mechanism of such AMPA responses or by glutamate spillover. The finding that AMPA autoreceptor responses are still present in mitral cell dendrites but cannot be evoked in a neighboring dendrite projecting to the same glomerulus in the Cx36 knockout mouse supports the argument that this coupling is electrical and depends critically on Cx36.

Interestingly, Cx36 is not required for all synchronized responses in pairs of mitral cells projecting to the same glomerulus. A type of synchronization that occurs on a much slower time scale can be elicited by application of a pharmacological blocker of glutamate uptake (TBOA), and this synchronized response is not uncoupled in the Cx36 knockout mouse. Here, glutamate spillover seems a good bet for underlying synchronization.

So how does synchronized firing in mitral cells projecting to the same glomerulus relate to the olfactory coding mechanism? Although this question has not yet been answered, the Cx36 knockout mouse shows great promise as a molecular tool to address this central problem. Genetic disruption of electrical coupling between pairs of mitral cells should now make it possible to begin to determine whether this manipulation has functionally relevant consequences for the coding of olfactory information. Of special interest would be experiments employing natural odor stimuli at physiologically relevant concentrations to replace current injection. Equally important would be experiments at the behavioral level, not unlike those that were used in mice with targeted deletions in specific olfactory signal transduction genes (Kelliher et al., 2003). Of course, all of this is easier said than done. It is clear that expression of Cx36 is not limited to mitral cells in the olfactory bulb and occurs also in other parts of the olfactory system (such as the main olfactory epithelium and the accessory olfactory system [Zhang and Restrepo, 2003]) as well as in other parts of the brain. A tissue-specific knockout mouse may be the answer to circumvent these problems. Tackling the functional significance of synchronized firing for odor coding will remain a challenge that is likely to generate exciting results for years to come.

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DOI 10.1016/j.neuron.2005.05.015

Conventional Synapses for Unconventional Cells

Powerful synapses between climbing fibers (CF) and Purkinje cells are crucial to cerebellar motor learning. In this issue of *Neuron*, Lin and colleagues provide compelling evidence for the existence of direct synaptic contacts between CFs and NG2-expressing glia cells, adding to the intrigue of neuro-glial interactions.

Neurons in the inferior olive form characteristic synapses with cerebellar Purkinje cells known as climbing fiber (CF) synapses (Eccles et al., 1964). In the adult brain, each Purkinje cell is contacted by a single CF that closely follows the arborizations of the proximal dendrites and forms synaptic contacts at hundreds of release sites (Palay and Chan-Palay, 1974). CF inputs provide an important error signal during planned or ongoing movements and help to induce long-term cerebellar learning. CF activation can control the strength of parallel fiber synapses, formed by granule cells, through long-term depression (Ito, 2001), and on short time scales through the release of endocannabinoids (Brenowitz and Regehr, 2005). However, it is unclear how the CF achieves and maintains its special relationship with a Purkinje cell and whether CF control of cerebellar function can be exclusively explained by its influence on Purkinje cells.

Over the past several years, attention has turned to glia cells as potential targets of synaptic inputs. Glia are intimately associated with synapses and can rapidly remove neurotransmitter from the synaptic cleft, thereby limiting spillover of transmitter to neighboring release sites (Marcaggi and Attwell, 2004). Glia also express a variety of ionotropic and metabotropic receptors and can therefore function as detectors of neural activity. In addition, several types of glia release transmitters in response to neural activity, which can locally modulate neuronal excitability and synaptic transmission (Auld and Robitaille, 2003).

The molecular layer of the cerebellar cortex contains multiple types of glia, including Bergmann glia and $NG2^+$ cells, a diverse class of progenitor cells named

after a proteoglycan they express (Figure 1A). Bergmann glia ensheath CF synapses, thereby isolating individual release sites. They express glutamate transporters near sites of release that help to terminate the glutamate signal in the synaptic cleft. They also express calcium-permeable AMPA receptors; however, because of their low affinity for glutamate and their extrasynaptic location, the means of activation of these receptors under physiological conditions have been poorly understood. CF EPSCs recorded from Bergmann glia and Purkinje cells differ in their calcium sensitivity and short-term plasticity (Matsui and Jahr, 2003; Matsui and Jahr, 2004). These differences have been shown to reflect the ectopic release of glutamate from CFs (i.e., from sites distinct from the active zone; Figure 1B, left). Ectopic release thus ensures that glial low affinity receptors located outside the synaptic cleft are efficiently activated. Converting the calcium-permeable AMPA receptors into calcium-impermeable forms via viral delivery of the GluR2 gene results in retraction of glial processes from the synapse (Iino et al., 2001). This suggests that ectopic release may be vital to the maintenance of Bergmann glia processes that surround CF synapses.

A more straightforward means for rapid neuron-to-glia signaling would of course be a conventional synaptic contact consisting of a presynaptic active zone and a postsynaptic density. Until recently, synaptic contacts dedicated exclusively to glia were unknown. However, Bergles and colleagues have identified such synapses in NG2$^+$ cells that are present in several regions throughout the developing and adult brain (Lin and Bergles, 2002). These cells share a number of properties with other types of glia. Their stellate shape is reminiscent of astrocytes, and their resting membrane potential is close to the K^+ equilibrium potential. Although they express Na^+ and K^+ channels, they do not generate action potentials. In contrast to other types of glia, NG2$^+$ cells do not contain the glial marker GFAP, they do not express glutamate transporters, and show no signs of gap junction-mediated dye coupling. Furthermore, NG2$^+$ cells do not participate in the ensheathment of synapses. Recent work has shown convincingly that NG2$^+$ cells in the hippocampus are directly contacted by both pyramidal cells (Bergles et al., 2000) and interneurons (Lin and Bergles, 2004).

In this issue of *Neuron*, Lin et al. (2005) provide further evidence that this type of direct signaling could be a general property of NG2$^+$ cells (Figure 1B, right). They recorded from NG2$^+$ cells in the molecular layer of cerebellar slices by making use of transgenic mice that express EGFP in cells in oligodendrocyte lineage. Activation of CF inputs resulted in synaptic currents exclusively mediated by AMPA receptors with rapid rise and decay kinetics. Cyclothiazide, which relieves AMPA receptor desensitization and increases the affinity of AMPA receptors for glutamate, only moderately enhanced synaptic currents onto NG2$^+$ cells. This suggests that, as for Purkinje cells, AMPA receptors on NG2$^+$ cells experience high and brief concentrations of glutamate following CF activation.

Are CF EPSCs in NG2$^+$ cells mediated by conventional synapses? To address this question, Lin and colleagues performed two manipulations aimed to reduce calcium influx into CF terminals. They found that both

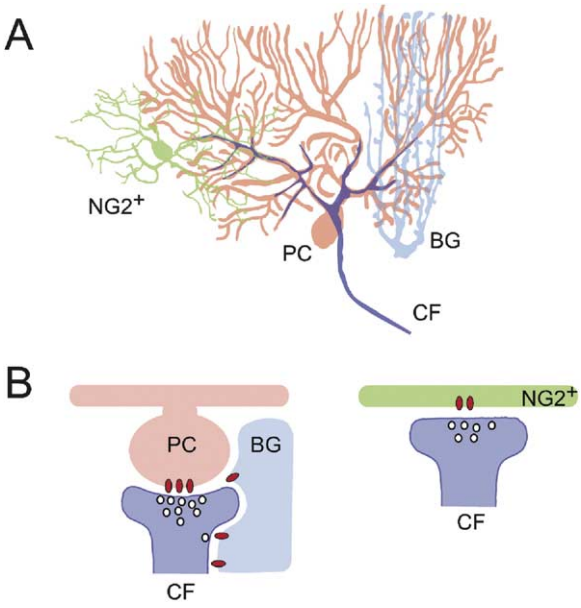

Figure 1. Three Postsynaptic Targets of Climbing Fibers

(A) CFs (dark blue) form synapses with Purkinje cells (red), Bergmann glia (light blue), and NG2$^+$ cells (light green).

(B) (Left) CFs release glutamate onto Purkinje cell dendrites via conventional synapses and onto Bergmann glia via ectopic release. The conventional synapse consists of an active zone with a cluster of docked and reserve vesicles (white circles) opposed by a postsynaptic density and a cluster AMPA receptors (dark red), whereas ectopic release occurs at sites distant from active zones. (Right) CFs form conventional synapses with NG2$^+$ cells.

presynaptic CB1 receptor activation and blocking N-type calcium channels reduced EPSCs only moderately at CF-NG2$^+$ cell synapses, similar to what is observed at the CF to Purkinje cell synapse. By contrast, the same manipulations more strongly attenuated the CF EPSC in Bergmann glia, which is mediated by ectopic release and is therefore more sensitive to manipulations of presynaptic evoked calcium levels. The authors conclude that CF EPSCs in NG2$^+$ cells are mediated by glutamate released from conventional synaptic contacts. Further support for anatomically defined CF-NG2$^+$ cell synapses comes from serial electron microscopy showing that the processes of NG2$^+$ cells are indeed contacted by vesicle-filled presynaptic endings.

Although there are many similarities between CF synapses on Purkinje cells and NG2$^+$ cells, the work by Lin and colleagues highlights some interesting differences. Purkinje cells typically only receive a single CF input in the mature animal, but the processes of NG2$^+$ cells extend over the arbors of several Purkinje cells and therefore receive multiple CF inputs. In contrast to CF synapses on Purkinje cells, CF-NG2$^+$ cell synapses are not ensheathed by Bergmann glia. Furthermore, AMPA receptors in NG2$^+$ cells are highly calcium permeable, suggesting that the functionally relevant postsynaptic response might be a rise in glial calcium levels.

What could be the functional role of the CF input to NG2$^+$ cells? These cells constitute a heterogeneous group of progenitors that may have distinct developmental potential. It is likely that CF activity influences

gene expression and ultimately helps to determine the fate of these cells. In addition, $NG2^+$ cells could release neurotransmitters or other trophic factors aimed at the stabilization of climbing fiber inputs. The challenge in the future will be to selectively disrupt synaptic signaling in these cells and test the functional consequences.

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DOI 10.1016/j.neuron.2005.05.017