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Short communication

Standardized crush injury of the mouse median nerve

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ABSTRACT

The employment of transgenic mouse models for peripheral nerve regeneration studies is continuously increasing. In this paper, we describe a standardized method for inducing a crush lesion in mouse median nerve using a non-serrated clamp exerting a crush compression force of 20.43 MPa for a duration of 30 s. Quantitative assessment of posttraumatic functional recovery by grasping test showed that recovery was very fast and mice returned to baseline performance already after 20 days only. Stereological analysis of nerve fibers distal to the crush lesion showed the presence of axons with a significantly smaller size and thinner myelin sheath in comparison to controls. This experimental nerve injury model is highly reproducible and the impact on animal well-being is minimal. Its employment can be particularly indicated for exploring the basic neurobiological mechanisms of peripheral nerve regeneration.

1. Introduction

Important progresses have been obtained over the last decades in the study of posttraumatic nerve regeneration and the strategies to promote this process and it is expected that the understanding of the neurobiological mechanisms that occur after injury can be used to design modern strategies for reconstruction after nerve trauma (Dahlin et al., 2009; Navarro, 2009). Whereas a clear prevalence in the selection of the rat for nerve regeneration studies can still be detected in the literature, recently the availability of a number of genetically modified mouse colonies is causing an increasing use of mice for the experimental investigation of neural repair in the peripheral nervous system (Tos et al., 2009). Most mouse studies so far have been based on sciatic nerve repair and regeneration (see Table I in Tos et al., 2009). In a recent paper, we have described the use of the mouse median nerve model for the experimental investigation of end-to-end nerve reconstruction (Tos et al., 2008). However, high-level microsurgical skills are required since the small size of the nerve requires performing 12-0 epineurial suture. To avoid this requirement, and also the related high variability in post-surgical outcome, a nerve crush lesion can be adopted in order to obtain complete axonal transection (without interrupting continuity of the nerve's connective tissue) (Bridge et al., 1994).

Since we have recently described a standardized procedure for median nerve crush lesion in the rat using a non-serrated clamp (Ronchi et al., 2009), the aim of this study was to adapt this procedure to the mouse median nerve, describing the pattern of posttraumatic regeneration and functional recovery by behavioral and stereological analysis.

2. Materials and methods

2.1. Animals and surgery

Five FVB adult male mice (Charles River Laboratories, Milano, Italy), weighing approximately 30 g, were used. Animal cages were housed in a temperature and humidity controlled room with 12–12 h light/dark cycles. The animals were fed with standard chow and water *ad libitum*. Measures were taken to minimize pain and discomfort taking into account human endpoints for animal suffering and distress. All procedures were performed with the approval of local Institution's Animal Care and Ethics Committee and in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). All surgical procedures were carried out under deep anaesthesia using ketamine (9 mg/100 g-body weight), xylazine (1.25 mg/100 g-body weight) and atropine (0.025 mg/100 g-body weight), intramuscular. The median nerve of the left forelimb was approached from the axillary region to the elbow and carefully exposed (Fig. 1A). The crush lesion was applied

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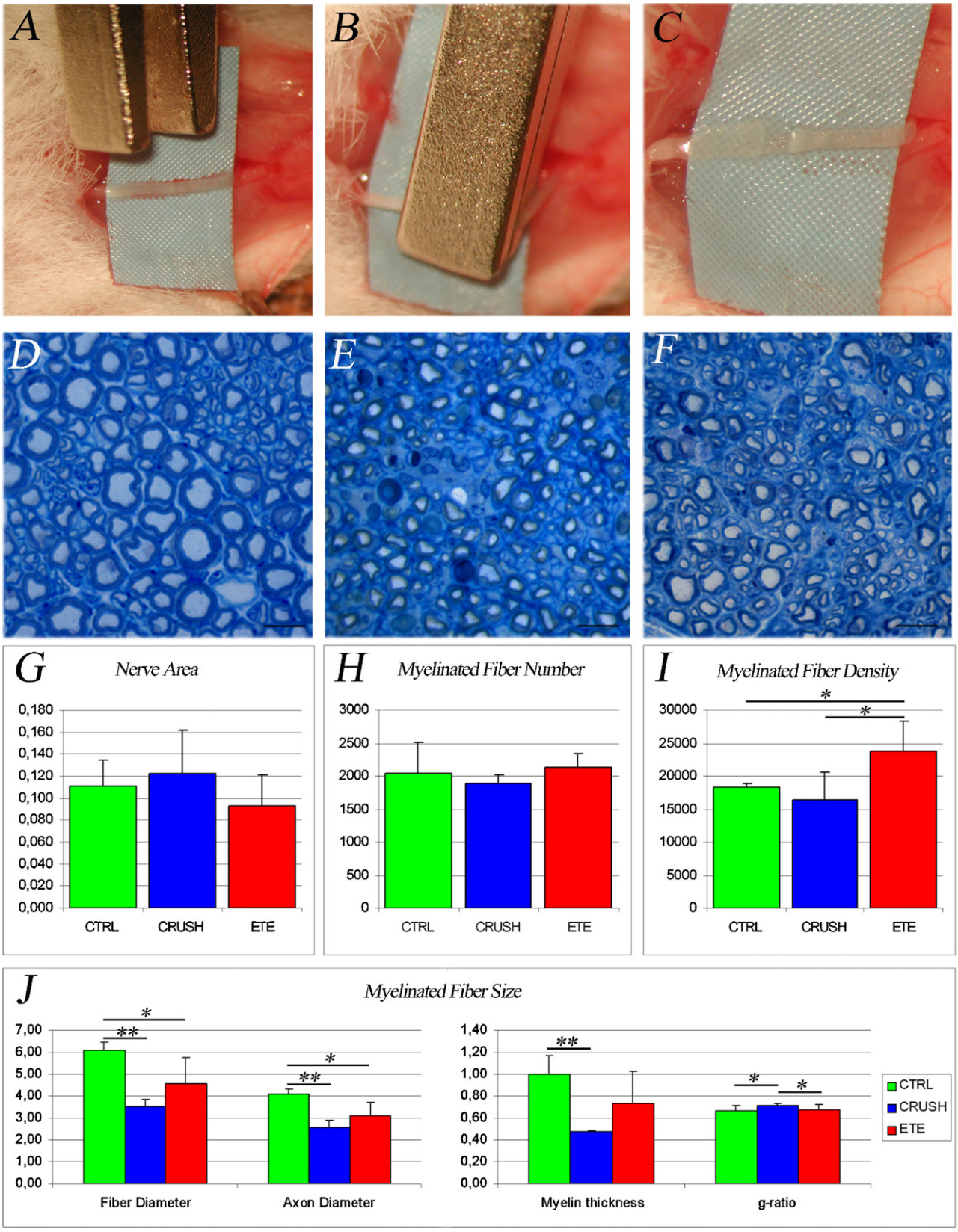

Fig. 1. (A–C) Steps for producing the crush injury using the non-serrated clamp. (D–F) High resolution light microscopy of nerve fibers from control (D), crush (E) and end-to-end repair (F) animal groups (scale bar = 10 μm). (G–J) Results of the design-based stereological estimation of control and regenerated nerve fibers: CTRL, control; CRUSH, crush injury; ETE, end-to-end nerve repair. $^{*}p < 0.05$; $^{**}p < 0.01$.