Gerdts, J., Summers, D.W., Milbrandt, J., and DiAntonio, A. (2016). Neuron *89*, 449–460.

Gilley, J., Orsomando, G., Nascimento-Ferreira, I., and Coleman, M.P. (2015). Cell Rep. *10*, 1974–1981.

Henninger, N., Bouley, J., Sikoglu, E.M., An, J., Moore, C.M., King, J.A., Bowser, R., Freeman, M.R., and Brown, R.H., Jr. (2016). Brain *139*, 1094–1105.

Hill, C.S., Coleman, M.P., and Menon, D.K. (2016). Trends Neurosci. *39*, 311–324.

Neukomm, L.J., and Freeman, M.R. (2014). Trends Cell Biol. *24*, 515–523.

Osterloh, J.M., Yang, J., Rooney, T.M., Fox, A.N., Adalbert, R., Powell, E.H., Sheehan, A.E., Avery, M.A., Hackett, R., Logan, M.A., et al. (2012). Science *337*, 481–484.

Schuldiner, O., and Yaron, A. (2015). Cell. Mol. Life Sci. *72*, 101–119.

Aligning a Synapse

Daniel Kerschensteiner[1,2,3,4,*]
[1]Department of Ophthalmology and Visual Sciences
[2]Department of Neuroscience
[3]Department of Biomedical Engineering
[4]Hope Center for Neurological Disorders
Washington University School of Medicine, Saint Louis, MO 63110, USA
*Correspondence: kerschensteinerd@wustl.edu
http://dx.doi.org/10.1016/j.neuron.2017.03.012

Synaptic communication requires alignment of transmitter release sites with transmitter receptors. In this issue of *Neuron*, Wang et al. (2017) show that α2δ4 subunits link calcium channels to a trans-synaptic complex with glutamate receptors at the visual system's first synapse.

If at night you worry about the state of this planet and look through a telescope for alternative destinations, you need the optical elements of your telescope and eye to be aligned. Then, as a few photons hit your retina, rod photoreceptors (rods for short) initiate signals that need to propagate across many synapses before you can identify the celestial light source. Like the propagation of photons through your telescope and eye, the propagation of signals across synapses requires alignment, between presynaptic sites of transmitter release and postsynaptic transmitter receptors. The molecular mechanisms that align synapses are mostly unknown. In this issue of *Neuron*, Wang et al. (2017) show that the auxiliary calcium channel subunit α2δ4 plays a critical role in aligning synapses between rods and rod bipolar cells (RBCs).

The synapses between rods and RBCs are spatially and molecularly complex (Figure 1). Rod axons end in a single spherule with a basal invagination in the outer plexiform layer. At the roof of each invagination, glutamate-filled vesicles are tethered to an electron-dense ribbon, and voltage-gated calcium channels are enriched in an active zone of the plasma membrane to which the ribbon is anchored. Calcium influx at the active zone triggers vesicle fusion and the release of glutamate into the synaptic cleft. RBC dendrites enter spherule invaginations, and metabotropic glutamate receptors (mGluR6) cluster at dendritic tips opposite presynaptic active zones. Signals from mGluR6 are relayed via second messengers to a cation non-selective channel (Trpm1), which depolarizes RBCs in response to light. In addition to RBC dendrites, horizontal cell axons penetrate spherules and provide inhibitory feedback to rods. Whereas many of the proteins involved in pre- and postsynaptic signaling in rods and RBCs, respectively, have been identified (Hoon et al., 2014), the mechanisms that align pre- and postsynaptic machineries remain obscure. This disparity is common to our knowledge of synapses across the nervous system.

A couple of years ago, Cao et al. (2015) conducted a proteomic screen for binding partners of mGluR6. The top hit in this screen was the leucine-rich repeat protein ELFN1. Cao et al. (2015) found that ELFN1 was expressed selectively by rods and reached across the synapse to interact with mGluR6 on RBCs. When ELFN1 was deleted (*ELFN1 KO* mice), synapses between rods and RBCs failed to form or were not maintained. Moreover, RBC dendrites, but not horizontal cell axons, were absent from rod spherule invaginations in *ELFN1 KO* mice (Cao et al., 2015). Although this study identified a trans-synaptic complex through which rods and RBCs interact, how presynaptic transmitter release sites are aligned with this complex and thus with postsynaptic receptors remained unclear.

The pore-forming Cav1.4 subunit of voltage-gated calcium channels is required for the structural and functional integrity of the presynaptic release machinery in rods. Mutations in Cav1.4 cause congenital stationary night blindness in people (Strom et al., 1998), and *Cav1.4 KO* mice exhibit deficits in signal transmission from rods to RBCs and in the assembly and/or maintenance of presynaptic ribbons (Mansergh et al., 2005). Cao et al. (2015) found

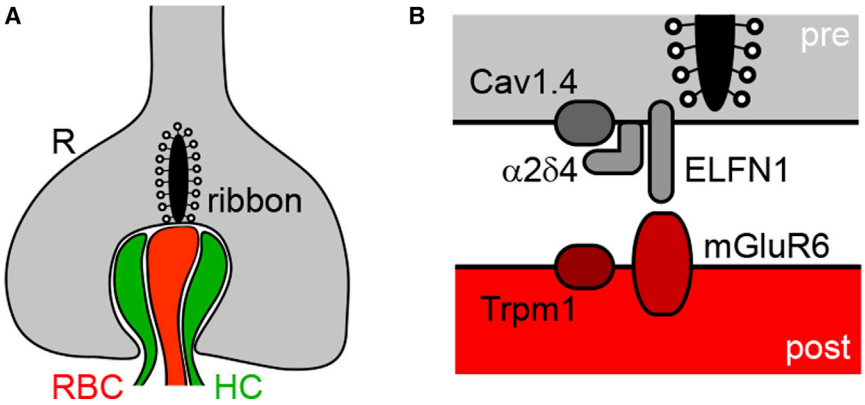

Figure 1. Schematic of the Synapse between Rods and Rod Bipolar Cells
(A) Rod spherule (R, gray) with a basal invagination containing a rod bipolar cell dendrite (RBC, red) and a horizontal cell axon (HC, green).
(B) Apposition of presynaptic (gray) and postsynaptic (red) specialization of rods and RBCs, respectively, illustrating the trans-synaptic molecular complex that aligns calcium channels at the ribbon release site with postsynaptic receptors.

that expression levels of ELFN1 were reduced in *Cav1.4 KO* mice and that its localization to presynaptic release sites was disrupted. Similarly, mGluR6 expression was reduced and receptors failed to cluster at RBC dendrite tips. Together, these observations revealed a functional link between the presynaptic organizer Cav1.4 and trans-synaptic ELFN1-mGluR6 complexes, but the molecular identity of this link was unknown.

Proteins of the α2δ family of auxiliary calcium channel subunits are split post-translationally into a large glycosylated extracellular α2 domain and a small membrane-associated δ domain, which are reconnected via disulfide bridges (Dolphin, 2012). α2δ subunits associate with pore-forming subunits of voltage-gated calcium channels in the endoplasmic reticulum, promote their trafficking to the plasma membrane, and regulate biophysical channel properties (Dolphin, 2012). As part of calcium channel complexes, α2δ subunits localize to extracellular face of presynaptic active zones, where they can interact with extracellular matrix proteins and postsynaptic binding partners to regulate synapse formation and plasticity (Dolphin, 2012). The importance of α2δ proteins is highlighted by their association with neurodevelopmental and neurological disorders. Patients with mutations in α2δ4 exhibit cone dystrophies and electrophysiological abnormalities indicative of disrupted rod-RBC signaling (Wycisk et al., 2006).

In this issue of *Neuron*, Wang et al. (2017) show that α2δ4 localizes to active zones of rods and cones. Using an impressive array of knockout mice, the investigators find that the synaptic localization of α2δ4 depends on the presence of Cav1.4, but is unaffected by removal of ELFN1, the active zone protein CAST, and components of the postsynaptic specialization, including mGluR6. Wang et al. (2017) next generate *α2δ4 KO* mice. In these mice, expression levels of Cav1.4, ELFN1, the ribbon protein CTBP2, and mGluR6 and other postsynaptic proteins are reduced. Patch-clamp recordings from rods and cones show that in addition to regulating the abundance of Cav1.4, α2δ4 shifts the voltage-dependent activation of calcium currents. Together, these observations suggest that α2δ4 interacts with Cav1.4 in rods and cones to regulate the amplitude and threshold of calcium influx and reveal that Ca1.4-α2δ4 channel complexes stabilize many proteins of the pre- and postsynapse.

Rod signals propagate across the retina through three alternative pathways that function at different light levels (Bloomfield and Dacheux, 2001). Near the threshold of vision (e.g., during stargazing), all rod signals are conveyed by RBCs (i.e., primary pathway). At slightly higher light levels, rod signals can flow indirectly through gap junctions with cones to cone bipolar cells (i.e., secondary pathway), or reach a subset of cone bipolar cells directly through basal contacts of their dendrites with rod spherules (i.e., tertiary pathway). In a behavioral assay, in which mice try to locate an escape platform in a water maze under different illumination conditions, Wang et al. (2017) find that the performance of *α2δ4 KO* mice is indistinguishable from wild-type littermates at all except the lowest light levels, which exclusively activate the primary rod pathway. Moreover, patch-clamp recordings reveal residual rod-driven light responses in cone bipolar cells, but not RBCs, of *α2δ4 KO* mice. Therefore, although α2δ4 interacts with Cav1.4 in rods and cones, it is required selectively for synaptic transmission from rods to RBCs and for vision mediated by the primary rod pathway.

Wang et al. (2017) explore the structural basis of this pathway-specific loss of function in *α2δ4 KO* mice. Using light and electron microscopy, they observe a series of anatomical deficits: (1) fewer rod axons correctly target the outer plexiform layer, (2) fewer rod axons terminate in spherules, (3) fewer rod terminals contain synaptic ribbons, (4) fewer RBC dendrites enter rod terminals, and (5) mGluR6 receptors fail to cluster on RBC dendrites. By contrast, the ultrastructure and molecular composition of synapses between cones and cone bipolar cells is largely unchanged in *α2δ4 KO* mice. It is possible that cones express additional α2δ proteins that compensate for the loss of α2δ4. Alternatively, the mechanisms of cone synaptogenesis may differ from those of rods, as suggested by the absence of ELFN1 from cones (Cao et al., 2015). In rods, however, α2δ4 is required, and Wang et al. (2017) show that it interacts via separate domains with Cav1.4 and ELFN1. Thus, α2δ4 appears to be the missing link between Cav1.4 channels, which organize presynaptic ribbon release sites, and trans-synaptic ELFN1-mGluR6 complexes, which recruit the postsynaptic machinery.

Specific connections between neurons are established and maintained by the combined actions of many proteins with distinct if overlapping functions. In the

CellPress

inner retina, for example, repulsive guidance cues regulate neurite lamination, adhesive cues maintain contact between appropriate synaptic partners, and proteins involved in transmitter release promote synaptic differentiation (Hoon et al., 2014). Given such division of labor, one might have expected deletion of α2δ4, which severs the link between pre- and postsynaptic organizers, to have limited consequences (e.g., misaligned synapses). Instead, Wang et al. (2017) show that loss of α2δ4 affects the expression, localization, and alignment of many synaptic proteins; the adhesion of synaptic partners; and neurite lamination. As a result, all synaptic communication between rods and RBCs is lost and *α2δ4 KO* mice cannot see in low light.

As with all exciting advances, the study of Wang et al. (2017) raises numerous new questions for future studies. Establishing the onset of deficits in *α2δ4 KO* mice could reveal whether α2δ4 is required for the development or maintenance of normal lamination patterns, invaginated contacts, and synapses between rods and RBCs. The order in which deficits appear could distinguish primary and secondary effects of α2δ4 (e.g., do rod axons retract after synapses are lost, or do they fail to reach appropriate laminar targets, preventing synapse formation?). For rods, an open question is to what extent Cav1.4-α2δ4 channel complexes organize presynaptic ribbons by mediating Ca^{2+} influx or by engaging in protein-protein interactions and serving as a structural scaffold and/or anchor. This could be addressed by replacing wild-type Cav1.4 with a non-conducting mutant.

α2δ subunits have now been shown to participate in complexes that align presynaptic calcium channels with postsynaptic transmitter receptors at two synapses: between inner hair cells and spiral ganglion neuron in the auditory system (Fell et al., 2016) and between rods and RBCs in the visual system (Wang et al., 2017). Both of these are ribbon synapses of non-spiking presynaptic neurons, which support gradual changes in transmitter release. It is tempting to speculate that these synapses require particularly precise alignment of release sites and receptors to transmit continuously varying (i.e., analog) signals accurately.

REFERENCES

Bloomfield, S.A., and Dacheux, R.F. (2001). Prog. Retin. Eye Res. *20*, 351–384.

Cao, Y., Sarria, I., Fehlhaber, K.E., Kamasawa, N., Orlandi, C., James, K.N., Hazen, J.L., Gardner, M.R., Farzan, M., Lee, A., et al. (2015). Neuron *87*, 1248–1260.

Dolphin, A.C. (2012). Nat. Rev. Neurosci. *13*, 542–555.

Fell, B., Eckrich, S., Blum, K., Eckrich, T., Hecker, D., Obermair, G.J., Münkner, S., Flockerzi, V., Schick, B., and Engel, J. (2016). J. Neurosci. *36*, 11024–11036.

Hoon, M., Okawa, H., Della Santina, L., and Wong, R.O. (2014). Prog. Retin. Eye Res. *42*, 44–84.

Mansergh, F., Orton, N.C., Vessey, J.P., Lalonde, M.R., Stell, W.K., Tremblay, F., Barnes, S., Rancourt, D.E., and Bech-Hansen, N.T. (2005). Hum. Mol. Genet. *14*, 3035–3046.

Strom, T.M., Nyakatura, G., Apfelstedt-Sylla, E., Hellebrand, H., Lorenz, B., Weber, B.H., Wutz, K., Gutwillinger, N., Rüther, K., Drescher, B., et al. (1998). Nat. Genet. *19*, 260–263.

Wang, Y., Fehlhaber, K.E., Sarria, I., Cao, Y., Ingram, N.T., Guerrero-Given, D., Throesch, B., Baldwin, K., Kamasawa, N., Ohtsuka, T., et al. (2017). Neuron *93*, this issue, 1359–1374.

Wycisk, K.A., Zeitz, C., Feil, S., Wittmer, M., Forster, U., Neidhardt, J., Wissinger, B., Zrenner, E., Wilke, R., Kohl, S., and Berger, W. (2006). Am. J. Hum. Genet. *79*, 973–977.

Undercover Power of Endocannabinoids: Postsynaptic Ion-Channel Modulator

Aya Matsui[1] **and Veronica A. Alvarez**[1,*]
[1]Laboratory on Neurobiology of Compulsive Behaviors, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, Maryland 20892, USA
*Correspondence: alvarezva@mail.nih.gov
http://dx.doi.org/10.1016/j.neuron.2017.03.014

In this issue of Neuron, Gantz and Bean (2017) show that the endocannabinoid 2-arachidonoyl glycerol (2-AG) can directly alter the properties of native ion-channel $Kv_{4.3}$ and accelerate the pacemaker activity of rodent dopamine neurons. These findings are one of the first demonstrations of postsynaptic, cell-autonomous actions of endocannabinoids in the mammalian brain.

The endocannabinoid 2-AG, which is the focus of the study, and anandamide are the two main endogenous ligands of the cannabinoid receptors CB_1 and CB_2 (Pacher et al., 2006). Endocannabinoids have pleiotropic functions within and outside the brain. They are involved in regulation of pain, sleep, eating, stress, immune response, inflammation, and liver function. Until now, CB_1 receptors were thought to mediate most of the effects of endocannabinoids in the brains of vertebrates, though CB_2 receptor expression in astrocytes, microglia, and other neurons has also been reported. Over a