cAMP but *not* cGMP signaling negatively regulates the repulsive response downstream of CaN. Such negative regulation is likely to provide a feedback control mechanism between the CaMKII and the CaN pathway: high $[Ca^{2+}]_i$ influx can activate adenylate cyclase (AC) in addition to CaMKII and CaN; AC functions to increase local cAMP level, which in turn inhibits the CaN pathway, thus ensuring the attractive responses. Similarly, increased cAMP was also able to switch netrin-mediated repulsion to attraction, although the effect was stronger than that of CaN inhibition, suggesting that cAMP may act both upstream and downstream of the Ca^{2+} signaling.

Unanswered Questions

The work by Wen et al. provided an answer to the puzzle: how does local elevation of Ca^{2+} mediate bidirectional growth cone responses in a context-dependent manner? They demonstrated that the dynamics of growth cone Ca^{2+} exerted its effect on turning by controlling the balances between the activity of the kinase, CaMKII, and the phosphatase, calcineurin. However, many questions remain unanswered.

The majority of the data in this paper as well as in the previous literature indicates that the levels of *relative* Ca^{2+} increases ($\Delta[Ca^{2+}]_i$) determine the difference between CaMKII and CaN activation. It is thus puzzling or even paradoxical that global lowering of the cytosolic Ca^{2+} led to *preferential* activation of CaN over CaMKII. Considering that the FLIP-induced focal elevations of Ca^{2+} were the same relative magnitudes and decayed with the same kinetics under both normal and low global $[Ca^{2+}]$ conditions (Zheng, 2000), the implication was that, under certain circumstances, the absolute Ca^{2+} concentration rather than $\Delta[Ca^{2+}]_i$ determined the activations of CaMKII and CaN. One possible explanation is that lowered resting Ca^{2+} may change the distribution patterns of CaN and CaMKII such that CaN is more available within the growth cones than CaMKII. Consistent with this idea, the subcellular localization of CaMKII is subjected to many regulations (Griffith et al., 2003). Future experiments using GFP-tagged CaMKII should allow the visualization of its distribution in growth cones extending in normal or calcium-free medium.

Another unsolved issue is the downstream targets of CaMKII and CaN. Wen et al. tested PP1, a known target activated by CaN. Sure enough, inhibition of PP1 had the same results as inhibition of CaN. Previous work by other groups have shown that CaMKII and CaN-PP1 can regulate the phosphorylation status of tubulin, the microtubule-associated proteins such as MAP2 and Tau, as well as the growth-associated protein GAP43, all of which can affect filopodia and growth cone motility (reviewed by Zheng et al., 1996; Gomez and Spitzer, 2000). But the full spectrum of CaMKII/CaN targets and the cascades leading to growth cone turning still remain to be uncovered. Moreover, it is not clear if or how the CaMKII/CaN switch mechanism interacts with the Rho family small GTPase signaling pathways (which control actin dynamics) to produce coherent turning. Further studies are needed to address these questions.

Finally, the upstream events from the detection of axon guidance cues to the generation of distinct patterns of Ca^{2+} waves are also largely unknown. cAMP/cGMP and IP3 have been implicated in transducing the signals of activated guidance receptors into the opening or closing of calcium channels either at the plasma membrane or in intracellular compartments (Song and Poo, 2001). However, a direct molecular link between receptor activation and a known second messenger system is still missing except for certain signaling aspects downstream of neurotrophin receptors. Much work lies ahead of us before we can fully unveil the directional signaling involved in axon guidance.

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Endocannabinoids: Losing Inhibition to Increase Learning Capacity?

Recent work has implicated endocannabinoids in various forms of synaptic plasticity. In this issue of *Neuron*, Chevaleyre and Castillo describe a new mechanism whereby a CB1 receptor-mediated LTD of inhibitory synaptic transmission facilitates the subsequent induction of LTP in a narrow band of synapses surrounding a region of potentiated synapses.

Endocannabinoids have been shown to be involved in the retrograde regulation of synaptic transmission at a variety of brain synapses, most commonly at inhibitory synapses. A common phenomenon is depolarization-induced suppression of inhibition (DSI), whereby a transient depolarization of a postsynaptic cell elicits calcium-dependent endocannabinoid production which activates CB1 receptors on inhibitory terminals to reduce GABA release (Diana and Marty, 2004). This short-term

plasticity, which typically lasts for 5–30 s, occurs in many brain regions, such as the cerebellum (Kreitzer and Regehr, 2001b) and hippocampus (Wilson and Nicoll, 2001). Endocannabinoids can also mediate depolarization-induced suppression of excitation (DSE; Diana and Marty, 2004; Kreitzer and Regehr, 2001a; Ohno-Shosaku et al., 2002). More recently, it has been shown that endogenous cannabinoid release is involved in the induction of longer lasting forms of plasticity, in particular, long-term depression (LTD) of excitatory transmission in the striatum (Gerdeman et al., 2002) and LTD of inhibitory synaptic transmission (I-LTD) in the hippocampus (Chevaleyre and Castillo, 2003).

In this issue of *Neuron*, Chevaleyre and Castillo (2004) have expanded on their earlier observations regarding I-LTD in the hippocampus. Previously, they demonstrated that high-frequency stimulation of Schaffer collateral-commissural fibers elicits a persistent decrease in GABA transmission (Chevaleyre and Castillo, 2003). The mechanism responsible involves a metabotropic glutamate receptor (mGluR)-mediated calcium-independent release of the endocannabinoid 2-arachidonoyl glycerol from CA1 pyramidal neurons, which acts as a retrograde messenger to reduce GABA release. They also provided evidence that I-LTD is involved in E-S coupling potentiation associated with long-term potentiation (LTP) at excitatory synapses; that is, the increase in the ability of an EPSP to fire an action potential after LTP has been induced. Their new study expands on the role of I-LTD in LTP induction to show a unique regulatory effect of I-LTD. Specifically, they describe a form of metaplasticity, whereby induction of I-LTD can prime synapses so that LTP of excitatory transmission can subsequently be induced by stimuli that were previously subthreshold for LTP induction.

Chevaleyre and Castillo demonstrate that I-LTD is spatially restricted to within 20 μm of the site of glutamatergic activation. By using two whole-cell electrodes placed short distances apart, they show that stimulation via one electrode produces I-LTD of IPSCs elicited by the second electrode when the two electrodes are 20 μm or less apart. When the distance is increased, no I-LTD is seen. A criticism of the earlier study (see Freund and Hájos, 2003) was that experiments were performed at 25°C, a below-physiological temperature which will affect diffusion of glutamate and endocannabinoids, and thus raised the possibility that I-LTD would not occur at physiological temperatures. However, the current study demonstrates that I-LTD still occurs at 35°C and is spatially constrained to within 40 μm of the site of activation, which thus strengthens the case for this form of plasticity being physiologically important.

The major observation of this new work is that I-LTD induced by endocannabinoid release primes nearby excitatory synapses for subsequent LTP induction. By investigating a range of stimulus frequencies, Chevaleyre and Castillo demonstrate that stimulation of Schaffer collateral-commissural fibers at 10 Hz (200 stimuli) induces I-LTD without overtly affecting excitatory transmission. However, the induction of I-LTD potentiates the LTP observed in response to subsequent weak theta burst stimulation. This priming effect is seen immediately after 10 Hz stimulation has been delivered and lasts for at least 1 hr. It has the same pharmacological profile as I-LTD, suggesting that I-LTD is indeed responsible for priming: both I-LTD and priming are blocked by a CB1 receptor antagonist; they are also sensitive to group I mGluR antagonists; they are insensitive to NMDA receptor antagonism; and both are absent in CB1 receptor knockout mice. This latter observation is particularly satisfying, as some effects of cannabinoids, such as the reduction in hippocampal CA1 excitatory transmission by the synthetic cannabinoid WIN 55,212-2 (Hájos et al., 2001), may be mediated by a novel cannabinoid receptor, as they are still present in CB1 knockout animals.

The spatial specificity of the priming effect is also similar to that of I-LTD; induction of I-LTD with one focal electrode primes LTP at excitatory synapses stimulated by another focal electrode 10 μm, but not 40 μm, away. As expected, the LTP observed when the two electrodes are10 μm apart is input specific (i.e., induction of LTP via one electrode does not affect transmission evoked by the second electrode). This implies that a small zone exists surrounding the site of LTP induction where synapses are primed by I-LTD for future LTP induction. Therefore, a subsequent weak, normally sub-LTP inducing, stimulus arriving at synapses in this zone could induce LTP. Consistent with this hypothesis, the authors show that LTP is associated with a long-lasting CB1 receptor-dependent facilitatory effect on surrounding synapses.

A priming effect of endocannabinoids on LTP induction in the hippocampus has been observed previously. Carlson et al. (2002) demonstrated that a subthreshold LTP-inducing stimulus delivered up to 30 s after DSI can induce LTP. The current study of Chevaleyre and Castillo add a further complexity to this story, as I-LTD and DSI, although both mediated by CB1 receptors and a decrease in GABA release, differ not only in their duration but also in their dependence on postsynaptic calcium rises and the nature of the endocannabinoid molecules involved (Chevaleyre and Castillo, 2003).

It is well established that a reduction in inhibitory transmission can facilitate the induction of LTP (Wigström and Gustafsson, 1983) by enhancing the synaptic activation of NMDA receptors (Herron et al., 1985). Moreover, it has been shown previously that physiological patterns of activation can depress synaptic inhibition to facilitate the induction of LTP. In particular, Davies et al. (1991) showed that $GABA_B$ receptor activation is critical for the induction of LTP by a primed-burst tetanus (four stimuli delivered at 100 Hz preceded by 200 ms by a single shock). In this case, the priming pulse causes the activation of presynaptic $GABA_B$ autoreceptors on inhibitory terminals which reduces GABA release during the subsequent four stimuli, and hence increases the level of NMDA receptor activation. The mechanism of priming described by Chevaleyre and Castillo differs from that involving activation of presynaptic $GABA_B$ autoreceptors, particularly as $GABA_B$-mediated priming occurs at short time intervals (ms to second time scale), whereas endocannabinoid-mediated I-LTD primes synapses for at least an hour. Furthermore, $GABA_B$ receptor antagonists totally block priming-induced LTP (Davies et al., 1991), whereas the current study shows that cannabinoid receptor antagonists do not block LTP induced by theta burst stimulation (see Figure 8 in Chevaleyre

and Castillo, this issue of *Neuron*). Thus, it may be that I-LTD is responsible for a more subtle modulation of LTP induction, in that it induces a form of metaplasticity that alters the parameters required for subsequent LTP induction.

The current study adds a new insight into the role of the endogenous cannabinoid system in synaptic plasticity. It shows that endocannabinoids can depress, for prolonged periods of time, synaptic inhibition in a small region surrounding the site of LTP such that subsequent, ordinarily subthreshold LTP stimuli in this surround can elicit LTP. Theoretically, this depression of inhibition could facilitate learning and memory. However, it remains to be seen whether a similar process actually occurs in vivo and, if so, what its role is.

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