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Brief Report

Co-induction of long-term potentiation and long-term depression at a central synapse in the leech

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Abstract

Most studies of long-term potentiation (LTP) have focused on potentiation induced by the activation of postsynaptic NMDA receptors (NMDARs). However, it is now apparent that NMDAR-dependent signaling processes are not the only form of LTP operating in the brain [Malenka, R. C., & Bear, M. F. (2004). LTP and LTD: An embarrassment of riches. *Neuron, 44*, 5–21]. Previously, we have observed that LTP in leech central synapses made by the touch mechanosensory neurons onto the S interneuron was NMDAR-independent [Burrell, B. D., & Sahley, C. L. (2004). Multiple forms of long-term potentiation and long-term depression converge on a single interneuron in the leech CNS. *Journal of Neuroscience, 24*, 4011–4019]. Here we examine the cellular mechanisms mediating T-to-S (T → S) LTP and find that its induction requires activation of metabotropic glutamate receptors (mGluRs), voltage-dependent Ca^{2+} channels (VDCCs) and protein kinase C (PKC). Surprisingly, whenever LTP was pharmacologically inhibited, long-term depression (LTD) was observed at the tetanized synapse, indicating that LTP and LTD were activated at the same time in the same synaptic pathway. This co-induction of LTP and LTD likely plays an important role in activity-dependent regulation of synaptic transmission.

Keywords: Metabotropic glutamate receptor; Voltage-dependent Ca^{2+} channel; Protein kinase C; Neuroplasticity; Invertebrate; Long-term potentiation; Long-term depression

NMDAR-dependent long-term potentiation (LTP) and long-term depression (LTD) are thought to be critical cellular substrates for mediating learning and memory because their initiation requires coincident activity in both the pre- and postsynaptic neurons (activity dependence) and the resulting changes are restricted to the co-activated synapses (synapse specificity). However, it is now clear that other molecules can perform coincidence-detection in place of NMDARs for both LTP and LTD (Anwyl, 2006; Malenka & Bear, 2004). This heterogeneity in cellular mechanisms mediating LTP and LTD, along with the structural complexity of the vertebrate brain, complicates efforts to determine the functional contribution of synaptic changes to learning-related changes in behavior. The medicinal leech (*Hirudo medicinalis*) has a number of properties that make it a useful model for studies of LTP and LTD. Most neurons in the leech CNS are large and easily visualized and there are far fewer neurons in the leech CNS (~400 neurons/ganglion with 21 body ganglia plus the head and tail ganglia (Muller, Nicholls, & Stent, 1981)) compared to a mammalian brain. Therefore, it is possible to record from the same, identifiable neuron from one animal to the next and to link changes in a given neuron to a specific behavioral function (Burrell & Sahley, 2005; Kristan, Calabrese, & Friesen, 2005). Furthermore, the cellular and molecular properties between leech and vertebrates neurons are highly conserved (Burrell & Sahley, 2001), so discoveries about neural function in invertebrates are relevant to understanding processes in vertebrate neurons.

LTP and LTD have been observed in two different synaptic connections in the leech CNS; those made by the touch (T) sensitive cells onto the S interneuron (S-cell)

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and by pressure (P) sensitive onto the same S-cell. The S-cell is thought to be critical for certain types of learning in the leech (Burrell, Sahley, & Muller, 2003; Modney, Sahley, & Muller, 1997). LTP in the P → S synapse is NMDAR-dependent, synapse-specific, and expressed postsynaptically (Burrell & Sahley, 2004). At the T → S synapse (Fig. 1A), which is the focus of this paper, tetanic stimulation simultaneously induces homosynaptic LTP (homLTP) in the tetanized synapse and heterosynaptic LTD (hetLTD) in the non-tetanized synapse (Fig. 1B; also see Burrell & Sahley, 2004). This pattern of homLTP and hetLTD (synapses consisting of different presynaptic cells, but the same postsynaptic target) has been observed in the CA1 (Lynch, Dunwiddie, & Gribkoff, 1977), CA3 (Kosub, Do, & Derrick, 2005) and dentate gyrus (Abraham & Goddard, 1983) regions of the hippocampus, the amygdala (Royer & Pare, 2003) and the visual cortex (Tsumoto & Suda, 1979). HomLTP at the T → S synapse is NMDAR-independent while T → S hetLTD is NMDAR-dependent (Burrell & Sahley, 2004). In this study, we examined underlying homLTP at the T → S synapse and discovered that inhibition of homLTP uncovered LTD in the same synapse that was apparently initiated in parallel with LTP in the tetanized pathway.

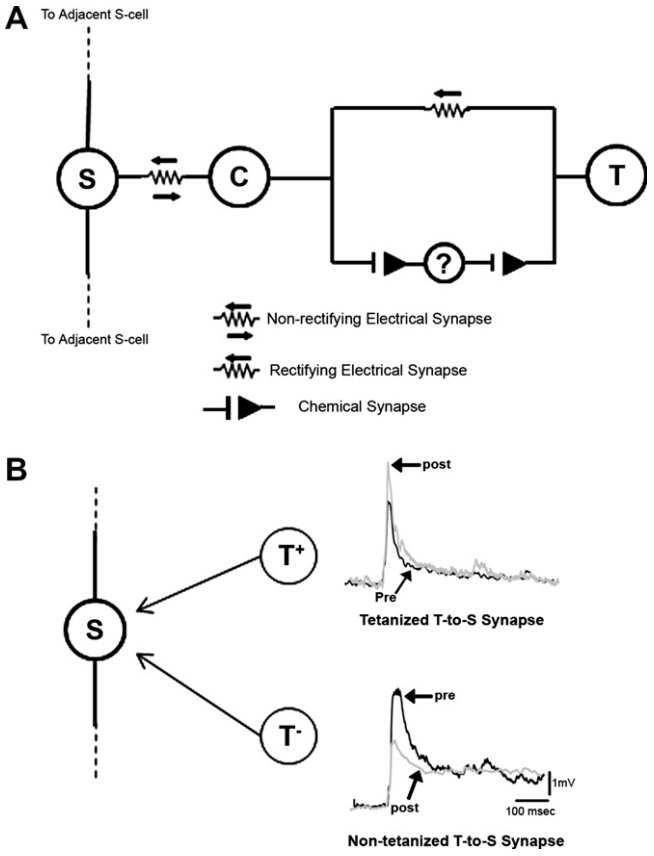

Fig. 1. (A) T → S synaptic circuit. The T → S synapse has both a monosynaptic electrical and polysynaptic chemical (glutamatergic) component (Muller & Scott, 1981; Li and Burrell, 2006). The "?" indicates the unknown neuron(s) that mediate the polysynaptic, chemical component of the T → S synapse. Nearly all synaptic input to the S-cell is routed through the coupling (C) interneuron. The S- and the C-cells are linked by a non-rectifying electrical synapse and the level of electrical coupling is so strong that EPSPs elicited in the C-cell are carried to the S-cell with minimal attenuation or delay, acting as monosynaptic EPSPs (Muller & Scott, 1981). The C-cells are not directly recorded from because they are on the opposite (dorsal) side of the ganglion. (B) Changes in T → S EPSP at the tetanized and non-tetanized synapse. *Left:* Diagrammatic representation of convergent inputs by the two T-cells, the tetanized (T^+) and non-tetanized (T^-), onto a single postsynaptic S-cell. *Right:* Traces labeled "pre" were recorded prior to tetanic stimulation and those labeled "post" were recorded 60 min after tetanus. Tetanization of the DP nerve elicited homLTP in the tetanized T → S synapse and simultaneously elicited hetLTD in the non-tetanized T → S synapse (same postsynaptic S-cell, different presynaptic T-cells).

To test the signaling pathways that mediate T → S LTP, individual ganglia were dissected from 3 g leeches obtained from a commercial supplier (Leeches USA Ltd.) and maintained in pond water (0.5 g/1 L H_2O Hirudo salt from Leeches USA Ltd.) at 18 °C with a 12 h:12 h light/dark cycle. Dissections and recordings were carried out in normal leech saline (in mM: 115 NaCl, 4 KCl, 1.8 $CaCl_2$, 1.0 $MgCl_2$ and 10 Hepes). Following dissection, ganglia were placed in a recording chamber under constant perfusion. Intracellular recordings from identified T- and S-cells were made using glass sharp microelectrodes connected to a bridge amplifier (BA-1; National Precision Instruments). A detailed protocol for inducing homLTP and hetLTD in the T → S synapse are described in Burrell and Sahley (2004). In brief, unitary excitatory postsynaptic potentials (EPSPs) were elicited in the S-cell by stimulation of the presynaptic neuron (the T-cell) prior to (pre-test) and 1 h after (post-test) tetanic stimulation of the dorsal posterior nerve root. Ten tetani were delivered at 10 s intervals, with each tetanus consisting of five stimuli delivered at 25 Hz (STG 1004 Programmable Stimulator; Multichannel Systems). Drugs were applied after the pre-test for 10 min with the tetanizing stimuli applied at the end of this period (all drugs were obtained from Sigma). T → S synaptic transmission was tested at the tetanized synapse (T^+) and the non-tetanized connection (T^-). Only two recordings (pre- and post-tetanus) were made because chronic (>10–15 min) recordings of the S-cell damage the interneuron (Burrell & Sahley, 2004).

In normal saline, tetanic stimulation elicited homLTP in the tetanized T → S synapse and hetLTD in the non-tetanized connection (Figs. 1B and 2), replicating the results obtained in Burrell and Sahley (2004). The T → S synapse is glutamatergic (Li and Burrell, 2006) and since T → S LTP is NMDAR-independent, the potential involvement of mGluRs was investigated. HomLTP was blocked in ganglia treated with 1 mM alpha-methyl-4-carboxyphenylglycine (MCPG, an antagonist of mGluR1, 2 and 5) during tetanic stimulation. MCPG did not block hetLTD, indicating that mGluRs contribute only to T → S homLTP and not to hetLTD (Fig. 2A), which has already been shown to be NMDAR-dependent (Burrell & Sahley, 2004). Surprisingly, homosynaptic LTD (homLTD) was observed in the tetanized T → S synapses (T^+) of the MCPG-treated ganglia in addition to the hetLTD at the non-tetanized syn-