Tensile Physiology: Measuring Force and Conduction in Peripheral Nerves Undergoing Controlled Stretch

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Abstract— **Tensile loading is a common physiological condition for peripheral nerves, but can also induce pathologic effects upon electrophysiological conduction. Functional deficits resulting from nerve elongation are not thoroughly understood. Using a computerized micromanipulator, load cell, and grease gap-recording chamber, a new system for tensile electrophysiology is proposed and demonstrated. This paper examines the effects of tension on conduction through guinea pig sciatic nerves. Nerves were stretched at 0.15 mm/s to 5%, 10%, 15%, and 20% beyond their physiological maximum. Results indicate minimal changes in conduction for 5% elongation. Further strain resulted in approximately linear increases in recorded force and decreases in conduction amplitude.**

Keywords-peripheral nerve; stretch; electrophysiology; tension; loading

I. INTRODUCTION

Peripheral nerves are unique soft tissues, anchored to the spinal column but capable of extensive movement throughout the body. Neurons in the periphery are capable of undergoing physiologic distension without effecting electrophysiological conduction. Nerves have redundant systems to allow for mild distensions, such as nonlinear axonal paths and natural tissue folds as evident in the bands of Fontana[1]. Nonetheless, stretch injury is a primary mechanism of peripheral nerve dysfunction, causing most nerve injuries in civilian populations[2]. Such injuries are common in sprains and dislocations, when peripheral nerves are subjected to forces or strains in excess of their physiological tolerance.

Establishing the functional limits for stretch tolerance is important for improving patient outcomes following nerve injury. Early work in this area primarily examined anatomical damage from tensile loading [1, 3]. However, recent work has shown that functional deficits occur prior to histological disruption[4]. Values ranging from 4% to 21% have been reported as the clinical threshold for sustained functional deficits [1, 5]. It is clear that the effects of tensile stress upon peripheral nerve conduction are not yet completely understood.

We are unaware of any other system for measuring electrophysiological conduction through peripheral nerves in tandem with tensile analysis *in vitro*. By using a computer controlled micromanipulator and load cell, it is possible to precisely control distension and measure forces in real time for nerves undergoing electrophysiological evaluation in a modified grease gap recording chamber[4]. This tensile electrophysiology setup allows force and displacement to be closely correlated with the conduction effects induced by controlled elongation.

This apparatus was tested on *ex vivo* guinea pig sciatic nerves. For supra-physiological strains of 5%, 10%, 15%, and 20%, it was possible to record position, tension, conduction amplitude, and signal latency with high fidelity. Results indicate a linear relationship between force and displacement. Similarly, there are proportionate decreases in compound action potential (CAP) amplitude conduction over the range of displacements tested. No significant effects were observed on conduction latency, except for nerves undergoing 20% strain.

II. MATERIALS AND METHODS

A. Nerve Isolation

Animal handling protocols were approved by institutional review (PACUC# 04-049). Female guinea pigs (250-400 g) were anesthetized by IM injection with ketamine (80 mg/kg) and xylazine (12 mg/kg). Transcardial perfusion with oxygenated Kreb's buffer solution removed blood. Sciatic nerves were exposed by incising the hamstring muscles. Hind limbs were flexed until the hip, knee, and ankle joints formed a 45º angle with respect to the body axis (Figure 1A). Nerves were then marked with spots of India ink 20 mm apart. This reference length (L_r) was assumed to correlate to maximum physiologic stretch.. Nerves were excised and stored ≥1 hour in cold, oxygenated Kreb's solution to allow biochemical recovery from surgical extraction.

B. Electrophysiology and Force Recordings

Electrophysiology was conducted using a modified grease gap recording device originally described by Li and Shi [4]. Briefly, an acrylic chamber was manufactured as shown in Figure 1B. Sciatic nerves were placed in grooves spanning the recording chambers. The smaller wells were filled with silicone grease to form a seal around the nerve and between wells. KCl (120 mM) was placed in the outer wells and

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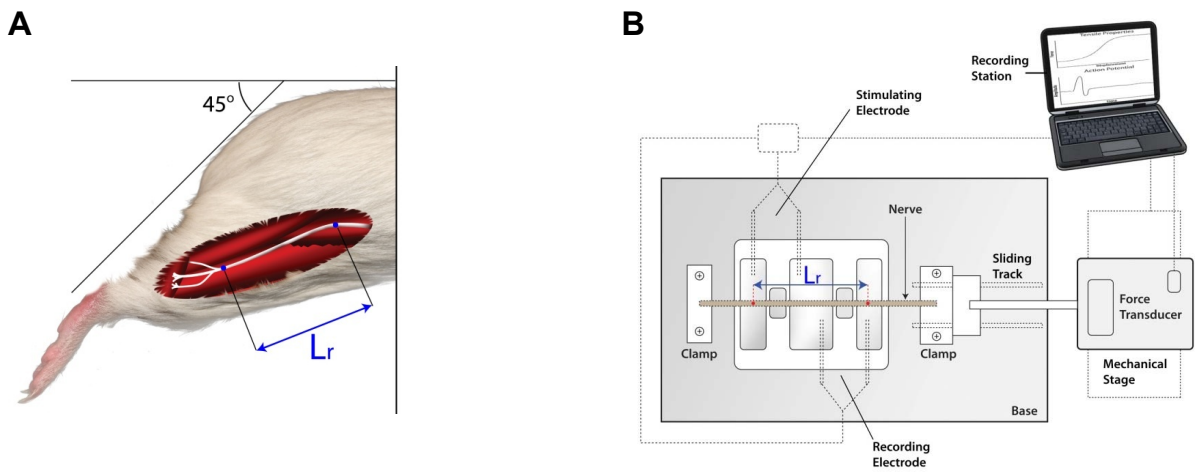

Figure 1. Tensile electrophysiology on guinea pig sciatic nerves. (A) The reference length (L_r) was marked on guina pig sciatic nerves at maximum physiologic elongation by stretching the hind limb to ~45 º and applying two spots of India ink. (B) Schematic of tensile electrophysiology apparatus including stimulating/recording electrodes, sliding track, force transducer, mechanical stage, and recording station.

oxygenated Kreb's solution was regularly refilled in the center. Paired Ag/AgCl electrodes in each well recorded electrophysiological conduction through the nerve without physical contact. Conduction recordings were made with a bridge amplifier (Neurodata Instruments) and output to a custom Labview interface (National Instruments). Compound action potential (CAP) amplitude and latency were recorded continuously as the sum of evoked potentials and lag between stimulus and CAP peak, respectively.

One end of the nerve was clamped to a stationary surface while the other was clamped atop a sliding track. The apparatus was linked to the force transducer (Honeywell Sensotec Model 11) which was clamped to a computer controlled mechanical stage (Model ESP100, Newport Corp.). Stage position and movement could be specified through a custom Labview program to control and record position and force. Nerves were stretch until the reference length (L_r) was obtained between the ink dots. This corresponds to maximum physiological stretch and was subsequently set as zero displacement for analysis. L_r was taken as the absolute length of the nerve for calculating strains.

Nerves were maintained at L_r for 5 minutes before stretch was initiated. Four nerves were stretched to strains of 5%, 10%, 15%, or 20%. Strain rate was set at 0.15 mm/s. The mechanical stage immediately reversed direction and returned nerves to their reference length. Electrophysiological monitoring was continued for 5 minutes following initiation of stretch. Electrophysiological results were normalized to their pre-stretch values.

C. *Statistical Analysis*

Electrophysiological data is reported as normalized means ± standard error. Pair wise comparisons between groups were accomplished using one-way ANOVA to compare means. P-values less than 0.05 were identified as statistically significant.

III. RESULTS

Electrophysiological data was measured before and after stretch, with values normalized to their pre-stretch values. CAP amplitude and latency are shown in Figure 2. For each stretch value, three nerves were tested. Average normalized amplitude was found to recover to 1.00±0.09, 0.87±0.01, 0.58±0.06, 0.17±0.03 after 5%, 10%, 15%, and 20% respectively. Similarly, latency values increased 6.15±3.63%, 2.25±3.47%, 5.37±1.60%, and 29.19±0.80% after strain.

Force transduction allowed for rapid analysis and visualization of the position and force data as the nerve underwent electrophysiological testing. Representative time-profiles of the exerted force are shown in Figure 3A. The maximum forces recorded were 1.20, 3.46, 4.15, 5.06N for 5%, 10%, 15%, and 20% stretch, respectively. Figure 3B also shows a force-displacement graph.

IV. DISCUSSION

Evaluating nerve responses to pathological stretch is relevant for clinical planning and neurophysiological modeling. Previous studies in this area have identified a range of thresholds for sustainable stretch without inducing long-term deficits. Authors have reported many different values for acceptable strains from which nerves may rapidly recover. These range from 4% to 21% elongation [5, 6]. However, comparing data between studies is complicated by variations in equipment, procedures, and model organisms. One highly variable element within these protocols is the starting length of the nerves used: in some experiments, reference lengths were marked on limbs at rest[6] or flexed to 90º [3]. However, these results do not account for the full extent of physiological stretch, and variations in nerve reference length can profoundly affect the results. Some groups have estimated maximum strain in peripheral nerve by fully extending limbs, roughly to 45º from the body axis [4, 7]. By utilizing this approach, we seek to eliminate one primary source for error. Differences in equipment account for some variations between experimental results. Computerized control of stage movement and real-time

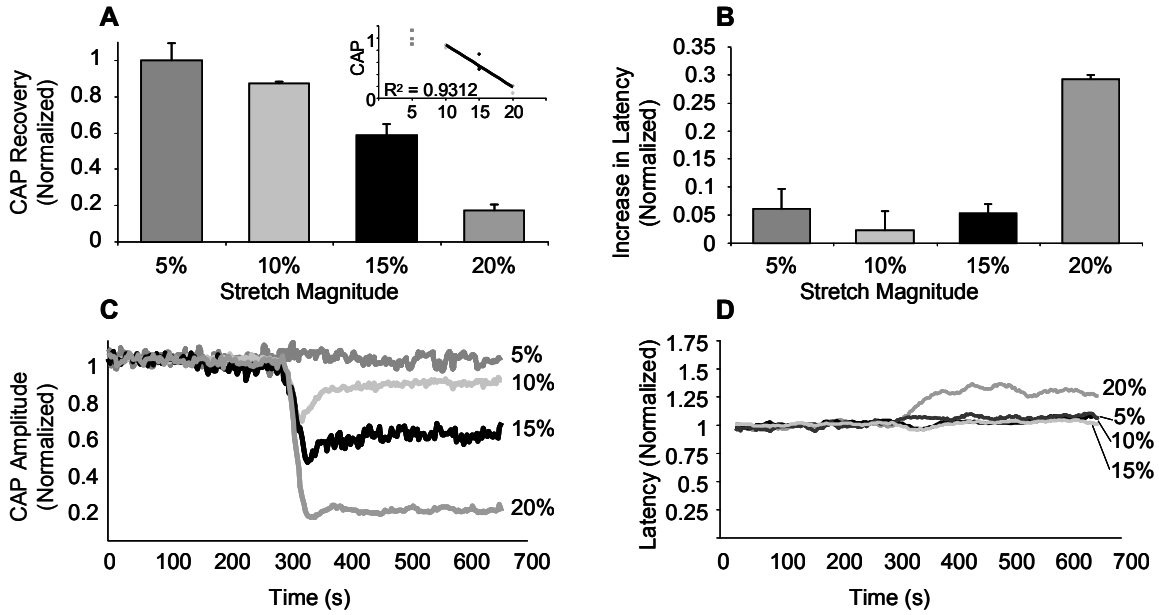

Figure 2. Electrophysiology. (A) CAP amplitudes were normalized after stretch to reveal a strong-dose response to strain. Except for the nerves experiencing 5% stretch, amplitudes were significantly decreased in all samples. All comparisons between stretch values were significant except between 5% and 10%. Inset shows the linear relationship between stretch magnitude and CAP amplitude (R^2=0.9312). (B) Latency values were increased for all stretch values, but statistical significance was only noted on the 20% strain. (C) Average CAP amplitude time profiles demonstrate a clear decrease with onset of stretch and gradual recovery. (D) Latency time profiles showed little effect except for nerves undergoing 20% stretch.

recording of sample strain, force, and electrophysiological conductance allows for gathering data with high accuracy and reproducibility. By utilizing the established guinea pig model, our results may be compared to other published data [4, 8].

Our results correspond well to published data. Driscoll estimated the threshold for peripheral nerve stretch effect upon CAP amplitude to be 8.8% [7]. As expected, Li and Shi reported no sustained change in electrophysiological conduction for nerves stretch 5% [4]. This finding is corroborated by our results showing no significant effect on CAP amplitude or latency after 5% strain. However, after 10% stretch, CAP amplitude was decreased 12.8±0.01%, indicating this value exceeds peripheral nerve's tolerance threshold. A linear trend of decreasing conduction amplitude with increasing strain was observed for 15% and 20% strains as well. This regular effect was not observed on conduction latency. There was no significant difference in latency after 5%, 10%, or 15% stretch, but 20% stretch elicited a 29.2±0.8% increase. This suggests that CAP amplitude and latency may have different thresholds for conduction deficits. Physiologically, amplitude is largely dependent on the number of intact axons in a sample. Axons may start suffering from ischemia or rupture at different degrees of strain, as each axon follows a unique, tortuous path [1]. As long as some axons remain intact, they will carry signal with the same speed as before stretch. Therefore, the influence of strain upon latency of conduction would not be expected until after amplitude effects were observed.

Tensile properties were recorded in real time, as shown in Fig. 4. Contrary to the predictions of Bueno and Shah, stretched nerves underwent linear increases in tensile force for all regions tested (Figure 3B) [9]. This variation may be attributable to differences in nerve extraction techniques resulting in reference lengths obtained at submaximal physiological extensions. The resulting slack from such extraction would correspond to the "toe" region reported by Rydevik [3]. A linear trend was also observed for the maximum force necessary to reach each strain, which correlates well with prior studies on nerve elasticity [1, 10]. Proportionate responses were also observed for changes in CAP amplitude in response to increasing levels of force (data not shown). The value of this device for tensile electrophysiology is demonstrated in Figure 4 with

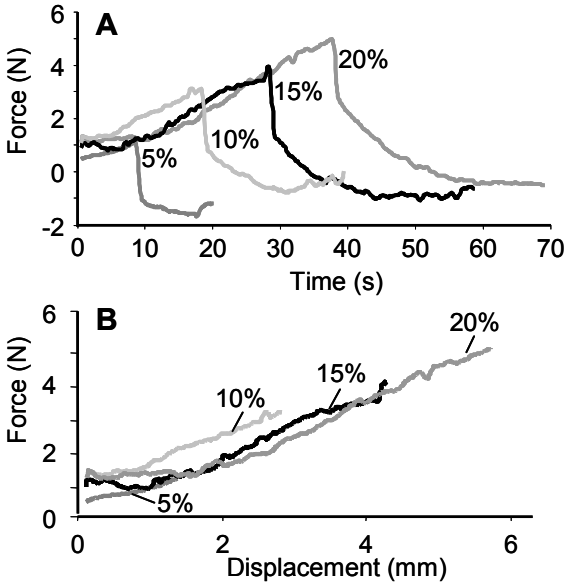

Figure 3. Force measurement and analysis. (A) Representative recordings of tension for nerves undergoing strain. (B) Force graphed with respect to stretch position demonstrates elastic behavior.

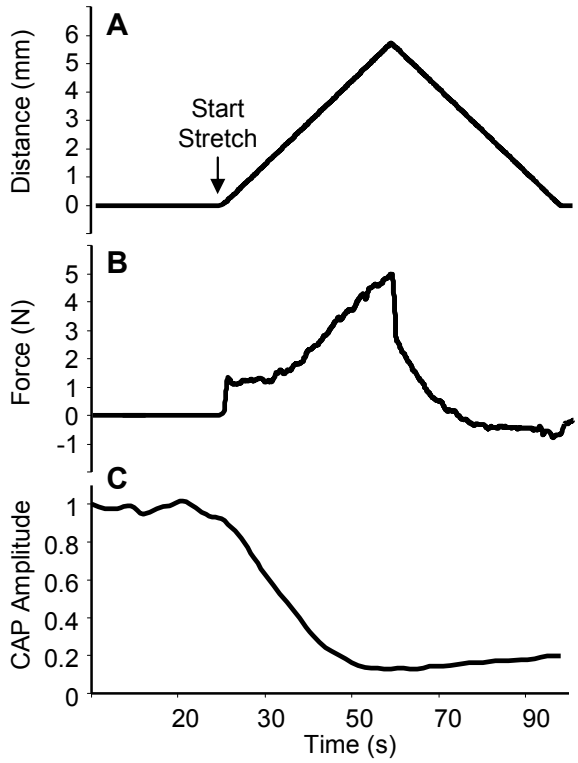

Figure 4. Correlated output. Representative traces for a nerve undergoing 20% strain with real time recording of (A) position, (B) tension, and (C) normalized CAP amplitude.

representative recordings of position, force, and electrical conduction. These data may be collected and processed simultaneously together for rapid and accurate analysis of the effects of strain on nerve function.

As demonstrated by the findings of conduction and tension, this tensile electrophysiology apparatus represents a robust system for examining the effects of stretch upon conduction. Computerized movements produce controlled nerve displacements, while real time recording of force and electrophysiology produces a robust picture of the mechanical and functional effects of stretch. This system is a notable improvement over that proposed by Li and Shi[4] in that strain may be controlled more accurately. Furthermore, tension levels are precisely recorded and displayed as the experiment proceeds. In addition to observing mechanical and electrophysiological effects of stretch, this system could be easily adapted to include histological analyses. Such protocols could include digital image correlation (DIC)[8] or conventional stains such as horseradish peroxidase (HRP) to examine the integrity of axonal membranes [11].

V. Conclusion

This paper proposes a new system for evaluating the response of peripheral nerves to physical stresses and strains. This tensile electrophysiology apparatus is used to evaluate the effects of 5%, 10%, 15%, and 20% supraphysiologic strains on guinea pig sciatic nerves. CAP amplitude was statistically unchanged at 5% strain, suggesting a minimum threshold between 5% and 10% for stretch effects on CAP amplitude. A linear trend was observed for decreases in conduction amplitude with increasing strain beyond 5%. Tensile analysis was performed and force values agreed with literature values. Forces varied linearly with strain, indicating elastic behavior of the peripheral nerves under this testing protocol. Overall, tensile electrophysiology was able to link functional deficits with physical parameters such as force and strain.

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