tactical pathfinding. Thus, axons from the VL, which normally project to the rostral cortex (motor cortex), target instead the parietal cortex (somatosensory cortex; caudal to the motor cortex) in the absence of ephrin-A5 and EphA4. This defect is observed with a low frequency in ephrin-A5 (35%), EphA4 (12.5%), and EphA7 (50%) single mutants, supporting the view that several EphA receptors and ephrin-A ligands are involved in vivo in setting up the topographic mapping of thalamocortical axons. Finally, analysis of thalamocortical connectivity in ephrin-A5/EphA4 double mutants at the time when thalamic axons are invading the ventral telencephalon showed that the topographic sorting of thalamic axons is already shifted in this intermediate region—additional evidence supporting the role of the ventral telencephalon in this process.

**ephrins Contribute to the Formation of the Cortical Somatotopic Map**

In addition to their role in establishing the topographic organization of thalamic projections to distinct cortical regions, Dufour et al. (2003) investigated whether ephrin-A/EphA interactions are required for topographic mapping within an individual cortical area. Ephrins have been previously shown to play a role in the formation of other intra-areal topographic maps (Wilkinson, 2001), but their implication in cortical mapping has remained elusive. Projections from the VB to the barrel field of the somatosensory cortex are less precisely organized in ephrinA5/EphA4 double mutants than in control mice, suggesting that the topographic arrangement of somatosensory projections from the dorsal thalamus to the cortex is compromised in the absence of normal ephrin-A/EphA signaling. These results are consistent with the postnatal pattern of expression of ephrin-A5 and EphA4 in the cortex and dorsal thalamus, respectively. Thus, although additional experiments need to be performed to extend these observations to other cortical regions, another important message of this paper is that the same set of mapping labels is used differentially for the generation of topographic maps among different thalamocortical projections (inter-areal topography) and within individual cortical regions (intra-areal topography).

**Open Questions**

The recent findings by the Polleux and Vanderhaeghen groups have opened new venues for understanding how thalamocortical mapping is established. For example, several questions are raised regarding the role of Ngn2 in specifying rostral thalamic axon responsiveness to ventral telencephalic cues. What are the downstream effectors of Ngn2 in the dorsal thalamus? Is Ngn2 sufficient to direct connections from any dorsal thalamic nucleus toward the rostral cortex? What factor(s) prevent the expression of Ngn2 in the caudal lateral dorsal thalamus, thus enabling this region to project toward the caudal cortex? The identification of ephrin-A ligands as mediators of the topographic mapping of thalamocortical axons, along with the high expression of EphA receptors in the rostral thalamus, would suggest that Ngn2 directly or indirectly induces the expression of these receptors in the thalamus. Consistent with this hypothesis, Kania and Jessell (2003) have recently shown that the establishment of topographic projections between spinal cord motor neurons and the limb mesenchyme is controlled, in part, through LIM homeo-

**“Resistant” Channels Reluctantly Reveal Their Roles**

Presynaptic calcium influx is mediated by a variety of different calcium channel subtypes with distinct pharmacological and biophysical properties. In this issue of *Neuron*, Dietrich et al. show that although Ca$_{v}$2.3 calcium channels do not contribute to fast transmitter release at hippocampal mossy fiber synapses, they play a specialized role in induction of multiple presynaptic forms of synaptic plasticity.
Calcium ions in nerve terminals are multipurpose signaling molecules that play crucial roles in evoking neurotransmitter release, regulating several forms of synaptic plasticity over different time scales, and activating biochemical cascades. Several types of voltage-gated calcium channels (VGCCs) control the presynaptic influx of calcium in response to action potentials. VGCCs were initially characterized based on their electrophysiological properties and sensitivity to selective antagonists (Catterall, 2000). The use of the toxins ω-conotoxin-GVIA and ω-agatoxin-IVA, selective for N- and P/Q-type calcium channels, respectively, established the presence of these channel types in presynaptic terminals and demonstrated their central role in transmitter release. Dihydropyridine-sensitive L-type calcium channels and low-threshold T-type calcium channels are not usually present in nerve terminals but mediate transmitter release at specialized synapses. A toxin-resistant component of presynaptic calcium current often remained in the presence of blockers of L-, N-, P/Q-, and T-type calcium channels, and this resistant calcium current was termed “R-type.” Many questions remain regarding the identity of R-type calcium channels and their role in presynaptic function.

Molecular identification of calcium channels based on their pore-forming α1 subunit suggested that α1E contributes to R-type current (Piedras-Renteria and Tsien, 1998; Tottene et al., 2000). R-type current, however, has been difficult to study because of the lack of a suitable blocker. The toxin SNX482 blocks heterologously expressed α1E but only partially blocks R-type current in cultured neurons (Newcomb et al., 1998). Ni2+ also partially blocks R-type current, and it can also affect other channels, such as T-type calcium channels. Most significantly, a substantial fraction of R-type current remained in knockout mice in which the gene for the α1E subunit, Ca2,3, was disrupted (Wilson et al., 2000). Thus, R-type current appears to be heterogeneous, and there is evidence that some R-type currents may be mediated by undefined calcium channel α1 subunits.

Several studies have shown that R-type calcium current is present in presynaptic terminals and have investigated their contribution to synaptic transmission. At the cerebellar parallel fiber to Purkinje cell synapse (Mintz et al., 1995) and at the calyx of Held synapse (Wu et al., 1998), R-type current mediates ~25% of the total presynaptic calcium influx, but R-type current alone produces synaptic currents that are reduced to 1%–2% of their control amplitudes. This indicates that calcium influx through R-type channels is inefficient at evoking transmitter release. These findings are consistent with the discovery that Ca2,3 channels lack the “synprint” domain that allows N and P/Q channels to interact with SNARE proteins and synaptotagmin, presynaptic proteins involved in vesicle fusion (Catterall, 2000). Accordingly, antibodies showed that Ca2,3 channels were diffusely distributed around the terminal, in contrast to the P/Q-type channels that colocalized with markers for release sites (Wu et al., 1999). These findings highlight questions about the role of R-type channels, in particular why these calcium channels are present in nerve terminals if they are poorly coupled to transmitter release. In this issue of Neuron, Dietrich and colleagues answer this question at mossy fiber terminals in the hippocampus (Dietrich et al., 2003).

A key aspect of their study is the recognition that in addition to rapidly triggering transmitter release, calcium ions entering a presynaptic terminal can act on longer time scales to produce several forms of synaptic plasticity. Calcium entering a nerve terminal during an action potential generates a high-concentration “microdomain” in the vicinity of the calcium channel. Calcium channels of the N and P/Q types are closely associated with proteins involved in vesicle fusion and are ideally situated to provide the highly localized calcium signals of 10 to 100 μM (CaCaM) that last for about a millisecond and rapidly trigger transmitter release (Figure 1). As calcium equilibrates throughout the terminal, a small (<1 μM) and slowly decaying calcium signal persists. This residual calcium (CaCaM) is responsible for several forms of presynaptic plasticity including (1) paired-pulse facilitation, an enhancement of synaptic strength observed with paired stimuli that lasts 10’s to 100’s of milliseconds, (2) frequency facilitation, a progressive enhancement of synaptic strength that occurs during repetitive stimulation at low frequencies, and (3) posttetanic potentiation (PTP), a form of synaptic enhancement that results from more prolonged stimulation and lasts for seconds or minutes. A multitude of studies have shown that CaCaM is responsible for these forms of plasticity (Zucker and Regehr, 2002). In addition, although multiple forms of mossy fiber long-term potentiation (LTP) have been described, at least one type of mossy fiber LTP requires elevation of presynaptic calcium (Castillo et al., 1994). These diverse actions of presynaptic calcium raise the intriguing possibility that different sources of calcium influx, possibly through different calcium channels or channels with different presynaptic localization, may play specialized roles. Ca2,3 channels in particular appear well suited for contributing selectively to CaCaM that underlies various forms of synaptic plasticity but not to CaCaM that drives transmitter release.

Dietrich et al. have used a variety of techniques to demonstrate a specialized role of R-type calcium channels at mossy fiber synapses in area CA3 of the hippocampus of adult mice. By imaging presynaptic calcium influx in mossy fiber terminals, they find that a fraction of calcium influx remains in the presence of toxins for N- and P/Q-type channels, indicating the presence of R-type current. In a Ca2,3 knockout mouse, the N- and P/Q-type channel toxins block most if not all of the calcium influx, indicating that in these terminals the R current is predominantly mediated by the Ca2,3 channels. Previous studies had shown that R-type calcium channels help to evoke transmission at mossy fiber synapses in young rats (Gasparini et al., 2001), but Dietrich et al. show that in adult mice, R-type calcium channels do not contribute to synaptic transmission. This is consistent with developmental changes in the participation of different channel types observed at other synapses. Their principal finding is that in the Ca2,3 knockout, although baseline transmission appears unaffected at the mossy fiber synapse, both LTP and PTP are significantly impaired. Although both pre- and postsynaptic Ca2,3 channels are affected in the knockout mouse, the alterations of LTP and PTP are likely to result from changes in presynaptic calcium channels because of...
the known role of presynaptic calcium in regulating these forms of plasticity. The authors go on to show that by increasing the frequency of their induction protocol, LTP and PTP in the knockout are restored. These results imply that calcium entering via Ca\textsubscript{2.3} channels doesn't contribute to the Ca\textsubscript{res} that drives release, but does contribute to the Ca\textsubscript{rel} that underlies LTP and PTP. In the Ca\textsubscript{2.3} knockout mouse, increasing the frequency of the tetanus enhances Ca\textsubscript{res} through the N- and P/Q-type channels, thereby restoring these forms of plasticity.

To further clarify the selective contribution of Ca\textsubscript{2.3} calcium channels to Ca\textsubscript{res}, the authors show that when N and P/Q channels are blocked, a single presynaptic action potential does not evoke any transmitter release. However, release begins to occur after a number of stimuli delivered at high frequency. This suggests that with repetitive stimulation, Ca\textsubscript{res} through R-type channels can accumulate to sufficient levels to weakly drive transmitter release. This conclusion is supported by the finding that release is almost completely blocked by N- and P/Q-channel toxins in the Ca\textsubscript{2.3} knockout even after extremely long high-frequency stimulation. As a further indication that Ca\textsubscript{2.3} channels may selectively contribute to Ca\textsubscript{res}, the authors show that the calcium influx through Ca\textsubscript{2.3} channels facilitates during stimulus trains. Thus, during stimulus protocols that induce PTP or LTP, the fraction of calcium influx through Ca\textsubscript{2.3} channels increases and Ca\textsubscript{res} becomes selectively enhanced relative to the Ca\textsubscript{rel} driving release that is produced only by other, non-Ca\textsubscript{2.3} channel types.

The authors have thus demonstrated a specialized function for Ca\textsubscript{2.3} calcium channels at mossy fiber presynaptic terminals in regulating synaptic plasticity. While calcium entering through Ca\textsubscript{2.3} calcium channels does not support rapid transmitter release, it is important because of its contribution to Ca\textsubscript{res}. As a result, Ca\textsubscript{2.3} channels contribute to PTP and LTP without affecting release probability. Remarkably, Dietrich et al. show that two forms of presynaptic short-term plasticity, paired pulse facilitation and frequency facilitation, were not altered in the Ca\textsubscript{2.3} knockout or when R-type channels were partially blocked with Ni\textsuperscript{2+}. This finding suggests that calcium contributes to paired-pulse facilitation and frequency facilitation by interacting with a site having different spatial localization or calcium sensitivity from sites regulating PTP and LTP. Additional studies are needed to further characterize specific molecular targets involved in the regulation of the many forms of presynaptic plasticity. Another possibility that remains to be explored is whether Ca\textsubscript{res} generated in the proximity of Ca\textsubscript{2.3} channels may also play a role in synaptic plasticity or activation of biochemical signaling pathways, in addition to its contribution to Ca\textsubscript{res} (see Figure 1). An interesting implication of this study is the possibility that presynaptic Ca\textsubscript{2.3} calcium channels could be targeted selectively by presynaptic modulation by G protein-coupled receptors or other signaling pathways, enabling selective regulation of specific forms of synaptic plasticity dependent on Ca\textsubscript{res}. The findings of Dietrich et al. have thus revealed new roles for the Ca\textsubscript{2.3} calcium channel in presynaptic function, while suggesting many directions for further studies.

Stephan D. Brenowitz and Wade G. Regehr
Department of Neurobiology
Harvard Medical School
220 Longwood Avenue
Boston, Massachusetts 02115

Selected Reading


