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A device for the electrophysiological recording of peripheral nerves in response to stretch

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Abstract

The functional consequences of nervous tissue subjected to mechanical loads are of vital importance in successful clinical outcomes and in tissue engineered applications. In this paper, we developed a new ex vivo device that permitted the recording of nerve action potentials while the nerve was subjected to elongation. Experimental results showed guinea pig nerves to possess an inherent tolerance to mild stretch. The mean elongation at which the compound action potential (CAP) amplitude began to decrease was found to be $8.3 \pm 0.56\%$. The CAP response to stretch was immediate beyond this threshold. After $17.5 \pm 0.74\%$ elongation, the CAP levels decreased to approximately 50% of its uninjured values. When allowed to relax, the CAP recovered almost completely within minutes. Based on the temporal scale of the CAP response and the presence of oxygen during testing, we conclude that the initial mechanism to CAP degradation cannot be ischemia. Since it is inherently difficult to study mechanical damage independent of hemodynamic factors in vivo, the developed model could be used to further elucidate the injury mechanisms of stretch-induced trauma. The design of the ex vivo chamber will also permit the administration and assessment of pharmacological agents on electrical conduction in various deformation conditions.

Keywords: Strain; Sciatic nerve; Grease gap recording; Compound action potential

1. Introduction

From a morphological perspective, peripheral nerves are naturally structured to withstand mild levels of stretch (Fig. 1A). The tortuous pattern of axonal elements and surrounding axolemma offer a protective mechanism against stretch injuries (Sunderland, 1990). Previously, there have been several studies attempting to define tensile loads that translate into measurable anatomical damage (Haftek, 1970; Rydevik et al., 1990; Sunderland, 1990; Spiegel et al., 1993; Ninan, 2003). However, it is reasonable to assume that functional damage may occur prior to any permanent morphological change. Using mostly in vivo models, later studies have aimed to characterize such conduction behavior during maintained levels of nerve stretch (Denny-Brown and Doherty, 1945; Kwan et al., 1992; Wall et al., 1992; Driscoll et al., 2002; Takai et al., 2002; Jou et al., 2000). Yet due to the confounding variables of in vivo models, it was difficult to determine the initial mode of injury. Indeed during nerve elongation, there is constriction of the vasculature and decreased blood flow within nervous tissue (Lundborg and Rydevik, 1973; Jou et al., 2000). The subsequent ischemia is sometimes believed to be the primary culprit leading to functional deficit, although some suggest there may be an earlier mechanism ascribed to mechanical deformation (Kwan et al., 1992; Ochs et al., 2000; Driscoll et al., 2002). We also hold the view that prior to ischemic injury there is a mode of mechanical injury that is responsible for the observed conduction block. However, reliable ex vivo models are still lacking. Thus, the present study attempts to elucidate the initial process of functional deficit through the development of an ex vivo measurement device. The implementation of our unique isolation chamber permits us to continuously measure compound action potentials (CAP) and collective cell membrane integrity while the nerve is subjected to stretch. This novel acute injury model also allows us to compare and contrast conduction block caused by tissue deformation to that of ischemic insult.

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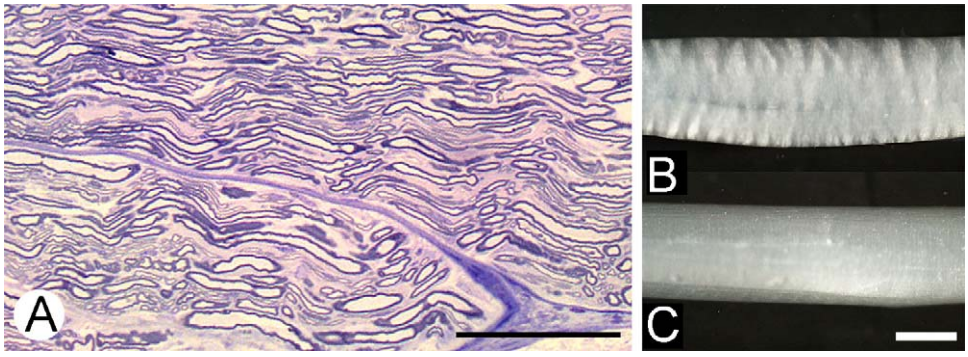

Fig. 1. (A) High magnification of guinea pig tibial nerve sectioned longitudinally. Samples were stained with toluidine blue as a marker for myelin. The histology reveals the natural wavy pattern typical of peripheral nerves. Bar: 100 μm. (B) Low magnification of guinea pig tibial nerve at rest (retracted) length. The vertical light and dark bands are an optical phenomena (bands of Fontana) caused by the microscopic undulations of the nerve architecture (A). (C) During stretch, the axonal undulations straighten and the vertical bands of Fontana disappear (Clarke and Bearn, 1972). Elongations at which these bands vanished were found to be $9.15 \pm 0.19\%$ ($n = 8$). These values are lower than the data reported by Pourmand, who observed the disappearance of the bands at 14% strain with rat sciatic nerves (Pourmand et al., 1994). Upon release of stretch the bands are restored as the nerve rebounds to initial length. Based on our observations, the disappearance of these bands may serve as an indicator for the onset of conduction block. Bar: 0.5 mm.

2. Materials and methods

2.1. Nerve isolation

All animals used in this study were handled in strict accordance with the National Institutes of Health guide for the Care and Use of Laboratory Animals. The experimental protocol was approved by the Purdue Animal Care and Usage Committee.

Adult female guinea pigs (ranging from 325 to 500 g) were anesthetized using ketamine (80 mg/kg) and xylazine (12 mg/kg) and perfused with 15 °C Kreb's solution. The sciatic nerve (~5 cm long) was then excised and allowed to retract in an oxygenated (95% O_2, 5% CO_2) Kreb's solution for 10 min. After 10 min, a pair of dye lines approximately 12 mm apart was made on the nerve. This 12 mm distance served as the retracted reference gauge length that was used for subsequent elongation calculations. After dye marking, the sciatic nerves were then split with a #12 scalpel blade to isolate the tibial and peroneal components. The nerves were re-incubated in the bubbling Kreb's solution for an additional hour to recover from the surgical process. The composition of Kreb's solution was as follows (mM units): 124 NaCl, 5 KCl, 1.2 KH_2PO_4, 1.3 $MgSO_4$, 2 $CaCl_2$, 20 glucose, 10 sodium ascorbate, and 26 $NaHCO_3$, equilibrated with 95% O_2, 5% CO_2 to produce a pH of 7.2–7.4.

2.2. Experimental chamber and setup

The current experimental apparatus is a modification of the double sucrose gap chamber first devised by Shi and Blight (1996). Briefly, an acrylic chamber 30 mm long was machined with five wells as shown in Fig. 2. The sucrose gaps from the original apparatus were substituted with grease insulator slots that separated the middle compartment from the end wells. Vacuum grease placed in these wells formed a leak proof barrier that separated the fluid reservoirs. Each end well was filled with isotonic (120 mM) potassium chloride, while the center reservoir contained Kreb's buffer solution. Excised nerves were placed in a 1 mm wide central channel that extended through the chamber. The nerve ends were fixed by a stationary and moveable clamp. The moveable clamp was attached to a rigid bar, which was in turn mounted to a digital force transducer (Mark 10). The force transducer was bolted to a motorized micromanipulator (Newport). A clear acrylic lid was set over the entire chamber to seal the setup (not shown) and a built in gas line was used to control the gaseous environment (Fig. 3). Two pairs of Ag/AgCl electrodes on opposite sides of the chamber served to stimulate and record the nerve tissue. The electrodes were not in direct contact with the nerves. All electrophysiology recordings were made using a bridge amplifier (Neurodata Instruments) and data analysis was performed with custom PC Labview software (National Instruments). Control trials with the experimental setup showed stable electrophysiology for over an hour (Fig. 4) and nerve

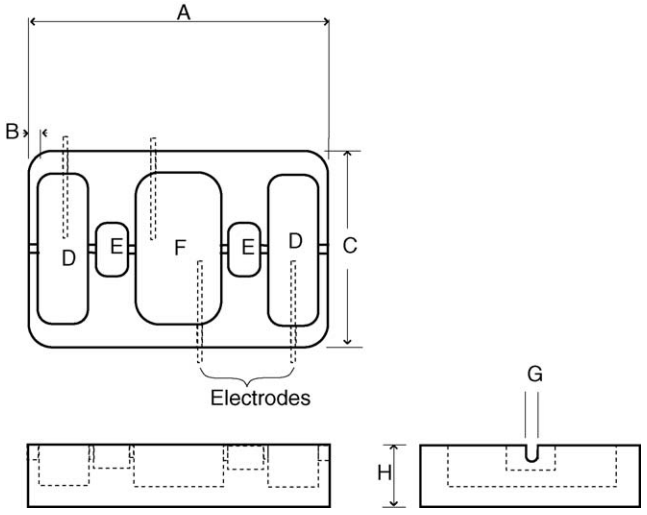

Fig. 2. Schematics of the acrylic isolation chamber that was modified from the version in Shi and Blight (1996). All dimensions given in millimeters. (A) 29, (B) 0.9 (typical), (C) 20, (D) 3.5 × 16.5 × 3 (length × width × depth), (E) 3.5 × 6 × 3, (F) 9.6 × 16.5 × 3.5, and (G) 1.0 DIA, 2 deep. The cover (not shown) was placed over the chamber and sealed with vacuum grease (Dow). The chamber is airtight except at the notches where the nerve exits the chamber. The covered chamber was found to keep oxygen tension at 26 ppm when measured with an oxygen sensor (WPI Inc.).