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.

Synapse-Specific Reinnervation in the Injured Brain

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Review of Ichikawa et al.

Functional deficits resulting from disease or injury to the mature CNS can be mitigated by endogenous repair mechanisms. The degree of normal circuit function restored by these mechanisms is largely controlled by factors that permit and restrict regeneration and reinnervation of synapses (He and Jin, 2016). One way to improve functional outcomes is to develop strategies to overcome barriers that restrict regrowth of damaged axons. A complementary approach is to encourage spared axons to reorganize their connections to restore circuit function. This form of reorganization is induced not only after acute injuries, but also during neurodegenerative diseases, such as Parkinson's disease and amyotrophic lateral sclerosis, where subsets of neurons and synapses degenerate and unaffected neurons reorganize in an attempt to maintain normal connectivity (Schaefer et al., 2005; Guo et al., 2015). Although the therapeutic potential of such reorganization is considerable, relatively little is known about the dynamics of synaptic changes that occur as part of these processes. To improve functional outcomes, more detailed information regarding the nature of the synaptic reorganizations initiated by spared axons is required.

A recent study published in *The Journal of Neuroscience* provided an in-depth analysis of the local synaptic reorganizations that occur along Purkinje cell dendrites following cerebellar injury (Ichikawa et al., 2016b). Using fluorescence imaging and serial section EM, Ichikawa et al. (2016b) examined how the distribution of excitatory parallel fiber and climbing fiber synapses changed along the dendritic arbor of Purkinje cells following the removal of a subset of parallel fiber innervation.

Cerebellar granule cell axons ascend toward the surface of the cerebellum and send off orthogonal branches that innervate the distal dendrites of Purkinje cells, whereas climbing fibers form synapses along the proximal dendrites. Ichikawa et al. (2016b) took advantage of this distinct innervation pattern to overcome two major challenges to the study of synapse-specific reinnervation. The first concerns how to cut a subset of axons within the dense wiring of the mature brain. Ichikawa et al. (2016b) made two parallel cuts in the superficial aspect of the cerebellar cortex to remove >60% of parallel fiber inputs to Purkinje cells within a small patch of cerebellar cortex. Importantly, climbing fiber innervation to Purkinje cells within the denervated zone remained intact, and the severed parallel fiber axons did not regenerate across the legion site, meaning that any subsequent functional recovery would likely result from local reorganization initiated by uninjured axons. The second obstacle concerns how to assess whether reinnervated synapses exhibit target specificity. Because parallel fibers and climbing fibers innervate the distal and proximal domains of Purkinje cell dendrites, respectively, with a small overlapping domain, the authors could determine whether the reinnervation process reestablished these appropriate domains.

Ichikawa et al. (2016b) observed that spared parallel fibers ultimately reinnervated the appropriate Purkinje cell domain in a process that progressed through three anatomically defined phases. The first stage, the "degenerative phase," occurred 1 d after parallel fiber transection and was characterized by the appearance of free spines (i.e., spines without a presynaptic partner). In addition to those vacated by degenerating parallel fiber axons, the authors speculate that some free spines appeared through *de novo* formation because they appeared deep into the climbing fiber domain (their Fig. 5*G*) without a change in the number of climbing fiber synapses (their Fig. 8*E*). Parallel fiber terminals innervated spines within the climbing fiber compartment, leading to an expansion in the parallel fiber/climbing fiber overlap domain. GABAergic presynaptic terminals were also found to ectopically innervate dendritic spines in both the parallel fiber and climbing fiber dendritic domains. The second stage, the "hypertrophic phase," was observed 6 d later and was characterized by the enlargement of parallel fiber terminals and the recovery of synaptic innervation and parallel fiber/climbing fiber compartmentalization. The enlargement of parallel fiber terminals was associated with their innervation of multiple free spines, which doubled their terminal-to-spine

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contact ratio (their Fig. 2*L*). Finally, the "remodeling phase" occurred over the next few weeks and was characterized by the extension of parallel fiber collateral sprouts, the shrinkage of presynaptic axons and terminals back to normal size, and the complete elimination of GABAergic spine synapses. Synapse numbers and parallel fiber/climbing fiber compartmentalization were fully restored, reflecting a pruning process that eliminated all remaining ectopic parallel fiber and GABAergic spine synapses.

The expansion and contraction of the parallel fiber/climbing fiber overlap domain during the reinnervation process (their Fig. 5*G*) are similar to the developmental segregation of parallel fiber/climbing fiber domains (Ichikawa et al., 2016a). The developmental pruning of parallel fibers from Purkinje cell proximal dendrites requires metabotropic glutamate receptor 1 (mGluR1) activity (Ichikawa et al., 2016a). mGluR signaling has also been implicated in homeostatic synaptic scaling in response to chronic activity perturbation (Hu et al., 2010), and the loss of mGluR1 activation in response to parallel fiber transection may underlie the early reorganization that is observed during the degenerative phase. Indeed, activity deprivation by chronic administration of tetrodoxin leads to the appearance of free spines, the expansion of the parallel fiber/climbing fiber overlapping domain, and the accumulation of the σ subunit of the glutamate receptor (GluD2) at parallel fiber and climbing fiber spines (Cesa et al., 2003). GluD2 is a potent synaptic organizing molecule (Matsuda et al., 2010; Uemura et al., 2010) that is normally only expressed along distal Purkinje cell dendrites at postsynaptic regions of the parallel fiber synapse to drive its formation and stability (Kurihara et al., 1997). The injury-induced loss of mGluR1 activity may therefore influence the accumulation of GluD2 at newly formed free spines and lead to their innervation by sprouting and enlarged parallel fiber terminals. Subsequent mGluR1 activity at these reinnervated synapses could then drive the pruning of ectopic parallel fiber synapses from the climbing fiber domain and lead to the reestablishment of pathway segregation.

GluD2 plays a major role in parallel fiber reinnervation, reflected by the absence of reinnervation after injury in GluD2 knock-out mice (Ichikawa et al., 2016b). Indeed, in unoperated GluD2-deficient mice, the state of Purkinje cell innervation by parallel fibers is remarkably similar to wild-type Purkinje cell innervation during the degenerative phase: there are fewer parallel fiber synapses, a corresponding increase in the number of free spines, and the parallel fiber/climbing fiber overlap domain is broadened. Transection of parallel fibers in GluD2-deficient mice resulted in an additional loss of parallel fiber innervation and an increase in free spines, but neither measure recovered at any point after the injury (their Figs. 2, 8).

Although it is clear that GluD2 is essential for the effective reinnervation of Purkinje cell dendrites by parallel fiber collateral axons, these observations raise questions regarding the relationship between the different phases of synaptic reorganizations. Is GluD2 generally required for synaptogenesis in both the hypertrophic and remodeling phases, or is it simply required for the transition between these phases? The transient appearance of GABAergic spine synapses is absent in GluD2 knock-out mice, suggesting that GluD2 also dictates the formation of these synapses. Do GABAergic synapses themselves play a role in regenerative phase transitions? A clear understanding of the relationship between each phase of reinnervation could inform the development of therapeutics that may promote the progression of similar phases in recovery-resistant systems.

A notable feature of the phase transitions described by Ichikawa et al. (2016b) is that parallel fiber collateral axons ultimately displace the enlarged parallel fiber synapses during the remodeling phase, even though both synaptic contact (their Fig. 2*J*) and parallel fiber/climbing fiber compartmentalization (their Fig. 5*I*) were reestablished during the preceding hypertrophic phase. Because the denervation surgery removed >60% of parallel fiber inputs, both the hypertrophic and remodeling phases require spared granule cell axons to more than double their complement of synapses. The hypertrophic phase accomplishes this by expanding the synaptic terminal itself, whereas the remodeling phase involves expansion of the axon arbor by collateral axon sprouting. Thus, the remodeling phase reestablishes a one-to-one relationship between the presynaptic terminal and the postsynaptic spine after the hypertrophic phase (their Fig. 2*L*). How this transition occurs is unknown, but it might involve calcium signals in Purkinje cell dendrites. Spines innervated by a common presynaptic terminal would likely be active simultaneously and may result in greater postsynaptic calcium elevation, which has been implicated in the development of synaptic connections in other brain regions (Lee et al., 2016). Presynaptic terminals that innervate multiple spines may be slightly weakened as a homeostatic response to heightened postsynaptic activity and could therefore be more sensitive to activity-dependent competitive displacement by reinnervating parallel fiber collateral sprouts during the remodeling phase.

Even after the three stages of recovery described by Ichikawa et al. (2016b) have occurred, cerebellar synaptic physiology might remain altered as a consequence of the degree of presynaptic expansion experienced by single granule cells. Because the synaptic vesicle cycle is a primary source of energy consumption during synaptic transmission (Rangaraju et al., 2014), doubling the number of output synapses could carry metabolic consequences that limit synaptic function. For instance, repetitive presynaptic activity might quickly deplete the energy stores of a neuron with an expanded terminal field, and information processing that relies on repetitive or tonic neurotransmission could be predominantly affected. Furthermore, evidence from peripheral systems suggests that the degree of presynaptic expansion is limited, possibly because single neurons are unable to maintain increasingly large numbers of synapses (Rafuse et al., 1992). These limitations would significantly constrain the potential for recovery following injury or during the progression of neurodegenerative disease.

This study by Ichikawa et al. (2016b) has contributed an important framework that deepens our understanding of the regenerative potential of the mature brain. We must continue to develop a clear understanding of how neural circuits reorganize in response to injury and disease to best harness the regenerative potential of neural systems and to most effectively improve their recovery.

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