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Regular Article

Activity dependent therapies modulate the spinal changes that motoneurons suffer after a peripheral nerve injury

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ABSTRACT

Injury of a peripheral nerve not only leads to target denervation, but also induces massive stripping of spinal synapses on axotomized motoneurons, with disruption of spinal circuits. Even when regeneration is successful, unspecific reinnervation and the limited reconnection of the spinal circuits impair functional recovery. The aim of this study was to describe the changes that axotomized motoneurons suffer after peripheral nerve injury and how activity-dependent therapies and neurotrophic factors can modulate these events. We observed a marked decrease in glutamatergic synapses, with a maximum peak at two weeks post-axotomy, which was only partially reversed with time. This decrease was accompanied by an increase in gephyrin immunoreactivity and a disintegration of perineuronal nets (PNNs) surrounding the motoneurons. Direct application of neurotrophins at the proximal stump was not able to reverse these effects. In contrast, activity-dependent treatment, in the form of treadmill running, reduced the observed destructuring of perineuronal nets and the loss of glutamatergic synapses two weeks after injury. These changes were proportional to the intensity of the exercise protocol. Blockade of sensory inputs from the homolateral hindlimb also reduced PNN immunoreactivity around intact motoneurons, and in that case treadmill running did not reverse that loss, suggesting that the effects of exercise on motoneuron PNN depend on increased sensory activity. Preservation of motoneuron PNN and reduction of synaptic stripping by exercise could facilitate the maintenance of the spinal circuitry and benefit functional recovery after peripheral nerve injury.

Introduction

Rehabilitation is one of the cornerstones of the treatment of injuries of the nervous system. It is assumed that repeated activity will reinforce the circuitry of the nervous system and facilitate functional recovery, by promoting structural plasticity and axonal growth. Therefore, either physical activity or exposure to enriched environment promotes neurite outgrowth and functional plasticity (Ghiani et al., 2007; Rampon et al., 2000; Sale et al., 2007; Vaynman and Gomez-Pinilla, 2005). Activity-dependent plasticity has been linked with changes in neurotrophin expression, neuronal growth genes and regulatory substances (Cobianchi et al., 2013; Molteni et al., 2004). However, other studies note the importance of a specific rehabilitation therapy to improve functional recovery after neural damage (García-Alías and Fawcett, 2012; Wang et al., 2011).

Exercise and other activity-dependent therapies have been extensively used to improve functional recovery after spinal cord injuries (Hutchinson et al., 2004; Ying et al., 2008) and peripheral nerve lesions (Al-majed et al., 2000; Asensio-Pinilla et al., 2009; Van Meeteren et al., 1997; Sabatier et al., 2008). However, just how these therapies can influence the plasticity of the central circuits where spinal motoneurons are involved is not clear. It is known that in the intact adult nervous system, the motoneurons in the ventral horn of the spinal cord are surrounded by perineuronal nets (PNNs) (Takahashi-Iwanaga et al., 1998) that restrict plasticity and play a key role in the maintenance of synapses (Kwok et al., 2011). It has been demonstrated that external stimulation, by increasing synaptic inputs, reduces PNN content, increasing the plastic abilities of cerebellar neurons and modulating the wiring of visual and somatosensory cerebral circuits (Corvetti and Rossi, 2005; Foscarin et al., 2011; McRae et al., 2007; Pizzorusso et al., 2002). Interestingly, task-specific rehabilitation increases the expression of PNN around decorticated spinal motoneurons (Wang et al., 2011), suggesting that PNN behavior in the spinal cord can be differentially regulated by injury and activity when compared to brain neurons.

After a peripheral nerve injury, the interruption between the axons and their target organs is accompanied by important changes at the spinal cord and supraspinal levels (Navarro et al., 2007). Axotomized motoneurons suffer massive stripping of their central

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synapses, related to the loss of trophic support from the muscle. Brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT3) are two key neurotrophins that regulate the synaptic plasticity, formation and density of synaptic innervation of motoneurons and, when exogenously delivered, are able to prevent/reverse the synaptic stripping suffered by axotomized motoneurons (Davis-López de Carrizosa et al., 2009; Novikova et al., 2000).

When neurons regenerate and reinnervate target organs, they partially recover their synaptic arbor, but in contrast to other excitatory and inhibitory inputs, muscle spindle Ia excitatory synapses, among the most affected by synaptic stripping, never recover baseline levels, even when the muscle spindle and the muscle are correctly reinnervated (Alvarez et al., 2011; Haftel et al., 2005). This reduced connectivity may explain the lack of recovery of a functional stretch reflex (Alvarez et al., 2011). The stretch reflex is the simplest circuit but it plays a key role in neuromuscular self-control. It is a monosynaptic reflex where Ia afferents from the muscle spindle excite motoneurons, innervating the same muscle. In fact, normalization of motor function requires not only specific reinnervation of peripheral target organs but also adequate reconnection of the central circuitry between sensory afferents and motoneurons (Alvarez et al., 2010). In contrast to the functional stretch reflex, its equivalent electrophysiological response, the H reflex, recovers after peripheral nerve injury and successful muscle reinnervation. In fact, there is a facilitation of this reflex, inversely correlated with the degree of reinnervation (Valero-Cabré and Navarro, 2001). Thus, the connection between motoneurons and sensory afferents measured by the H reflex does not guarantee a functional stretch reflex. The lack of correlation between the H reflex and the stretch reflex after injury suggests that peripheral axotomy favors an inadequate reorganization of the central circuitry, which can be detrimental to functional recovery. Interestingly, physical exercise and other activity-dependent therapies reduce the facilitation of the H reflex observed after nerve injuries (Asensio-Pinilla et al., 2009; Udina et al., 2011b; Vivó et al., 2008), probably by modulating plasticity of spinal circuits.

Maintenance of neurotrophic support and activity in the neural circuits after lesions can be a key-point in reducing the plastic changes that neurons suffer due to the loss of synaptic and neurotrophic inputs. Therefore, supply of trophic factors and appropriate training and/or provision of afferent inputs to spinal neurons may help to prevent these changes. The aim of this study was to analyze the changes that axotomized motoneurons suffer after peripheral nerve injury and how the addition of trophic factors into the injured stump and provision of activity-dependent strategies modulate these events. A better understanding of these changes would make it easier to determine the best protocol to improve functional outcome.

Material and methods

Experimental animals

Adult female Sprague Dawley rats (n = 74, 8 weeks old; 250–300 g) were housed with free access to food and water at room temperature of 22 ± 2 °C under a 12:12-h light–dark cycle. All experimental procedures were approved by the ethics committee of our institution and followed the European Communities Council Directive 86/609/EEC. Animals were studied in four groups (see Table 1). For all the surgical interventions, rats were anesthetized by intraperitoneal administration of ketamine (0.9 ml/kg; Imalgen 2000) supplemented with xylazine (0.5 ml/kg; Rompun 2%).

Retrograde labeling

To identify motoneuron pools from tibialis anterior (TA) and gastrocnemius medialis (GM) muscles, retrograde tracing was applied to the muscle 1 week before any intervention. Bilaterally, two retrotracers, True Blue Chloride (TB, Setareh Biotech) and Fluorogold (FG, fluorochrome), were applied to identify both motoneuron pools in the same animal. Firstly, the muscle was exposed by making a small cut to the skin, and two injections (2.5 μl/injection) were distributed throughout the body of the muscle with a glass pipette using a Picospritzer. In a first set of experiments, both tracers were used in the two muscles, and after corroborating that the results were similar, further experiments were performed applying FG in TA and TB in GM muscles.

Surgical procedure

Under anesthesia, the sciatic nerve was exposed at the mid-thigh and cut by using microscissors. In a first group of animals (n = 16), the transection was not repaired. In another group of animals (n = 16), the proximal and distal stumps were rejoined with two epineural sutures. Afterwards, muscles and skin were sutured in layers, iodine povidone was applied to the wound, and the rats were allowed to recover in a warm environment under close observation. Animals were followed for 1, 2, 4 and 8 weeks after injury (n = 4 for each time and each condition).

Neurotrophic factor application

In another group of animals (n = 4 for each time and condition) we applied BDNF, NT3, or a mixture of the two to the injury site by repairing the transected nerve with a silicone tube filled with a collagen matrix enriched with these factors. The tubes were filled with a

Table 1
Experimental design. Groups and treatments applied. The animals were divided into 4 experimental groups: 1. Time course of spinal synaptic changes. Sciatic nerve was cut and left unrepaired or repaired by direct suture. Animals were sacrificed at 4 different times post-injury. 2. Influence of trophic support on the synaptic changes. BDNF, NT3 or a mixture of the two neurotrophic factors was applied at the injury site using a silicone tube. 3. Influence of treadmill running exercise on the synaptic changes. Two days after nerve repair animals were submitted to LTRP or HTRP, for 1 or 2 weeks. 4. Suppression of homolateral sensory inputs. After L3 to L6 rhizotomy, half of the animals were submitted to HTRP and the other half was left untrained.

Experimental group	Injury	Lesion/treatment	Follow-up
1. Time course of spinal synaptic changes (n = 32)	Sciatic nerve section	No repair	1 week
	Sciatic nerve section	Direct suture	2 weeks
			4 weeks
			8 weeks
2. Influence of trophic support on the synaptic changes (n = 20)	Sciatic nerve section	Silicone tube with BDNF	1 week
	Sciatic nerve section	Silicone tube with NT3	2 weeks
	Sciatic nerve section	Silicone tube with BDNF + NT3	
3. Influence of treadmill running exercise on the synaptic changes (n = 16)	Sciatic nerve section	Direct suture/LTRP	1 week
	Sciatic nerve section	Direct suture/HTRP	2 weeks
4. Suppression of homolateral sensory inputs (n = 6)	L3–L6 rhizotomy (intact sciatic nerve)	Untrained	2 weeks
	L3–L6 rhizotomy (intact sciatic nerve)	HTRP	