Journal Club

Editor's Note: These short, critical reviews of recent papers in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

Early Events of Synapse Disassembly in the Damaged Retina

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Review of Dunn

In mammalian retina, the gradual degeneration of photoreceptors is a hallmark of several genetic disorders that ultimately lead to blindness. Over long time periods, the depletion of photoreceptors dramatically influences downstream retinal circuitry by causing dendritic remodeling and disassembly of synapses with postsynaptic bipolar cells (Strettoi et al., 2003). Animal models of retinal degeneration successfully recapitulate the gradual decline of photoreceptor populations. However, the global scale of photoreceptor death and unpredictable time course of degeneration in these models limits their utility in studies of disassembly at single synapses. The ability to control the degeneration of individual photoreceptors would provide a powerful tool for investigating consequent effects on surviving retinal circuits at high spatial and temporal resolution.

How does the loss of individual photoreceptors immediately affect postsynaptic bipolar cells? In a recent issue of *The Journal of Neuroscience*, Dunn (2015) addressed this question by imaging glutamate receptor expression and dendritic morphology in bipolar cells following laser-induced ablation of individual presynaptic cone photoreceptors in a double transgenic mouse retina. In this retina, cones containing medium wavelength-sensitive opsin (M cones) express GFP and a subset of ON cone bipolar cells (type 6) express td Tomato. This dual genetic method of cell type identification permitted targeted laser ablation of single cones in conjunction with high-resolution imaging of postsynaptic bipolar cells. Within 24 h of cone ablation, the live retina was fixed and a combination of immunohistochemistry and confocal microscopy was used to visualize changes in a bipolar cell's metabotropic glutamate receptor type 6 (mGluR6) expression and dendritic morphology.

Dunn (2015) first asked whether ablation of all M cones presynaptic to a single type 6 bipolar cell elicited changes in postsynaptic mGluR6 receptor expression and dendritic morphology within 24 h. Immunohistochemistry revealed that dendritic mGluR6 receptor expression decreased to somatic expression levels in the bipolar cell postsynaptic to the ablated cones, suggesting that complete loss of cone input can rapidly alter glutamate receptor allocation at cone-to-ON cone bipolar cell synapses. There were no major differences in dendritic tip morphology at the time points tested, suggesting that the drastic dendritic remodeling observed previously (Strettoi et al., 2003) begins >24 h after ablation. However, because Dunn (2015) did not perform quantitative measurements of dendritic structure, it is difficult to interpret this negative result. For example, the bipolar cell dendritic claw shown in their Figure *2A* (right) appears to redistribute its center of mass toward its intact presynaptic cone inputs following each successive cone ablation. Quantitative analysis of dendritic morphology (for review, see Uylings and van Pelt, 2002) may assist in identifying subtle changes that occur shortly after presynaptic cone ablation.

Next, to determine whether mGluR6 expression is independently regulated at individual cone-to-ON cone bipolar synapses after cone ablation, Dunn (2015) eliminated one or a subset of cones presynaptic to a single bipolar cell. mGluR6 receptor expression decreased at bipolar cell dendritic tips postsynaptic to ablated cones but not at those postsynaptic to intact cones, suggesting that postsynaptic mGluR6 expression is regulated independently at individual synapses onto a single bipolar cell.

Dunn (2015) also performed finer analysis of the time course of mGluR6 receptor loss by varying the interval between cone ablation and tissue fixation. This experiment revealed that postsynaptic mGluR6 receptor loss begins >50 min after ablation of a presynaptic cone and progresses with a time constant of ~2 h. Dendritic mGluR6 expression declined to somatic levels by 3 h after ablation. Together, these experiments

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illustrate synapse disassembly at the cone-to-ON cone bipolar synapse as a process of remarkable spatial and temporal regulation.

Does the loss of cone glutamate release and the consequent decline in glutamate receptor binding explain the rapid decrease in dendritic mGluR6 receptor expression? To answer this question, Dunn (2015) tested whether pharmacological occupation of mGluR6 receptors preserves mGluR6 expression in dendritic tips of bipolar cells postsynaptic to ablated cones. Application of an mGluR6 agonist and antagonist, either simultaneously or separately, during and after cone ablation, failed to rescue mGluR6 expression in bipolar cell dendrites. These findings suggest that the loss of glutamatergic cone input to bipolar cells does not primarily explain mGluR6 receptor loss. However, because bath application of mGluR6 agonists and antagonists lacks the spatiotemporal patterning of synaptic glutamate release in the outer plexiform layer, these experiments cannot rule out the possibility that patterned glutamate release may influence postsynaptic mGluR6 expression in bipolar cell dendrites. Focal glutamate uncaging experiments could clarify the role, if any, of patterned glutamate release in regulating mGluR6 expression in bipolar cells.

Dunn's (2015) laser illumination protocol, combined with genetically encoded fluorescent protein, enables cell-type-specific ablation with superior spatiotemporal control compared with previous methods. However, the maximum time scale over which changes in retinal circuitry can be monitored using this protocol is limited by the ability to maintain the health of the retina following its isolation. Therefore, this technique likely cannot yet replace genetic ablation methods in studies of retinal synapse disassembly spanning weeks or months. The maximum spatial scale of inducible changes in retinal circuitry may be similarly limited by the challenge of serially targeting and ablating large numbers of homotypic neurons that provide convergent input to a common postsynaptic neuron. In particular, laser ablation of substantial fractions of presynaptic inputs to single wide-field retinal ganglion cells may prove difficult.

To what extent does laser-induced ablation faithfully model photoreceptor death in blinding genetic disorders? A preponderance of evidence suggests that the mechanisms of photoreceptor death in these various disorders converge upon apoptotic pathways (Chang et al., 1993; Portera-Cailliau et al., 1994). Interestingly, near-infrared laser illumination can induce apoptosis by generating reactive oxidative species that in turn activate caspase-dependent pro-apoptotic pathways (Tirlapur et al., 2001; Yoon et al., 2015). This suggests a potentially apoptotic mechanism of cell death in the laser ablation protocol introduced by Dunn (2015). Additionally, the observation that mGluR6 receptor loss in bipolar cells occurs no earlier than 50 min and with a time constant of 2 h following cone ablation may reflect the evolution of apoptosis in the ablated cell (Elmore, 2007). However, this delay and time course are also consistent with the kinetics of postsynaptic events that follow acute, necrotic death of a presynaptic cone. Ultimately, additional study of the mechanism of laser-induced photoreceptor ablation will be instructive in guiding interpretation of these results and evaluating the utility of this method in modeling photoreceptor degeneration.

What mechanisms might underlie mGluR6 loss at the cone-to-ON cone bipolar synapse? As suggested above, disruption of the precise spatiotemporal pattern of glutamate release onto postsynaptic bipolar cell dendritic tips may promote mGluR6 loss in a manner that cannot be rescued by bath application of pharmacological agents. Previous work by Dunn and colleagues (2013) revealed mGluR6 loss at dendritic tips of type 6 ON cone bipolar cells of dark-reared mice, suggesting that a light-dependent mechanism controls the localization and maintenance of mGluR6 at this synapse, at least during retinal circuit development. Because maximal glutamate release from photoreceptors occurs in the absence of light, the authors proposed that strong, sustained activation of mGluR6 during dark-rearing resulted in the subsequently observed decrease in synaptic mGluR6 allocation at ON cone bipolar cell dendritic tips. However, while glutamatergic neurotransmission from presynaptic cones is an attractive candidate for such a light-dependent mechanism, unidentified signaling pathways could also transform photon flux into changes in postsynaptic mGluR6 expression and localization. Furthermore, distinct pathways could converge upon a common mechanism that mediates the mGluR6 loss reported by Dunn and colleagues (2013) and Dunn (2015). Studies of protein–protein interactions involving mGluRs have identified several partners that bind to intracellular regions of mGluRs, including scaffold proteins that may stabilize mGluRs at synapses by promoting the formation and maintenance of macromolecular complexes (Hall and Lefkowitz, 2002; Enz, 2007). Other binding partners have been shown to regulate mGluR internalization and trafficking (Francesconi et al., 2009; Hong et al., 2009). Glutamate, cell adhesion molecules, or neurotrophic factors could act as trans-synaptic elements of signaling pathways that modify these protein–protein interactions at the cone-to-ON cone bipolar synapse.

Several key questions must be resolved en route to understanding synapse disassembly in the damaged retina and, more broadly, in the CNS. What is the presynaptic mechanism governing the allocation of postsynaptic mGluR6 receptors in ON cone bipolar cells? How do changes in receptor expression during synapse disassembly correspond to specific physiological and behavioral deficits? Do other synapses in the CNS exhibit immediate changes in postsynaptic receptor expression after loss of individual presynaptic inputs? Given the substantial progress that remains to be made in answering these and related questions, the findings reported by Dunn (2015) constitute an important advance toward an understanding of the events of synapse disassembly in the CNS.

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