STDP has been already described in many areas of the brain, but so far it was not known whether it occurs at hippocampal mossy fiber synapses made between granule cells and CA3 pyramidal cells (Mf–CA3 synapses). In adult animals, Mf–CA3 synapses are glutamatergic synapses, but during the first week of development, GABA is the main neurotransmitter released (Safiulina et al., 2006). These synapses display several unique features, including low basal release probability, pronounced paired-pulse facilitation, and frequency facilitation. Moreover, Mf–CA3 synapses also display a particular form of LTP that is expressed presynaptically and is independent of NMDA receptor activation. Conversely, Mf–CA3 synapses are commonly assumed not to express the classical NMDA-dependent LTP associated with postsynaptic AMPA-EPSCs.

At Mf–CA3 synapses, whether presynaptic LTP depends on postsynaptic induction mechanisms has been a matter of debate. Williams and Johnston (1989) and Yeckel et al. (1999) argued that presynaptic LTP requires postsynaptic increases in Ca^{2+} and metabotropic glutamate receptor (mGluR) activation. The dependence of mossy fiber presynaptic LTP on postsynaptic mechanisms was further supported by the observation that pairing presynaptic stimulation of mossy fibers with CA3 pyramidal cell depolarization induces LTP at Mf–CA3 synapses (Urban and Barrionuevo, 1996). However, Mellor and Nicoll (2001) showed that mossy fiber LTP is independent of postsynaptic Ca^{2+} and mGluRs receptor activation, arguing that the postsynaptic cell does not participate in inducing synaptic plasticity at Mf–CA3 synapses. Regardless of this controversy, the question that remains unanswered is whether mossy fibers present a Hebbian-like form of LTP.

In a recent study published in *The Journal of Neuroscience*, Sivakumaran et al. (2009) elegantly explored the presence of STDP at hippocampal Mf–CA3 pyramidal cell synapses. They examined this issue during an early postnatal period (postnatal day 2–5) when these synapses are thought to operate mainly through depolarizing $GABA_A$ synaptic currents (Safiulina et al., 2006). Mossy fiber $GABA_A$-mediated synaptic potentials or currents (Mf-GPSPs or Mf-GPSCs) were evoked at 0.05 Hz from a holding potential of −70 mV in the presence of DNQX (20 μM) and D-AP5 (50 μM) to block AMPA- and NMDA-mediated synaptic responses, respectively. Using a paired-pulse stimulation recording paradigm (interstimulus interval 50 ms), the authors recorded from what they classified as "presynaptic silent synapses." These correspond to synapses at which a synaptic response was detected following the

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second stimulus of a pair, but not after the first stimulus. Semantically, the term "silent synapses" should not be used to refer to synapses with a very low probability of release, which are not very active, but also not silent. Although we agree that the difference between the two is subtle and that the term "silent synapses" is commonly used to refer to these synapses with very low probability of release, it is important to distinguish these from postsynaptically silent synapses whose definition is not dependent on the rate of stimulation. Maybe it is more appropriate to use the definition "whispering" synapses, a term already adopted by Voronin and Cherubini (2004).

By applying a pairing paradigm (10 postsynaptic spikes evoked at 0.1 Hz with afferent stimulation) between presynaptic and postsynaptic activity, the authors observed STDP in immature Mf–CA3 synapses. The observed activity-dependent changes in synaptic efficacy strongly relied on the temporal relationship between presynaptic and postsynaptic activation, with a critical window for STDP similar to that described for most glutamatergic and GABAergic synapses. If a positive pairing of postsynaptic spikes 15 ms after single Mf-GPSCs was used, LTP was observed; however, if postsynaptic spikes preceded Mf-GPSCs by 15 ms, LTD was induced.

Coincident presynaptic and postsynaptic activity had been shown previously to modulate synaptic strength at GABAergic synapses when GABA is still depolarizing (Woodin et al., 2003). However, in the previous study, the modulation of synaptic strength was related to a calcium-dependent decrease of the K^+–Cl^- cotransport activity. This seems not to be the mechanism involved in the activity-dependent modulation of GABAergic synaptic responses at immature mossy fiber synapses, since no change in GABA equilibrium potential was observed after pairing.

Interestingly, both LTP and LTD appeared to be induced postsynaptically and expressed presynaptically. In support of the presynaptic expression, the authors observed that LTP was followed by a decrease in paired-pulse ratio, decrease in the number of failures, and increase in the CV^{-2} (inverse squared value of coefficient of variation), whereas LTD was accompanied by opposite changes in these parameters. However, as mentioned by the authors, these parameters should be analyzed with caution, because insertion or removal of postsynaptic receptors could in the same circumstances produce the same effects.

The authors observed that LTP induction by the pairing protocol led to synapse "fully speaking," further supporting the presynaptic locus of expression of the observed LTP. Moreover, in a recent paper the authors showed that the "whispering" state of mossy fiber synapses at this age seems to be related to presynaptic tonic $GABA_B$ receptor activation inhibiting synaptic release (Safiulina and Cherubini, 2009). In future work, it would be interesting to investigate whether "fully speaking" synapse induced by the pairing protocol operates by modulating tonic $GABA_B$ receptor activation.

Mechanistically, Sivakumaran et al. (2009) found that STDP-LTP of GPSCs depended on postsynaptic calcium rise, postsynaptic protein kinase A (PKA) activity, and TrkB [brain-derived neurotrophic factor (BDNF) receptor] receptor activation. The authors hypothesize that the pairing protocol induced an activity-dependent release of BDNF from CA3 pyramidal cells, which in turn acted presynaptically at mossy fiber terminals to increase probability of release. Consequently, exogenous BDNF application did indeed increase synaptic efficacy at immature Mf–CA3 pyramidal cell synapses through a presynaptic mechanism required on PKA activity. To further support BDNF action through presynaptic TrkB receptors it would have been interesting to test the effect of exogenous BDNF application while blocking postsynaptic PKA activity. This experiment would rule out the possible modulation of synaptic transmission by BDNF acting on postsynaptic TrkB receptors as it has been suggested by Huang et al. (2008). While there are substantial evidence that BDNF can act as a retrograde messenger in several other synapses (Poo, 2001), this is the first time that such a phenomenon is reported to occur at Mf–CA3 synapses.

Surprisingly, when the authors blocked LTP induction by using a TrkB receptor antagonist or by reducing postsynaptic calcium rise using an antagonist of voltage-dependent L-type calcium channels (nifedipine) or with patch pipettes containing the calcium chelator 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA), they observed LTD of Mf-GPSCs instead. Although the nature of LTD obtained with BAPTA and with nifedipine is not clear, it could simply result from blockade of a calcium-dependent protection mechanism against a decrease in Cl^- conductance as proposed by others (Woodin et al., 2003). The authors stated that the persistent synaptic depression obtained by blocking TrkB receptors was also of presynaptic origin because of the reduced number of successes and the significant increase in paired-pulse ratio. It might suggest the presence of a second pathway of postsynaptic/presynaptic communication that apparently does not depend on postsynaptic calcium rise and BDNF as a retrograde messenger. An interesting possibility would be to test the role of mGluRs, since this synapse can also release glutamate at this age (Safiulina et al., 2006).

An important point that we would also like to underline concerns the induction protocol of STDP. Immature Mf–CA3 synapses are normally "whispering" synapses (i.e., no postsynaptic response is triggered at the first stimulus). How is it then possible to explain that isolated presynaptic stimulations used during the pairing protocol, which should not trigger a postsynaptic response, give rise to these forms of plasticity? Indeed STDP at immature Mf–CA3 synapses requires both presynaptic and postsynaptic activity. A possible explanation is that another (a second) neurotransmitter is released by mossy fiber stimulation during the pairing protocol. Again, glutamate seems like a good candidate. An intriguing possibility is that glutamate released from Mfs during the pairing protocol would activate postsynaptic mGluRs, inducing increase of intracellular calcium levels and in turn BDNF release. In their experiments the authors had blocked ionotropic glutamate receptors, but not mGluRs, leaving this possibility open. It would be of great importance to verify the possible role of these receptors in STDP.

In conclusion, the work of Sivakumaran et al. (2009) describes for the first time a Hebbian-like form of synaptic plasticity at immature hippocampal mossy fiber synapses. This form of plasticity may contribute to the shaping of neuronal connectivity before the establishment of the adult neuronal circuits. Interestingly this new form of plasticity can result in both LTP and LTD, depending on the precise timing of presynaptic versus postsynaptic activity, which are both postsynaptically induced and presynaptically expressed. The current work raises also the need to readdress the role of the postsynaptic component in modulating synaptic plasticity induction at mossy fiber synapses in adult animals.

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