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Mammalian dendritic regrowth: a new perspective on neural repair

This scientific commentary refers to 'Insulin signalling promotes dendrite and synapse regeneration and restores circuit function after axonal injury', by Agostinone *et al.* (doi:10.1093/brain/awy142).

Neuroscientists studying injuries and diseases of the CNS have largely focused on mechanisms of neuronal dysfunction or death and of axon degeneration; and reciprocally, on developing strategies to maintain cellular function and to promote axon regeneration (Fig. 1). We now know the pathways that lead to cell death or dysfunction in multiple models, and promising treatment strategies for neuroprotection and/or cellular replacement are under development. Because communication within the CNS occurs largely through axodendritic synapses, it is also essential to protect or restore the axons and dendrites of surviving neurons. Axons are commonly compromised in CNS injuries and diseases, and therapies that address physical or chemical barriers to growth, as well as neuron-intrinsic, glial, and inflammatory regulators of axon growth, have emerged in animal studies, with a few potential treatments having entered clinical trials. Dendrites are likewise affected by CNS injury and disease, and several CNS diseases are known to directly affect synaptic structures. Importantly, however, the structural degeneration of dendrites has been little studied, and their regeneration relatively neglected. In this issue of *Brain*, Agostinone and co-workers help to fill this gap by identifying an intracellular signalling pathway that underlies injury-induced dendritic retraction and growth in the mammalian visual system, and by demonstrating a relatively straightforward strategy to restore dendritic structure and circuitry (Agostinone *et al.*, 2018).

Using an *in vivo* model of traumatic nerve injury, this article provides evidence that the dendritic and somatic atrophy that occur in retinal ganglion cells (RGCs) after injury to the optic nerve can be reversed with insulin acting through the mTOR pathway. The rodent optic nerve injury model closely mimics clinical optic nerve trauma and is a useful model for clinical glaucoma. This model has also helped generate strategies to address other types of CNS injury. The visual system is particularly well suited to investigating neural responses to injury, with the dendrites, somata, and axons of RGCs occupying distinct zones in the retina (inner plexiform layer, retina ganglion cell layer, and nerve fibre layer) and optic nerve, which are relatively accessible CNS structures. Current consensus from optic nerve injury, other glaucoma models, and clinical data is that dendritic retraction is an early event in glaucomatous pathology, beginning before somatic loss, axon pathology, and overt functional changes. In agreement with this literature, Agostinone *et al.* demonstrate that RGC dendritic complexity is reduced by 3 days after optic nerve transection, as determined using four common histological measures of genetically-labelled dendritic trees: total dendritic length, dendritic field

Glossary

Dendritic complexity/morphology: In adult mammals, the shape and location of dendritic arbors can be used to identify neuronal subclasses (including RGCs). Dendritic morphology is commonly assessed using measurements of dendrite length, area, branching, and Sholl analysis, which involves counting the number of intersections between dendrites and concentric rings centred at the soma. Dendritic arbours shrink and lose complexity, or degenerate, in injury and disease. Along with dendritic morphology, circuit function requires the structural and functional integrity of synaptic connections.

area, number of dendrite branches, and Sholl analysis.

To date, most studies of endogenous and treatment-induced dendritic regrowth have used invertebrate peripheral sensory neuron injury models. Recent experiments in *Caenorhabditis elegans* described spontaneous dendrite regeneration (Oren-Suissa *et al.*, 2017), while experiments in *Drosophila* confirmed and extended these findings and have begun to elucidate the underlying mechanism, which appears to involve the PTEN/PI3K/Akt/mTOR pathway and bantam microRNA (Song *et al.*, 2012) and to be activity-dependent (Thompson-Peer *et al.*, 2016). Two recent, intriguing papers make use of *in vivo* two-photon microscopy to show dendritic growth in the brains of adult rodent models. Zhao *et al.* (2017) provide 3 h of time-lapse images of a single, normal, mostly morphologically stable dendrite in the mouse motor cortex. They contrast this to images of a single dendrite after nanosurgical ablation, showing a dynamic spontaneous morphological response, although the ultimate result is increased distance between the proximal and distal ends of the severed dendrite. These data hint at an exciting potential for regrowth while remaining in line with other literature suggesting that the summation of endogenous signals present during injury and disease results in dendritic arbour retraction in mammals. Paveliev *et al.* (2016) present images and quantification of spontaneous and treatment-induced dendritic growth in mouse somatosensory cortex after brain prick injury. However, because therapy with pleiotrophin began immediately after injury, it is difficult to distinguish protective from regenerative effects.

The experiments by Agostinone *et al.* address regeneration by showing that daily insulin treatment starting 3

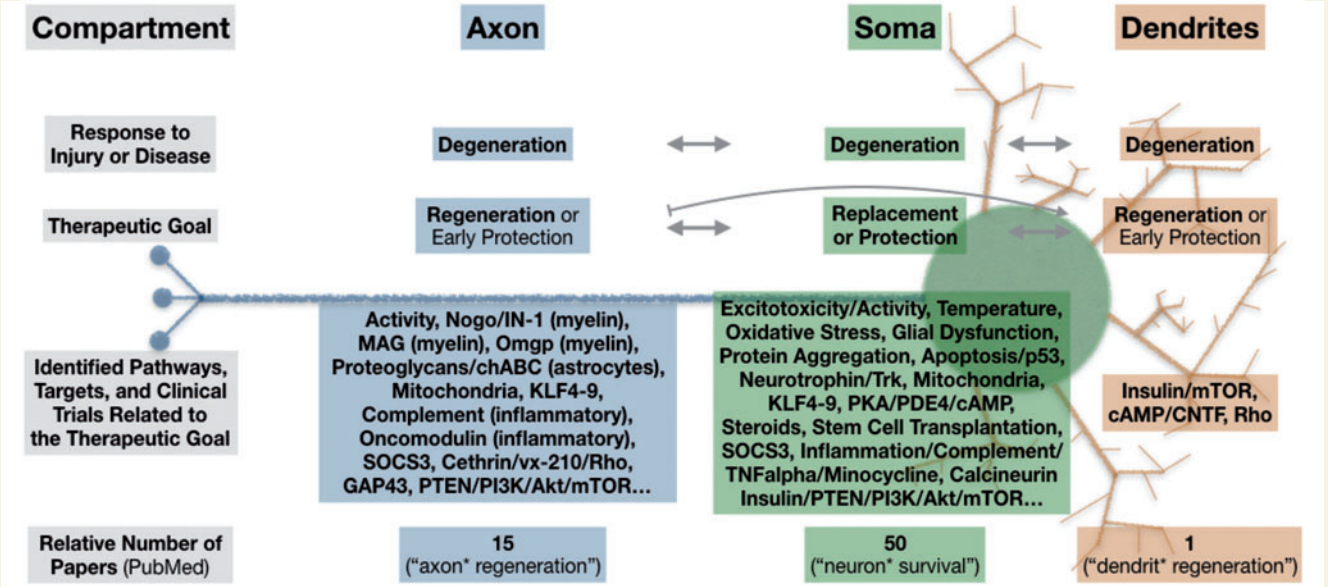

Figure 1 Little is known about adult mammalian dendrite regeneration. Along with somata and axons, dendrites degenerate in CNS injuries (including optic nerve transection) and diseases (including glaucoma). Despite the obvious importance of dendrites to CNS function, and the interdependence (arrows) between neuronal compartments for both degeneration and regeneration/protection/replacement, much more research has been directed toward axon regeneration and soma replacement/protection. A simplified measure of this disparity is the relative number of publications found on PubMed (search terms in parentheses, asterisk represents wildcard), which reveals that for every one dendrite regeneration paper, there are ~15 publications on axon regeneration and 50 for neuron survival. In this issue of *Brain*, Agostinone *et al.* identify a role for insulin/mTOR signalling in dendrite regeneration. Note common targets, including mTOR, as well as unique targets, and possible antagonism of dendrite growth on axon growth (bar-headed line). This simplified diagram does not include data from non-mammalian or developmental models and is not exhaustive. Axon and dendrite protection strategies likely require very early clinical intervention and are therefore noted but not emphasized here.

days post-injury successfully reverses the loss of dendritic complexity, with multiple dendritic measures returning to normal. Importantly, the timing of insulin intervention to several days post-axotomy—after dendrite retraction has begun—allows the authors to conclude that insulin promotes regrowth of dendrites, as opposed to promoting dendritic stabilization or preventing dendritic loss after optic nerve transection. Given the characterization of dendritic pathology as very early events that occur before clinical presentation in many CNS injuries and diseases, and the resulting timing of intervention, dendritic regrowth is a more relevant therapeutic target than dendritic protection. Another rodent visual system study with similarly delayed treatment identified other dendritic growth strategies [blocking Rho GTPase; elevating ciliary neurotrophic factor (CNTF) plus cAMP], albeit in the presence of a peripheral nervous system graft, and found that while the agents used had effects, the resulting dendritic morphology was abnormal (Drummond *et al.*, 2014). Interestingly, the outcome of the present study appears to be a restoration of normal morphology. This study's elegant, simple design allows for a conceptual distinction between preservation and restoration, with consequences for treatment identification, characterization, and clinical administration, and should be considered for future studies on mammalian dendrite repair.

Agostinone and colleagues further show, using siRNA-mediated knockdown techniques, that the various insulin-mediated dendritic effects require two separate mTOR signalling complexes, mTORC1/Raptor and mTORC2/Rictor, and that pharmacological inhibition of all mTOR signalling fully blocks the dendritic growth effects of insulin. Yet while this paper appears to be alone in describing a partial mechanism for regrowth of dendrites in the mammalian CNS, the key proteins and pathway identified are not new to us. Insulin or insulin-like growth factor-1 (IGF1) have been reported to be neuroprotective in this and other rodent CNS injury models. Agostinone *et al.* confirm previous reports with data showing insulin-, and specifically, mTOR-mediated neuroprotection of RGCs at subacute time points (7 and 14 days post-injury), and extend the published time course for this model by reporting significant neuroprotection with reduced effect sizes at more chronic time points (28 and 42 days). These more chronic data are key to any treatment aimed at clinical translation, especially given that most neuroprotective effects in this model have been disappointingly transient, including those of mTOR as shown here, making these data impressive but imperfect. This reinforces the need for neuroprotective treatments (or combinations of treatments) with long-term effects, and the development of improved slow-release or repeated administration strategies.

The PTEN/PI3K/Akt/mTOR pathway mechanism, shown here to be required (mTOR) for insulin-induced dendritic regrowth, is shared not only with that for dendritic development, invertebrate dendritic regeneration, and neuroprotection, but also with a well-described mechanism for CNS axon regeneration (Park *et al.*, 2008). The suggestion of a common pathway for soma protection, axon regeneration, and dendrite regeneration in multiple CNS injury models may have notable implications for clinical therapeutic manipulations of this pathway. The larger literature strongly suggests some interdependence of neuronal compartments for maintenance, degeneration and regrowth. Because intact somata, axons, and dendrites are all essential for neuronal communication and CNS function, a shared pathway is an exciting prospect and should be highlighted as a general target, meriting even more research in this area.

In contrast, other evidence points to an antagonism between dendrite growth and innervation versus axon growth or regeneration (Cull, 1974; Goldberg *et al.*, 2002; Francis and Freeman, 2016). Such antagonism may or may not play a role in the current work by Agostinone *et al.*, but axonal regeneration requires a different injury model and was not investigated here. However, this issue has clear implications for therapeutic development for any disease or injury with axonal pathology, including glaucoma. The present study provides limited evidence of insulin-mediated synapse restoration and improvement in retinal circuit function at 7 days post-injury, which may be indicative of local recovery, but the study did not address perception or behaviour, which would require regeneration of injured axons and which are critical issues for clinical therapies. Future studies will be required to determine the full effect on the larger system, perhaps using a glaucoma model in which, during a therapeutic window that needs to be defined, retinal axons are compromised but still present. In addition, if the potential inhibitory action of dendrites on axons is confirmed in future studies, careful attention to the timing of intervention may improve outcomes.

Although much remains to be explored, the translational potential of insulin treatment for glaucoma is exciting. This potential may even reach beyond glaucoma to other CNS injuries and diseases. Enthusiasm is based on the strong, partially sustained neuroprotection and neuro-restoration after injury, the current clinical use of insulin, the post-injury timing of administration, and the eye drop route of administration, which all seem favourable for translation. Moreover, prying open the figurative window to the vastly under-explored but crucial research topic of dendrite regeneration, and demonstrating the potential for treatment-induced dendrite regrowth, are important steps forward.

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A broader view of dementia: multiple co-pathologies are the norm

This scientific commentary refers to 'Neurodegenerative disease concomitant proteinopathies are prevalent, age-related and APOE4-associated', by Robinson *et al.* (doi:10.1093/brain/awy146).

Alzheimer's disease and other dementias are defined by hallmark protein abnormalities found in brain tissue post-mortem. Despite increasingly accurate diagnosis of primary pathology in life, treatments targeting the underlying protein abnormalities in Alzheimer's disease have so far failed to work. Why is dementia proving so hard to treat? One argument is that treatments are given too late in the course of illness—by the time of diagnosis, disease has progressed for a decade or more, has initiated self-perpetuating secondary processes and is no longer modifiable. A related, but distinct argument is presented in this issue of *Brain* by Robinson and co-workers, who demonstrate concurrence of multiple different abnormal proteins in dementias such as Alzheimer's disease, hinting at the likelihood that treatment might require a multi-pronged approach (Robinson *et al.*, 2018).

Accuracy of clinical diagnosis

Brains from 766 people who had died with dementia, and age-matched controls, were classified using standard diagnostic criteria as having either a neurodegenerative disease or minimal pathology. As in previous studies, the clinical and neuropathological diagnoses did not always agree. For example, high levels of Alzheimer's disease pathology at post-mortem examination had only a 79% sensitivity and 59% specificity for a clinical diagnosis of Alzheimer's disease. The figures are in keeping with those of other large clinicopathological studies (e.g. Beach *et al.*, 2012). Fourteen per cent of brains with high levels of Alzheimer's disease pathology came from patients who had been diagnosed clinically as having frontotemporal dementia. This highlights the complexity and imprecision of clinical diagnosis, even in the best centres, and the importance of moving to molecular markers of disease processes that cause dementia to ensure the right patients enter the right trials.

Ubiquity of tau

A striking finding was that neuronal accumulation of tau protein was almost universal, even in the minimal pathology group, within which neurofibrillary tangles were found in 93% of brains. Neurofibrillary tangles were present in at least 88% of brains with all other primary pathologies, and some of these—frontotemporal dementia and Lewy body diseases in particular—had tau co-pathology in 100% of cases. The prevalence and extent of neuronal tau pathology increased with age and incipient Alzheimer's disease. Robinson *et al.* did not address the relevance of astroglial tau, which also increases with age. One argument is that neuronal