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Functional and Mechanical Evaluation of Nerve Stretch Injury

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Abstract Peripheral nerves undergo tensile loading in common physiological conditions, but stretch can also induce nerve pathology, impairing electrophysiological conduction. The level of strain nerves can tolerate and the functional deficits which result from exceeding this threshold are not thoroughly understood. To examine these phenomena, a novel system for tensile electrophysiology was created using a grease gap-recording chamber paired with a computerized micromanipulator and load cell. Guinea pig sciatic nerves were stretched beyond their maximum physiologic length to examine the effects of tension on signal conduction. Mechanical and electrophysiological data such as load, position, compound action potential amplitude, and signal latency were recorded in real-time. While 5% strain did not affect conduction, further elongation decreased amplitude approximately linearly with strain. These experiments verify the findings of prior studies into nerve stretch, and demonstrate the utility of this apparatus for investigating the mechanical and electrophysiological properties of nerves undergoing strain.

Keywords Peripheral nerve · Stretch · Electrophysiology · Tension · Loading

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Introduction

Despite being anchored to the central nervous system, peripheral nerves are capable of extensive movement without affecting electrophysiological function. However, supraphysiological distension can impair signal conduction and disrupt nerve anatomy [1, 2]. The non-linear path axons follow through peripheral nerve sheaths may be one mechanism by which nerves elongate without injury, as axons may straighten without undue damage [3]. Nonetheless, stretch injury is a primary mechanism of peripheral nerve dysfunction, causing most nerve injuries in civilian populations [4]. These injuries commonly occur in longitudinal mechanical trauma, such as sprains and dislocations. Characterizing the functional tolerances of peripheral nerves for stretch is a step toward understanding the mechanisms of mechanical nerve injury and may be useful in improving patient diagnosis and treatment following nerve trauma.

Early studies in nerve stretch injury investigated the anatomical disruptions induced by tension. Sunderland and Rydevik found that peripheral nerves' mechanical properties derive from the connective tissue of the perineurium [1, 5]. Further studies have shown that electrophysiological deficits occur at stretches below the distension at which histological disruptions have been reported [6, 7]. The degree of strain at which sustained functional impairment is incurred has been reported as values ranging from 4% to 21% [1, 3]. Further research is necessary to clarify the effects of tensile stress on peripheral nerve function.

The variance in the mechanical and electrophysiological results for studies of nerve deformation may be due to differences in equipment and testing procedures. Here we propose a system for precisely controlling mechanical deformation while simultaneously recording the resulting

tension and deficits in electrophysiological conduction through ex vivo peripheral nerves. For this purpose, we utilized an apparatus in which a load cell can record the tensile load on an isolated nerve while a computer controlled micromanipulator stretched the nerve at a constant rate until reaching the desired strain ratio. A modified grease-gap recording chamber was used to measure compound action potential (CAP) amplitude and latency without interfering with nerve distension [7]. The mechanical data were recorded in real-time and paired with the electrophysiological results, enabling the displacement and force to be closely correlated with the resulting effects on conduction. This system offers the benefits of automated control of nerve distension and allows for more rapid analysis when compared to prior ex vivo models [2, 7]. Analyzing the consequences of nerve elongation is important for understanding stretch pathology and may assist in patient diagnosis.

Stretch induced conduction deficits were examined in guinea pig sciatic nerves using this experimental apparatus. The level of tension which nerves experience physiologically was recreated prior to elongation. Position, tension, CAP amplitude, and latency were recorded while nerves were stretched to supraphysiologic strains of 5%, 10%, 15%, and 20%. As expected, the CAP amplitude was found to decrease when nerves were subjected to higher levels of elongation and force. Latency was not significantly affected by the lesser strain ratios, but a significant increase was noted in conduction time for nerves at 20% supraphysiologic strain.

Materials and methods

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 45° angle with respect to the body axis (Fig. 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 h in cold, oxygenated Kreb's solution to allow biochemical recovery from surgical extraction.

Electrophysiology and force recordings

Electrophysiology was conducted using a modified grease gap recording device originally described by Li and Shi [7]. Briefly, an acrylic chamber was manufactured as shown in Fig. 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, isolating the wells containing conducting solutions. KCl (120 mM) was placed in the outer wells and 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 secured 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) on a computer controlled mechanical stage (Model ESP100, Newport Corp.). Stage position and movement were specified through a custom Labview

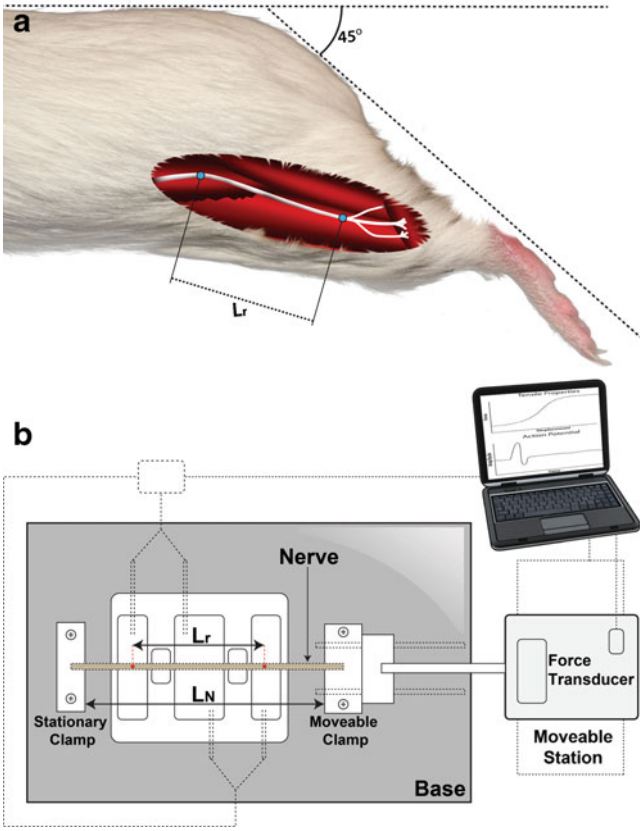

Fig. 1 Nerve extraction and testing. **a** Guinea pig limbs were flexed to 45° to maximize physiologic elongation. The reference length (L_r) was marked by applying two spots of India ink. **b** Schematic for computer controlled tensile electrophysiology apparatus including stimulating/recording electrodes, force transducer, mechanical stage, sliding track, and recording station. Nerves were pre-stretched to restore the reference length and the new gauge length (L_N) of the nerve was measured for mechanical calculations

program to control and record position and force. Nerves were stretched until the reference length (L_r) was obtained between the ink dots. This corresponds to maximum physiological stretch, and both the displacement and tension values were set as zero for analysis. The total distance between stationary and mobile clamps was measured for each nerve, and defined as the gauge length ($L_{N)}$ for calculating strains.

Nerves were maintained at the gauge length for 5 min before stretch was initiated to allow nerve conduction to stabilize. Nerves were stretched 5%, 10%, 15%, or 20% greater than L_N, with three nerves in each group. The strain rate was set to 0.15 mm/s, or approximately 3% strain/min—a rate comparable with other nerve elongation studies [2, 7]. Upon reaching maximum elongation, the mechanical stage immediately reversed direction and returned nerves to their physiologic length. The tension on each nerve and the total change in length were recorded every 100 ms for the duration of experiment. Nerve conduction monitoring was continued for 5 min following stretch to record nerve recovery. For comparison and analysis, electrophysiological results were normalized to their pre-stretch values.

Mechanical analysis

The initial length of the nerve (L_N) measured before testing was used for calculations of strain. Engineering strain values are referred to as strain throughout this document. These were defined as the change in length divided by the original gauge length and set to 5%, 10%, 15% or 20%. The necessary deformations for each nerve were calculated by multiplying these values by the L_N and were input into the controlling Labview program. The highest level of force recorded by the transducer was reported as the maximum tension for each nerve sample. Another crucial mechanical parameter is the total energy absorbed by the nerve to reach a given strain. The quantity of work done was calculated as the summation of the products of force by change in distance for each recorded time point until maximum elongation was obtained.

Additional mechanical analyses examined the elastic regime of tissue deformation using conventional stress-strain mechanics. These analyses included calculations of engineering stress and Young's modulus. The engineering stress values were calculated by dividing the recorded force by the average cross-sectional area. However, obtaining the cross-sectional for each nerve stretched proved difficult, as peripheral nerve is a soft, compressible tissue and deforms readily under compression and tension. Accordingly, an average cross-sectional area was obtained by imaging histological sections of guinea pigs of comparable weights to those used in the elongation experiments. The Young's modulus was calculated as the slope of the linear portion of the stress-strain diagram for each nerve.

Statistical analysis

Electrophysiological data is reported as normalized means± standard error of the mean, as is convention [2, 7, 8]. Mechanical data is reported as normalized means±standard deviation. Pair wise comparisons between groups were accomplished using one-way ANOVA to compare means. Comparisons resulting in p-values less than 0.05 were identified as statistically significant.

Results

Electrophysiological data was measured before and after stretch, with values normalized to their pre-stretch values. CAP amplitude and latency are shown in Figs. 2 and 3, respectively. Following 5%, 10%, 15%, and 20% supra-physiologic strain, mean conduction amplitude were found to be 100±9%, 87±1%, 58±6%, 17±3% of the pre-stretch value, respectivelys. 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 Fig. 4a, while Fig. 4b charts the force vs. displacement. The maximum forces recorded were 1.27±0.55, 2.85±0.69, 3.91±0.23, and 5.21±0.77 N for 5%, 10%, 15%, and 20% stretch, respectively. Histological sections revealed an average cross-sectional area of 1.135±0.234 mm^2, which was used to calculate values for stress and Young's Modulus. Figure 5 shows the average values of force, work, and Young's Modulus for each level of strain. Hysteresis was observed for all samples, and Fig. 6 displays representative stress-strain plots.

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 [1, 9]. Comparing data between different studies is complicated by variations in equipment, procedures, and model organisms. Discrepancies in equipment account for some of the variation between experimental results. Computerized control of stage movement and real-time recording of sample strain, force, and electrophysiological conductance allows

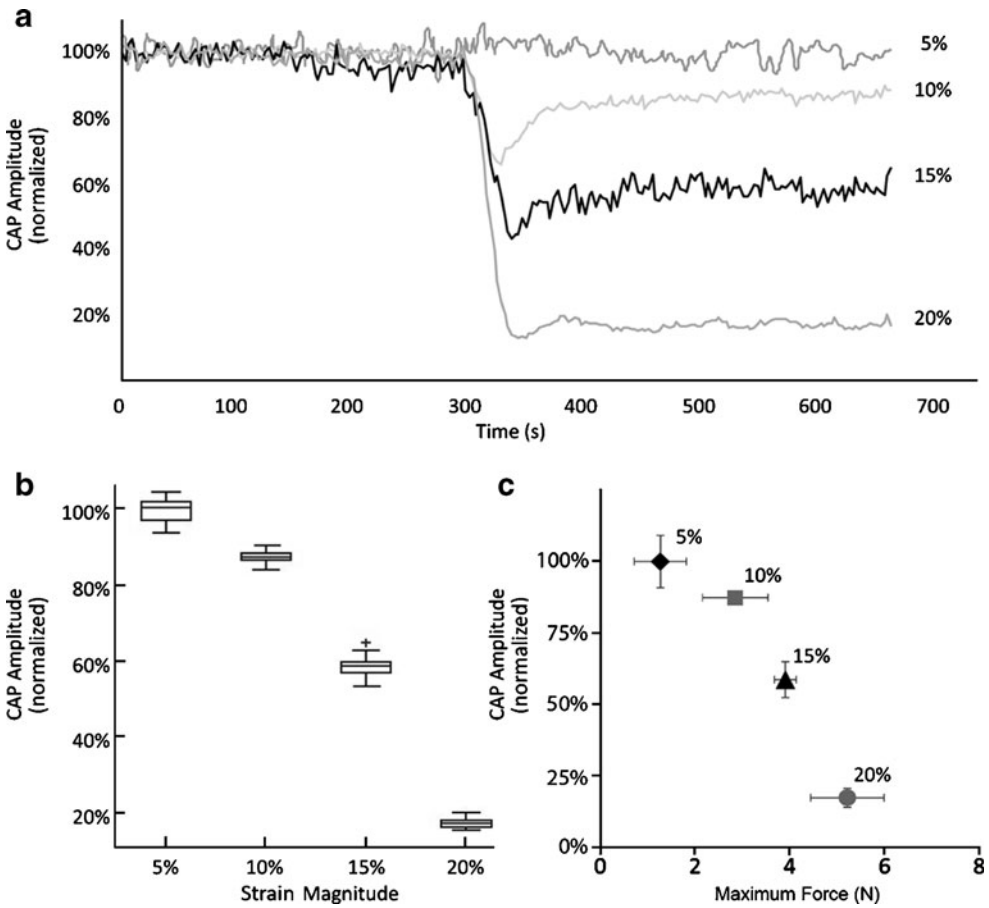

Fig. 2 Effect of strain on CAP amplitude. **a** Time course of average CAP amplitude 5 min before and after stretch shows the effects of strain on conduction. The nerve strained 5% was unaffected, while conduction decreased rapidly in the nerves stretched 10% and 15%. These nerves gradually recovered to 87±1% and 58±6% of their pre-stretch values. Nerves stretched to 20% strain displayed little recovery, only 17±3% of the amplitude prior to distension. **b** Box and whisker plot of CAP recovery following stretch demonstrated significant decreases in conduction amplitude for each level of strain. **c** A linear relationship was observed between stretch magnitude and amplitude of the recovered CAP (R^2=0.949). The *vertical error bars* represent the standard error of the mean, and *horizontal bars* indicate standard deviation

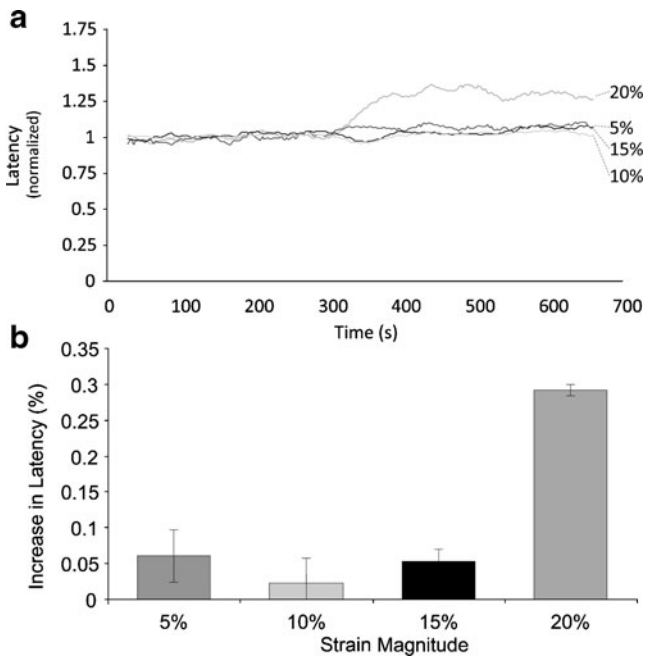

Fig. 3 Effect of strain on CAP latency. **a** Time profiles of conduction latency showed little change except for nerves stretched 20% beyond their physiologic length. **b** Average latency values were increased for all stretch groups, but statistical significance was only observed on the 20% strain. *Error bars* represent the standard error of the mean

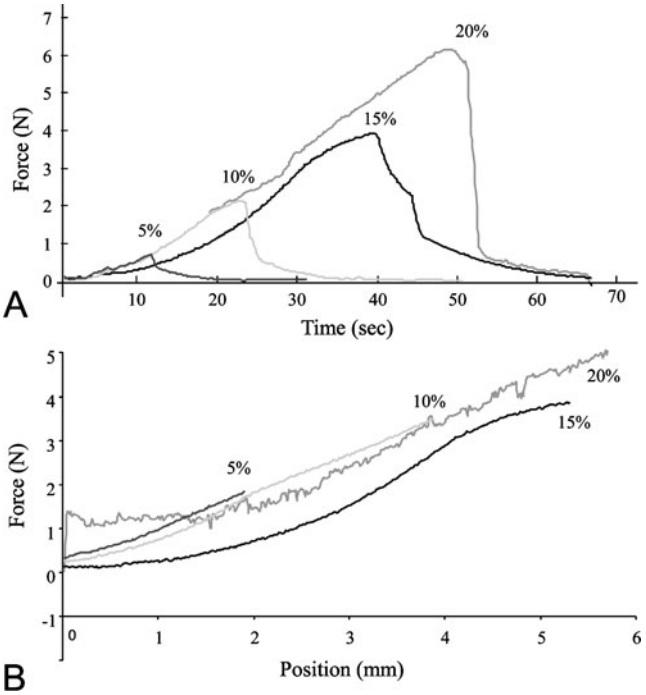

Fig. 4 Force recordings. **a** Representative time profiles of observed tension showed a regular pattern of tensile elongation. **b** Representative force-position diagrams indicate a roughly elastic deformation for the nerve tissue

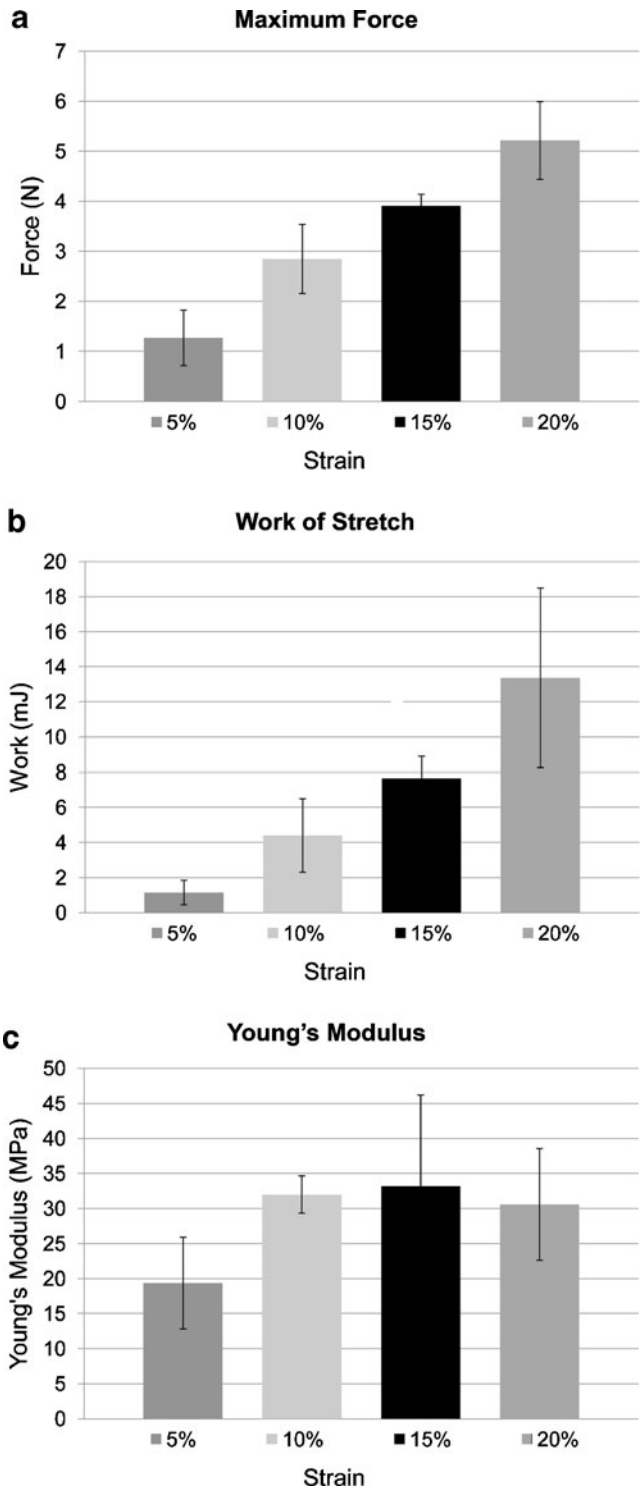

Fig. 5 Mechanical data. **a** The average of the maximum forces necessary to reach each strain level increased linearly (R^2=0.994). **b** Similarly, the average work done on the nerves was obtained by integrating the force-position plots and was found to increase linearly with increasing stretch (R^2=0.978). **c** Average values of the elastic modulus were similar, except for the 5% strain level, indicating this deformation was insufficient to fully enter the elastic domain. All error bars indicate standard deviation

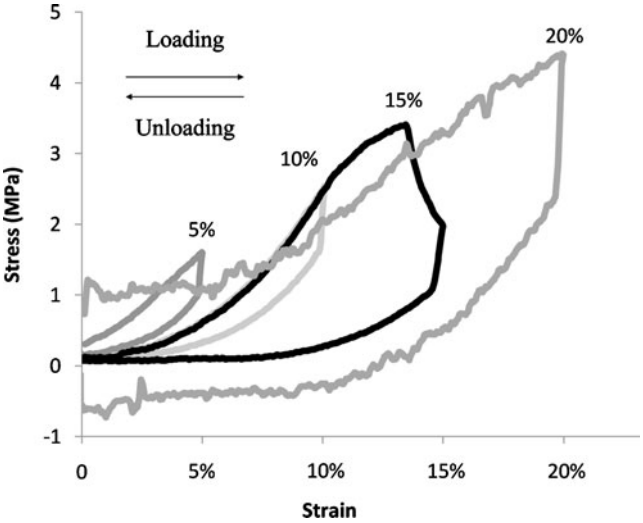

Fig. 6 Nerve deformation hysteresis. Representative stress-strain plots of nerves undergoing controlled stretch exhibited different levels of tension for a given distension when the nerve was being stretched or relaxed. The degree of hysteresis increased with increasing strain, and may indicate destruction of the neural connective tissue

for gathering data with high accuracy and reproducibility. Another highly variable element within the published protocols on nerve stretch is the starting length of the nerves used: in some experiments, reference lengths were marked on limbs at rest [9] or flexed to 90° [5]. 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 45° from the body axis [7, 10]. By utilizing this approach, we seek to eliminate one primary source for error, and to account for the discrepancy between reported values. By utilizing the established guinea pig model for peripheral nerve study, our electrophysiological results may be compared to other published data to validate this system for studying nerve stretch injury [2, 7].

Through our technique, the minimum threshold for nerve stretch prior to functional deficit was found to be between 5% and 10%. These values correspond to several of those reported in the literature. Driscoll estimated the threshold for peripheral nerve stretch effect upon CAP amplitude to be 8.8% [10]. Similarly, Li and Shi reported no sustained change in electrophysiological conduction for nerves stretched 5%, and observed effects on amplitude at an average strain of 8.3% [7]. This finding is corroborated by our results showing no significant effect of 5% strain on CAP amplitude or latency. However, after 10% stretch, CAP amplitude was decreased to 12.8±0.01%, indicating this value exceeds peripheral nerve's mechanical tolerance. The degree of strain was proportional to the decrease in conduction amplitude (CAP Amplitude=−5.25×Strain

Ratio +1.35, R^2=0.949). 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 statistically significant 29.2±0.8% increase. These results correspond well to the observations of Bain et al. who found that low levels of strain did not increase latency compared to controls in guinea pig optic nerves [1].

We hypothesize that the discrepant thresholds are due to the differences in physiologic parameters recorded by the two tests. Given that the intensity of measured CAP amplitude is known to be dependent on number of excited axons, any change in the CAP amplitude when under a constant level of stimulation may be due to decreases in the number of intact, functional axons. Increasing strain disrupts the connective tissues that protects axons from mechanical trauma—particularly the perineurium—while elastic deformation of nervous tissue decreases cross-sectional area, compressing axons [3, 5, 11]. These phenomena would lead to a progressive decrease in functional axons with increasing strain. However, as long as some axons remain intact, they will conduct signals with the same speed as before stretch. The latency of compound action potential is a measure of the time between stimulation and recording, and does not directly account for the number of stimulated nerve fibers. Therefore, the influence of strain upon latency of conduction would not be expected until after amplitude effects were observed.

In these experiments, nerves were tested while undergoing strain ex vivo, suspended in oxygenated Kreb's solution. The resulting decreases in conduction amplitude were comparable to literature values for in vivo and ex vivo experiments [2, 7, 12]. Lundborg and Rydevik attributed the stretch-induced impairment of nerve conduction to decreases in intraneurial circulation, which has been supported by other work [6, 11, 12]. While reductions in blood flow are known to affect nerve function, this phenomenon cannot be the sole mediator of acute stretch injury, as similar results are here demonstrated in ex vivo situations where blood supply is not a factor.

Tensile properties were recorded in real time, as shown in Fig. 4. Contrary to some published accounts, stretched nerves underwent linear increases in tensile force for all regions tested (Fig. 4b) [5, 13]. This variation from the literature may be attributable to differences in nerve extraction techniques: variations between reference lengths obtained at maximal physiological extension and relaxed position will greatly alter calculations of nerve strain. The slack resulting from testing beginning with at submaximal distension would correspond to the "toe" region of early stretch, after which other groups have observed linear, elastic behavior at strains above 20% the relaxed nerve length [5, 12]. A linear trend was also observed for the maximum force necessary to reach each strain, which correlates well with prior studies on nerve elasticity (Maximum Force=25.79×Strain+0.09, R^2=0.995) [3, 14]. A similar pattern was observed for the work required to reach each level of strain, with a very linear (R^2=0.978) relationship for increasing energy for each level of strain. These trends are to be expected as the tissue deforms in predictably increasing patterns for materials with spring-like properties which approximate Hooke's Law (force= material spring constant×displacement). Increasing the strain or distance traveled results in higher maximum forces as the deviation from L_N increases, and the summation of the work differential necessarily increases as well. No such trend was observed for the Young's modulus, which was approximately constant for 10%, 15%, and 20% strains, though a statistically smaller value was observed for the 5% value. A non-variant stiffness is expected for similar materials undergoing regular elastic strains. The diminished stiffness for the 5% strain may be attributed to strains insufficient to enter the elastic portion of their deformation profile, but the observed elastic modulus is reported here for purposes of comparison. These relationships were observed for four levels of strain, at a set rate of distension. Future work in this area could examine the effect of strain rate on nerve mechanical and electrophysiological properties.

A stress-strain analysis of the tensile data (Fig. 6) reveals distinct behaviors for the nerves as they underwent strain and relaxation. Such hysteresis is to be expected when testing soft tissues such as nerve, which are known to be viscoelastic. The differences may be due to changes in tissue organization, such as the straightening of nerve fibers and the rupture of endo- and perineurium. Such an effect would be expected to be cumulative, increasing the hysteresis of the tension levels as the maximum strain level increases. This effect was indeed observed, and is evident on the representative stress-strain graph shown in Fig. 6. The negative force values recorded for the 20% strain were unexpected, but have been previously observed in relative tension and compression of neural tissues [15].

When the post-stretch CAP amplitude is plotted against the maximum recorded force, (Fig. 2c) the destructive effect of increasing tension is apparent. This corroborates the findings of Kwan et al. who found that increasing stress resulted in conduction deficits irrespective of the degree of strain [12]. As reported by others, conduction deficits were induced far below the ultimate strength of the nerve [5, 12]. This supports the hypothesis that the perineurium protecting individual nerve fascicles fails before the epineurium encapsulating the whole nerve trunk. However, our previous study identified the maximum tension tolerated by guinea pig peripheral nerves to be below the values recorded here [14]. This discrepancy may be due to differences in the hydration of the nerve: in the prior work, nerves were tested vertically and dried rapidly, whereas in

these experiments the nerves were tested horizontally and maintained in solution throughout the experiment. Hydration has been shown to have a strong effect on the mechanical properties of collagen, the primary component of neural connective tissue [16].

The value of this experimental apparatus lies in the rapid and simultaneous recording of position, force, and electrical conduction. These data may be collected and processed together for accurate analysis of the effects of strain on nerve function. Mechanical distortions may be more precisely controlled than in prior ex vivo experiments[2, 7]. Future uses of this device could include examining the effect of strain rate on nerve conduction, elucidating the relationship between tensile force and conduction deficits, and electrophysiological evaluation of nerves undergoing prolonged strain.

As demonstrated by the findings of conduction and tension, this apparatus for tensile electrophysiology is a robust system for examining the effects of stretch upon conduction. Computerized movements yield 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 [7] 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) [2] or conventional histological techniques such as horseradish peroxidase (HRP) stains to examine the integrity of axonal membranes [8].

Conclusion

This paper proposes an automated system for evaluating the response of peripheral nerves to longitudinal strain. An apparatus for tensile electrophysiology was designed, and used to evaluate nerve response to 5%, 10%, 15%, and 20% supraphysiologic strains. Conduction amplitude was statistically unchanged at 5% strain, but diminished at 10% strain, suggesting a minimum threshold for stretch effects on CAP amplitude somewhere between these values. For strains greater than 5%, the decreases in conduction amplitude were proportional to increasing degrees of strain. The levels of force required to reach each strain increased linearly with increasing distension, indicating elastic behavior of the guinea pig sciatic nerves for strains less than 20%. Overall, this testing regime successfully demonstrated a quantitative relationship between the physical parameters of stretch and the resulting functional deficits. Such information may prove useful in understanding the pathology of mechanical trauma to nerves and eventually may inform patient diagnosis and treatment.

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