

# Reaching for the brain: stimulating neural activity as the big leap in optic nerve regeneration

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*Comment on:* Lim JH, Stafford BK, Nguyen PL, *et al.* Neural activity promotes long-distance, target-specific regeneration of adult retinal axons. *Nat Neurosci* 2016;19:1073-84.

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In the August issue of *Nature Neuroscience*, Lim and co-workers reported on their study of axonal regeneration in the adult optic nerve, revealing that combined activation of mammalian target of rapamycin (mTOR) signaling and enhancing neural activity leads to successful restoration of axonal trajectories to visual brain centers (1). Here, we shed light on their breakthrough findings and how these may represent a major leap forward in the search for novel strategies for central nervous system (CNS) repair.

Along the line of evolution, animals have lost the capacity to repair their CNS. The presence of axonal outgrowth-repressing molecules, the lack of neurotrophic factors and axonal guidance cues, and the repressed intrinsic growth state of adult neurons, have turned the adult mammalian CNS into a hostile environment for regenerating neurons/axons. Because of this limited ability of self-repair, in conjunction with the lack of preventive treatments, CNS injury and diseases lead to irreversible, often progressive debilitation and have a tremendous impact on the patients' quality of life. Furthermore, given the rising life expectancy and the progress in other medical fields (e.g., cardiovascular and cancer treatments), neurodegenerative diseases have turned into an increasing socio-economical challenge and research into "healthy aging" has finally gained attention of policy makers.

In recent years, the focus of neuroregenerative research has somewhat shifted from manipulating extrinsic inhibitors

of axonal regeneration to reprogramming the intrinsic growth capacity of neurons. Various experimental paradigms in animal models of CNS injury were tested with varying success in order to rebuilt axonal trajectories, and the optic nerve has proven a particularly valuable research model in this context. Research into several signaling pathways for intrinsic growth control (e.g., JAK/STAT, PI3K/Akt/mTOR and MAPK/ERK) has led to the identification of multiple molecules that regulate axogenesis. Among them, the mTOR kinase stands out as one of the most promising factors to promote CNS regeneration. Upon axonal injury, mTOR activity is markedly reduced and maintenance of pre-injury mTOR activity levels, e.g., via inhibition/downregulation/deletion of phosphatase and tensin homolog (PTEN)—which at least in part acts by enhancing mTOR activity—induces axonal regeneration in the adult mammalian CNS. Nevertheless, most potent regeneration has been obtained by combining mTOR activation with other treatments affecting intrinsic or extrinsic factors, such as SOCS3 deletion, c-myc overexpression, and hyper IL-6 expression (2-4). Although these combinatorial experimental treatment paradigms can induce robust sprouting of retinal ganglion cell axons and navigation beyond the optic chiasm, correct navigation and synapse formation with their proper brain targets seems still out of reach.

With their study, Lim *et al.* (1) add a novel strategy to this growing list of pro-regenerative interventions, i.e.,

increasing electrical activity of retinal ganglion cells to enhance regrowth of their axons. This builds upon a large body of evidence—both from preclinical work and clinical trials—for its success in peripheral nerve regeneration, where electrical stimulation is a well-known treatment to promote neural regeneration and functional recovery (5,6). The molecular mechanism of this stimulated peripheral nerve regeneration is believed to largely revolve around an elevation of cyclic AMP (cAMP) in response to electrical stimulation, which activates a variety of pathways, including the CREB transcription factor, which leads to subsequent upregulation of neurotrophic factors and their receptors, as well as various other growth associated genes (7-9). Interestingly, evidence for a neuroprotective and regeneration-promoting effect of electrical stimulation has also been found in the retina, thereby suggesting similar mechanisms to be at play in the CNS. Indeed, in an *in vitro* study, Goldberg *et al.* (10) demonstrated that retinal ganglion cell survival and neurite outgrowth greatly increase upon electrical stimulation. Similarly, stimulating retinal neurons with a transcorneal electrode proved to increase *in vivo* survival and axon preservation following optic nerve axotomy and crush, respectively (11,12).

In their manuscript “Neural activity promotes long-distance, target-specific regeneration of adult retinal axons”, the research group led by prof. Huberman integrated two experimental paradigms in order to create a synergistic effect on axonal regeneration in the optic nerve. They cleverly combined activation of mTOR signaling with visual stimulation to boost neuronal activity in the damaged neurons. Whereas mTOR activation appeared a prerequisite for axonal outgrowth, enhancing neural activity of the injured neurons proved to be the indispensable trigger in this study to not only induce axonal outgrowth but also navigation and target reinnervation. Furthermore, with an elegantly designed set of experiments in which neural activity was either abolished or promoted by use of Designer Receptors Exclusively Activated by Designer Drugs (DREADD)-based chemogenetic tools, Lim *et al.* were able to pinpoint neuronal spiking as the driving force for this regeneration-promoting effect. Notably, although their combined treatment did successfully induce axonal outgrowth beyond the optic nerve lesion site, it appeared only modestly effective in doing so—e.g., more robust sprouting is seen upon *pten* deletion. What is so striking about this study, however, is that these axons were capable of long-distance navigation and even reinnervation of several nuclei of the central and accessory visual

system. Besides the work of the Benowitz (13) and Park laboratories (14), who reported axonal regeneration up to the superior colliculus and suprachiasmatic nucleus, respectively, no study ever described a successful reinnervation of as many visual system nuclei as now observed by Lim *et al.* Tracing studies of genetically labelled retinal ganglion cell subtypes, moreover, confirmed that their axons travelled to the proper target neurons in the brain, while partial recovery of some vision-driven behaviors confirmed that at least part of them successfully established new synapses. Overall, these data provide the first indication of an experimental treatment that can overcome (some aspects of) optic nerve injury-induced blindness by reactivating the intrinsic growth potential of retinal ganglion cells. Notably, whereas the first part of the study stands out for its exquisite experimental design and cutting-edge technology, the second half—describing long-distance regeneration to and functional recovery in several brain targets—is somewhat more exploratory and comes with a few limitations. In particular, the possibility that spared rather than regenerating axons are (at least in part) responsible for the observed effects has not been convincingly excluded. The critical reader would therefore be looking forward to follow-up studies with a deeper focus on the timing and success of target reinnervation, e.g., with increased animal numbers, extensive visual testing before, immediately after and at late time points post lesion, and additional electrophysiological read-outs in the brain target areas.

The novel experimental paradigm to enhance visual system repair introduced by Lim *et al.* provides an outlook on what future CNS regenerative therapies may look like. Visual stimulation—as a proxy for enhancing neuronal electrical activity—initiates intracellular signaling mechanisms for cell survival and neurite outgrowth. By influencing protein synthesis, modification and activation, with or without altering gene expression, it enhances an endogenous network of signaling cascades and therefore elicits a more potent response than any other intervention targeting—or should one say, outbalancing?—a single molecule or pathway. Furthermore, its effect is likely to be multifactorial, potentially not only affecting the intrinsic growth capacity of a neuron. Electrical stimulation of neurons has indeed been suggested to also alter their metabolic regulation, neurotrophin secretion, receptor profile, responsiveness of growth cones to extrinsic growth/guidance cues, synapse formation/stabilization, etc. (15). This fits the idea that a future CNS restorative therapy

will highly likely be a combinatorial treatment, tackling the multiple underlying causes of the adult CNS poor regenerative capacity. Notably, in line with this multi-target approach, a prerequisite for successful axonal regeneration is to make sure that a sufficient population of neurons survives the initial insult, where after they can regrow their axons. Besides being a fruitful approach for axonal regeneration, the treatment paradigm presented by Lim and co-workers also proved to have a profound neuroprotective effect (with a 30% increase in retinal ganglion cell survival), which could have added to the robust axonal regeneration that they observed.

At the same time, the study also unveils some challenges that still stand in between the successful development of therapies for CNS restoration. Mirroring the time course of axonal regeneration, a therapy would have to induce neuroprotection followed by axonal sprouting, elongation and navigation, and target reinnervation. With most of the research still focusing on robust initiation of axon outgrowth, the current thinking is that promoting correct navigation over long distances and anatomical decision points, is the next big challenge in this research field. The data presented by Lim *et al.*, however, suggest that axonal guidance might be less of an issue than expected. Again, the cellular and molecular mechanisms that have been tied to enhancing neuronal activity via electrical stimulation, might explain. These include alignment of astroglial processes—which serve as guiding scaffolds for growing axon growth along them—favoring growth cone navigation over stalling/retraction, enhancing the intrinsic sensitivity of a neuron to growth-promoting factors and cues. Or, alternatively, as suggested by the authors, ligands and receptors that mediate developmental axon navigation in the CNS may still be present and/or become upregulated upon injury. This finally leads us the ultimate stages of CNS repair: synapse formation and refinement, as well as remyelination. The study of Lim *et al.* did not explore these in depth, yet the varying degree of functional recovery for different visual tasks—corresponding to different brain nuclei in the visual pathway—that they observed, clearly indicates that more work is needed. Of note, adding to the holistic approach of neural activity stimulation, neuron-to-target activity is important for stabilizing synapses.

To conclude, this report extends the existing literature on the beneficial effects of neural activity on neuroprotection and axonal regeneration, which all used electrical stimulation (15). Over the last decade, new methods to artificially control neural activity have become available,

such as optogenetics and chemogenetics using DREADDs. Both methods show great promise by allowing genetic targeting of specific cell populations, which might prove to be the next scientific breakthrough—given that activity patterns will likely need be tailored to neuronal subtypes to further increase efficacy—and a significant advantage over non-specific electrical stimulation. Whereas these methods are well-established tools to dissect neural circuitry, this report now also highlights their potential in the fields of CNS regeneration and protection (16,17). The take-home message of the article by Lim *et al.* thus is a message of hope, pointing out that a fairly easy-to-translate approach such as neural stimulation may force a breakthrough in the fight for sight and CNS repair in general.

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### Footnote

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

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