Shape-shifting at a cerebellar synapse allows submillisecond signaling

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Synaptic currents become faster with age. A new study uses electron microscopy, physiology and modeling to show that the progressive speeding of AMPA receptor–mediated synaptic currents during development results from changes in the structure of the synapse rather than the composition of postsynaptic receptors.

As any surfer can attest, the timing and duration of a wave makes all the difference in its impact. Likewise, synaptic responses vary dramatically in their time course, and these differences are central to the plasticity of neural networks. The amplitude and duration of synaptic currents influence the extent of interaction among discrete inputs, the amount and duration of calcium influx into the neuron, and the temporal precision with which action potentials are initiated. During development, there is a remarkable change in the time course of synaptic currents, with most becoming faster with age; at mossy fiber (MF) to granule cell (GC) synapses in the cerebellum, miniature excitatory postsynaptic currents (mEPSCs), the response of synapses to single release events, last only about half as long in adults as they do during the first week of life. Despite the profound influence that these changes have on developing circuits, the cellular adaptations responsible for speeding up EPSCs remain poorly understood, largely because of the great number of potential sites for modification and the limited accessibility of most synapses.

In this report, Cathala and colleagues[1] use the combined power of single-cell electrophysiology, electron microscopic reconstruction and kinetic modeling to reveal how changes in the size and packing of glutamatergic synapses in the cerebellum alter the shape of currents at individual synapses.

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At a variety of excitatory and inhibitory synapses, age-dependent speeding of synaptic currents is achieved through changes in the expression of postsynaptic receptors, which are modified through the assembly of different combinations of subunits[2,3]. Although the expression of subunits that allow AMPA receptors to desensitize rapidly increases in cerebellar GCs during development[4,5], this period of kinetic refinement is also associated with dramatic alterations in cell shape, synaptic structure and synaptic density[6] (**Fig. 1a**,**b**), suggesting that morphological changes might also contribute to the observed changes in EPSC time course. MFs provide the sole excitatory input to GCs, and they extend large presynaptic terminals that form several synaptic junctions

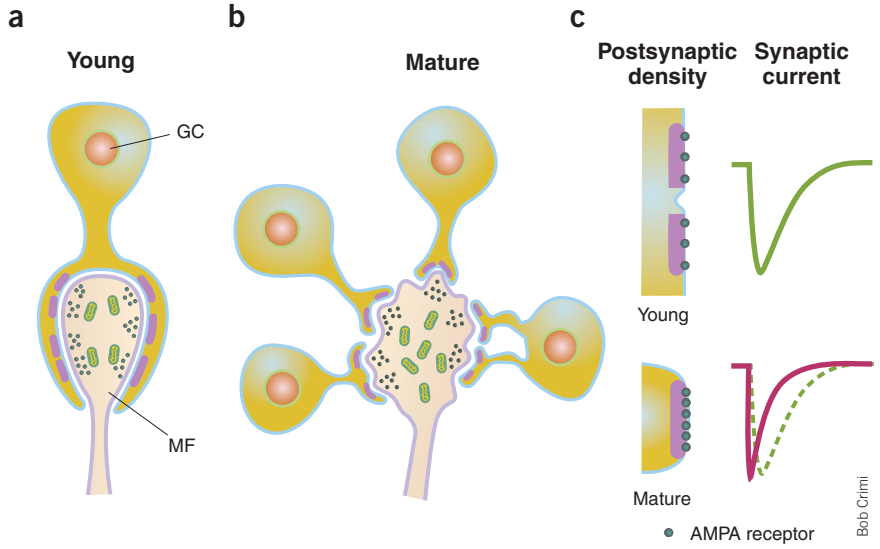

Figure 1 Developmental changes in the structure of MF-GC synapses in the cerebellum lead to shorter synaptic currents. (**a**) MF terminals in the developing brain form numerous synapses with the cup-shaped dendrites of granule cells. Synapses in 1-week–old animals are more than fourfold larger than in adults, and the extensive synaptic area is often interrupted by perforations. (**b**) With development, the dendrites of granule cells in contact with the MF terminal narrow and become claw-shaped. Synapses at this age are smaller and more uniform in shape and are located farther away from neighboring synapses. (**c**) Cathala *et al.* suggest that the faster rise time of synaptic currents in adults results from changes in the area of the postsynaptic density and density of AMPA receptors, whereas the speeding of the decay is due to an increase in routes for glutamate to escape from the synapse and a decrease in the probability that glutamate released at one synapse will interact with receptors at neighboring synapses (cross-talk). Panels **a** and **b** were adapted from ref. 6.

with the short dendrites of GCs (**Fig. 1**). The close proximity of MF synapses to the soma (where responses are measured) allows AMPA receptor currents to be recorded with high fidelity, free from distortions in shape[7]. In the current study, the authors took advantage of the exceptional accessibility of MF-GC connections to compare the properties of AMPA receptors at MF synapses in the young (1 week old) and mature (2–3 months old) cerebellum.

By restricting their analysis to mEPSCs to avoid complications arising from differences in the probability of release and release rates among groups of synapses, the authors found that manipulations such as increasing the temperature, which accelerates receptor gating and speeds clearance of glutamate by transporters, and depolarizing the cell, which slows receptor gating, exerted proportionally similar effects on mEPSCs at the two ages. Furthermore, the ability of AMPA receptors to pass current in the outward direction did not change with age, as expected if the same number of GluR2-containing receptors were present. Using peak scaled nonstationary fluctuation analysis, a method that makes it possible to extract information about the properties of individual receptors that underlie a synaptic current[8], they found that the unitary conductance of AMPA receptors was also similar at both ages, suggesting that most receptors remain GluR2/GluR4 heteromers throughout development. Thus, at first blush the AMPA receptors in young and mature GCs looked quite similar.

Alternative splicing can yield GluR2 and GluR4 subunits with long ('flip') or short ('flop') C termini that vary in their rates of desensitization and sensitivity to cyclothiazide, a compound that inhibits AMPA receptor desensitization. Although prior studies indicate that the expression of GluR4-flop increases in GCs with age[4], mEPSCs recorded from young and mature synapses were slowed to a similar extent by a concentration of cyclothiazide that should effect primarily flip subunits. These results presented a conundrum: although there seemed to be a global change in the expression of receptors, which would predict a shift from slower to faster gating receptors, the composition of receptors giving rise to mEPSCs remained largely unchanged through development.

If the receptors are the same, how then do the synaptic currents become faster with age? The authors explored whether the stimulus itself, the glutamate transients in the synaptic cleft, had different spatial and temporal profiles at these two ages. In a key series of experiments, they found that application of kynurenate, a low-affinity competitive antagonist of AMPA receptors that has been used to derive the time course of glutamate at hippocampal synapses[9], resulted in a selective speeding of mEPSC decay in the younger animals. This phenomenon could be explained if glutamate were present for a prolonged period in the cleft of immature synapses[10]; when kynurenate is present, the ability of glutamate to bind to receptors within the cleft or perhaps at neighboring sites is reduced[9]. However, this behavior could also be caused by changes in distribution of AMPA receptors.

If the same number of AMPA receptors were spread over a larger area, mEPSC kinetics should be slowed, as the receptors distant from the site of release would be exposed to a lower peak concentration of glutamate, resulting in a subtle but significant delay in activation relative to receptors nearest to the site of fusion. Using high-resolution immunogold labeling of AMPA receptors and serial reconstructions of cerebellar tissue, the authors made three significant discoveries. First, the density of AMPA receptors was threefold lower in the young synapses; second, young synapses had larger postsynaptic densities (PSDs), which were often interrupted along their length by perforations that create a tortuous path for glutamate to travel; and third, the effective distance between neighboring synapses was shorter in the developing cerebellum. This change from large, complex synapses where AMPA receptors are spread over a larger area and glutamate is more likely to be trapped, to more discrete, circumscribed synapses where glutamate has the opportunity to bind a population of receptors nearly simultaneously before rapidly diffusing away, could account for the observed changes in mEPSC time course.

As it is not yet possible to modify the shape of synapses in a controlled manner *in situ*, the authors turned to modeling to assess whether these changes in morphology could quantitatively account for the acceleration of mEPSCs. They combined a 2-D model of cerebellar space to approximate the restricted diffusion of glutamate in the developing neuropil and a 3-D model to approximate diffusion in adult tissue with a kinetic scheme for AMPA receptor gating that has been validated at MF-GC synapses[11]. The authors found that a ~2.5-fold decrease in the size of the PSD, which mimicked the age-dependent transformation, resulted in an acceleration in the rise time of the simulated mEPSC comparable to that observed experimentally. Furthermore, the increase in diffusional sinks—routes for the escape of glutamate—in the 3-D model prominently decreased the decay time of the simulated mEPSC, although this speeding was still less than that observed during development.

Finally, the authors considered whether spillover of glutamate from neighboring synapses[12], which would be more prominent in younger animals because of the closer packing of synapses[6], could also extend the time course of mEPSCs. Indeed, when several small, slowly rising and decaying glutamate transients were added to the direct response, the mEPSC time course was prolonged further at young but not mature synapses. Although certainly not a unique solution, a close fit to the observed differences in mEPSCs between young and mature synapses was obtained by surrounding a simulated young synapse by four identical synapses located 800 nm away. Thus, the observed decrease in PSD size could account for the decrease in rise time, and the combined influence of the increase in diffusional sinks and intersynaptic distance could account for the acceleration of mEPSC decay time. Excitatory signaling in GCs could, however, be under some homeostatic control, as mEPSC amplitude remained constant during development despite these remarkable morphological transformations.

The near-universal acceleration of synaptic currents among connections that use different transmitters, rely on different mechanisms of transmitter clearance and have dramatically different structures suggests that longer responses may be essential for both stabilizing nascent junctions and initiating subsequent morphological and functional refinements of the synapse. Indeed, the prolonged synaptic currents in developing GCs produce a longer depolarization[13] that may allow greater relief of Mg^{2+} block of NMDA receptors. However, it remains to be determined whether enhanced Ca^{2+} influx through NMDA receptors in young GCs acts in a self-limiting way to trigger the structural changes responsible for accelerating EPSC time course[14].

The shortening of EPSC time course in adults enhances the precision with which MF inputs trigger action potentials in GCs[13]. Long-term adjustments in motor output are thought to require modification of the strength of GC parallel fiber synapses formed with Purkinje neurons; because long-term depression at these synapses is induced only when parallel fiber input is coincident with climbing fiber input, the precise timing enabled by these structural refinements of MF synapses is likely to have a wide-ranging impact on cerebellar function. Furthermore, these results raise the possibility that the extensive morphological changes that occur in other synapses during development, in

response to learning tasks[15] and after brain injury and disease also may produce significant changes in the time course of synaptic responses.

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A no-Wnt situation: SFRPs as axon guidance molecules

Uwe Drescher

SFRPs are endogenous inhibitors of Wnt signaling. New work shows that, independently of its interaction with Wnts, SFRP1 can act as a repulsive guidance molecule for retinal axons on their way to the tectum, signaling through the receptor Fz2.

The mind-boggling complexity of vertebrate CNS connectivity suggests that a large number of axon guidance molecules must direct initial wiring during development. However, the boom in identifying axon guidance activities from various assay systems ebbed in the mid-1990s, revealing only a few families of axon guidance molecules[1]. Large-scale screens did not yield the anticipated vast numbers of guidance cues, either. At present, the 'reverse' approach is proving more successful: that is, the investigation of candidate molecules for which, for example, a graded expression pattern or a known role in cell migration suggest a possible axon guidance function. In this way, classical morphogens such as sonic hedgehog, Wnts and BMPs were shown to act as axon guidance cues[2]. Now Rodriguez *et al.*[3] from the laboratories of Paola Bovolenta and Christine Holt demonstrate the guidance function of a molecule known as secreted frizzled related protein-1 (SFRP1).

SFRP1 is part of a large protein family whose members are expressed in complex patterns throughout brain development[4]. In previous studies, Bovolenta and co-workers observed that SFRP1 consistently enhanced neurite extension[5]. Now Rodriguez *et al.*[3] show, using classical axon guidance assays, that asymmetrically presented SFRP1 can also reorient the growth of retinal ganglion cell axons. This is an essential characteristic for an axon guidance molecule. On a laminin substrate, SFRP1 repelled retinal axons. As SFRP1 and laminin are coexpressed in tissues surrounding the pathway of retinal axons from the eye to the tectum, Rodriguez *et al.* propose that SFRP1 acts as a repellent to guide retinal axons from the eye to the tectum. In accordance with this hypothesis, retinal axons left their normal pathway to the tectum and went off-target when deprived of SFRP1-mediated positional information—as done here by soaking an exposed brain preparation in SFRP1. In this respect, SFRP1 resembles the Slit proteins, which also guide retinal axons by 'surround repulsion[1]', suggesting synergistic effects of Slits and SFRP1.

Rodriguez *et al.* further show that SFRP1 action is sensitive to changes in substrate or cyclic nucleotide concentration and to inhibition of protein synthesis and proteasome degradation, all effects that are also observed for other axon guidance molecules[1]. SFRP1 activity could be blocked by pertussis toxin,

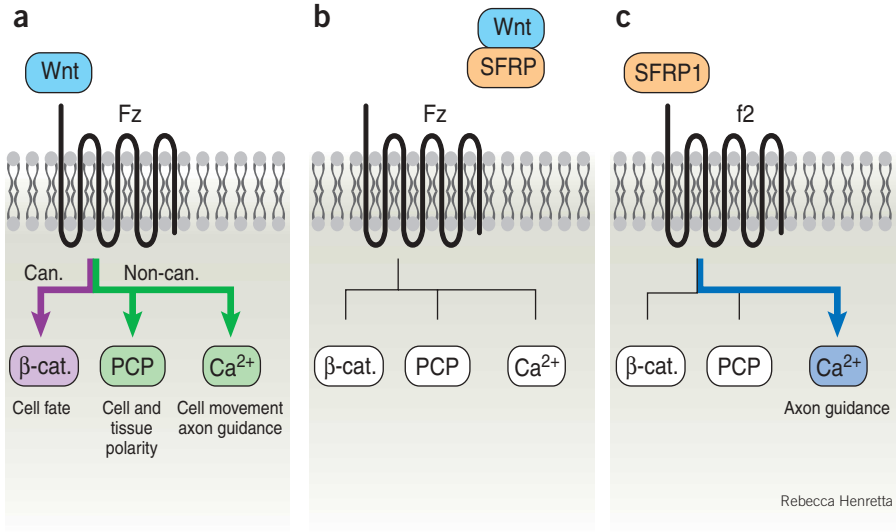

Figure 1 SFRP1 functions as an axon guidance molecule. (**a**) The three main branches of the Wnt signaling pathway: the 'canonical' or β-catenin pathway regulates cell fate decisions, the PCP (planar cell polarity) or JNK pathway is involved in determining cell and tissue polarity and the Ca^{2+} pathway contributes to cell movement and axon guidance. (**b**) In cases of coexpression, SFRPs can bind to Wnts, thus blocking binding of Wnts to Fz receptors and abolishing all Wnt signaling. (**c**) Binding of SFRP1 to Fz2 results in activation of the Ca^{2+} pathway. The effects of SFRP1 on axon growth and growth cone turning are sensitive to changes in cyclic nucleotides, pertussis toxin (suggesting the involvement of G protein–coupled receptors) and, seemingly, calcium calmodulin kinase II (CaMKII).

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