

● PERSPECTIVE

Territory maximization hypothesis during peripheral nerve regeneration

Territory awareness refers to the notion that an organism lives in a territory, considers this territory its own, and prevents entry of other organisms. Generally, an organism maximizes its territory for best survival advantages, which subsequently allows for species continuation. Axonal sprouting occurs when peripheral nerves regenerate. The distal regenerated nerve fibers significantly outnumber those at the proximal end of the donor nerve, which has long been termed the “multiple amplification” phenomenon (Yin et al., 2013). Previous studies have shown that sprouting and multiple amplification occur during peripheral nerve regeneration. The regenerated nerve fibers greatly outnumber the original fibers of the donor nerve, thereby filling the entire physiological space of the acceptor nerves and allowing for the peripheral neurons to maximize innervation to the target organs. However, the role of the fibers in the amplification processes remains to be shown, as well as the factors involved in amplification. Additionally, very little is understood about how the fibers serve as donor nerves to repair peripheral nerve injury, whether there is a biological motive for the regeneration process, and whether the peripheral neurons have their own behavioral pattern. Further investigation might offer different perspectives about peripheral nerve regeneration and the potential application in disease treatments.

We hypothesized that there is a biological motive of the peripheral nerves to maximize their “territory” during regeneration. Individual peripheral neurons are thought to consider target organs as their territories, thereby maximally regenerating new fibers to innervate those territories. During regeneration of the injured peripheral nerves, neurons generate numerous axonal sprouts (growth cones) at the stump. The number of sprouts can be significantly greater than that of original nerve fibers. These new sprouts attempt to grow into and fill the Bungner bands to eventually regain control of the target organs (Navarro et al., 2007; Wang et al., 2010).

Preliminary studies have shown that multiple amplification occurs in peripheral nerve restoration (Jiang et al., 2007; Yin et al., 2013). The proximal rat tibial nerve segments, which have a variable number of axons, were used as donor nerves, and a biodegradable chitin conduit was used to bridge these segments to the distal stump of the same tibial nerve. After 12 weeks, the number of myelinated axons was quantified, showing an increased ratio of regenerative myelinated axon numbers to proximal donor axon numbers, with an estimated maximum value of approximately 3.3 (Yin et al., 2013). The results showed that as the ratio of donor to acceptor nerve fibers increased, amplification of nerve regeneration also increased; additionally, the amplification effect only occurred when there was a greater number of acceptor nerve fibers compared with donor nerve fibers at the proximal end. We postulated that distal stumps with sufficient endoneurial tubes for growth of new nerve fibers are a prerequisite for the amplification effect in regenerated nerve fibers. The endoneurial tubes at the distal stumps and the corresponding targets are considered to be obtainable territories of the peripheral nerve fibers. The neurons were

hypothesized to maximize the number of axonal sprouts for maximal influence.

Multiple amplification has been observed at every section level of the peripheral nerve trunk. In our previous work using a rabbit model, the proximal, intermediate, and distal nerve segment of the median nerve served as the donor nerve, and the restored ratio of the number of donor fibers to recipient fibers was 1:4. At 3 months post-surgery, the number of regenerated fibers was quantified, revealing a 2.9-ratio of the number of regenerated nerve fibers to donor nerve fibers in all three groups (Wang et al., 2009).

Multiple amplification also occurred in a delayed repair model of the peripheral nerve, which has been shown to be similar to clinical conditions. The distal tibial nerve stumps, which served as receptor nerves, were denervated for 1, 2, 4, and 8 weeks prior to repair with partial, freshly transected, proximal, peroneal nerve stumps. Results revealed a multiplication ratio of 2.33, 2.32, 2.25, and 1.45, respectively (Yin et al., 2013).

Similarly in previous end-to-side neurotomy model, multiple amplification still existed in the side of distal tibial nerve stump to preserve the structure of denervated muscle (Liu et al., 2015). The sural nerve which was used as the donor nerve generated numerous axonal sprouts. These myelinated nerve fibers grown into target organs and maximized its territory for preventing the atrophy of muscle fibers and motor end plate (Li et al., 2014).

Results from these studies demonstrate that peripheral nerves always generate more sprouts during regeneration and are able to retain the new nerve fibers to a large extent. Therefore, we hypothesized that territory maximization effects exist during regeneration of peripheral nerves. Individual peripheral neurons are thought to treat the target organs at terminals as their territories and subsequently form new fibers to maximally innervate those territories.

According to our hypothesis, peripheral nerve neurons have corresponding targets at nerve terminals, such as muscles and skin, which are considered to be their territories. An internalized territory awareness could allow an organism to maximize its territories whenever possible. For peripheral neurons, an increased number of regenerated nerve fibers could increase their influences over the targets organs at their terminals.

When injuries occur to peripheral neurons, including epineurium injury, perineurium injury, and even damaged nerve continuity, the peripheral nerves lose connection to their original territories. When seeking more territories, the branches of regenerating nerve fibers proliferate and new sprouts continuously grow to regain eventual control over the original targets (Ide, 1996; Jianping et al., 2012). In some instances, such as repair of large nerves with smaller ones or the repair of multiple nerves with a single one, territory awareness of the peripheral nerves is maximized. As long as the distal acceptor nerves have sufficient endoneurial tubes for the new nerve branches to grow into, the retention of a greater number of nerve fibers for forming mature structure is potentially possible; the regenerated nerve fibers might even outnumber the donor nerve fibers at the germinal site, which is termed the “amplification effect” (An et al., 2015; Kou et al., 2015). However, metabolism and the axonal transportation capacity of neurons are limited, and individ-

ual neurons are unable to provide sufficient material and energy to a large number of branched nerve fibers, which results in limited controllable territories for that neuron. Therefore, branch proliferation of the regenerated nerve fibers is limited, which is called the “amplification limit” (Yin et al., 2014).

Rational utilization of territory maximization effects during peripheral nerve regeneration offers new options for repairing injured peripheral nerves. Our preliminary studies have focused on peripheral nerves from a rat model. Whole or partial median nerves at the proximal end served as donor nerves to repair median nerves and whole ulnar nerves at the distal end in two different experiment groups. The number of nerve fibers, nerve conduction velocity, and corresponding muscle contraction strength were measured to evaluate nerve reconstruction. Results showed nerve amplification rates of 1.39 and 1.79, respectively. Additionally, conduction velocity of the median and ulnar nerves was 24.4 ± 5.9 and 30.7 ± 11.2 m/s, and 24.6 ± 5.3 and 30.3 ± 7.2 m/s, separately. These results showed that the target organs of the donor and recipient nerves achieved functional recovery (Yin et al., 2011a, b). In another study, the anterior pronator teres nerve (donor) of the Rhesus monkey was used to repair the ulnar nerve (recipient). After 6 months, motor nerve conduction velocity was 22.63 ± 6.34 m/s, and fiber numbers for donor and recipient nerves were $1,657 \pm 652$ and $2,661 \pm 843$, respectively, with a multiplication ratio of 1.61 (An et al., 2015). These results suggested that the peripheral nerves sought to control more distal target organs (“territories”) by increasing the number of new nerve fibers during regeneration.

We hypothesized that the peripheral nerves maximize the “territory” effects during regeneration. Individual peripheral nerves are thought to consider target organs as their territories and, therefore, maximally regenerate new fibers to innervate those territories. These effects could be utilized to repair injured peripheral nerves. After the peripheral nerves expand their territories, functional recovery is dependent on the regulation of those territories. In other words, once tissue continuity is established following amplification of peripheral nerve fibers, functional remodeling in the spinal cord and brain is still needed to achieve effective control of the target organs. We have already designed the retrograde tracing of rat spinal motor neurons using fluorescent dyes, while marking the motor end plate by α -bungarotoxin to investigate the variation in the nervous system. Further studies are needed to explore the mechanism of functional remodeling.

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References

- An S, Zhang P, Peng J, Deng L, Wang Z, Wang Z, Wang Y, Yin X, Kou Y, Han N, Jiang B (2015) Motor function recovery during peripheral nerve multiple regeneration. *J Tissue Eng Regen Med* 9:415-423.
- Ide C (1996). Peripheral nerve regeneration. *Neurosci Res* 25:101-121.
- Jiang BG, Yin XF, Zhang DY, Fu ZG, Zhang HB (2007) Maximum number of collaterals developed by one axon during peripheral nerve regeneration and the influence of that number on reinnervation effects. *Eur Neurol* 58:12-20.
- Jianping P, Xiaofeng Y, Yanhua W, Zhenwei W, Yuhui K, Chungui X, Peixun Z, Baoguo J (2012) Different multiple regeneration capacities of motor and sensory axons in peripheral nerve. *Artif Cells Blood Substit Immobil Biotechnol* 40:309-316.
- Kou YH, Zhang PX, Wang YH, Chen B, Han N, Xue F, Zhang HB, Yin XF, Jiang BG (2015) Sleeve bridging of the rhesus monkey ulnar nerve with muscular branches of the pronator teres: multiple amplification of axonal regeneration. *Neural Regen Res* 10:53-59.
- Li Q, Zhang P, Yin X, Han N, Kou Y, Jiang B (2014) Early sensory protection in reverse end-to-side neurotaphy to improve the functional recovery of chronically denervated muscle in rat: a pilot study. *J Neurosurg* 121:415-422.
- Liu HF, Chen ZG, Shen HM, Zhang H, Zhang J, Lineaweaver WC, Zhang F (2015). Efficacy of the end-to-side neurotaphies with epineural window and partial donor neurectomy in peripheral nerve repair: an experimental study in rats. *J Reconstr Microsurg* 31:31-38.
- Navarro X, Vivó M, Valero-Cabré A (2007) Neural plasticity after peripheral nerve injury and regeneration. *Prog Neurobiol* 82:163-201.
- Wang J, Zhang P, Wang Y, Kou Y, Zhang H, Jiang B (2010). The observation of phenotypic changes of Schwann cells after rat sciatic nerve injury. *Artif Cells Blood Substit Immobil Biotechnol* 38:24-28.
- Wang YH, Zhang DY, Zhang PX, Yin XF, Kou YH, Wang J, Yu K, Li X, Zhang HB, Jiang BG (2009) Amplification effects in nerve regeneration after different segment injury: experiment with rabbit median nerve. *Zhonghua Yi Xue Za Zhi* 89:1645-1649.
- Yin XF, Kou YH, Wang YH, Zhang PX, Zhang DY, Fu ZG, Zhang HB, Jiang BG (2014) Morphological study on the collaterals developed by one axon during peripheral nerve regeneration. *Artif Cells Nanomed Biotechnol* 42:217-221.
- Yin XF, Kou YH, Wang YH, Zhang P, Zhang DY, Fu ZG, Zhang HB, Jiang BG (2011a) Can “dor+rec neurotaphy” by biodegradable chitin conduit be a new method for peripheral nerve injury. *Artif Cells Blood Substit Immobil Biotechnol* 39:110-115.
- Yin XF, Kou YH, Wang YH, Zhang P, Zhang HB, Jiang BG (2011b) Portion of a nerve trunk can be used as a donor nerve to reconstruct the injured nerve and donor site simultaneously. *Artif Cells Blood Substit Immobil Biotechnol* 39:304-309.
- Yin XF, Kou YH, Zhang PX, Wang YH, Jiang BG (2013) Characteristics of peripheral nerve collateral multiplication and its potential use in neural trauma. *Chin Med J (Engl)* 126:1598-1599.