SCIENTIFIC COMMENTARIES

An intriguing way to enhance rehabilitation of grasping in rats after spinal cord injury

This scientific commentary refers to 'Eliciting inflammation enables successful rehabilitative training in chronic spinal cord injury', by Torres-Espín *et al.* (doi:10.1093/brain/awy128).

Rehabilitation after nervous system injury, such as spinal cord injury or stroke, can improve outcomes when undertaken in the acute or chronic phase (Ward, 2017) but it rarely restores all lost function in humans. Rehabilitation can be exhausting and the dose of rehabilitation available to each patient is also limited by its expense: adjunct therapies that enhance rehabilitation would thus be of great value. Animal studies indicate too that the effectiveness of rehabilitation declines with time since spinal cord injury. In this issue of *Brain*, a team led by Karim Fouad reports perhaps surprisingly and counter-intuitively, that treatment with bacterial endotoxins (lipopolysaccharides; LPS) can enhance medium or high intensity rehabilitation in rats with spinal cord injuries that occurred 2 months previously (Torres-Espín *et al.*, 2018).

In fact, this study is an extension of work dating back many decades that indicates that bacterial endotoxins (by themselves or in combination with other treatments) can improve anatomical and functional recovery after acute spinal cord injury (reviewed in Popovich *et al.*, 2012). In the present study, adult rats received mid-cervical (C4) unilateral dorsolateral quadrant spinal cord injuries that persistently impaired their ability to grasp and retrieve sugar pellets with their previously-preferred paw. Eight and 11 weeks later, rats received intraperitoneal injection of saline or LPS at doses that induced effects within the cervical spinal cord (e.g. microglial activation), but induced only mild sickness for <2 days (body temperature was typically increased only ∼1°C). From 8 weeks, rats received either low, medium or higher doses of rehabilitation of pellet retrieval for an additional 7 or 8 weeks.

The key message of the paper is that medium or high (but not low) doses of rehabilitation led to greater success in pellet retrieval in rats treated with LPS than with saline. In animals treated with LPS and high doses of rehabilitation, wrist movements during retrieval ('supination') were more normal; anterograde tract tracing showed increased sprouting of the injured corticospinal tract in the cervical segment rostral to the injury; and microstimulation of motor cortex evoked greater output to the contralateral (disabled) posterior forelimb muscles.

So how does LPS enhance rehabilitation in rats after spinal cord injury? This is a complex question that will take many years to address adequately as we do not yet know how even an intact system reaches and grasps. But given the animal data showing that rehabilitation as a monotherapy is more efficient after acute spinal cord injury than after chronic injury, then perhaps, when given after chronic injury, LPS enhances rehabilitation by recapitulating some of the inflammatory components of the acutely injured state. Acutely, spinal cord injury generates damage associated molecular pattern molecules (DAMPs) and opens the blood–brain barrier, facilitating the influx of inflammatory peripheral cells into the injury site. Both beneficial and harmful effects have been attributed to the presence and activation of microglia, macrophages and lymphocytes. At the injury site, activated immune cells contribute to the production of cytokines, proteolytic enzymes and matrix metalloproteinases inducing a reactive process of secondary cell death, leading to increased cavitation and cyst formation, and exacerbating neurological dysfunction. Paradoxically, inflammation can also be beneficial. The effects of macrophages at the injury site depend on the balance of macrophage subtypes (often dichotomized as M1 or M2 but shown by transcriptomics to be spectral). 'Classically activated (M1)' macrophages are pro-inflammatory and contribute to glial scar formation and production of pro-inflammatory cytokines, reactive oxygen species, nitric oxide and proteolytic enzymes that lead to extracellular matrix degradation and tissue damage. 'Alternatively activated (M2)' macrophages are anti-inflammatory, provide neural and axonal trophic support, partially degrade the glial scar and induce inflammation resolution, thus contributing to

wound healing and tissue remodelling. Despite M2 macrophage activities, the resolution of inflammation after spinal cord injury is incomplete and the presence of pro-inflammatory macrophages is maintained for long periods (reviewed by Gensel and Zhang, 2015).

To enhance rehabilitation, what are the key receptors to which LPS must bind and where are they? This is not yet known, but others have shown that LPS binds to a co-receptor complex—including CD14, LPS-binding protein and TLR4—on microglia/macrophages, activating them via TLR4 for pathogen clearance and boosting the innate immune response. LPS-activated macrophages acquire a pro-inflammatory phenotype (M1) and secrete oxygen free radicals, IL-1, IL-6, IL-12, TNF-alpha, inducible nitric oxide synthase and chemoattractants that recruit additional leucocytes, possibly creating a neurotoxic environment. But the presence of a pro-inflammatory mediator at the site of injury is not sufficient reason to assume that it will have detrimental effects, and evaluation in experimental animals is necessary to determine whether this is in fact the case. The experimental conditions, such as time and dose of LPS injections, as well as the injury model, seem to be crucial in determining whether the overall effect will be neurotoxic, neuroprotective or neuroreparative.

Other authors have also reported beneficial effects of single or multiple intraperitoneal injections of low doses of LPS and other similar inflammatory agents when applied in the acute phase after a lesion. Guth and colleagues (1994) observed reduced cavitation and ingrowth of a greater number of axons, frequently gathered into fascicles, after spinal cord injury and LPS treatment in rats. Vallières and collaborators (2006) observed accelerated myelin clearance from white matter tracts undergoing Wallerian degeneration after spinal dorsal hemisection following daily systemic injections of LPS; the injections recruited activated macrophages to the injury site, although this was not sufficient to promote regeneration of injured sensory axons. Fouad's work goes beyond this by showing that LPS can enhance recovery after chronic spinal cord injury when combined with medium or high intensity rehabilitation.

How did LPS and high intensity rehabilitation enhance corticospinal tract sprouting, neural output to affected forelimb muscles and functional recovery? In this study, fluorescently-labelled LPS was detected in macrophages in the liver and at the site of injury, but not in the spinal cord one segment rostral to the injury site and not in cortex. If LPS activates microglia/macrophages at the injury site, might some of the mediators liberated by these cells cause sprouting of corticospinal axons? Perhaps LPS-induced inflammation stimulated sprouting of axons and then rehabilitation was important for axonal pathfinding and for strengthening of functionally useful connections. This refinement may happen only to circuits related to the rehabilitated task, as Torres-Espín et al. found no improvement in other tasks. In the future, transsynaptic tracing and chemogenetic silencing strategies could be used to identify the pathways that confer improved hand and arm movements after LPS and rehabilitation. Others have shown that application of LPS to motor cortex does not induce regeneration of corticospinal axons through or distal to a C3/4 lesion site (Hossain-Ibrahim et al., 2006). If LPS did not induce regeneration of corticospinal axons around or through the injury site in Torres-Espín's work, then one possibility is that corticospinal sprouts rostral to the injury formed synapses on short propriospinal neurons in upper cervical (C3–C4) segments that project to lower (C6–T1) segments in the white matter spared ventral to the C4 dorsolateral injury (Bareyre et al., 2004) (Fig. 1A and B). Another possibility is that the non-lesioned corticospinal tract sprouted collaterals across the midline below the level of the spinal cord injury to form synapses on premotor interneurons (Fig. 1C and D) as the spared corticospinal tract is known to sprout in response to various potential therapies for unilateral spinal cord injury, including LPS plus neurotrophin-3 (NT-3; Chen et al., 2008). In a series of papers, David Shine's group cut one corticospinal tract in the brainstem of rats and then overexpressed NT-3 in lumbar motor neurons on the more disabled side. NT-3 induced sprouting of uninjured corticospinal axons across the midline but only when applied within 2 weeks of injury and not when applied 4 months after injury (Chen et al., 2006) unless LPS was injected intraperitoneally (Chen et al., 2008). The parallel between Shine's work and Fouad's work is that LPS synergized with other therapies (NT-3 or rehabilitation,
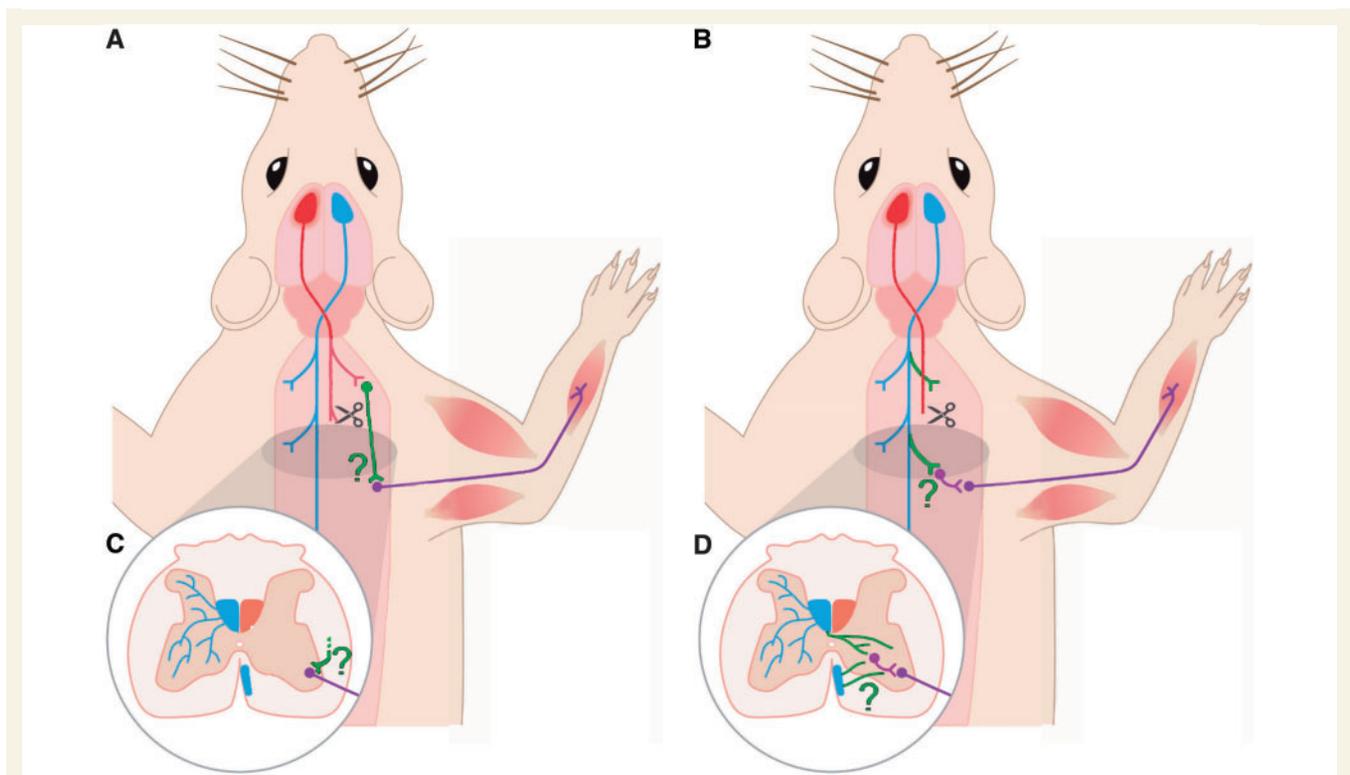
Figure 1 Lipopolysaccharides and high intensity rehabilitation may induce new circuit formation. (**A** and **C**) Torres-Espín *et al.* (2018) show in rats that high intensity rehabilitation of grasping initiated 8 weeks after dorsolateral cervical spinal cord injury is more effective when combined with intraperitoneal injection of LPS. Enhanced sprouting of the affected corticospinal tract rostral to the injury (red sprouts in **A**) was accompanied by increased output to affected forelimb muscles perhaps via spared short propriospinal neurons (green neuron in **A** and **C**), the axons of which travel in white matter ventral to the injury site. (**B** and **D**) Future research may show whether other pathways are also involved. For example, the spared corticospinal tract from the other hemisphere (blue pathway in **B** and **D**) may sprout axons across the spinal midline (green sprouts in **D**) to form synapses on premotor interneurons involved in grasping. This kind of neuroplasticity has been shown before in the lumbar spinal cord when rats are treated intraperitoneally with LPS and intraneural injection on the more-disabled side of an adenoviral vector encoding NT-3, 4 months after unilateral corticospinal tract axotomy in the brainstem (Chen *et al.*, 2008).

respectively) in the chronic phase after injury (Chen *et al.*, 2006, 2008).

To achieve high levels of NT-3 expression in motor neurons, Shine's group applied an adenoviral vector encoding human preproNT-3 to the sciatic nerve on the more disabled side, having cut the nerve to ensure efficient uptake of the vector. It is interesting that this proinflammatory stimulus (the delivery of a non-self transgene using an immunogenic vector combined with a proinflammatory nerve injury) was insufficient by itself to promote corticospinal tract sprouting in their chronic paradigm, which indicates that there is something rather special about the stimulus delivered by LPS.

Could LPS be used to enhance neurorehabilitation in people? People with spinal cord injury are often immunocompromised and elevated temperature can exacerbate cell death, so exposure of vulnerable subjects to a proinflammatory pyrogen might be ruled out for the acute phase. In the past, more than 100 people with acute or chronic spinal cord injury or disease have been dosed with preparations of bacterial polysaccharides although these case histories were only superficially documented (Friedlander and Bailey, 1953). To our knowledge there have been no clinical trials of LPS for spinal cord injury, although clinical trials of LPS have been run for other conditions. A fuller understanding of the mechanisms by which LPS induces benefit might enable a targeted approach that can generate intellectual property that could enable expensive clinical trials: engineered antibodies or small molecules might be developed against the key receptor/cell type.

In closing, it is worth emphasizing that evidence continues to accumulate that intensive rehabilitation by itself improves outcome even in people with chronic neurological injuries (Ward, 2017). More therefore needs to be done by healthcare systems to make intensive rehabilitation more widely available to those who need and want more. In the longer term, adjunct drug or neurostimulatory therapies may enable greater gains to be made.

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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