Journal Club

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Depression in the Fly

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Review of Guo and Zhong (http://www.jneurosci.org/cgi/content/full/26/15/4004)

A PubMed search for long-term depression (LTD) finds 8703 articles, reflecting the interest this putative mechanism for learning and memory generates among neuroscientists. LTD has been characterized in the mammalian hippocampus and cerebellum, and its induction involves regulation of protein phosphorylation by protein kinases and phosphatases (Mulkey et al., 1994; Linden and Connor, 1995).

In arthropods, short-term depression at low-frequency stimulation has been reported at the crayfish (Silverman-Gavrila et al., 2005) and *Drosophila* neuromuscular junction (NMJ) (Wu et al., 2005). However, Guo and Zhong (2006), in their recent *Journal of Neuroscience* article, now report LTD properties at *Drosophila* larval NMJs. Their study provides a direct demonstration that the highly conserved protein kinase B/Akt, known to modulate neurotransmitter receptors (Hou and Klann, 2004), is required for induction of LTD but not short-term plasticity. *Drosophila* NMJs resemble mammalian central glutamatergic synapses in that they share various signaling pathways underlying LTD (Guo and Zhong, 2006). This preparation offers advantages for exploring the molecular mechanism of LTD because presynaptic boutons at identifiable synapses are readily accessible for electrophysiological analysis, unlike most synaptic boutons in the mammalian brain. Combined with powerful genetic and biochemical manipulation, this may allow a multilevel integrative approach for understanding gene function in synaptic physiology and behavioral plasticity.

In their experiments, Guo and Zhong (2006) found that LTD was induced at *Drosophila* NMJ of muscle M12 by 30 Hz stimulation for 20 s in 0.4 mM external [Ca^{2+}] [Guo and Zhong (2006), their Fig. 1*A–C* (http://www.jneurosci.org/cgi/content/full/26/15/4004/F1)] after 5 min of baseline stimulation at 0.05 Hz. At low-frequency stimulation [Guo and Zhong (2006), their Fig. 1*A*,*B* (http://www.jneurosci.org/cgi/content/full/26/15/4004/F1)], excitatory junction current (EJC) amplitude decayed slightly after long recording periods, but to a lesser extent than during LTD induced at high frequency. This is most likely attributable to a reduction in the quantal amplitude [Guo and Zhong (2006), their Fig. 4*A*,*C* (http://www.jneurosci.org/cgi/content/full/26/15/4004/F4)]. Quantal content in controls remained stable [Guo and Zhong (2006), their Fig. 1*C* (http://www.jneurosci.org/cgi/content/full/26/15/4004/F1)].

Next, the authors showed that stimulation frequency rather than the total number of stimuli was crucial for LTD induction [Guo and Zhong (2006), their Fig. 1*D* (http://www.jneurosci.org/cgi/content/full/26/15/4004/F1)]. LTD was induced at 20 Hz stimulation for 20 s, and it reached a plateau at 30–50 Hz. In interpreting their results, the authors carefully addressed three concerns: (1) that LTD can be induced at different muscle fibers with different dynamics (LTD and short-term depression at M12 and only LTD at M4 and M6) [Guo and Zhong (2006), their Fig. 3 (http://www.jneurosci.org/cgi/content/full/26/15/4004/F3)]; (2) that LTD did not result from action potential failures; and (3) that muscle contraction resulting from LTD induction did not disrupt NMJ function [Guo and Zhong (2006), their Fig. 2 (http://www.jneurosci.org/cgi/content/full/26/15/4004/F2)].

In a variety of synapses, LTD can be generated presynaptically by a reduction in release probability, postsynaptically by activation of postsynaptic glutamate receptors, or a combination of the two. Using quantal and miniature EJC analysis, as well as exogenous glutamate applications, Guo and Zhong (2006) [their Fig. 4*E* (http://www.jneurosci.org/cgi/content/full/26/15/4004/F4)] report that *Drosophila* LTD likely results from a reduced number of vesicles in each evoked response rather than postsynaptic changes [Guo and Zhong (2006), their Fig. 4*F* (http://www.jneurosci.org/cgi/content/full/26/15/4004/F4)].

To investigate the molecular mechanism of LTD, the authors combined electrophysiology with genetic analysis of mutants with impaired learning, abnormal synaptic function, or deregulated signal transduction. LTD was not significantly affected in mutants with disrupted synaptic transmission or short-term plasticity such as *rutabaga*, *dunce*, or *latheo* but was strongly impaired in *akt* mutants (with mutations in the gene encoding protein kinase B/Akt [Guo and Zhong (2006),

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their Fig. 5*B* (http://www.jneurosci.org/cgi/content/full/26/15/4004/F5)]. LTD was rescued in *akt* mutants by acutely induced expression of the normal *akt* transgene. The degree of LTD rescue appeared to be proportional to the amount of *hsp70–akt* expression [Guo and Zhong (2006), their Fig. 7*C* (http://www.jneurosci.org/cgi/content/full/26/15/4004/F7)], but because no *akt* null allele is available, the authors were not able to address whether LTD would be abolished by complete loss of Akt proteins. This does not dilute the significance and importance of the study, and it does not alter the main finding of the critical role of Akt in mediating long-term plasticity.

Guo and Zhong (2006) further clearly demonstrated that Akt is expressed at *Drosophila* NMJs [their Fig. 9 (http://www.jneurosci.org/cgi/content/full/26/15/4004/F9)] using a polyclonal antibody against *Drosophila* Akt. The immunoreactivity was present in wild type and reduced in mutant NMJs.

To determine whether the impaired LTD was a result of defective synaptic transmission, or abnormal short-term plasticity, they examined spontaneous and evoked synaptic transmission, short-term facilitation, and post-tetanic potentiation in *akt* mutants [Guo and Zhong (2006), their Fig. 8 (http://www.jneurosci.org/cgi/content/full/26/15/4004/F8)]. They found that LTD involved different mechanisms than short-term plasticity because LTD was unaffected in learning and memory mutants that have impairments in short-term synaptic plasticity, such as *rutabaga* and *dunce*. Akt was not essential for basal synaptic transmission and short-term plasticity because *akt* mutants showed normal short-term facilitation and post-tetanic potentiation [Guo and Zhong (2006), their Fig. 9 (http://www.jneurosci.org/cgi/content/full/26/15/4004/F9)].

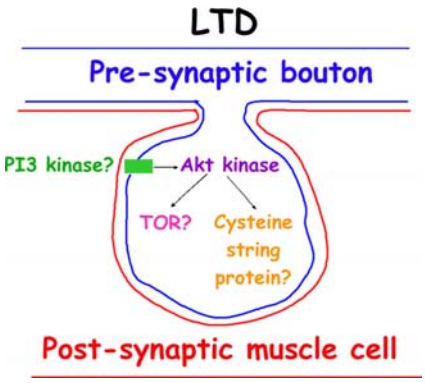

Figure 1. A putative mechanism of LTD at *Drosophila* NMJs might involve the activation of phosphatidylinositide (4,5)-bisphosphate kinase (PI3-kinase) leading to the production of phosphatidylinositide 3,4,5-trisphosphate, which promotes the phosphorylation and activation of a serine/threonine-kinase called protein kinase B (also known as Rac or Akt). Akt might regulate exocytosis via the phosphorylation of cysteine string protein (Evans et al., 2006) or target of rapamycin (TOR) proteins involved in translation and transcription and therefore in protein synthesis. The cysteine string protein has several independent synaptic functions such as affecting synaptic growth, evoked release, thermal protection of evoked release, and intraterminal calcium levels at rest and during stimulation (Bronk et al., 2005).

LTD depended on external $[Ca^{2+}]$ [Guo and Zhong (2006), their Fig. 1*E* (http://www.jneurosci.org/cgi/content/full/26/15/4004/F1)]. The inverse correlation between $[Ca^{2+}]$ and LTD was similar in *akt* mutants and in wild-type [Guo and Zhong (2006), their Fig. 6 (http://www.jneurosci.org/cgi/content/full/26/15/4004/F6)]. This relationship is remarkable and raises questions about whether Ca^{2+}/CaM-activated phosphatases are also involved in LTD. It will be interesting to investigate in additional experiments whether *Drosophila* LTD is mediated by similar upstream phosphatidylinositide (4,5)-bisphosphate kinases and downstream (target of rapamycin) signaling of Akt (Fig. 1) as reported in hippocampal LTD (Hou and Klann, 2004).

The results support the hypothesis that the properties of LTD at *Drosophila* NMJs are similar to LTDs reported in various preparations including an inverse relationship with $[Ca^{2+}]$ and kinase involvement. In summary, the authors provided the first detailed analysis of LTD at *Drosophila* larval NMJs. Their data convincingly demonstrated that at these synapses, LTD is a presynaptic phenomenon that is directly mediated by Akt and depends on Ca^{2+}, phosphorylation, and high-frequency stimulation.

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