in normal mice nearly all of the axon shafts project through this region. A conditional deletion of *plexinA1* in sensory neurons but not spinal cord resulted in the same aberrant trajectory, arguing that plexinA1 expression in sensory neurons is required.

Curiously, plexinA1 is not required for the development of normal projections of proprioceptive axon collaterals into the ventral spinal cord, where many of these fibers make direct synaptic contacts with motoneurons. Despite their aberrant pathway through the medial portion of dorsal spinal laminae, proprioceptive axons in *plexinA1*-deficient mice terminate in the same regions as in wild-type mice. Judging by the intensity of PV staining in the intermediate and ventral cord, there was no obvious deficit in these projections, and the authors note no particular behavioral phenotype that would suggest a major disruption of sensory input to the cord. Comparatively little is known concerning how proprioceptive afferents are guided down to ventral laminae of the spinal cord. Neurotrophin-3 (NT3) has been proposed as a chemoattractant for these axons (Genç et al., 2004; Ringstedt et al., 1997), as NT3 is expressed in the ventral cord and stimulates growth and branching of trkC+ axons in vitro (Lentz et al., 1999). This idea is difficult to test genetically, however, because NT3 is required for expression of the ETS gene *ER81*, which itself is required for the normal ventral projection of proprioceptive axon collaterals (Patel et al., 2003). Indirect evidence suggests that attractive cues from the ventral cord are not required; these afferents grow ventrally into cord segments in which the ventral half has been replaced by an inverted dorsal half (Sharma and Frank, 1998). A satisfactory explanation of how these ventral projections develop awaits further rigorous tests such as those made in the current report.

Finally, the authors demonstrate that the presence of proprioceptive axons in the dorsal laminae seen in *plexinA1*-deficient mice is correlated with the exclusion of two classes of small-caliber (unmyelinated and thinly myelinated) cutaneous sensory axons from their normal target region in the dorsal cord. At birth, the projections of $IB4^+$ (unmyelianted) and $vGlut1^+$ (thinly myelinated) cutaneous axons were normal in $plexinA1^{-/-}$ mice, but after 1 week, the terminal arbors of these axons were absent from the medial portion of dorsal laminae. The correlated presence of proprioceptive axons and absence of cutaneous axons in this area in $plexinA1^{-/-}$ mutants suggest that it is the presence of the proprioceptive fibers that is responsible for the exclusion of these two classes of cutaneous afferents. Although proprioceptive axons might repel cutaneous axons directly, the delayed exclusion of cutaneous axons in *plexinA1* mutants is temporally correlated with the aberrant migration of large numbers of oligodendrocytes into this region. Oligodendrocytes are known to be inhibitory to the growth of certain classes of sensory neurons (He and Koprivica, 2004). Thus, the authors suggest that the exclusion of cutaneous axons from dorsal laminae may be mediated by repulsive cues emanating from the oligodendrocytes that migrate with the misdirected proprioceptive axons.

Oligodendrocyte-mediated exclusion might contribute to the development of axonal projections in wild-type mice as well. The presence of myelinated proprioceptive axons and their associated oligodendrocytes in ventral laminae of the spinal cord could block postnatal growth of cutaneous afferents into the ventral cord, keeping them confined to the dorsal horn. This could provide an explanation for why blockade of npn-sema3D signaling does not result in the invasion of the ventral cord by cutaneous axons. As the authors conclude, programs of axon exclusion are important determinants of the patterning of sensory afferent projections.

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They're Plastic, but They Recycle

Dendritic spines form and grow during hippocampal long-term potentiation (LTP). In this issue of *Neuron*, a new study by Park et al. uses both serial reconstruction electron microscopy and time-lapse imaging to show that plasma membrane for such spine expansion is trafficked from recycling endosomes that reside locally at the spines themselves.

Neuroscientists have long conjectured that information processing and storage might involve the physical growth of synapses—even before the experimental means to rigorously test this idea became available. Not long following the discovery of LTP in the hippocampus and its potential role as a cellular substrate of memory, researchers began to test whether growth of dendritic spines underlies tetanus-induced synaptic

plasticity. Thus, early efforts combined high-frequency synaptic stimulation with post-hoc analyses of spine morphology. However, such efforts faced two challenges: the need for high-resolution imaging, which at the time could only be achieved using electron microscopy, and the need to distinguish specifically those synapses that received the stimulus, since only a fraction of synapses within a small region are activated by any given electrode. Although several studies found spine changes associated with LTP, early results were mixed and fell short of establishing a clear relationship between spine growth and plasticity (Yuste and Bonhoeffer, 2001).

This situation began to change when advances in fluorescence imaging techniques enabled investigators to track changes at individual synapses. Studies from several laboratories found that spines expanded, lengthened, or grew de novo following a plasticity-inducing stimulus. Not only did spines grow during LTP, but they also added filamentous actin and new AMPA receptors (Tada and Sheng, 2006; Matsuzaki, 2006).

Several candidate mechanisms for modifying spine actin during LTP have been identified (Calabrese et al., 2006; Tada and Sheng, 2006; Carlisle and Kennedy, 2005). But where does the membrane used to support spine growth come from? The laboratories of Michael Ehlers and Kristin Harris have teamed up to provide some satisfying answers (Park et al., 2006 [this issue of *Neuron*]). It seems that the spines themselves contain all the needed manufacturing equipment and an abundant supply line for boosting their plasma membrane upon demand. Furthermore, spines reuse material that has already visited the plasma membrane. The key orchestrator is the recycling endosome.

Mammalian cells are efficient recyclers. Certain transmembrane receptors are known to be reused hundreds of times. Endocytic trafficking uses complex, interconnected pathways to transport membrane and proteins to various destinations (Maxfield and McGraw, 2004). First, membrane invagination occurs, often—but not always—through clathrin-coated pits. These pinch off (becoming "primary" endosomes) and fuse with one another to form sorting endosomes. From here, material is sorted either back to the membrane, to late endosomes (often destined for degradation in lysosomes), or to another intermediate organelle called the endocytic recycling compartment (ERC). The ERC sends material either to the plasma membrane or to the *trans*-Golgi network. Together, the sorting endosome and the ERC are sometimes referred to as "recycling endosomes," as depicted in Figure 3 of Park et al. (2006). Each of these trafficking steps is finely controlled by specialized families of proteins, including endophilin, dynamin, amphiphysin, AP2 and other adaptor molecules, small GTPases of the Rab and Arf families, epsins and espsin homology domain proteins, and SNARE complexes (Maxfield and McGraw, 2004). Phospholipids, and lipid kinases and phosphatases, also play crucial roles (Di Paolo and De Camilli, 2006). Park et al. (2006) utilize the fact that transport to recycling endosomes depends on the SNARE protein syntaxin13, and transport from the ERC to the plasma membrane requires Rab11 and the Eps15-homology domain protein EHD1/Rme1.

First, Park et al. (2006) showed, using either live-cell imaging or electron microscopy in three distinct types of preparations from hippocampus (dissociated cultures, acute slices, and in vivo), that endosomal compartments are within or beneath nearly all spines. Spines with endosomes tended to be larger than those that lacked them. The authors calculated that the surface area of internal endosomal membranes nearly equals that of the spines themselves and therefore represents an adequate resource for spine growth.

They next demonstrated that spine maintenance requires recycling endosomes. Park et al. (2006) accomplished this by transfecting neurons with mutant or truncated versions of the proteins Rab11a, EHD1/Rme1, or syntaxin13, and showed that spine numbers drastically decreased. Conversely, they showed that overexpression of wild-type versions of these proteins caused a doubling of spine numbers. In an elegant series of time-lapse studies, they used the cell permeant TAT sequence to carry mutant syntaxin13 across the dendritic membrane and showed, thereby, that inhibition of endosomal recycling can induce spine shrinkage and collapse within an hour.

These experiments implicate recycling endosomes as the key source of membrane that maintains spines at steady state, but what about during LTP? Park et al. (2006) induced LTP in dissociated hippocampal cultures using a chemical stimulus (elevated glycine, which abruptly enhances NMDA receptor activation by endogenous glutamate). They observed that, as in hippocampal slices stimulated synaptically, chemical LTP is associated with an increase in the size and number of spines. However, these increases were prevented when endosome recycling was blocked. Finally, the authors demonstrate, using a pH-sensitive form of GFP tagged to transferrin receptors, that LTP triggers exocytosis of recycled vesicles within spines. In individual spines, the timing of exocytosis correlated with the emergence of new spines or the expansion of existing spines, implying a cause-effect relationship.

Remarkably, the chemical LTP stimulus also induced, within minutes, a translocation of endosomes (tagged via trasferrin reuptake) from the base of the spine toward and into the spine head. Time-lapse imaging of fluorescent transferrin receptor revealed the budding off of presumptive recycled vesicles that moved toward the spine plasma membrane. This relocalization of endosomal compartments after LTP was confirmed using serial section EM of hippocampal slices in which potentiation was induced by theta-burst stimulation.

These exciting new studies answer several key questions and raise intriguing new ones. Are membrane recycling pathways in dendrites similar to those in other cells, or are there specialized mechanisms? Are endosomal compartments anchored to the base of spines, and if so, how? What mechanism either releases them or promotes their expansion into spine heads during LTP? In addition to AMPA receptors, what other cargo is to be found in recycling endosomes in spines? Do such cargoes regulate synapse stability or adhesion? How is membrane addition coordinated with actin remodeling? Spines are heterogeneous in terms of their protein (and probably lipid) composition, so how does endosomal trafficking relate to the variety of spine

characteristics? Finally, LTP is triggered through calcium-dependent mechanisms. Does calcium regulate endosomal trafficking in spines, and if so, what are its downstream effectors? Long-term depression (LTD), which is in some sense the opposite of LTP and is induced by different patterns of synaptic activity, is linked to spine shrinkage (Zhou et al., 2004). Does LTD involve changes in endosomal recycling in spines and/or translocation of recycling compartments (see Brown et al. [2005])? Spines may be avid recyclers, but there is still much to be learned about their local ecology.

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Sleepy Dialogues between Cortex and Hippocampus: Who Talks to Whom?

During NREM sleep, neocortical neurons undergo near-synchronous transitions, every second or so, between UP states, during which they are depolarized and fire actively, and DOWN states, during which they are hyperpolarized and completely silent. In this issue of *Neuron*, Isomura et al. report that slow oscillations of membrane potential occur near-synchronously not only in neocortex but also in entorhinal cortex and subiculum. Within the hippocampus proper, pyramidal neurons lack the bistability of UP and DOWN states, but their firing is strongly modulated by cortical activity during the UP state. Intriguingly, many hippocampal neurons fire during the cortical DOWN state. Thus, during sleep UP states, the cortex can talk to the hippocampus, but it is unclear whether the hippocampus talks back.

The slow oscillation (SO)—a near-synchronous alternation of UP and DOWN states at around 0.8 Hz that occurs in virtually all excitatory and inhibitory cortical neurons—underlies the most pervasive and powerful of all EEG rhythms: the slow waves of NREM sleep (Steriade, 2006). The SO has several intriguing features. First, it is the default mode of activity of cortical circuits: it is seen not only in the sleeping cortex, but it persists after thalamectomy, in isolated cortical slabs, and even in cortical slices, being initiated, maintained, and terminated through the interplay of intrinsic currents and intracortical network interactions.

Second, the SO enforces a unique state of near-absolute synaptic stillness, for a good fraction of a second, over the entire cortical mantle. This forced inactivity, which invariably follows any form of activation of cortical neurons into an UP state, spontaneous or triggered by stimuli, is a remarkable expression of the intrinsic bistability of cortical networks in states of reduced consciousness, such as deep NREM sleep and certain forms of anesthesia.

Third, the SO behaves like a traveling wave: an UP state is ignited locally by the activation of local populations of neurons, more often than not in prefrontal cortex, after which it invades progressively other cortical areas over tens to a few hundred milliseconds (Massimini et al., 2004). Indeed, though born and discovered in the neocortex (Steriade, 2006), in recent years the SO has traveled steadily to conquer many other brain regions. Currently, we know that the SO also entrains the thalamus, the basal ganglia, the paleocortex, and the hippocampus. And now, using multiple intracellular and field potential recordings in the rat, Isomura et al. (2006) have nicely demonstrated a sequential propagation of cortically generated UP and DOWN states through the entorhinal cortex and the subiculum down to the dentate gyrus. Exactly how the SO might travel from one area or structure to the next is still unclear. Although corticocortical and corticofugal connections acting upon already primed neuronal targets are a likely mechanism, it is possible that subcortical structures, such as the thalamic reticular nucleus, may also play a role.

Fourth, the slow oscillation is responsible for grouping most other sleep rhythms (Steriade, 2006). Thus, the onset of the UP state in the cerebral cortex sends a strong volley of spikes down to the GABAergic neurons of the reticular thalamic nucleus, which in turn trigger recurring sequences of spindle oscillations in thalamocortical neurons. Similarly, the depolarized UP state of the SO favors the intermittent appearance of wakefulness-like fast rhythms during sleep. Isomura et al. (2006) as well as Mölle et al. (2006) have now shown that this grouping role extends to the hippocampus, where sharp wave-ripple complexes reliably follow cortical SO after a delay of tens of milliseconds.

Fifth, though SO are the largest of neural waves, and though they invade the cortex a thousand times a night, it is not clear whether they serve any function at all—apart from making us less conscious. However, we know that SO underlie EEG slow wave activity (0.5–4.5 Hz)—a reliable indicator of sleep need that increases with time awake and decreases during sleep. Thus, an attractive function for the SO itself, or for the