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Review Article

# The use of the rat as a model for studying peripheral nerve regeneration and sprouting after complete and partial nerve injuries

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ABSTRACT

Rat models of complete and partial injuries are the most frequently used models for analysis of the cellular and molecular processes of nerve regeneration and axon sprouting. Studies of nerve regeneration and axon sprouting after complete and partial nerve injuries, respectively, are reviewed. Special consideration is made of the peripheral nerves chosen for the studies and the outcome measures that were utilized in the studies. The studies have made important contributions to our knowledge of the degenerative and regenerative processes that occur after the peripheral nerve injuries, why functional recovery is frequently compromised after delayed surgery, the positive effects of neurotrophic factors on nerve regeneration after delayed nerve repair or after insertion of autografts between transected nerve, and how axon regeneration may be accelerated by brief periods of electrical stimulation and/or by administration of androgens.



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## 1. Introduction

The capacity of the peripheral nervous system to regenerate lost axons after nerve injuries is well recognized and frequently contrasted with the inability of injured neurons to regenerate their axons within the non-permissive growth environment of the central nervous system. Yet the frequent inability of injured peripheral nerves to restore function even after microsurgical repair, is less well recognized by the neuroscience community. It is an important reason for the community to continue to investigate the basis for this poor recovery of function as well as to develop methods to promote effective nerve regeneration and functional recovery.

The rat is the most frequently used animal model of peripheral nerve injury. The injuries include those that isolate all or some of the axons from the cell bodies of the neurons, complete and partial nerve injuries, respectively. Many peripheral nerves have been used as the model system for these studies, those in the hindlimb being the most frequently used. Although the facial nerve and the nerves in the forearms have been used in different studies, the nerve of choice depends primarily on the outcome measures used to determine regenerative success. A brief overview of the response of neurons and glial cells of the peripheral nervous system is given before considering rat models in the context of the research questions that have been addressed. The discussion is directed at the insights that have been generated into the biology of nerve regeneration and our quest to determine the basis for the poor functional recovery after peripheral nerve injuries.

## 2. The transient response of neurons and glial cells to injury

### 2.1. Neurons

The motor and sensory neurons that are disconnected from their target organs by nerve injury have been referred to as *axotomized* (Fu and Gordon, 1997). The axotomized neurons undergo morphological or chromatolytic changes that vary in extent in different neurons (Lieberman, 1971). The nucleus moves to an eccentric position and there is dissolution of Nissl bodies starting near the nucleus and extending peripherally toward the plasma membrane. These changes underlie the change in gene expression of the neurons. These changes have also been regarded as a switch from the normal transmitting state of neurons to a growth mode with upregulation of cytoskeletal proteins actin and tubulin that mediate axonal growth and with downregulation of neurofilament that accounts for the reduced diameter of the disconnected axons in the proximal nerve stump (Gordon, 1983; Gordon and Stein, 1982b; Tetzlaff et al., 1988). The neurons also upregulate several neurotrophic factors including nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF), the more heterogeneous neurons within the dorsal root ganglia demonstrating preferential expression of some but not other neurotrophic factors (Sebert and Shooter, 1993; Verge et al., 1996). The expression of growth associated genes is transient however, the expression of cytoskeletal proteins and neurotrophic factors for example, decaying as a function of time (Boyd and Gordon, 2003b; Gordon and Tetzlaff, 2015; Gordon et al., 2015).

### 2.2. Wallerian degeneration and Schwann cells

Distal to a crush or transection injury, the axons within the endoneurial tubes are separated from their cell bodies and undergo axonal fragmentation known as Wallerian degeneration (Waller, 1850). Calcium waves from the injury site down the distal nerve stump play a key role in executing a proximo-distal degeneration of the axons by activating the protease calpain that breaks down the neurofilaments of the cytoskeleton (Lubinska, 1977; Vargas et al., 2015; Yang et al., 2013). The myelin and axonal debris are phagocytosed within the first few days by the Schwann cells themselves and thereafter by the blood-born macrophages that enter through the permeabilized nerve-blood barrier and perform the bulk of the phagocytosis (Avellino et al., 1995, 2004; Beuche and Friede, 1984; Gaudet et al., 2011; Gordon, 2015; Hirata and Kawabuchi, 2002; Rotshenker, 2011; Shamash et al., 2002). In the rat, the time course of these events is a protracted period of at least 3 weeks in which myelin and axon debris are slowly removed (Fig. 1) after which the macrophages leave the distal stump (Avellino et al., 1995; You et al., 1997). During the course of Wallerian degeneration, the Schwann cells undergo a transition from a myelinating state to a growth supportive state in which they assume an elongated shape as they undergo mitosis and line the endoneurial sheaths as the bands of Bungner (Jessen et al., 2015). In the growth-permissive state, the denervated Schwann cells within sensory pathways express neurotrophic factors that include the neurotrophins NGF, BDNF, and neurotrophin 4/5, the insulin growth factors 1 and 2 (IGF-1,-2), whilst glial derived neurotrophic factor (GNDF) and pleiotrophin (PTN) are expressed primarily by the Schwann cells within motor pathways (Boyd and Gordon, 2003b; Hoke et al., 2006). Only the p75 receptor and not the trk receptors for the neurotrophins is expressed on the Schwann cells whilst the receptors for other neurotrophic factors such as GDNF and EGF are expressed on the membranes of the denervated Schwann cells (Fig. 1) (Boyd and Gordon, 2003b; Hoke et al., 2006). Microarray analyses have revealed hundreds of genes that are expressed in Schwann cells after denervation, most of whose functions remain to be determined (van Kesteren et al., 2011). Presently, it is the expression of the neurotrophic factors that has concerned the most recent research with particular emphasis on the transient nature of the expression of these factors after their rapid upregulation; the elevated expression declines within a month of injury (Figs. 1 and 2) (Brushart et al., 2013; Hoke et al., 2006).

### 2.3. Denervated end organs

The muscle(s) and sense organs that are denervated by the nerve injuries undergo atrophic changes. These are often regarded as progressing inevitably to replacement with fat (Lundborg, 2004).