NEURAL REGENERATION RESEARCH

**PERSPECTIVE**

**Delayed peripheral treatment with neurotrophin-3 improves sensorimotor recovery after central nervous system injury**

Neurotrophin-3 (NT3) is a growth factor found in many body tissues including the heart, intestines, skin, nervous system and in skeletal muscles including muscle spindles (Murase et al., 1994). NT3 is required for the survival, correct connectivity and function of sensory (“proprioceptive”) afferents that innervate muscle spindles; these neurons express receptors for NT3 including troponymcin receptor kinase C. These proprioceptive afferents are important for normal movement (Boyce and Mendell, 2014) and signals from muscle spindles are important for recovery of limb movement (e.g., after spinal cord lateral hemisection) (Takeoka et al., 2014). The level of NT3 declines in most tissues during postnatal development: its level is low in adult and elderly humans and other mammals (Murase et al., 1994). Elevation of NT3 has been shown to improve outcome in various animal models of neurological disease and injury. For example, many groups have shown that delivery of NT3 directly into the central nervous system promotes recovery after spinal cord injury but this often involved invasive routes or gene therapy (Boyce and Mendell, 2014; Petrosyan et al., 2015; Wang et al., 2018).

Our lab began evaluating NT3 in a rat model of focal stroke about 10 years ago. The work was inspired by experiments from Prof. David Shime’s lab (Zhou et al., 2003) involving a model of injury created by transection of one corticospinal tract in the medullary pyramids (pyramidotomy; Figure 1A). Viewed from the perspective of a spinal cord, unilateral pyramitodamy has much in common with unilateral cortical stroke: one corticospinal tract is spared entirely while the other is partially or completely lesioned, resulting in a (predominantly) contralateral deficit in sensorimotor function (Figure 1B). Prof. Shime’s team showed that a spared corticospinal tract could be induced to sprout across the midline of the spinal cord into the affected side when NT3 was expressed in lumbar motor neurons (Zhou et al., 2003). Specifically, 2 weeks after unilateral pyramitodamy, to enhance expression of NT3 by lumbar motor neurons on the affected side, they applied an adenosine viral vector encoding the rat preproNT3 gene (which encodes a precursor to the mature NT3) to DRG (either by transport of the growth factor in axons or via the blood stream). We are still not sure why in our experiments retrograde transport of the AA virus was poor. However, in both experiments, the level of NT3 in the injected muscles was very high and we found evidence for transport of NT3 protein across the midline of the spinal cord or DRG. Injections of AA expressing human preproNT3 (AAV1-hNT3) into hindlimb muscles 24 hours after stroke again induced sprouting of the spared corticospinal tracts into the affected spinal cord and improved sensorimotor recovery in elderly rats (on the horizontal ladder and also using a test of forelimb cortical function) (Duricki et al., 2016). As in our previous experiment, there was underlying expression of the human NT3 mRNA in the spinal cord or DRG. We are still not sure why in our experiments retrograde transport of the AAV was poor. However, in both experiments, the level of NT3 in the injected muscles was very high and we found evidence for transport of NT3 protein to DRG (either by transport of the growth factor in axons or via the bloodstream). We concluded tentatively that synthesis of human NT3 in the spinal cord and DRG of rats might not be necessary for sensorimotor recovery after stroke, after all but that peripheral elevation of NT3 might suffice.

Accordingly, we set out to test a new hypothesis: that infusion of NT3 protein into affected forelimb muscles would be sufficient to promote sensorimotor recovery after unilateral cortical stroke. We induced unilateral cortical ischemia in adult rats and, 24 hours later, implanted a catheter into the triceps brachii muscle on the affected side (Figure 1C). A subcutaneously implanted osmotic minipump infused NT3 protein (gift of Genentech) or vehicle intra-arterially to both the affected and unlesioned forelimbs. Dr. Jeff Petruska and other colleagues were studying the transport of various adeno-associated viral vectors (AAVs) from hindlimb muscles to lumbar motor neurons and dorsal root ganglia (DRG). They showed that AAV serotype 1 underwent retrograde transport more effectively than AAV2 or AAV5 and that injection of AA1 into the human preproNT3 (AAV1-hNT3) into hindlimb muscles could modify lumbar motor neuron properties and responses (Petruska et al., 2010).

We wondered whether it might be possible to induce sprouting of the spared corticospinal tract in a rat model of unilateral cortical stroke by injecting AA1-hNT3 (relative to AA1-GFP) improved sensorimotor outcome after delivery into affected biceps brachii and triceps brachii 24 hours after focal ischemic cortical stroke. After unblinding the treatment groups, it was apparent that NT3 modestly improved the accuracy of limb placement during walking on a horizontal ladder with irregularly spaced rungs. Moreover, anterograde tracing showed that the less-affected corticospinal tract sprouted across the midline and into the affected spinal cord (Duricki et al., 2016). However, to our surprise there was little evidence for expression of the transgenes in the spinal central spinal cord: 1) The human NT3 transcript can be distinguished from the rat NT3 transcript by qRT-PCR but we found little evidence for human NT3 mRNA in the spinal cord or DRG (Duricki et al., 2016; Kathe et al., 2016); 2) We did find a few motor neurons and DRG neurons positive for green fluorescent protein but the number was considerably smaller than others had shown convincingly.

Not being sure what to make of this, we decided to repeat the experiments, except using elderly rats (because stroke primarily affects the elderly) and with a slightly higher dose of AA1 (7 x 10^10 viral genomes rather than 3 x 10^10 viral genomes). Injections of AA1 encoding hNT3 into forelimb muscles 24 hours after stroke again induced sprouting of the spared corticospinal tracts into the affected spinal cord. We ran a blinded, block-randomized study to determine whether AA1-hNT3 improved sensorimotor outcome after delivery into affected biceps brachii and triceps brachii 24 hours after focal ischemic cortical stroke. After unblinding the treatment groups, it was apparent that NT3 modestly improved the accuracy of limb placement during walking on a horizontal ladder with irregularly spaced rungs. Moreover, anterograde tracing showed that the less-affected corticospinal tract sprouted across the midline and into the affected spinal cord (Duricki et al., 2016). However, to our surprise there was little evidence for expression of the transgenes in the spinal central spinal cord: 1) The human NT3 transcript can be distinguished from the rat NT3 transcript by qRT-PCR but we found little evidence for human NT3 mRNA in the spinal cord or DRG (Duricki et al., 2016; Kathe et al., 2016); 2) We did find a few motor neurons and DRG neurons positive for green fluorescent protein but the number was considerably smaller than others had shown convincingly.

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**Figure 1** Twenty-four hour-delayed, peripheral treatment with neurotrophin-3 induces neuroplasticity and enhances sensorimotor recovery after corticospinal tract axotomy or cortical stroke in rats.

(A) Unilateral pyramitodamy involves transection of the corticospinal tract in the medullary pyramids (injury location denoted by scissors) and results in near-total loss of corticospinal axons on one side (red and white dots) but a preservation of the corticospinal tract from the other hemisphere (blue).

(B) Unilateral ischemia in motor cortex induced by endothelin-1 (red) led to a partial (20%) reduction in corticospinal axons to the contralateral spinal cord (red dots). Injection of AA1 encoding NT3 into biceps brachii and triceps brachii resulted in sprouting of the spared corticospinal tracts into the denervated hemiscord and sensorimotor recovery in adult and elderly rats (Duricki et al., 2016). (C) After unilateral cortical ischemia, infusion of NT3 protein into triceps brachii resulted in sprouting of the spared corticospinal tracts into the denervated hemiscord and sensorimotor recovery in adult rats (Duricki et al., 2019). (D) Bilateral pyramidotomy involves section of both corticospinal tracts in the medullary pyramids. Other sensorimotor pathways (not shown) are spared but little or no corticospinal inputs to the spinal cord remain. Injection of AA1 encoding NT3 into multiple forelimb flexors reduced spasm, normalised H reflexes to a hand muscle and improved sensorimotor recovery (Kathe et al., 2016). Figures reproduced from Duricki et al. (2016, 2019) and Kathe et al. (2016) under the Creative Commons Attribution License. NT3: Neurotrophin-3; AAV: adeno-associated viral vector.
muscularly for 1 month. Anterograde tracing revealed that the intact corticospinal tracts sprouted into the affected cervical hemisected and spared the spinal cord, for example, from sensory afferents or motor neurons. We have discovered that DRG neurons modify their gene expression profile after bilateral pyramidotomy (Kathe and Moon, 2018) and that NT3 normalises some of these gene expression changes. For example, one of these genes, Vgf, codes for a secreted molecule: it would be interesting to determine whether this protein induces corticospinal axon sprouting. NT3 can have side effects: Unlike other neurotrophins, NT3 does not cause pain in humans (Duricki et al., 2016) which contributes to abnormal movement. We have shown that intramuscular injection of AAV1-hNT3 normalised a reflex to a paw muscle (Kathe et al., 2016). We propose that, primarily, NT3 normalises various sensory reflexes and that, secondarily, this leads to plasticity in other pathways which, together, improve movement.

**How did peripheral infusion of NT3 induce sensorimotor recovery?** One possibility is that sensorimotor recovery is a consequence of the spared (and sprouted) corticospinal tract obtaining some control over the spinal cord controlling the affected limb. However, an additional possibility is that NT3 normalises propriospinal reflexes that control muscles on the affected side. After stroke, pyramidotomy or spinal cord injury, propriospinal afferents below the injury can sprout and propriospine reflexes are modified (Kathe et al., 2016) which contributes to abnormal movement. We have shown that intramuscular injection of AAV1-hNT3 normalised a reflex to a paw muscle (involving low threshold afferents including propriospine afferents) after bilateral pyramidotomy in adult rats. This was accompanied with reduced spasm and improved walking on a horizontal ladder (Kathe et al., 2016). We propose that, primarily, NT3 normalises various sensory reflexes and that, secondarily, this leads to plasticity in other pathways which, together, improve movement.

**References**


C-Editors: Zhao M, Li JY; T-Editor: Jia Y