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A New Locus for Synaptic Plasticity in Cerebellar Circuits

Experimental and computational analyses of cerebellar function indicate that excitatory synapses onto deep nucleus neurons are likely to be a critical site of plasticity during motor learning. In this issue of *Neuron*, Pugh and Raman report that unconventional stimulus protocols can drive synaptic plasticity in the deep cerebellar nuclei.

What are the cellular mechanisms of learning? Despite decades of intense focus on hippocampal synaptic plasticity and spatial learning, the links between long term synaptic potentiation and depression (LTP and LTD, respectively) and learning remain elusive, predominantly because the consequences of hippocampal neuronal firing for specific behaviors are not known. In contrast, a wealth of information about the role of cerebellar activity in motor control and motor learning make cerebellar circuits attractive sites for analyzing how cellular mechanisms of plasticity act within well-defined neural networks to mediate behavioral learning and memory storage.

Until recently, efforts to pin down the engram, or site of memory storage, in cerebellar circuits have focused primarily on plasticity at the synapse between parallel fibers and Purkinje cells. While cerebellar LTD does seem critical for short-term learning, recent evidence indicates that motor memories can be formed even in mice with impaired or absent parallel fiber LTD (De Zeeuw and Yeo, 2005). Furthermore, inactivation of cerebellar cortex in animals that have undergone long-term training does not prevent expression of motor memories (Kassardjian et al., 2005; Shutoh et al., 2006). Available evidence indicates that sites downstream of Purkinje cells must contribute to memory storage (du Lac, 1995; Medina et al., 2000).

Purkinje cells influence behaviors exclusively via inhibitory synapses onto neurons in the deep cerebellar and vestibular nuclei (Figure 1). Within the deep nuclei, large excitatory neurons project to a variety of premotor structures while intermingled inhibitory neurons provide local inhibition and a major feedback projection to the inferior olive. Deep nucleus neurons receive excitatory drive from collaterals of pontine mossy fiber axons that continue to the cerebellar cortex and synapse onto granule cells, whose parallel fiber axons in turn provide a major excitatory input to Purkinje cells. (A sparse set of excitatory inputs to the deep nuclei from climbing fiber collaterals is not shown.) Until now, the known forms of plasticity in the deep nuclei were limited to inhibitory synaptic plasticity at the Purkinje cell to deep nucleus synapse and intrinsic excitability changes in deep nucleus neurons (Aizenman et al., 2000). Despite efforts by several groups, no one had reported plasticity at the remaining major input, the mossy fiber synapse (although LTP and LTD of the homologous synapse in the vestibular nucleus has been reported [Grassi and Pettorossi, 2001]). In a groundbreaking paper in this issue of *Neuron*, Pugh and Raman (2006) demonstrate induction of LTP of excitatory inputs to the deep nuclei, while Zhang and Linden (2006), in a complementary study in the *Journal of Neuroscience*, report induction of LTD at the same synapse.

Conventional LTP induction methods developed for the hippocampus are ineffective in deep cerebellar nucleus neurons, which fire tonically at high rates that are modulated by increases and decreases in Purkinje cell inhibition. Pugh and Raman were able to design an effective method of inducing plasticity by simulating activity patterns that are likely to occur in vivo during a well-studied form of associative learning, classical conditioning of the eyeblink response (Medina et al., 2000). During classical eyeblink conditioning, cerebellar mossy fibers are activated by the conditioned stimulus, usually a tone, and in turn increase activity in Purkinje cells (which fire tonically at high rates), with the result that deep nucleus neurons receive nearly simultaneous excitation and inhibition. Powerful Purkinje cell synapses, many of which target the soma and axon initial segment, would be predicted to dominate this interaction (Medina et al., 2000; Telgkamp et al., 2004). However, the arrival of the unconditioned stimulus, an air puff, produces an increase in climbing fiber activity with a resulting brief burst in Purkinje cells, followed by relative quiescence, a condition that promotes postinhibitory rebound firing in deep nucleus neurons. Pugh and Raman sought to mimic these conditions by pairing synaptic stimulation of mossy fibers with *hyperpolarization* of the postsynaptic deep nuclear neuron, such as would occur during Purkinje cell inhibition. Through an elegant set of experiments exploring the parameter space of plasticity induction, Pugh and Raman demonstrate that synaptic activity must precede postinhibitory rebound currents to produce LTP.

Two features of this induction protocol are salient and unusual. First, bath application of the NMDA receptor antagonist CPP prevents LTP, despite the fact that the pairing of synaptic stimulation with postsynaptic hyperpolarization would normally not be expected to yield significant postsynaptic NMDA receptor activation because of the failure to relieve a voltage-dependent Mg^{2+} block. However, Pugh and Raman demonstrate that NMDA receptors at this synapse can pass significant amounts of current at hyperpolarized potentials—in fact, 20% of the EPSC amplitude at −65 mV is due to current through the NMDA receptor, while as much as 50% of the EPSC is due to NMDA receptors by the end of stimuli trains such as those used to induce plasticity in their protocol. These data suggest that the NR2D subunit, which confers much weaker Mg^{2+} block than the more common NR2A or NR2B subunits, is a major component of NMDA receptors at this synapse, consistent with previous reports (Anchisi et al., 2001).

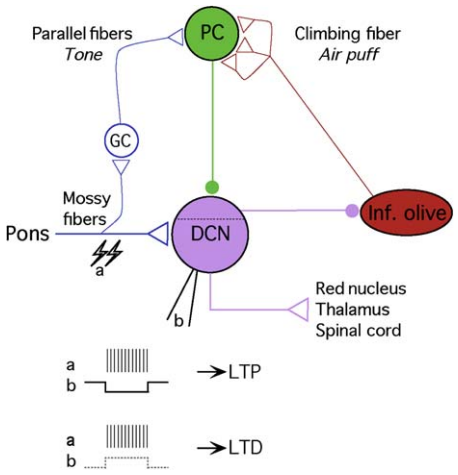

Figure 1. The Simplified Cerebellar Circuit

Deep cerebellar nucleus neurons (DCN, purple) receive excitatory mossy fiber inputs from pontine nuclei and inhibitory input from Purkinje cells (PC, green). Mossy fibers, which carry information about the conditioned stimulus, also synapse on granule cells (GC, blue), which give rise to parallel fibers. The inferior olive (red), which carries information about motor error (or unconditioned stimulus), sends climbing fibers to Purkinje cells and in turn is inhibited by a subset of inhibitory neurons in the DCN. Large excitatory projection neurons in the DCN send their output to a variety of motor and premotor areas, including those listed.

Second, the rebound currents following hyperpolarization turn out to be an absolute requirement for potentiation; when the authors eliminate rebound currents by recording in voltage-clamp and using current ramps to slowly depolarize the neuron, LTP is abolished. It is unclear precisely what component of the rebound current is critical for potentiation, although the authors demonstrate that action potentials per se are not needed. Low-threshold calcium currents, which are required for plasticity of Purkinje cell synapses onto deep nucleus neurons (Aizenman et al., 2000), are likely to play a key role. Pugh and Raman show that synaptic stimulation preceding, but not following, rebound firing is effective in potentiating the synapse. This suggests the intriguing possibility that a specific temporal pattern of calcium entry (NMDA receptor followed by voltage-gated calcium channels) is required for the induction of LTP. Determining the precise somatodendritic locations and nature of the requisite calcium signals will be an important challenge for future studies.

A nice counterpoint to these results comes from Zhang and Linden (2006), who show that pairing synaptic stimulation with postsynaptic *depolarization* produces LTD. They demonstrate that the postsynaptic depolarization is not necessary for LTD to occur; instead, LTD relies on an mGluR-dependent slow EPSC that arises at the synapse during high-frequency stimulation. As with the case for LTP, postsynaptic BAPTA eliminates LTD; however, NMDA receptor antagonists have no effect, while mGluR1 (but not mGluR5) antagonists prevent LTD. Zhang and Linden take the further step of determining that protein synthesis is required for LTD to develop, while transcription is not. They speculate that their protocol may tap into the ERK or rapamycin-sensitive pathways downstream of mGluR1 activation; it will be interesting to see what specific proteins are required for LTD expression and whether LTP also depends on protein synthesis. Curiously, the two studies report different results in the response to mossy fiber stimulation alone: Pugh and Raman see no change in synaptic efficacy, while Zhang and Linden find that this protocol produces LTD. Differences in postsynaptic activity may account for the discrepancy: in the first instance, cells were maintained in current-clamp during synaptic stimulation, while in the second, cells were held in voltage-clamp with both sodium and potassium channels blocked.

A few aspects of these intriguing studies preclude making direct links to behavioral learning at this stage. Both studies were performed in young animals in which the cerebellar circuit appears to be insufficiently developed to permit eyeblink conditioning to occur (Nicholson and Freeman, 2003). Although the heavy myelination of the deep nuclei in older animals is problematic for slice physiology, it will be important to determine whether LTP and LTD can be induced in deep nucleus neurons of mature animals. In addition, although deep nucleus neurons typically fire spontaneously in vivo at high rates, in both studies, neurons were held in voltage-clamp for the duration of pre- and postinduction synaptic tests (Pugh and Raman returned to current-clamp during induction, while Zhang and Linden maintained voltage-clamp throughout). The calcium dependence of plasticity in tonically firing neurons, such as those in cerebellar circuits, can differ substantially from that in quiescent neurons (Coesmans et al., 2004). In spontaneously firing vestibular nucleus neuron (brainstem homologs of deep nucleus neurons), neuronal hyperpolarization (such as occurs during voltage-clamp) reduces calcium levels with consequent reductions in CaMKII activity, thereby driving plasticity of intrinsic excitability (Nelson et al., 2005). Furthermore, postsynaptic action potentials following induction of synaptic plasticity can reduce or eliminate the synaptic changes (Zhou and Poo, 2004). Thus, holding neurons in voltage-clamp for synaptic testing, particularly those that fire at high tonic rates, may drive important cellular changes that affect the biochemical state of the cell and its available forms of plasticity. Finally, because intermingled GABAergic neurons that inhibit the inferior olive are also targets of Purkinje cell inhibition (De Zeeuw and Berrebi, 1995), it is possible that one or both of the forms of plasticity discussed here might extend to inhibitory neurons, with far-reaching implications for studies of acquisition and extinction (Medina et al., 2000). These possibilities can be addressed in future studies aimed at identifying the relationship between mossy fiber synaptic plasticity and postsynaptic activity, ideally in identified neuron subtypes in older animals.

Models of cerebellar learning predict that Purkinje cell activity instructs plasticity at the mossy fiber to deep nuclear neuron synapses, and that this plasticity contributes both to the acquisition and extinction of classically conditioned responses (Medina et al., 2000) and to motor learning in the vestibulo-ocular reflex (Raymond et al., 1996). In this view, Purkinje cells in cerebellar cortex are responsible for accurate timing of behaviors by sculpting the firing of deep nucleus neurons (or their equivalents in the vestibular nuclei). Behavioral results consistent with

this hypothesis were obtained in mice deficient in parallel fiber LTD, which can learn to blink to a tone following classical conditioning but exhibit inaccurate blink timing (De Zeeuw and Yeo, 2005). The two studies discussed here provide the first firm physiological evidence for the predicted forms of plasticity at the mossy fiber to deep nuclear synapse. It is intriguing to speculate that the LTP described by Pugh and Raman could participate in acquisition of eyeblink conditioning, while the LTD described by Zhang and Linden may play a role in extinction. Other forms of cerebellum-dependent learning, including adaptation of the vestibulo-ocular reflex or of reaching movements, are likely to rely on similar forms of plasticity. Because so much is known about the downstream effects of deep nucleus neuronal activity, the consequences of experimental manipulations that specifically enhance or abolish each type of synaptic plasticity can be assessed in behaving animals, providing one of the rare opportunities to forge a clear link between physiological plasticity and behavioral learning in vertebrates.

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Progress in Understanding Spatial Coordinate Systems in the Primate Brain

A new study in this issue of *Neuron* shows that when monkeys reach to a visual target, neurons in the dorsal premotor cortex compare the location of the target, the hand, and the point of visual fixation. The neurons therefore encode space through a combination of eye-centered and hand-centered coordinates.

We act on the world by reaching, grasping, manipulating, looking, avoiding, and performing hundreds of other actions on the objects around us. These behaviors depend on computing the relative spatial locations of objects and body parts. How does the brain coordinate spatially accurate behavior? The dorsal premotor cortex (PMd) of the monkey brain, and more specifically the caudal division of PMd (PMDc; see Figure 1), is densely connected to a network of motor structures, including the spinal cord, and is involved in the control of reaching. In a new study, Pesaran et al. (2006) show in this issue of *Neuron* that PMDc may guide the arm by means of a simultaneous comparison of hand location, eye location, and target location. Here I outline some of the previous experimental steps in understanding the representation of space in parietal and frontal cortical areas and discuss how the present finding significantly extends this line of research.

Retinal Receptive Fields Modulated by Extraretinal Factors

One of the first accounts of how neurons represent space was proposed by Andersen et al. (1985). They described visually responsive neurons in area 7a of the posterior parietal lobe of monkeys. Like classical visual neurons at most stages of the visual system, each neuron in area 7a had a visual receptive field on the retina. The magnitude of the response of a 7a neuron, however, was modulated by the angle of the eyes in the orbit. When the eyes were angled one direction, the neuron might become relatively unresponsive. When the eyes were angled another direction, the neuron might become highly responsive to visual stimuli. The two pieces of information that influenced the neurons, the location of the stimulus on the retina and the location of the eyes in the orbit, could in principle provide the location of an object with respect to the head.

Further work by Andersen and colleagues (Brotchie et al., 1995; Snyder et al., 1998) revealed that not only the angle of the eyes in the orbit, but the angle of the head on the trunk, and vestibular information about the position of the head in the world, also modulated the responsiveness or the "gain" of neurons in posterior parietal areas. From this work, a general model of spatial coding emerged. In this model, neurons have receptive fields on the retina, explicitly encoding space in eye-centered coordinates. The response gain of the neurons, however, is modulated by additional spatial factors. As a result, the pattern of activity across a population of neurons carries information about the location of a visual stimulus with respect to the eye, the head, the trunk, and the external world.