Neuron, Vol. 44, 743–748, December 2, 2004, Copyright ©2004 by Cell Press

Selective Reduction of Weak Synaptic Activity Awakens Dormant Synapses

Slutsky et al. (this issue of *Neuron)* report that by selectively filtering out low-level uncorrelated synaptic activity at NMDA receptors in hippocampal cultures they can unlock a large reserve of quiescent synapses and make them available for potentiation with theta burst stimulation. These findings differ from previously reported activity-dependent mechanisms in that inactivity does not necessarily increase synaptic activity globally.

In both the developing and mature nervous system there are clear-cut rules for the maintenance and induction of synaptic plasticity. Synapses that have coincidental activity are strengthened through Hebbian mechanisms. Classically, this relies on the voltage-dependent unblocking of NMDA receptors by Mg^{2+} ions, which occurs if synaptic activity and postsynaptic action potentials are closely spaced in time. Since strengthened synapses are more active, they in turn are also likely to have coincident activity. This positive reinforcement would result in an extremely unequal distribution of synaptic strength if there were not homeostatic mechanisms. Homeostatic plasticity provides the necessary global negative feedback to ensure that neurons maintain an optimal distribution of synaptic strength and activity (Turrigiano and Nelson, 2004). The present work of Slutsky et al. (2004) has taken a new approach and examines what determines the number of synapses available for Hebbian modification within a given neuron.

The authors used FM dye-based imaging techniques to show that mature cultured neurons clearly did not live up to their full potential, as they only used about half of their synapses during physiological levels of activity. In keeping with previous findings, optical estimates of release probability (P_r) at single synaptic terminals indicated that the majority of the terminals had a low P_r (Ryan et al., 1996; Murthy et al., 1997). Surprisingly, these low P_r synapses were found to be *resistant* to potentiation when strong plasticity inducing theta burst stimulation (TBS) protocols were applied. At first glance, this appears to contradict previous studies that describe an inverse relationship between short- and long-term plasticity and P_r; where synapses that release the least have the most to gain (Ryan et al., 1996; Murthy et al., 1997). However, Slutsky et al. made a key observation that reconciles these differences. They found that ongoing synaptic activity strongly decreased the susceptibility of the low P_r synapses to plasticity. Intriguingly, suppressing action potentials with sodium channel blockers (1 μM tetrodotoxin [TTX]) for as little as 4 hr dramatically increased the ability of synapses to be potentiated. Indeed, this potentiation was found to be inversely proportional to P_r. Moreover, the short-term TTX treatment did not alter basal P_r or the number of active synapses, suggesting that the underlying mechanisms of these actions were distinct from the homeostatic-based increases in presynaptic function observed with prolonged TTX treatment (>2 days; Murthy et al., 2001). Such a robust activity-dependent gating of synaptic plasticity could be one way in which neural connections gain stability, as background activity reduces the number of synapses that are available for modification by experience.

The release of brain-derived neurotrophic factor (BDNF) is strongly implicated in hippocampal plasticity, especially in potentiation triggered by specific patterns of activity including TBS (Kang et al., 1997). Slutsky et al. thus focused on BDNF and assessed its role in TBS-induced plasticity that is sensitized by brief inactivity due to TTX treatment. In a set of clear-cut experiments, they demonstrated that a brief application of the BDNF neutralizing antibody blocked TBS-induced potentiation in TTX-treated cultures. They also showed that the application of BDNF mimicked and occluded the effect of short-term inactivity on potentiation, confirming its importance in this phenomenon. What remains obscure is the connection between short-term inactivity and the sensitization of the BDNF pathway. Does ongoing activity decrease the release of BDNF or affect its detection? This is a critical missing link in our understanding of the mechanisms governing the gating of plasticity. Future experiments involving the direct measurement of BDNF release and activation of its receptors during TBS will shed light on this issue.

Regarding mechanism, Slutsky et al. show that a decrease in Ca^{2+} influx was pivotal in translating reduced synaptic activity to the biochemical processes underlying the sensitization to TBS. The effect of reducing Ca^{2+} influx by inhibiting overall activity with TTX, or by directly antagonizing voltage-gated L-type Ca^{2+}channels or NMDA receptors, was qualitatively similar. It is important to note that in contrast to TTX, which reduces ongoing activity and multiple downstream messengers, L-type channel blockers have little effect on synaptic activity and block only a subset of calcium current associated with activity (Liu et al., 2003). This finding suggests that a specific $[Ca^{2+}]_i$ pathway, similar to that previously reported for activating immediate early gene expression (Murphy et al., 1991), reduces the number of potentially plastic synapses. In addition, this result is consistent with a recent report in which the activation of L-type channels in dendrites of CA1 pyramidal neurons were implicated in the modulation of long-term plasticity (Yasuda et al., 2003). Although the increased sensitivity in a vast reservoir of dormant synapses to TBS induced by a short-term reduction in Ca^{2+} influx is unprecedented, there were limitations. If treatment with agents that reduced Ca^{2+} influx was extended for up to 48 hr, no gating of theta burst-induced plasticity was observed. Apparently, the lack of an effect of chronic activity reduction was in part due to the activation of homeostatic plasticity mechanisms, which occluded potential theta burst-induced plasticity. Evidence for this included a general increase in baseline presynaptic function.

So then, how could one turn on plasticity permanently without inducing homeostatic plasticity? Slutsky et al. devised an ingenious approach to solve this dilemma. Since biochemical processes are tuned to both the amplitude and frequency of Ca^{2+} signals, they specifically blocked weak synaptic events that would evoke low levels of Ca^{2+}, but not stronger correlated activity that would drive higher intracellular Ca^{2+} levels. They achieved this selective inhibition by taking advantage of the voltage-dependent properties of NMDA receptor $[Mg^{2+}]_o$ block. Increasing $[Mg^{2+}]_o$ levels from 0.8 to 1.2 mM did not change the excitability or resting state of neurons, but strongly reduced NMDA receptor-mediated currents and the associated Ca^{2+} changes near resting membrane potential (by ~60%). However, at more depolarized potentials, in a range where the Mg^{2+} block was minimal, increases in $[Mg^{2+}]_o$ attenuated NMDA receptor activity to a lesser extent. Hence, the authors inferred that weak NMDA receptor-mediated EPSPs and their associated Ca^{2+} entry would be blocked, while the correlated activity that is strongly depolarizing and drives large Ca^{2+} influxes would remain relatively unaffected by increased $[Mg^{2+}]_o$. In contrast to the effects of global reduction of Ca^{2+} entry, which were lost after 2 days, this filtering of low-level NMDA receptor activity by modest increases in $[Mg^{2+}]_o$ led to a relatively long-lasting enhancement of low P_r synapse recruitabilty by theta burst stimulation. In addition, these effects of increasing $[Mg^{2+}]_o$ were reversible. The finding that a relatively small change within the physiological concentration range of this mineral can impact the degree of NMDA receptor activity and have a significant effect on the gating of plasticity is unprecedented. However, future experiments will need to exclude other potential targets of Mg^{2+} action, including voltage-gated Ca^{2+} channels (Yasuda et al., 2003), and indeed show that uncorrelated activity is blocked by this treatment.

The mechanisms underlying the unlocking of synapses by elevated $[Mg^{2+}]_o$ maybe multifaceted. Although the authors do not make it clear whether it involves the sensitization of the BDNF pathway, they find a new twist to this phenomenon when they look at the expression of NR2B. This was made in an attempt to compare their cultures to the developing nervous system, where immature, highly plastic synapses express NR2B. They found that increased $[Mg^{2+}]_o$ led to a relative increase in the role of NR2B receptors in NMDA receptor-mediated EPSCs. This increase in NR2B EPSCs was shown to be in part responsible for the gating of low P_r synapses by subsequent theta burst. In some ways, these results differ from recent studies showing that NR2B receptor expression is necessary for long-term depression and not long-term potentiation (Liu et al., 2004; Massey et al., 2004). However, the results are consistent with previous findings showing that ectopic expression of NR2B can increase learning and memory as well as LTP (Tang et al., 1999).

Clearly, more work is necessary before we fully understand the implications of the current findings for plasticity in the mature nervous system. The intricate connections between correlated bursting activity, BDNF, and NMDA receptor function still need to be elucidated. Future directions will also involve selectively manipulating the levels of uncorrelated versus correlated NMDA receptor-mediated activity in vivo. Although more work needs to be done, Slutsky et al. have led us through some of the tortuous interactions of pathways initiated by dynamic Ca^{2+} signals and have provided us with the first glimpse of the process by which synaptic plasticity can be gated at quiescent synapses.

The authors (G.A. and T.H.M.) are supported by grants from Canadian Institutes for Health Research.

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Neural Processing at the Speed of Smell

Olfaction is typically described as behaviorally slow, suggesting neural processes on the order of hundreds of milliseconds to seconds as candidate mechanisms in the creation of olfactory percepts. Whereas a recent study challenged this view in suggesting that a single sniff was sufficient for optimal olfactory discrimination, a study by Abraham et al. in this issue of *Neuron* sets out to negate the challenge by demonstrating increased processing time for discrimination of similar versus dissimilar stimuli. Here we reconcile both studies, which in our view together support the notion of a speed-accuracy tradeoff in olfactory discriminations