Femoral nerve regeneration and its accuracy under different injury mechanisms

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Abstract  
Surgical accuracy has greatly improved with the advent of microsurgical techniques. However, complete functional recovery after peripheral nerve injury has not been achieved to date. The mechanisms hindering accurate regeneration of damaged axons after peripheral nerve injury are in urgent need of exploration. The present study was designed to explore the mechanisms of peripheral nerve regeneration after different types of injury. Femoral nerves of rats were injured by crushing or freezing. At 2, 3, 6, and 12 weeks after injury, axons were retrogradely labeled using 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (Dil) and True Blue, and motor and sensory axons that had regenerated at the site of injury were counted. The number and percentage of Dil-labeled neurons in the anterior horn of the spinal cord increased over time. No significant differences were found in the number of labeled neurons between the freeze and crush injury groups at any time point. Our results confirmed that the accuracy of peripheral nerve regeneration increased with time, after both crush and freeze injury, and indicated that axonal regeneration accuracy was still satisfactory after freezing, despite the prolonged damage.

Key Words: nerve regeneration; peripheral nerve injury; chemotactic regeneration; retrograde labeling; selective nerve regeneration; functional recovery; NSFC grant; neural regeneration

Funding: This research was supported by the National Natural Science Foundation of China, No. 81360194, and a grant from the National Basic Research Program of China, No. 2014CB542200.


Introduction  
Five percent of patients in trauma centers are affected by peripheral nerve injury (Belkas et al., 2004; Taylor et al., 2008). Since most of these patients are of prime working age, such injuries pose a serious economic burden to society (Whitlock et al., 2009). Restoration of function after peripheral nerve injury remains one of the toughest challenges in surgery. Despite the development of microsurgical equipment, with which it is now possible to suture the damaged nerves accurately within a short time after injury, complete functional recovery after peripheral nerve damage has not yet been achieved (Eser et al., 2009). Various methods have been attempted (Vetter et al., 2010; Bosse et al., 2012; Seidel et al., 2013), but none have obtained complete success, mainly because the mechanisms underlying selective peripheral nerve regeneration remain poorly understood (Calvo et al., 2012; Jesuraj et al., 2012).

To achieve full recovery from nerve injury, high accuracy nerve innervation is just as important as robust axonal regrowth (Ruiter et al., 2011). Axons regenerated from proximal motor nerve stumps should grow into the motor nerve pathways in the distal stump, and vice versa. However, little is known about the mechanism underlying the misdirection of regenerating axons. To achieve accurate regeneration and full functional recovery, more research must be carried out to explore how the regeneration accuracy of axons changes after injury. Novel experimental approaches, such as retrograde labeling (Hoke et al., 2006), have provided us with new tools to observe this process. We have used this approach in the present study to investigate the regeneration of axons after crushing or freezing, two common types of peripheral nerve injury, with the aim of providing new insight into chemotactic regeneration.

Materials and Methods  
Animals  
A total of 92 healthy male Sprague-Dawley rats, aged 8 weeks and weighing 250 ± 20 g, were provided by the Experimental Animal Center of the Chinese PLA General Hospital. The study was approved by the same institute’s Animal Ethics Committee in China.

Establishment of animal models  
Animals were anesthetized by intraperitoneal injection of 10% chloral hydrate (0.3 mL/100 g) and placed on an operating table in the supine position. A surgical incision was