Sensory and Motor Systems

# Terminal Schwann Cells Lead Synapse Remodelling following Injury[1,2]

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### Significance Statement

This commentary article describes the importance and significance of the article recently published by the Kang and colleagues in *The Journal of Neuroscience* in 2014. Kang and colleagues provided new features of injury induced synapse remodelling. This commentary article summarizes the important findings of Kang and colleagues with the appropriate commentary. In addition to the article by Kang and colleagues, many other very exciting and recent studies about synapse remodelling in peripheral and central nervous system have also been included.

Although the neuromuscular junctions (NMJs) of adult animals are stable in their structure, the structure of the synapse may alter under certain circumstances: on account of development, aging (Kang and Lichtman, 2013), disease, and reinnervation after injury (Rich and Lichtman, 1989). Axon regeneration in the peripheral nervous system (PNS) after injury is well known, and is greatly supported and served by Schwann cells (SCs), glial cells in the PNS. Terminal Schwann cells (TSCs) that cover motor nerve terminals are known to extend their processes and lead regenerating nerve terminals to reinnervate adjacent postsynaptic sites after injury. Once the axons have been restored to their synaptic sites, remodeling of the contacts takes place. Structural changes in the synaptic site after reinnervation are associated with the speed of reinnervation. Fast reinnervation leads to modest remodeling, whereas delayed reinnervation results in more structural changes at the synapse (Rich and Lichtman, 1989). Important questions in all these events are how TSCs take the lead to regenerate synapse and how they play a role in synapse remodeling?

Kang and colleagues (2014) sought to answer these questions by sequential vital imaging of mice expressing GFP in their SCs and CFP in their motor neurons. They performed lateral, medial, and double nerve crush to achieve variable periods of denervation. As a consequence of different denervation methods, differential axon terminal branching patterns and directions of the reinnervation were observed (Kang et al., 2014, their Table 2). To explore the role of TSCs in synapse remodeling, Kang and colleagues (2014) first studied the behavior of the TSCs and regenerating axons during synapse remodeling. On denervation, TSCs occupy the synaptic area, but gradually abandon a variable portion of the acetylcholine receptors (AChRs) depending on the length of the denervation period (Kang et al., 2014, their Fig. 2); however, what drives TSCs to abandon AChR sites remains unclear. Multiple reinnervation, which is known to occur during reinnervation after injury, was observed here (Kang et al.,

2014, their Fig. 5*A*). AChRs that were isolated from the others and lacked SC coverage were susceptible to loss (Kang et al., 2014, their Figs. 4, 6), suggesting that AChR loss after reinnervation is associated with the abandonment by SCs (Kang et al., 2014 their Fig. 5). Here, it is clear that besides inducing and guiding regenerating axons, TSCs also affect postsynaptic receptor sites during regeneration and remodeling. Since postsynaptic receptor loss is the consequence of the remodeling process after injury, it is clear that there will be more receptor loss in the aged animals where junctions are already fragmented. Thus, it is interesting to know how TSCs lead synapse remodeling in older mice following injury. Nevertheless, this study raises the question of how the absence of SC coverage at denervated postsynaptic sites leads to receptor loss. Here, it seems that in reinnervated receptors, innervating axons cause elimination of unoccupied receptor sites by reorganizing the postsynaptic sites to coincide with the nerve terminal location, although the exact factors that contribute to the postsynaptic receptor loss as a consequence of synapse remodeling remains unclear. The major questions here are how TSCs sprouting is induced after injury, and what are the driving factors behind this sprouting? A possible explanation could be that the induced expression of ErbB2 in SCs enhances sprouting of TSCs in a manner reminiscent of the sprouting that occurs after denervation. This indicates that NRG/ErbB signalling may be involved in TSC sprouting, and it could be evaluated by inactivating ErbB receptors on TSCs after nerve injury (Hayworth et al., 2006).

During synapse remodeling, branches of axon terminals in regenerating axons initially followed the SC processes within the old synaptic sites (Kang et al., 2014, their Fig. 3), and formed escaped fibers (Kang et al., 2014, their Figs. 3*A*, 4, 8). In some cases, reinnervation stimulated sprouting of SCs that appeared to reinnervate previously abandoned receptors (Kang et al., 2014, compare their Figs. 3*B*, 4). A similar event can be seen in frogs where TSCs extend their processes on the arrival of regenerating axons. In addition, TSCs in frogs express neuronal agrin, which may induce AChRs clustering under TSCs sprouts.

Addition of new AChRs was observed during the reinnervation process after injury (Rich and Lichtman, 1989), and can also be seen in Kang et al. (2014, their Fig. 8). Regenerating escaped fibers contained synaptic vesicles and seemed to be capable of forming a functional synapse (Kang et al., 2014, their Fig. 9). In their study, Kang and colleagues (2014) focused entirely on the morphologic aspects of TSC-mediated synapse reinnervation. Physiology of the reinnervated synapses was not examined here, and it would be of great interest to examine it further. One important question: how do the SCs communicate to the axons during regeneration after denervation? Recently, it was proposed that dedifferentiated SCs secrete exosomes that are internalized by axons. Growth cone morphology is then shifted into the pro-regenerating phenotype by the exosomes (Lopez et al. 2013).

Findings from the TSCs are relevant to astrocytes, which are glial cells in the CNS and responsible for the portion of synapse remodeling and mediating synapse elimination in the developing and adult brain. Developing mice lacking

*Megf10* and *Mertk* phagocytic pathways failed to refine retinogeniculate connections and returned an excess number of synapses. Moreover, in adult mouse brain, astrocytes continued to engulf both excitatory and inhibitory synapses, suggesting that astrocytes are the critical players in the synapse remodeling (Chung et al. 2013). Since astrocytes have been shown to be involved in synapse remodeling, it is very interesting to know whether astrocytes also regulate injury-induced synapse remodeling in the CNS. Furthermore, like TSCs, astrocytes are also needed for the formation and maturation of synapses in the CNS. In cultured retinal ganglionic cells, removal of astrocyte feeding layers leads to decreased synapse numbers, suggesting that astrocytes are needed for synapse maintenance (Slezak and Pfrieger, 2003). Unlike PNS axon regeneration, capability of the CNS in axon regeneration is extremely poor due to diminished intrinsic regenerative capacities and lack of glial support (Lopez et al. 2013; Zainul, 2013). Despite positive roles of astrocytes in the CNS, astrocytes do not seem to be a favorable candidate in CNS axon regeneration due to the formation of glial scar, which is the physical barrier through which axons cannot further elongate. Astrocytes in glial scars become hypertrophic and release proteoglycans that contribute to the poor CNS axon regeneration. However, ablation of proliferating reactive astrocytes disrupted scar formation that consequently causes intensified inflammation, tissue damage, demyelination, and impaired functional recovery. It was reported in the early 1980s that the peripheral nerve environment, mainly composed of SCs, is more favorable for the CNS axon regeneration than CNS milieu. After such an excellent finding, SCs transplantation into the injured CNS is being extensively explored as an approach to overcome the problem of poor CNS regeneration (Deng et al., 2014). Moreover, transplantation of genetically modified SCs to secrete neurotrophin and chondroitinase better improved axon regeneration and functional recovery (Kanno et al., 2014). Interestingly, in a recent study, astrocytes revealed increased phosphorylation expression by Cdc2 and integrin activation, which are positively associated with the increased neurite outgrowth (Toy and Namgung, 2013). Recently, the FDA has approved clinical trials for SC transplantation in spinal cord injury. Further studies about the roles of astrocytes and comparing the common features shared with SCs may provide better understanding of the facts and factors that contribute to the poor regenerative response in the CNS.

In addition to synapse remodeling, TSCs also participate in the long-term maintenance of adult NMJs, although the maintenance mechanism as such is still not fully understood (Reddy et al., 2003). It is of great interest to know whether the TSCs provide mechanical support or trophic support (Feng and Ko, 2008). It has been shown that mature SCs occupy segregated territories, and such segregation is absent during development, lost after injury, and swiftly restored after reinnervation following injury. The territory of a single TSC is constrained by competition with its glial neighbors and axon terminal, but not by axonal activity, revealed by laser ablation of single SCs and axons (Brill et al., 2011). It has been observed that TSCs and nerve terminal sprouting is absent in muscle after injury when nitric oxide is blocked (Marques et al., 2006); however, the primary source of nitric oxide is not

known since it is expressed by TSCs, nerve, and muscle. Moreover, NRG-1 signaling is upregulated in SCs after derivation, suggesting that NRG-1 signaling may be responsible for inducing SCs sprouting (Carroll et al., 1997). Furthermore, TSCs seem to suppress nerve terminal plasticity when Semaphorin 3A is selectively expressed in TSCs only at fast twitch muscle fibres after nerve injury (De Winter et al., 2006). A plethora of studies demonstrate that denervated SCs express a number of genes, for example, *DCC* and *Uncoordinated* (*Unc*)*5H2*, which are receptors of netrin-1, osteopontin, and SC secretory apolipoprotein that positively regulate functional recovery after PNS injury (Zainul, 2014). To study the overall function of the SCs in mammalian NMJs, it would be interesting to perform loss-of-function studies with gene knock-outs that lead to the ablation of SCs, or else to employ a complement-mediated cell lysis approach (Reddy et al., 2003). This would enable us to better understand the functions of SCs in axon regeneration and synapse remodeling. One problem, however, is that the mice lacking SCs die immediately after birth, which would mean resorting to genetically altered mice with the reduced expression of SCs. It is known that physical activity determines the functioning of the NMJs and, interestingly, it has been recently reported that a muscle-specific increase in the expression of peroxisome proliferator-activated receptor γ co-activator 1α (PGC-1α) significantly promoted NMJ remodeling even when physical activity was not induced. The plastic changes in the NMJs were not restricted to the postsynaptic sites, but also modulated presynaptic morphology and function, thus confirming the pivotal role of skeletal muscle in NMJ remodeling (Arnold et al., 2014). However, Arnold and colleagues (2014) did not examine injury-induced NMJ remodeling, and it is known that the incomplete recovery following peripheral nerve injury can result in changes in the distribution of muscle fiber-types (Mendler et al., 2008). Based on the findings by Arnold and colleagues (2014), it would be highly interesting to examine whether SCs are involved or whether PGC-1α has direct, SC-independent effects on reinnervation in adult mice with PGC-1α overexpression after denervation.

In summary, Kang and colleagues (2014) demonstrated the cause of synapse remodeling and provided evidence for new features of the synapse remodeling after denervation. What is abundantly clear from their elegant work is that TSCs actively play a pivotal role in synapse remodeling following nerve injury. Their findings nevertheless raise many questions for future consideration, such as why the SCs abandon their previous synaptic sites, and what is the mechanism that leads to the failure of reinnervation at certain synaptic sites. Molecular signaling pathways that are responsible for the interactions among SCs, axon terminals, and the postsynaptic muscle fibres during synapse remodeling also remain to be further elucidated. Unmasking the underlying molecular mechanism will add vital insights to our understanding of the biology of synapse remodeling. Moreover, a therapy could be developed for shortening the denervation period or for promoting TSCs maintenance without axonal trophic support, which would be essential for reducing growth of SCs away from the synapses and could consequently promote actual synapse recovery.

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