Editorial

Retinal ganglion cells regenerate long-distance axons through neural activity stimulation and find their way back to the brain

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Human central nerve system (CNS) is an extremely complex and delicate structure. While regeneration is possible in some reptiles and fish CNS, the regeneration capacity seems completely lost in adult mammals. Therefore, the classic concept is that once neurons in mammal CNS are damaged in injury or disease, they cannot regenerate themselves anymore. Although we have known this feature of mammals CNS for quite some time, many scientists have never given up their dreams in finding the "Elysium" for CNS regeneration. In newborn children and in very rare cases of adult humans, we do see, amazingly, some reports that show successful regeneration of CNS neurons or their axons (1). From a clinical perspective, however, regeneration of neuron or its axon is still not good enough. The key issue to address is to reestablish neural circuit connections with functional neural electrical activities.

We know that neural retina is an extension of CNS brain. The model of eye-to-brain visual pathway, consisting of retinal ganglion cell (RGC) and subcortical targets is a quite popular model investigating neural axon regeneration. Previous study demonstrates that electrical stimulation can shape corticospinal (CS) axon outgrowth and augment connections after injury (2). Similarly, another study using RGCs shows that electrical stimulating accelerates axonal outgrowth *in vitro* (3). Interestingly, electrical stimulates works through the electrical activity of RGCs. Consequently, Lim *et al*. translate these findings and uses electrical stimulating into visual signal in their study, which is a potent stimulus that closely mimics genuine visual signals of the eye (4). After the optic nerve is crushed, the adult mice are exposed to high-contrast visual stimulation daily for 3 weeks. Amazingly, RGCs axons are observed to regenerate over long distance into the brain. To confirm whether RGCs axon regeneration is initiated by electrical activity from visual stimulation, RGCs are being either chemo genetically activated or silenced. The distance of regeneration is proportional to the RGC activity level; the effect of visual stimulation on RGC axon regeneration is abolished by RGC silence, while increased activity leads to better regeneration.

Optic nerve axon regeneration has been investigated for many years. The following factors are shown to be related to the capacity of regeneration, including cell-intrinsic signals, transcription factors and their inhibitors, receptors to cell-extrinsic inhibitors and intraocular inflammation (5). Among these factors, PTEN and SOCS3, as cell-intrinsic suppressors, are the most promising. PTEN deletion with SOCS3 deletion, in the presence of CNTF, successful induces a longer distance axonal regeneration (6,7). Recently, more attention is directed at mTOR, a downstream molecule within the PI3K/PTEN/mTOR pathway. The mTOR signaling pathway has a pivotal role in numerous cellular processes, including axonal regeneration. Strengthening mTOR signaling shows increased axons regeneration in optic nerve lesion (8). Therefore, cRheb1, which is a positive regulator

of mTOR signaling, is introduced into Lim's study. In the present of visual stimulation in addition to Rheb1, axon regeneration reaches a longer distance. Unfortunately, the stimulation of mTOR signaling in RGCs is not the "cure" for regeneration. RGC axons still fail to pass beyond the mid-optic nerve and optic chiasm.

Following the thinking that optic nerve is an extended part from CNS, Lim and his colleagues were enlightened by the rehabilitation treatment of limb paralysis caused by spinal cord injury. It has been reported that forced use of an impaired limb promotes sprouting of CS axons (9). By suturing shut the non-lesioned eye, the lesioned eye was forced to be biased used. Surprisingly, the cocktail scheme combining visual stimulation with cRheb1 plus biased use led to axonal regeneration extended through optic chiasm, down the optic nerve and back to the brain.

As a milestone that long-distance regeneration of RGC axons to the brain is possible, the next critical question "where would the axon go" is on the table. A new transgenic mouse line is adopted in the study, in which Cochlin-GFP (CoCH-GFP) is used to label a specific subtype of RGC that densely innervate the vLGN, dLGN, OPN and SC. These RGCs avoid the SCN, MTN and intergeniculate leaflet (IGL). After crushing the optic nerves in these transgenic mice, the regenerated CoCH-GFP + RGC axons are found to reach the vLGN, dLGN, OPN and SC, bypassing the SCN, the nucleus of the optic tract (NOT) and the MTN. This result indicates that RGCs are remarkably capable of navigating the axons back to and re-innervate their original targets in the brain. Furthermore, the rebuilding of visual function is also detected in these mice.

The remarkable advantages in this study bring the scientists closer to goal of curing nerve injuries. Especially, it is a sparkling strategy to apply unilateral lid suture in order to force the biased use of the other eye, reminding us the developmental connections between CNS and optic nerve. This work is promising in the entire field of neural regeneration in the CNS. Forcing the use of an impaired limb promotes CS axonal regeneration, not only by activity stimulation, but also by biased use. Considering these results in spinal injury, in terms of optic nerve, the activity stimulation is translated into visual signal and the biased use is achieved by unilateral lid suture, similar to the eye patch in amblyopia.

However, since the optic nerve crushing model is used, the result probably means more to patients with axon injury. As to glaucoma, it is still unknown whether the transplanted RGCs can integrate into the host retina or regenerate axons. In the optic nerve-crushing model, the connection between photoreceptors and RGC cell bodies are still intact. This is the structural foundation of "visual stimulation". However, in glaucoma, the RGCs are dead. If the transplanted RGCs cannot reconnect to photoreceptors, it is meaningless to provide the visual stimulus. On the other hand, the previous *in vitro* study shows that the regeneration capacity of RGCs is lost when dendritic grows and synaptic inputs expand (3,10). Moreover, during the course of development, RGC axons were guided by the recognitions of receptors expressed on growth cones and their ligand molecules, such as Cadherin 6 (11) and Eph (12,13). Another study in spinal cord injuries showed that the axonal re-innervation is guided chemotropically (14). As the ganglion cell bodies are already ruined in glaucomatous retina, it is in doubt that whether the new RGCs still have the memory to differentiate into the specific missing RGC subtypes and then recognize the guided signals and go back to the right targets. The current study also suggests that the time window critically affects the capacity of RGCs axon regeneration after crush injury. We do have the concerns in this study whether the axons are completely crushed and the debris disappeared before regenerating axon finding their paths across the abandoned field through the optic nerve. Are there any essential guiding structures or molecules presented within optic nerve which help the regenerating axons finding home to their brain targets? Or otherwise, are there any possibilities that some axons are partially damaged and survive the crush, while they are the ones regain their anatomical and functional integrity afterwards.

Nevertheless, the current study provides us with an exciting new strategy investigating RGCs exon regeneration. It is our genuine wish that more and more studies are completed based on this model, which will unequivocally confirm the regenerating capacity that we have been dreaming for in decades. If the injured axons in optic nerve are truly capable of re-growing back to the brain, this could be promising for some clinical patients, though there is a long way to go. We wish that strategy for axon regeneration in the current study can be translated into numerous studies using stem cell transplantation. Hopefully, neural activity stimulation and mTOR pathway modulation can be helpful to regenerating axons from RGCs derived from transplanted stem cells.

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None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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