PERIPHERAL EFFECTS OF NEURAL CONDUCTION VELOCITY ON THE FEMORAL CUTANEOUS NERVE OF NEW ZEALAND WHITE RABBITS

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Angela Forrest

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Dr. Gerald Timm

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Abstract

Acute graded compression can be caused by many factors from chronic acute pressure or a repetitive pressure applied to the nerve. Both are predicted to cause a decrease in the compound nerve action potentials (CNAP's). The change in compound nerve action potential detects an alpha change in the nerve fiber. This type of injury can occur in multiple different facets from over exertion on the nerve through a blunt force trauma or a repetitive occurrence through exercise. A repetitive force was applied to the posterior femoral cutaneous nerve of New Zealand White Rabbits. The method used was ten seconds of applied pressure followed by thirty seconds where no pressure was applied. This was done with different pressures ranging from 372.25 mmHg through 2,154.29 mmHg. This method consisted of a 12 French 5 cc silicon catheter and a gortex animal stent placed around the rabbit's posterior femoral cutaneous nerve. The analysis consisted of time to peak, latency, and peak amplitude in reference to the compound nerve action potentials. The investigation focused on a repetitive injury other than a chronic injury and found that there was not a significant change in the time to peak and latency. There was however a significant change in peak amplitude before failure of the balloon portion of the catheter caused inconsistent results.

Table of Contents

List of Tables

List of Figures

Background

There are many studies that include acute compression, electrophysiologic, histopathologic, and peripheral nerve injury that focus on damaging the nerve and then trying to repair the nerve. The clinical picture of a nerve compression depends on several factors, including the anatomical location of the lesion as well as the severity and duration of the trauma [1]. There were many groups that damaged the nerve without knowing the severity of the damage that was done to the nerve. "One group focused on a cuff that consisted of two balloons held in a fixed apposition to one another by metal frames, one under and the other over the mobilized peroneal nerve. Then the cuff was inflated, the nerve was compressed between the balloons [4]." Another used an asymmetric clip that compressed the cross section of the femoral nerve [21]. Then the voltage was applied between 200-400 mV to see the affect the asymmetric clip had on the femoral nerve. A stimulatory effect of a low level laser used for dermatology and carpal tunnel were tested. "The aim if this study was to examine the effect of low level laser treatment in recovery of motor function, neurophysiological activity and histological changes following a standardized compression injury known to cause extensive axonal destruction in a sciatic nerve [9]." All of these groups focused on damaging the nerve and then trying to repair the nerve. It is known that traumatic lesions of the nerve usually result in structural changes in the axon with or without separation of its supporting connective tissue. Most prior work in the reaction of peripheral nerve injury has been related to chronic or subacute injury either by imaging clinical or chronic neurophysiology. It is known that compression of peripheral nerves may induce impairment of nerve function [4]. Few studies have investigated the

neurophysiologic effects of compression over shorter time scales [3]. In previous studies no one has known what it takes to damage the nerve, and once the nerve is damaged how long it takes the nerve to fully restore if in fact it does. Previous research groups damaged the nerve and then tried to develop means to repair it. Previous groups were not interested in establishing the exact force levels that were required to produce irreversible injury. This study is intended to determine at precisely what force levels are required to produce irreversible neural injury to the nerve. At what level under constant compression from pressure will the conduction velocity start to decrease.

The mechanisms of acute compression injuries are not commonly understood when compressing peripheral nerves, which includes both sensory and motor nerve fibers. This compression may induce impairment of nerve function. Hearing, touching, vision, and feeling pain are all sensory input and are sensory nerve fibers. Motor nerve fibers are nerve impulses that come out from the brain or the spinal cord. "Acute compression may induce block of conduction which sometimes persists long after trauma [1]." It has been reported that peripheral injury causes the compound muscle action potentials (CMAPs) to decease and weaken the muscle [2-6], but the mechanism of determining what the compression weakens or impairs in the nerve in terms of its conduction velocities has not been well investigated. In compound nerve action potentials (CNAP's) the myelinated fibers each action potential propagates from one node to the next with a rate proportional to fiber diameter [2,5,6,10,12,15]. In unmyelinated fibers conduction velocities vary in proportion to the square root of the fiber diameter [2,5,6,10,12,15]. The aim of this project is to investigate the effects of compression on the functional status of

peripheral nerve in terms of its conduction velocities in well-controlled animal experiments. The central hypothesis behind the proposed research is to damage the nerve to see the peak to peak change, latency, and/or an amplitude change.

Urinary control is maintained by the interaction of sensory and motor fibers in the pudendal and pelvic nerves, in which the nerve originates in the S2, S3, and S4 segments of the spinal cord. The primary hypothesis is that acute crushing with added weight and duration will inhibit nerve fibers from activating, resulting in a slower conduction velocity. Then with repetitive compression it will lead to even slower conduction velocities.

During vigorous activities we anticipate that these neural structures are under high stress levels, which may disrupt the urinary control reflexes. This research is designed to reproduce the stress levels expected to be experienced during vigorous physical activity. We feel that urinary incontinence is strongly correlated with the forces experienced by the body during motion or exercise. We feel these forces would be consistent with the force we are using to determine at what point the conduction velocity starts to decrease. Our goal is to show that urinary incontinence is strongly correlated with the forces experienced by the body rather than fatigue. We are trying to figure out at exactly what point the conduction velocities start to decrease because this would be justification for a urine leakage study.

Methods and Materials

All of the animal experiments were in accordance with the Institutional Animal Care and Use Committee (IACUC) within the University of Minnesota on the use of laboratory animals.

These animals were housed at Diehl Hall. All efforts were made to minimize the animals' suffering and reduce the number of animals used. New Zealand White Rabbits were used with an average weight between 8-12 pounds. The rabbits were weighed each day of surgery and administered with Ketamine/Acepromazine/Xylazine (rabbit cocktail) per Table 1.

Scheduled Medications and Dose/Dosages (interval, Dose/Dosage, Route)				
Medication	**Purpose**	**Dose/Dosage**	**Route**	**Frequency/Duration**
Ketamine Xylazine * Acepromazine	Sedative	35 mg/kg 5 mg/kg 0.75 mg/kg	IM	single dose
Thiopental Sodium	Anesthetic	2-4 mg/kg	IV	to effect
OR Propofol	Anesthetic	1-2 mg.kg	IV	to effect

Table 1: Rabbit Cocktail

The rabbit cocktail was administered by an intramuscular (IM) injection with a 22-25 gauge needle. Following sedation the ear and both forelegs of the rabbit were shaved. A Teflon IV catheter was placed in the ear of the rabbit. Once the catheter was placed a 0.9% Sodium Chloride solution was administered throughout the procedure. This was to ensure that the rabbits held all of their biological fluids during the procedure. A warming blanket was used to ensure that the rabbit would stay at a constant room temperature of 37 °C. Then the rabbits were placed on a ventilator and administered 4 L/min of oxygen with 2.5% isoflourane. Bipolar electrodes were attached to monitor an electrocardiogram (ECG), saturated oxygen, and temperature. These readings were recorded every fifteen minutes per Experimental Surgical Services (ESS) standard operating procedures.

Once the rabbits were placed in position one thigh was cut down to expose the posterior femoral cutaneous nerve and a piece of plastic was placed behind the nerve for insulation. Then

a gortex animal stent was placed around the nerve with a 12 French 5 cc silicon catheter. Once the nerve, stent, and catheter were placed together as seen in Figure 1, the stent was then sewn shut so it would hold pressure as the catheter balloon inflated. Recording and stimulating bipolar stainless steel electrodes were placed roughly 5 centimeters (cm) apart on the posterior femoral cutaneous nerve to allow for direct stimulation and recording on the nerve. There was a third bipolar stainless steel electrode attached to the rabbits for the purpose of a ground electrode.

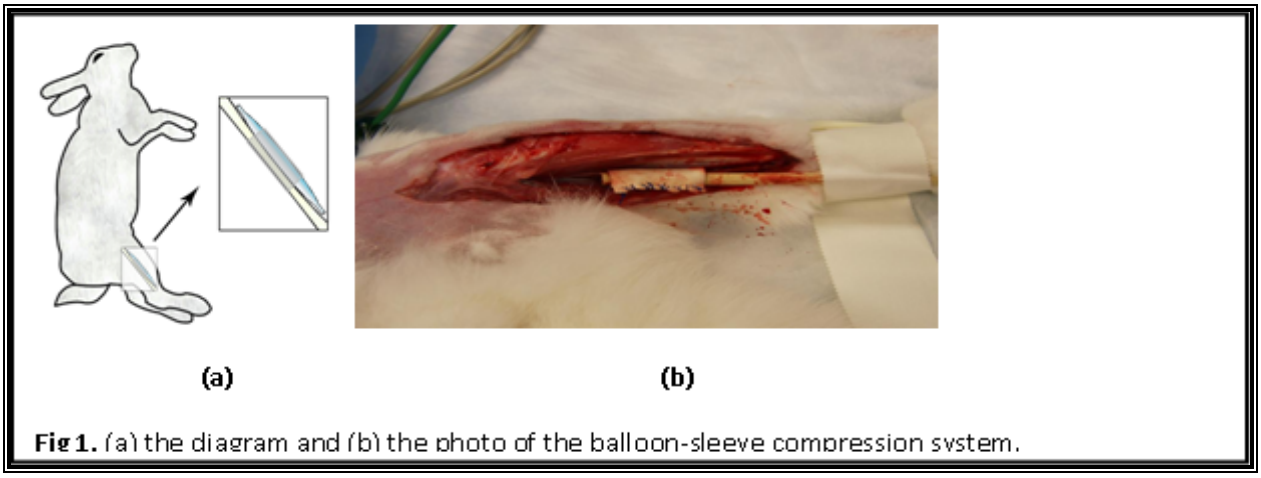

Figure 1: The placement of the, nerve, stent, and catheter

Once the electrodes were in position a 3 way stopcock, a pressure transducer (Cole-Parmer), and a 3 milliliter (ml) syringe were attached to the catheter. Connected to the pressure transducer was a Chart 7 (ADInstruments) program. The power lab, and the stimulus isolator stimulated the nerve, and the animal bio amp captured the nerve action potentials. To record the small compound nerve action potentials a signal averager, needle electrode, and the animal bio amplifier were needed. With the signal averager the shape and the duration of the CNAP's could be seen. The Chart 7 software was set to deliver a single square wave pulse with 0.1

millisecond (msec) width at 1 hertz (Hz). The stimulus intensity was 3 mA. The Chart 7 program recorded the pressure being applied to the nerve and the action potential of the posterior femoral cutaneous nerve after it was stimulated by the electrode. An example of these outputs from the Chart 7 program can be seen in Figure 2.

Once everything was in place the posterior femoral cutaneous nerve was stimulated and recorded for one minute with no pressure. After one minute was completed 0.25 ml of saline was inserted into the balloon of the catheter and the CNAP's were recorded for ten seconds. This was followed by the saline being released out for 30 seconds. After 30 seconds then 0.50 ml of saline was inserted in the balloon of the catheter for ten seconds and then released over 30 seconds. This process was repeated in increments of 0.25 ml with a maximum of 3 ml (0.25-3.0 ml) of saline inserted into the balloon of the catheter. The decrease in conduction velocities, time to peak, and latency were examined using the Chart 7 program. Shown in Figure 2 in the upper portion is the change in pressure. The blue portion is the onset of stimulation, and the green portion is the compound nerve action potential. In Figure 2 the x-axis is time in seconds and the y-axis is in volts.

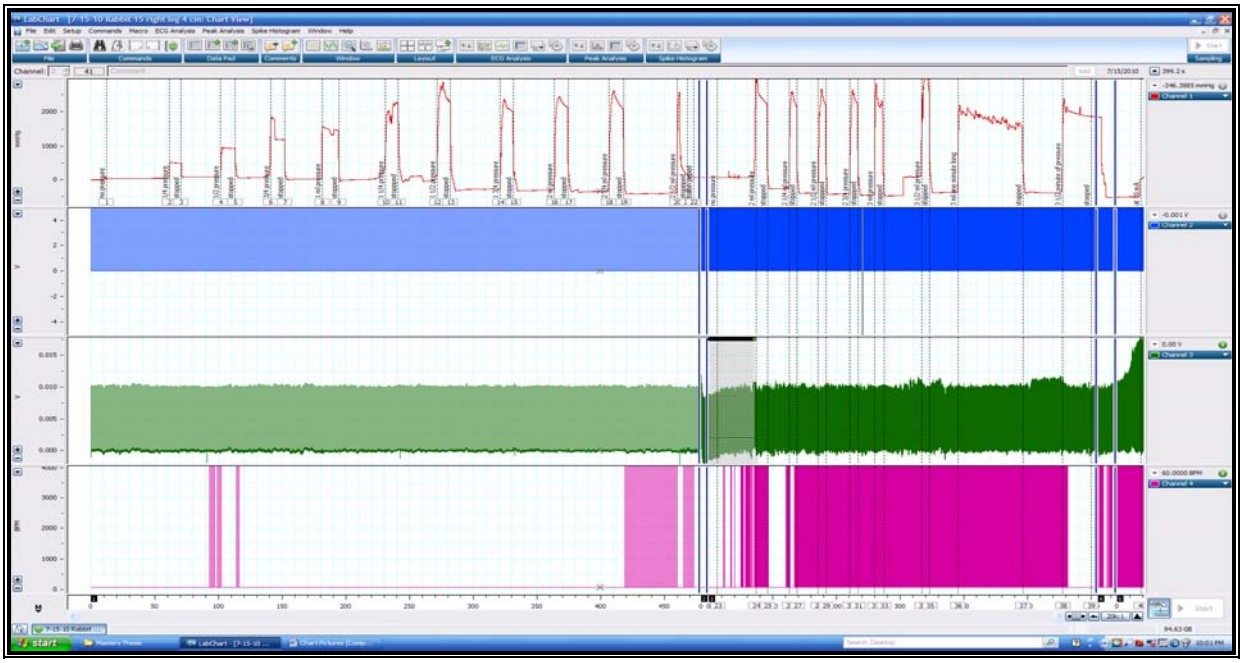

Figure 2: The change in Pressure, Onset of Stimulations, and CNAP's

Figure 3 is an example of the onset of stimulation and the compound action potential at a fifteen times zoom from Figure 2. The average from each recording ranging from the initial minute of recording to the on and off periods was taken. Seen in Figure 3 above the onset of stimulation and the peak of the CNAP's the Chart 7 program recorded each one of these points. We averaged each of these data points for each section of data.

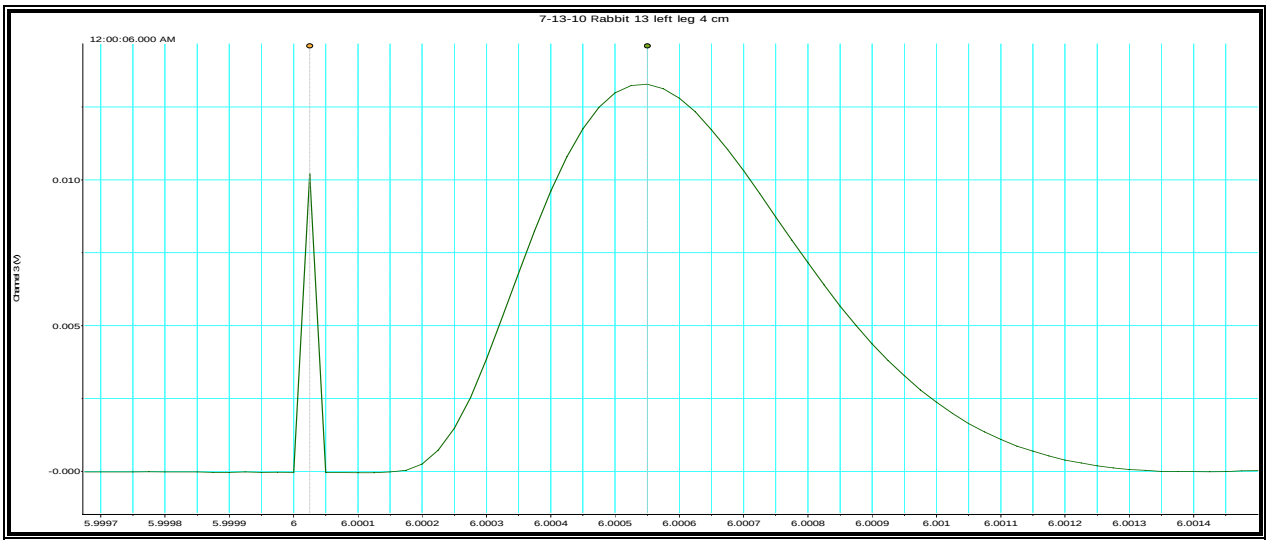

Figure 3: Onset of stimulation and CNAP

Before the final compression apparatus was constructed there were other apparatuses that were tried. The first one was a compression mechanism that is comprised of two parts; a stationary lower potion that was placed under the nerve and an upper portion consisting of variable incrementally compressive weights. As larger amounts were added the upper portion would compress against the nerve. Weight was added in five gram increments. This was found not to work because of where the nerve was placed inside the rabbit. The nerve was placed between two main muscle groups and there was no way to compress the nerve from the top as the muscle groups were in the way. Then it was determined that the nerve must be compressed from the side. The second technique that was tried was a pair of tweezers that were coated with silicon material. Silicon material coated the tips of the tweezers because metal is a conductor and could not record the action potential of the nerve. Inside the tweezers was a piezoresistive force sensor that measured the resistance. This single element force sensor acts as a force

sensing resistor in an electrical circuit. Every time the tweezers were pushed together the force sensor would measure the resistance. This method was not found to be accurate enough to produce reliable results. Then a third apparatus was tried that consisted of two plastic supports that would apply pressure to the nerve. Then the supports would be sewn together and the catheter would be inflated. The downfall to this apparatus was that the supports were not able to apply consistent pressure, however form this idea we came to the compression apparatus that ultimately was used for the completion of this study.

Results

The compound nerve action potentials were examined throughout the stimulation process at the different pressure levels. In Figure 4 the time to peak changes were investigated and found that there was no significant change. The medians stayed relatively the same. This was also the case with latency which can be seen in Figure 5. There was almost absolutely no change in latency. With repetitive compression the time lapse of pressure being applied was not long enough to see a change in time to peak and latency. In many chronic studies it took hours to change the compound nerve action potential at a pressure of 400 mmHg.

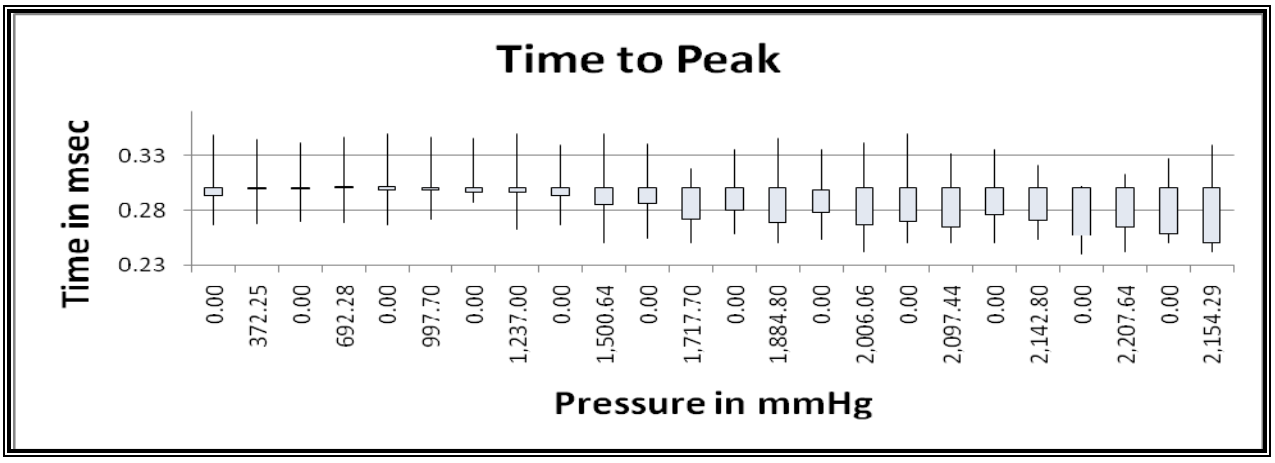

Figure 4: Time to Peak Change

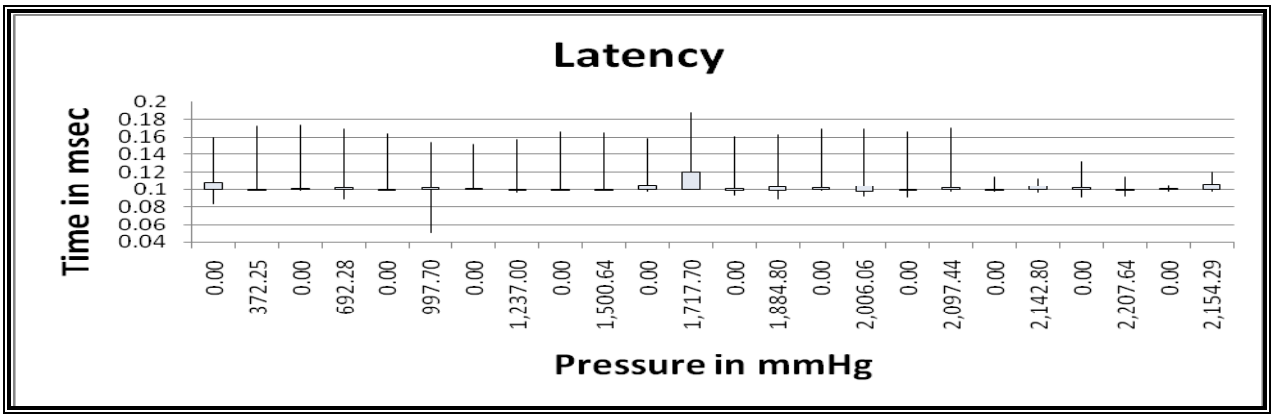

Figure 5: Change in Latency

When testing the pressure of the catheter inside the stent and then the catheter outside the stent by itself there was a significant change in the recordings of the pressure which can be seen in Figure 6. After 1.50 mL of saline was inserted into the catheter the elasticity of the catheter changed. Where the saline was held in the balloon of the catheter there was so much strain being put on the balloon of the catheter that the material of the catheter started to change characteristics as seen in Figure 6. The average pressure of the catheter outside the stent was a consistent linear

line where the catheter inside the stent varied. Because the strain was so high it caused a deformation in the uniqueness of the plastic and the balloon was not able to go back to its original state and therefore causing variation in pressure. This could also be why the time to peak and latency did not change.

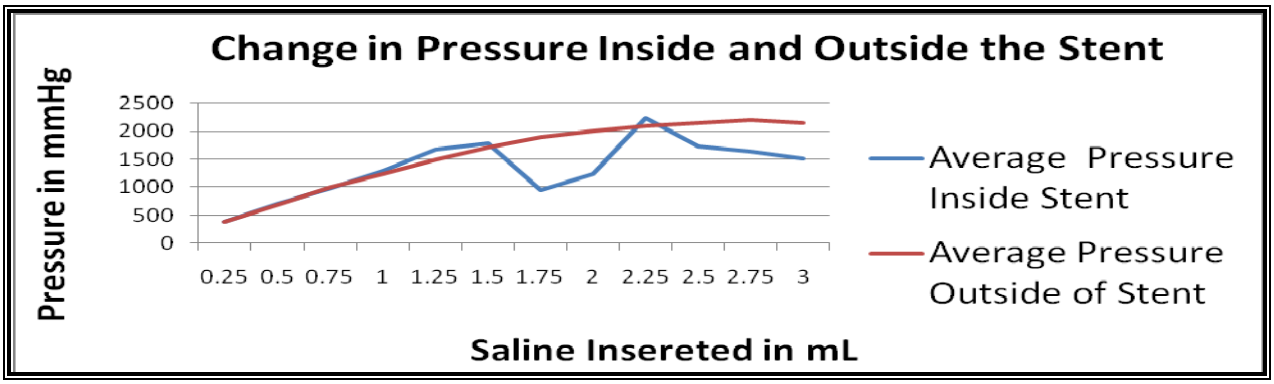

Figure 6: Change in Pressure Inside and Outside the Stent

There was a significant change in peak amplitude at the pressure of 1,717.00 mmHg. Before that pressure decrease the peak amplitude stayed at a relative constant as seen in Figure 7. Also seen in Figure 7 the peak amplitude varied from 4.89 to 6.44 millivolts which proves that the elasticity of the balloon of the catheter was failing. The results were inconsistent after that pressure point.

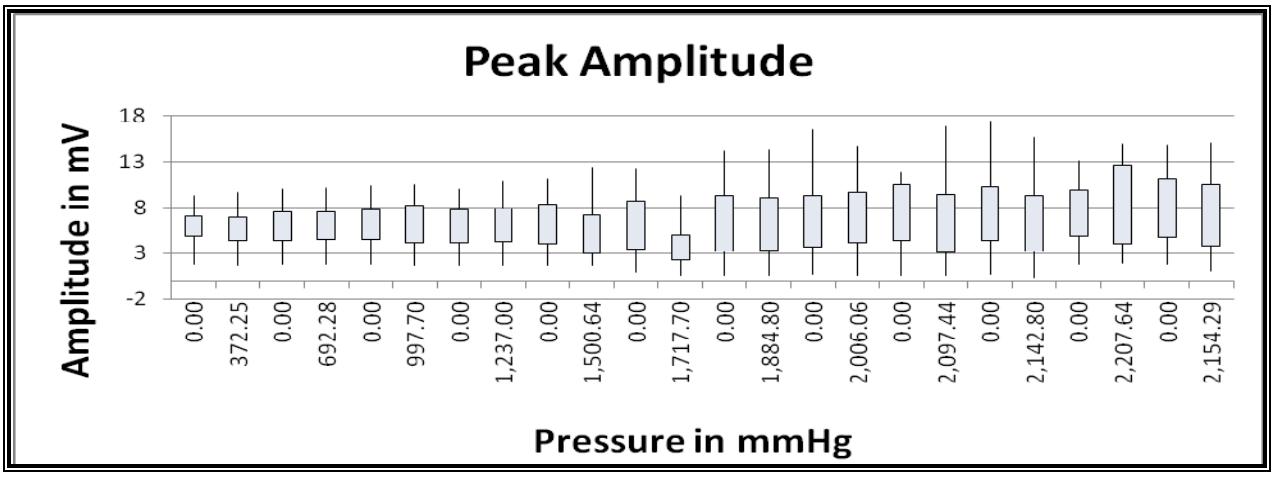

Figure 7: Changes in Peak Amplitude

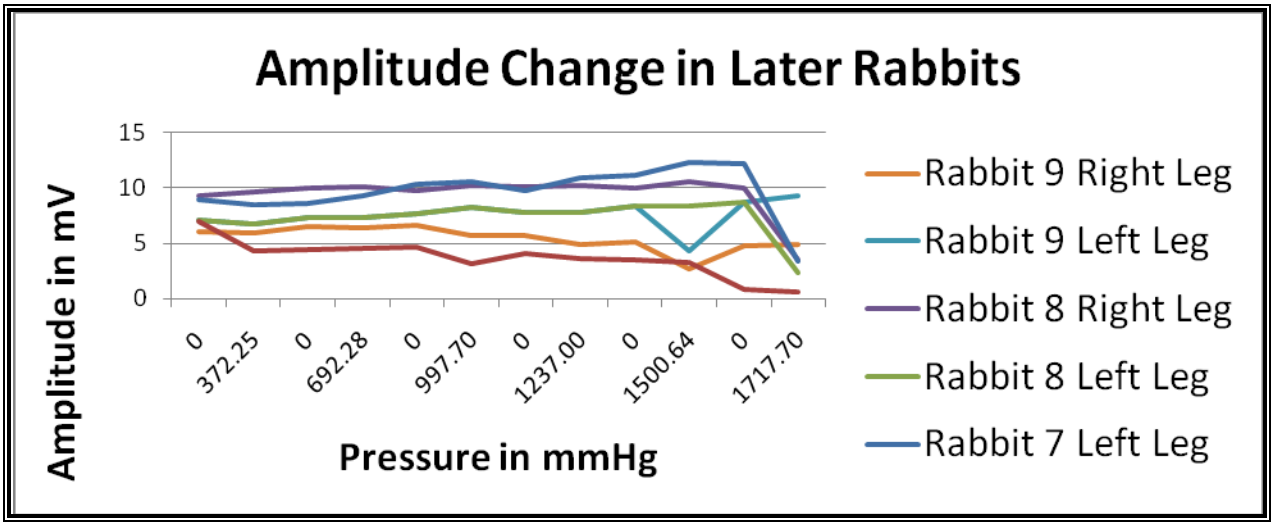

Figure 8: Graphical Change in Pressure in Later Rabbits

The data from Figure 8 was taken from later rabbits in the study. This data clearly shows that there was a decrease in the amplitude of the posterior femoral cutaneous nerve. We believe that after 1,717.70 mmHg of applied pressure the characteristics of the balloon of the catheter started to fail.

Discussion

Repetitive forces to the posterior femoral cutaneous nerve may not cause neural injury. Most neural injury studies are associated with chronically damaging the nerve and then trying to repair that nerve. Repetitive neural compression has had limited investigations done. Few studies if any have been done to see exactly at what point neural injury occurs. There was no change in conduction velocities in the time to peak and latency, however change did occur in peak amplitude. Why we did not see a change in latency is because with repetitive compression the compound action potential produced in chronic nerve compression faster fibers (mostly myelinated) determine the onset of latency and hence the velocity of the nerve [24]. When the nerve fibers are stimulated for longer periods of time the faster myelinated fibers start to lose functionality that results in slower conduction velocities. When chronic nerve compression was investigated it showed a change in conduction velocities over longer periods of time. "The chronic section of the peripheral processes of sensory neurons causes a decrease in conduction velocity for both the central and peripheral processes, and these changes were associated with changes in the properties of the cell body [2]." Studies have shown that the nerve function changes at two months, electrophysiological changes can only occur at four to six months, and structural changes only to the nerve after six months [3,6,8,10]. The two factors that affect a decrease in conduction velocity during compression are blood supply and perfusion. This is done by applying pressure to the nerve and observing when the conduction velocity of the nerve starts to decrease. "The morphology of the peripheral nerve responses is formed by the contribution of the different populations of nerve fibers of varying diameters within the

peripheral nerve bundle. The amplitude of the nerve response is actually a representation of the various groupings of fiber populations having differing diameters and degrees of myelination [24]."

The height which is the nerve's amplitude is represented in the axon bundles that make up the nerve. When the axon is injured at the nerve bundle through compression it can affect the number of axons responding during stimulation of the nerve. The can cause a decrease in peak amplitude of the evoked action potentials. "Therefore this reduction of the nerve conduction amplitude indicated the degree of axonal injury in the nerve bundle [24]". This is what happened when we saw a decrease in peak amplitude at a pressure of 1,717.70 mmHg. "The smaller diameter of the nerve axons accounted for the decrease in amplitude of the compound action potential while maintaining a normal nerve conduction velocity. The lack of change of the nerve conduction velocity would indicate that the axonal injury had not affected the faster (mostly militated) fibers contained in the nerve bundle [24]".

Accounting for these factors, this is why we did not see a decrease in time to peak and latency. With the method of ten seconds of applied pressure followed by thirty seconds where no pressure was applied the pressure was not applied for a long enough time to see a change in the compound action potential of the axon fibers of the nerve. The time to peak and latency follow along the same pathways and therefore this constitutes not seeing a decrease in either factor. The peak amplitude change occurs because there is a change in the decrease in the smaller diameter axons then the lager axons. The force levels we applied under a short time did not change the

axon fibers quick enough to see a change but with a longer applied force the axons have time to change.

Future Investigations

Options for future investigations could include using a larger animal. With the rabbit's foreleg there is limited space and a larger animal would provide the space needed to apply an adequate force. Applying longer pressure to the animal would give the opportunity to see when exactly the changes occur in time to peak, latency, and peak amplitude. Once the three variables are known then you can apply a repetitive force that is slightly lower than the force that it takes to decease the time to peak, latency, and peak amplitude. Instead of applying pressure for ten seconds on and then thirty seconds off the force being applied should be in minutes instead of seconds and then the period where no pressure is being applied should be in shorter intervals.

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