Lateral thinking: CaMKII uncouples kainate receptors from mossy fibre synapses

Bryan A Copits and Geoffrey T Swanson*

Department of Molecular Pharmacology and Biological Chemistry, Northwestern University, Feinberg School of Medicine, Chicago, IL, USA.

*Correspondence to: gtswanson@northwestern.edu

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Alteration of the efficacy of excitatory synaptic transmission between neurons is a critical element in the processes of learning, memory, and behaviour. Despite decades of research aimed at elucidating basic cellular mechanisms underlying synaptic plasticity, new pathways and permutations continue to be discovered. Carta *et al* (2013) now show that activation of the calcium/calmodulin dependent kinase II (CaMKII) induces an unusual postsynaptic form of long-term depression (LTD) at the hippocampal mossy fibre synapse by promoting

lateral diffusion of kainate receptors (KARs), a family of ionotropic glutamate receptors (iGluRs) that influence pyramidal neuron excitability. This report therefore reveals a new and mechanistically unique way of fine-tuning synaptic plasticity at this central synapse in the hippocampus.

Information transfer within the nervous system is regulated at the synaptic level by diverse cellular mechanisms. Synaptic efficacy is not static (i.e., it is 'plastic'), and the capacity to adjust the strength of communication between neurons in a

Figure 1 Kainate receptor-dependent plasticity mechanisms at the hippocampal mossy fibre-CA3 synapse. (A) Activation of presynaptic receptors enhances glutamate release from the mossy fibre terminals. (B) A spike-timing-dependent plasticity protocol known to activate postsynaptic CaMKII results in long-term synaptic depression. CaMKII phosphorylates the GluK5 kainate receptor subunit, which uncouples the receptor from PSD-95 in the postsynaptic density. This leads to an increase in receptor mobility and diffusion away from the synapse. (C) Low-frequency stimulation of mossy fibres and activation of postsynaptic group 1 mGluRs leads to activation of PKC, which promotes the association of SNAP-25 to the GluK5 kainate receptor subunit and the subsequent endocytosis of synaptic receptors.

network has been shown to be a critical component of diverse aspects of brain function that include many forms of behavioural learning (Martin *et al.*, 2000). The complex means by which neurons adjust their synaptic properties in response to changes in local and global activity in the central nervous system has been the subject of intensive investigation spanning multiple decades (Malenka and Bear, 2004; Feldman, 2009). Nonetheless, new mechanisms underlying plasticity of excitatory and inhibitory synaptic transmission continue to be elucidated; these can vary depending on the experimental parameters for induction of plasticity, the particular type of synapse under investigation, and even the prior history of activation at the synapse. Long-term potentiation (LTP) and LTD of excitatory synaptic transmission are two well-known phenomena in which efficacy is increased or decreased, respectively, and at many synapses in the CNS occur through concomitant alterations in the number of postsynaptic iGluRs. The movement of excitatory receptors in and out of synapses, and more generally to and from the neuronal plasma membrane, is dictated by their association with a wide variety of scaffolding and chaperone proteins, whose interactions are often controlled by various protein kinases (Anggono and Huganir, 2012).

It is generally appreciated now that long-term synaptic plasticity can be elicited by a variety of mechanisms even within a single type of synaptic connection. In addition to postsynaptic alterations in receptor content, for example, synaptic efficacy can also be tuned by regulated alterations in the probability of vesicular release of the neurotransmitter. Until recently, this presynaptic form of plasticity was thought to be the exclusive mechanism for altering excitatory synaptic strength at a morphologically unusual synapse in the hippocampus formed between large bouton-like presynaptic terminals arising from granule cell axons, or mossy fibres, and proximal dendrites on CA3 pyramidal neurons (Nicoll and Schmitz, 2005). These synaptic connections allow for single dentate granule cells to profoundly influence the likelihood of action potential firing in CA3 pyramidal neurons in a frequency-dependent manner, and for that reason have been referred to as 'conditional detonator' synapses (Henze *et al.*, 2002). The precise mechanisms that lead to increased vesicular release probability following LTP-inducing stimulation of mossy fibre axons, including a potential role for retrograde signalling, remain the subject of debate, although there is general consensus that activation of presynaptic protein kinase A (PKA) is a key step in this form of synaptic plasticity (Figure 1A). Enhancing release probability impacts signalling through all three types of iGluRs present at mossy fibre synapses—AMPA, NMDA, and KARs. Recently, however, novel postsynaptic forms of mossy fibre plasticity were discovered in which induction protocols specifically increased the number of NMDA receptors (Kwon and Castillo, 2008; Rebola *et al.*, 2008) or decreased the number of KARs (Selak *et al.*, 2009), expanding the mechanistic repertoire at this historical site of focus of research on presynaptic LTP. Alterations in the synaptic content of particular iGluRs could serve as an additional means to fine-tune synaptic integration at the mossy fibre—CA3 synapse and therefore have important consequences for hippocampal network excitability.

In this issue, Carta *et al.* (2013) identify a new postsynaptic mechanism for shaping mossy fibre plasticity that is specific to synaptic KARs, which serve to influence temporal integration of synaptic input as well as pyramidal neuron excitability through modulation of intrinsic ion channels. The authors paired postsynaptic depolarization of CA3 pyramidal neurons with a precisely timed presynaptic release of glutamate in a pattern that is known to produce LTP at many central synapses (Feldman, 2012). At mossy fibre synapses, however, this form of spike-timing-dependent plasticity (STDP) instead caused LTD of KAR-mediated excitatory synaptic potentials (KAR-LTD) while leaving AMPA receptor function unaltered (Figure 1B) (Carta *et al.*, 2013). Using a series of genetic and pharmacological manipulations, Carta *et al.* (2013) found that KAR-LTD was dependent upon the activation of postsynaptic KARs themselves, a rise in postsynaptic Ca^{2+} , and CaMKII phosphorylation of a specific protein component of synaptic KARs, the GluK5 subunit. Unlike other mechanisms of postsynaptic mossy fibre plasticity, KAR-LTD was independent of NMDA or metabotropic glutamate receptor activation. Most surprisingly, KAR-LTD did not require receptor endocytosis from the plasma membrane, as is the case with most other forms of postsynaptic depression of excitatory transmission, including a distinct form of KAR-LTD reported previously (Selak *et al.*, 2009) (Figure 1C). Instead, CaMKII-mediated phosphorylation of GluK5 subunits likely uncoupled receptors from the postsynaptic scaffolding protein PSD-95, which then led to enhanced lateral diffusion of KARs out of mossy fibre synapses. As KAR endocytosis was not altered in mossy fibre STDP, the activity-dependent reduction in KAR signalling was effectively limited to those receptors in the synapse. A molecular replacement strategy was employed using biolistic-based expression of mutant KARs in cultured hippocampal slices prepared from KAR knockout mice, which allowed Carta *et al.* (2013) to corroborate their detailed biochemical studies by showing that reconstituted KAR currents in CA3 neurons expressing recombinant GluK5 phosphorylation site substitutions were unable to express KAR-LTD. In summary, KAR-mediated activation of CaMKII leads to phosphorylation of the GluK5 subunit and subsequent KAR-LTD through enhanced lateral mobility of synaptic receptors (Figure 1B).

These findings are intriguing for several reasons. Most notably, they stand in stark contrast to studies in which CaMKII activation primarily triggers potentiation, rather than depression, of excitatory synaptic transmission at other synapses (Lisman *et al.*, 2012). CaMKII recently was shown to cause diffusional trapping of AMPA receptor complexes within the postsynaptic density following phosphorylation of a closely associated auxiliary subunit, stargazin (Opazo *et al.*, 2010), which is precisely the opposite of the effects of activation of the enzyme on KAR mobility at mossy fibre synapses. Further, these divergent consequences are both dependent upon carboxy-terminal PDZ interactions with scaffolding proteins, although in each case further research is needed to dissect out the relevant binding partners that control lateral mobility. It is of interest that KAR-LTD required synaptic activation of KARs to initiate signalling via CaMKII, which implies a tight coupling exists between KARs and the holoenzyme in the mossy fibre postsynaptic density.

This observation also raises the possibility that activated CaMKII could phosphorylate other targets to effect other, yet-to-be-discovered, changes in synaptic function. Finally, the report by Carta *et al* expands our understanding of how excitatory synaptic transmission is fine-tuned at an important central synapse and underscores the fact that even well-trod ground (or synapses) continue

to yield surprises that inform our understanding of the remarkable mechanistic diversity underlying synaptic plasticity in the CNS.

Conflict of interest

The authors declare that they have no conflict of interest.

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