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Effects of Sepsis on Nerve Evoked Responses

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EFFECTS OF SEPSIS ON NERVE EVOKED RESPONSES

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By

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Kevin Richard Novak ENTITLED Effects of Sepsis on Nerve Evoked Responses BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science.

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Abstract

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Sepsis and SIRS (systemic inflammatory response syndrome) have become two expensive and complicated problems seen in the intensive care unit (ICU). These two illnesses have been known to cause dysfunction with excitable tissues in the body. Encephalopathy, neuropathy, and myopathy are the three biggest. In this paper we discuss the development of an animal model of sepsis and the neurological complications sepsis brought about. Nerve conduction studies showed increased durations on compound muscle action potential, and decreased amplitude as well as increased duration and latency on sensory nerve action potentials. These results were not consistent with the two most common neuropathies, demyelinating and axonal. Collaborative efforts with the Cope lab found that action potential amplitudes of individual axons could be improved by delivering a hyperpolarizing current. This data is supported by similar findings in muscle fibers by the Rich lab.

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I. Introduction

Sepsis is a problem that has plagued many critically ill patients in intensive care units for years. A 1995 study found that 751,000 cases of sepsis occur in the United States with 215,000 of these cases resulting in death (Remick 2007). Current studies have found that the number of deaths associated with severe sepsis each year is rising. Sepsis is now responsible for the same number of annual deaths as acute myocardial infarction (Remick 2007). With increased medical care sepsis survival time has been prolonged but still has a 30 – 50% mortality rate (Bolton 2005). Not only is sepsis associated with a high mortality rate, it is also expensive. The average cost for one case of sepsis is $22,100 hitting the United States with an average annual bill of $16.7 billion (Remick 2007).

Sepsis is associated with severe inflammatory response syndrome or SIRS. Patients diagnosed with SIRS exhibit physiological changes including changes in body temperature, increased heart and respiratory rates, the need for mechanical ventilation, and changes in white blood cell counts (Bolton 2005). The inflammatory response is necessary to remove unwanted infectious organisms from the body (Remick 2007). The proper inflammatory response will remove the pathogen without damaging the body (Remick 2007). However, bacterial, fungal or viral infection are not the only causes of SIRS. Major mechanical, thermal, or chemical trauma can also lead to the onset of SIRS (Bolton, 2005 #4). Sepsis is SIRS induced by an infection, and is commonly seen in patients with critical illness (Bolton 2005; Remick 2007). A critically ill patient is defined as being septic with multiple system organ failure (Bolton 2005). SIRS induced by

bacterial infection may be initiated by the presence of gram negative bacteria, gram positive bacteria, or both (Bolton 2005). In most cases of sepsis the source of infection is from the lungs as a result of pneumonia, or the peritoneal area (Herridge, Cheung et al. 2003; Khan, Harrison et al. 2006; Remick 2007).

Many complications accompany sepsis and SIRS. Some complications affect nervous tissue in both the peripheral and central nervous systems. These include polyneuropathy and encephalopathy (Latronico, Fenzi et al. 1996; Trojaborg, Weimer et al. 2001; Bednarik, Lukas et al. 2003; Bolton 2005; Khan, Harrison et al. 2006). Complications also to occur in muscle tissue (Latronico, Fenzi et al. 1996; Lacomis, Petrella et al. 1998; Lacomis, Zochodne et al. 2000; Trojaborg, Weimer et al. 2001; Bednarik, Lukas et al. 2003; Bolton 2005; Khan, Harrison et al. 2006). Mainly, these complications occur in skeletal muscle resulting in a myopathy (Latronico, Fenzi et al. 1996; Lacomis, Petrella et al. 1998; Lacomis, Zochodne et al. 2000; Trojaborg, Weimer et al. 2001; Bednarik, Lukas et al. 2003; Bolton 2005; Khan, Harrison et al. 2006). Some recent studies have found sepsis and SIRS to possibly affect cardiac muscle tissue as well (Rich, McGarvey et al. 2002; Khan, Harrison et al. 2006).

Critical illness polyneuropathy (CIP) occurs in 50% - 70% of patients diagnosed with SIRS (Bolton 2005). This makes CIP a common neuromuscular disorder occurring in ICUs. One of the first studies to recognize CIP took place between 1977 and 1981. In this study five ICU patients were observed in an intensive care unit with difficulty weaning from mechanical ventilation and weakness in the limbs. Comprehensive electrophysiological and morphological studies determined the condition to be a primary distal, axonal degeneration of motor and sensory nerve fibers. Nutritional deficiency,

collagen vascular disease, toxicity from antibiotics or heavy metals, and spinal cord ischemia were ruled out as causes of fiber degeneration. The toxic effects accompanying sepsis are believed to have induced the polyneuropathy (Bolton 2005).

A classical case of CIP presents with a decrease in compound muscle action potential (CMAP) amplitude as well as a decrease in sensory nerve action potential (SNAP) amplitude (Latronico, Fenzi et al. 1996; Lacomis, Zochodne et al. 2000; Trojaborg, Weimer et al. 2001; Bolton 2005). These findings are obtained with the use of nerve conduction studies. Biopsies of both sensory and motor axons show fiber loss consistent with primary axonal degeneration, with a majority of fiber loss occurring in the distal segments of axons. Not all pathologic studies of nerve have shown a loss of nerve fibers though. Some cases present with the classic CIP symptom, decreased CMAP and SNAP amplitude, but pathologic studies of the nerve biopsy show all nerve segments to be normal (Latronico, Fenzi et al. 1996; Bolton 2005).

Abnormalities in muscle tissue are also seen with sepsis and SIRS. Critical illness myopathy (CIM) presents very similar to CIP (Bednarik, Lukas et al. 2003). Distinguishing between the two can be difficult if they present at the same time (Bednarik, Lukas et al. 2003). Nerve conduction studies are also used to diagnose CIM. CMAP changes seen in CIP are also seen in CIM, the decrease in CMAP amplitude being the most noticeable (Latronico, Fenzi et al. 1996; Lacomis, Zochodne et al. 2000; Trojaborg, Weimer et al. 2001; Bednarik, Lukas et al. 2003)(12). Sensory nerve conduction studies do not present any changes since only muscle tissue is affected in a CIM. In order to verify a diagnosis of CIM electromyography (EMG) studies are needed (Lacomis, Petrella et al. 1998). Small motor units and early recruitment on EMG are seen

in classic cases of CIM. Muscle biopsies are performed and while not always noticed necrosis and myosin loss are seen, as well as elevated levels of creatine kinase (CK) (Lacomis, Petrella et al. 1998; Trojaborg, Weimer et al. 2001). Still some patients present with decreased CMAP amplitudes and small motor units with early recruitment on EMG but yet upon muscle biopsy the tissue appears normal.

Critical illness myopathy and neuropathy are not the only change seen in patients with sepsis or SIRS. Most cases also present with septic encephalopathy, an early neurological complication seen to occur before CIP in 70% of patients that present with both conditions (Bolton 2005). Classic cases of septic encephalopathy present with normal cerebral spinal fluid and neurological imaging, as well as normal brain tissue upon autopsy. The only abnormalities seen are in the electroencephalogram. The severity of this encephalopathy has been correlated to the severity of CIP (Bolton 2005). The more deterioration noticed on the Glasgow Coma Scale, the more severe the CIP.

The final change accompanying sepsis and SIRS deals with cardiac muscle tissue. Decreases in QRS amplitudes have been noticed in electrocardiogram (EKG) readings of septic patients (Rich, McGarvey et al. 2002). The QRS amplitudes have been seen to improve drastically with recovery from sepsis (Rich, McGarvey et al. 2002; Khan, Harrison et al. 2006). QRS amplitude improvement is similar to the improvement noticed with SNAP and CMAP recovery.

Changes in skeletal and cardiac muscle tissue as well as changes in the central and peripheral nervous tissue have all been related to sepsis and SIRS. These changes do not always present at the same time, and in some cases not all changes may present

(Trojaborg, Weimer et al. 2001; Bednarik, Lukas et al. 2003; Khan, Harrison et al. 2006). We believe all of these tissues are in some way becoming electrically inexcitable due to the onset of sepsis. With this in mind there are two possible explanations. The changes seen in peripheral nerves, central nervous system, cardiac and skeletal muscles are all the result of one syndrome with a common connection to sepsis, or these four tissues are experiencing changes produced independent of one another. To test these two hypotheses we developed an animal model of sepsis using rats. This model will be used to test the electrical excitability of these four tissues and the effect that sepsis has on them.

II. Methods

Anesthesia

Anesthesia was induced in a chamber infused with five percent isoflurane in oxygen. After induction, the rat was removed from the chamber and was placed in a nose cone that delivered two percent isoflurane in oxygen. The rat remained anesthetized using the nose cone for all electrophysiologic studies and surgical procedures.

Temperature

Temperature was taken using a rectal thermometer and was maintained at 37^{o}C +/- 1°C using a heating pad for all electrophysiologic measures. The thermometer and heating pad were both removed during, and replaced after, each recording to ensure that electrical current was not interfering with the recordings.

Electrophysiological Measurements

Each measurement was taken twice, once before and once 1 to 4 days after the cecal ligation and puncture procedure. This made it possible to use each rat as its own

control to minimize variability in electrophysiologic measures. The time between the initial and terminal recordings was based on the severity of sepsis. We attempted to take the terminal measurements when the animal was severely septic, and would still be able to survive anesthesia for the terminal measurements.

EKG

The rat's right lower leg and both upper legs were shaved from the knee to the point where they meet the torso. Electro-gel was placed on all the shaved recording regions. EKG leads were then attached at these sites with alligator clips over the gel. Recordings were taken from the different leads, printed and then analyzed using calipers to determine the height of the QRS peak.

ABR (*Auditory Brainstem Response*)

The rats were prepped for ABR recording by shaving the head behind the ears, as well as under and between the ears. A small section on the back just above the tail was also shaved. Subdermal recording electrodes were placed at both right and left mastoids processes, and at the vertex of the head between the ears. A ground electrode was placed parallel to the vertebral column on the back close to the base of the tail. Small amplifiers were placed in the ears of the rat and generated clicking sounds. ABRs where obtained by 100 μs rarefaction clicks delivered at 31.7 per second to both ears through ear buds. The sound filters were set to decline frequencies higher than 3000 Hz and lower than 30 Hz. The recordings were done in an alternative phase with the subdermal electrodes placed in an A2-CZ-A1 fashion. Each individual ABR recording was the average of 2000

stimuli. All recordings had a rejection factor placed at 15 μV. The trace was printed and the latencies were measured from peak to peak.

Sensory Nerve Conduction

The rat was placed on its back with the tail extended. All electrodes were placed on the right side of the tail. Care was taken to avoid puncture of any blood vessels. The cathode recording electrode was placed at the base of the tail and the anode recording electrode was placed 1 cm distal. A ground electrode was placed 2 cm distal to anode recording electrode. The stimulating anode electrode was placed 2 cm distal to the ground electrode and the stimulating cathode electrode was 1 cm distal to the stimulating anode electrode. The stimulus current was increased during each recording until the response no longer increased in amplitude. The data from these recordings were used for latency, duration, and amplitude of the SNAP. Before removing the electrodes a permanent black marker was used to mark the location of the electrode placement. This allowed for identical placement of the electrodes during the terminal recording of sensory nerve conductions.

Motor Nerve Conduction

The right leg was completely shaved and the cathode and anode stimulating subdermal electrodes were placed on either side of the sciatic notch. The ground electrode was placed in front of the right thigh. The anode recording electrode was placed near the surface of the tibialis anterior muscle midway between the knee and ankle, while the cathode recording electrode was placed near the Achilles tendon. The recordings were performed until the amplitude no longer increased. The data from these

recordings were used for latency, duration, and amplitude of the CMAP. Before removing the electrodes a permanent black marker was used to mark the location of the electrodes. This allowed for identical placement of the electrodes during the terminal recording of motor conductions.

Surgical Procedure and Treatment of Postoperative Pain Management

We followed the cecal ligation and puncture surgical procedure mentioned in literature by Otero-Anton and Singleton. (Otero-Anton, Gonzalez-Quintela et al. 2001; Singleton and Wischmeyer 2003). The abdomen was shaved and a 2 cm midsagittal incision was made through the skin. The skin was then separated from the muscular wall around the incision. Next a 2 cm incision was made along the linea alba deep enough to enter the abdominal cavity. The cecum was located and removed from the abdomen. The cecum was ligated approximately half way between its tip and the ileum with 3.0 suture silk. After ligation of the cecum a through and through hole was made using an 18 gauge needle. The cecum was placed back into the abdominal cavity as close to its original position as possible before closing the incision. The muscular part of the incision was closed using 3.0 suture silk, while the skin portion was closed using surgical staples.

Upon complete closure of the incision a 0.1 mL dose of buprenorphine was administered subcutaneously to accommodate the first six to eight hours of pain that followed the surgical procedure. For continuous relief of pain after the buprenorphine effects had diminished, oxymorphine was continuously infused through the use of an Alzet 2mL osmotic pump. The osmotic pump delivered the oxymorphine at a rate of 30μg/kg/hr. The osmotic pump was inserted into the abdomen through the incision made

for the cecal ligation and puncture procedure. After inserting the pump, the wound was incision was closed as mentioned above. The isoflurane was then removed and the rat was given 100% oxygen for five minutes before being allowed to breathe air. After the surgery the animal was placed into a cage and was closely monitored until it became active. Half of the cage floor was heated using a heating pad and the rat was placed on this warm section until it became fully conscious. After consciousness was regained the heating pad was removed and the cage was returned to the designated room. The cage was outfitted with corncob bedding and food and water were made available.

Observation

Rats that under went the cecal ligation and puncture procedure were placed in a climate and humidity controlled room. Observation began six hours after the procedure was finished and rats continued to be observed every four hours after that. Things such as voluntary movement, piloerect hair, stance and agitation were observed and recorded to monitor the level of pain. If high levels of pain were observed 0.1 mL of buprenorphine was administered.

Terminal Electrophysiologic Studies

When the rat had piloerect hair, a hunched stance, little to no movement, and lack of response to touching and handling the rat was considered to be severely septic and was taken for terminal electrophysiologic studies. After induction of anesthesia, EKG, ABR, sensory and motor nerve conductions, were repeated. Many rats had a decreased rectal temperature when septic. If this was the case a heating pad was used to warm the animal to 37°C +/- 1°C. Random animals had blood drawn before they were euthanized. The

drawn blood was cultured to verify the presence of bacteria. After completion of the terminal recordings the animal was euthanized using carbon dioxide.

Pathology and Electrolytes

Following carbon dioxide euthanasia the tibialis anterior and gastrocnemius muscles were rapidly dissected and snap frozen in supercooled isopentane. Muscle histology was done on 8 micron frozen cross sections stained with hematoxylin, eosin, and with enzyme histochemistry for reduced nicotinamide adenine dinucleotide (NAHD), to differentiate fiber types. For nerve histology, the entire limb was separated from the animal and immersion fixed for at least 7 days in phosphate buffered 4% glutaraldehyde, pH 7.4. Sural and tibial nerves were dissected and embedded in epon resin. One micron cross sections were stained with toluidine blue for light microscopic analysis. All pathology studies were done at Emory University's School of Medicine Department of Neurology. These same animals had one milliliter of blood drawn by cardiac ventricular puncture at the time of muscle dissection. Electrolytes were measured using a Vet Scan Chemistry Analyzer.

Seven Day Study

Six of the 29 rats that were used for nerve conduction studies were allowed to survive for 7 days. Nerve conductions were taken from the rats on days 0, 2, 3, and 7. After the seventh day, half of these rats had nerve and muscle tissues harvested and sent for pathology at Emory University's School of Medicine Department of Neurology.

III. Results

Establishing a septic model with changes in nerve conductions

The goal was to determine whether severe sepsis induced changes in nerve evoked responses. To test this hypothesis it was necessary to induce severe sepsis and to have rats live long enough to develop changes in nerve evoked responses. In order to achieve this, a cecal ligation and puncture procedure was developed. A laparotomy was performed followed by ligation of the cecum with 3.0 silk sutures. The cecum was then punctured with a needle of a specific gauge. The time and severity of sepsis onset depended on the gauge of the needle, the number of punctures, the tightness of the ligation, the amount of cecum ligated, the management of pain, and the hydration of the animal. To perfect the septic model 76 rats where used. Five were used as controls, seven were used for auditory brainstem responses, and twenty nine were used for nerve conductions.

The first four surgeries were an attempt to determine the needle gauge needed to induce severe sepsis. Higher gauged needles produced smaller holes which allow fewer bacteria to exit the cecum. These first four surgeries used a 16 gauge needle. After 96 hours the rats showed no signs of sepsis and no changes were observed in the nerve evoked response studies. In an attempt to speed up the onset of sepsis a lower gauge needle was used to increase the hole size in the cecum. Three attempts were made using a 14 gauge needle, three using a 12, and two using a 10 gauge needle. Still all of these rats showed little to no physical signs of sepsis and no changes occurred in the nerve evoked responses. The two rats that received a puncture from the 10 gauge needle were submitted for an immediate necropsy after being euthanized. The necropsy revealed that the ligation around the cecum was not tight enough. The blood flow was not being restricted and the puncture was healing.

Again a 16 gauge needle was used to puncture the cecum of four rats. The ligation was tightly applied to ensure the restriction of blood flow. This resulted in the death of all four rats 24 to 30 hours after the surgery. In order to prolong the life span and delay the onset of severe sepsis the needle gauge was increased, reducing the size of the hole in the cecum. Two rats received punctures made with an 18 gauge needle; again the ligation was applied tightly to ensure no blood flow. After 36 hours both rats were taken for a second recording and then euthanized. The results showed only a small change in the nerve evoked response studies. None of the results were significant, suggesting that a 36 hour time point was not long enough for nerve evoked changes to occur. So again the needle gauge was increased.

The cecum of three rats were punctured using a 19 gauge needle. Again the ligation was tightly applied and the blood flow was restricted. The rats were monitored and after 45 hours they were taken for the terminal recordings and were then euthanized. Changes were noticed in the nerve conductions and ABR of all three rats. Another group of three rats underwent the same procedure using a 20 gauge needle. This was done in attempted to see if even longer delay in septic onset would lead to greater changes in the nerve evoked responses. The three rats that received punctures from the 20 gauge needle were taken for their second recordings after 60 hours. Major changes were observed in the duration of motor nerve conductions, amplitudes of sensory nerve conductions and in the ABR's of these rats.

The CLP (cecal ligation and puncture) procedure was continued in this manner. The next group of 6 rats all received identical surgical procedures using a 20 gauge needle and tight ligation. This group of rats were all found deceased after 24 hours.

Another set of 4 rats had received surgeries using the 19 gauge needle and tight ligation, but again these animals were all found deceased after 24 hours. In the next set of 4 rats 2 received punctures from 18 gauge needles and 2 received punctures from 16 gauge needles. The following day all 4 rats were deceased.

We next looked to see if these new results were due to the amount of cecum that was ligated. If too much was ligated then the cecum would rupture, releasing too much bacteria at once. This overwhelms the body and the rats would die rapidly. Another group of 6 rats underwent surgeries all receiving punctures from a 19 gauge needle. Two rats received a 50% ligation, 2 rats received a 60% ligation, and 2 rats received a 70% ligation. At 24 hours all of the 70% ligated rats were deceased. After 48 hours both 60% ligation animals were deceased. Both of the 50% ligation rats survived till 72 hours, but minor changes were noticed in the nerve evoked responses. Puncturing with a 19 gauge needle and ligating 50% provided the long survival times but not large enough changes in nerve evoked responses.

After consulting the onsite veterinarian it was determined that the rats might be experiencing excessive pain and discomfort. The recommendation was to try using a different drug for pain and change the location of the osmotic pump for drug delivery. Pain scoring was performed on the animals every 6 – 8 hours to determine which drug and delivery method was the greatest pain reliever.

A set of 8 rats all underwent surgery with a 19 gauge needle and tight ligation of 50% of the cecum, 4 rats received subcutaneous osmotic pumps with fentanyl and 4 received subcutaneous osmotic pumps with oxymorphine. All of the rats lived for the

same time period but it was observed that the rats that received oxymorphine had lower pain scores. Pain scores were taken before the surgeries and every 6 – 8 hours after the surgeries. They were based on porphyrin staining, pica, movement, lethargy, piloerect fur, and aggressiveness. Another set of 8 animals underwent the same surgical procedure, 4 received intraperitoneal oxymorphine osmotic pumps and 4 received subcutaneous oxymorphine osmotic pumps. Lower pain scores were observed in the rats with intraperitoneal oxymorphine delivery.

The surgical procedure now included using a 19 gauge needle, tightly ligating 50% of the cecum, and providing pain relief using an osmotic pump to deliver oxymorphine intraperitoneally. In an attempt to reduce further complications, 4mL of saline were administered daily in order to reduce hypotension and dehydration. Eight rats received the procedure and all survived for 96 hours. Minimal changes were observed in the nerve evoked responses. After euthanizing the rats at 96 hours peritoneal fluid and blood were drawn using a sterile syringe and cultured. The cultures came back positive for both gram negative and gram positive bacteria, informing us that sepsis had been induced, but not severe enough to create nerve evoked changes. The needle gauge was changed from 19 to 18, and two punctures were made instead of the previous one. This induced sepsis severe to create changes in nerve evoked responses without an early mortality. This allowed us to study changes in nerve evoked responses after the onset of sepsis.

The CLP model established for producing severe sepsis involved tightly ligating 50% of the cecum, puncturing the cecum twice with an 18 gauge needle, providing pain relieve intraperitoneally with 30 μg/kg/hr oxymorphine using an osmotic pump, and

administering 4mL of saline daily. In all the experiments below, the rats underwent this treatment procedure.

Nerve Conductions

All of the nerve conductions were performed using a Nicolet Viking Quest nerve conduction system. This system was used to collect data for both sensory and motor nerve conductions. After performing the nerve conductions latency, duration, and amplitude were recorded, analyzed, compared with statistical software, and exposed a paired T-test.

Motor Latency

Latencies in compound muscle action potentials (CMAP) taken from septic rats showed no significant changes in animals surviving 26 – 85 hours after the surgical procedure. The average latency before the surgery was 1.064 +/- 0.022 and after was 1.025 +/- 0.026, giving a p value of 0.66.

No significant changes were observed in CMAP latencies of control rats over a 72 hour period. At time zero the latency was 1.1 +/- 0.063 and after 72 hours the latency was 1.1 +/- 0.032, giving a p-value of 1.

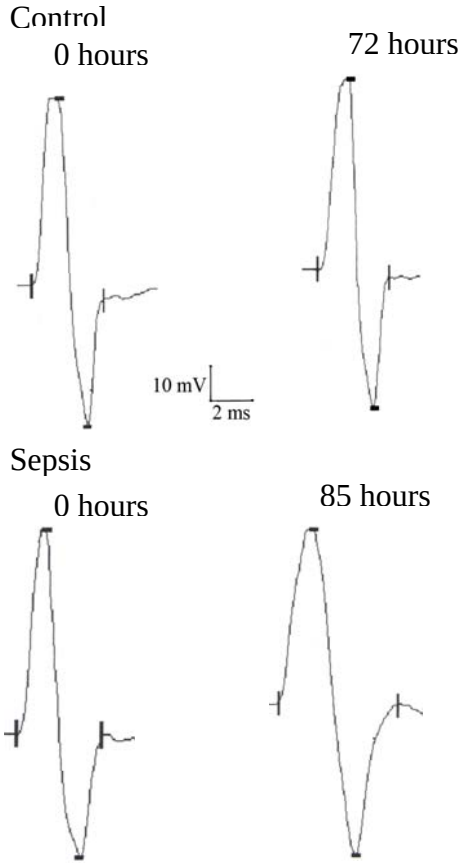
Figure 1 shows before and after traces taken from both a control rat and a septic rat. In all four traces the latency was measured from the start of the trace to the first vertical bar. No significant changes are seen in the latencies of control or septic animals.

Motor Duration

CMAP durations taken from the septic rats showed significant changes for rats surviving 26 – 85 hours after the surgical procedure. The average motor duration recorded before sepsis was 3.914 +/- 0.064 and after the onset of sepsis the terminal recording was 4.368 +/- 0.118 with a p value of 0.002.

Control rats showed no significant change in CMAP duration. Presenting an average initial recording of 3.72 +/- 0.136 and 72 hours later the duration was 3.84 +/- 0.112 giving a p-value of 0.6.

Figure 1



The traces shown in figure 1 show the increase in CMAP duration observed in the septic rat. Duration was measured between the two vertical bars. The control data has no change in duration between the trace taken at time zero and the trace taken at 72 hours. While the data collected from the septic rat shows an approximate double in duration.

Motor Amplitude

The differences in amplitudes obtained from motor nerve conductions performed on septic rats were not significant. CMAP amplitude before the surgery was 89.097 +/- 1.681 and after the onset of sepsis were 87.522 +/- 2.509, giving a p-value of 1.

Data collected from the control rats also showed no significant change in CMAP amplitude. The first recording measured 96.022 +/- 3.978, followed by a second recording taken 72 hours later which measured 96.574 +/- 3.426. When these two pieces of data were compared and gave a p-value of 0.9.

CMAP amplitudes shown in figure 1 were measured between the two horizontal bars. There is not significant change in the amplitude of neither the control nor the septic traces.

Sensory Latency

Sensory nerve action potentials (SNAP) latencies had significant changes in septic rats surviving 26-85 hours. Average sensory nerve latency before sepsis was 0.821 +/- 0.038 and after the onset of sepsis was 0.955 +/- 0.039 with a p value of 0.0027.

SNAPs performed on control rats however exhibited no significant changes in latency. The average latency found in the control rat at time zero was 0.9 +/- 0.045 and after 72 hours was 0.78 +/- 0.049 with a p-value of 0.09.

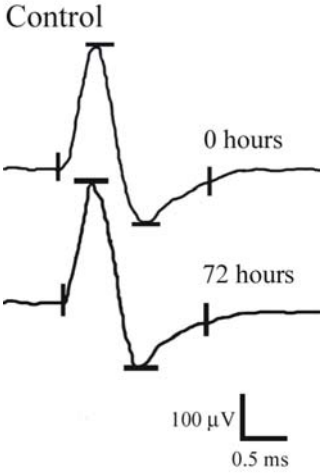
SNAP traces from both a control rat and a septic rat are shown in figure 2. In all the traces the latency is measured from the beginning of the trace till the first vertical bar. The control trace shows no change in latency over a 72 hour period while the septic trace shows a significant increase in latency.

Sensory Duration

SNAP durations from septic rats showed significant results for animals surviving 26 – 85 hours after surgery. Average SNAP duration before the surgery was 0.783 +/- 0.025 and after the onset of sepsis was 0.952 +/- 0.039 with a p value of 0.000003.

SNAP durations obtained from control rats also had no significant change. At time zero the SNAP duration of control rats was 0.8 +/- 0.045 and after 72 hours were 0.78 +/- 0.037 giving a p-value of 1.

Figure 2



Traces for SNAP durations are shown in figure 2. The duration was measured between the two vertical bars. There were no changes in the duration of the control traces, the space between the two vertical bars were not significantly different. There were however significant increases in the distance between the vertical bars of septic traces.

Sensory Amplitude

Amplitudes for sensory nerve conductions taken from septic rats did show a significant change (figure 2). Average SNAP amplitude before the onset of sepsis was 0.219 +/- 0.013 and after the onset of sepsis were 0.163 +/-0.013. When compared these two measures gave a P value of 0.015.

SNAP amplitude for the control rats did not exhibit significant changes over a 72 hour period. Average amplitude from the first recording was 0.228 +/- 0.025 and from the terminal recording 72 hours later was 0.215 +/- 0.011 giving a p-value of 1.

The SNAP amplitudes in figure 2 were measured between the two horizontal bars. There are no changes in the amplitude of the control traces, but a significant decrease is noticed between the amplitudes the septic traces.

EKG

Due to technical problems only lead II of the Physiocontrol LifePak 6 EKG machine gave consistent recordings. The results obtained from lead II did not provide significant results for the QRS amplitude, giving a p-value of 0.32. There was however one case where a large drop did occur in QRS amplitude but this was considered an isolated case and did not affect the significance of the data as a whole.

Auditory Brainstem Response (ABR)

Auditory brainstem responses were administered to 7 rats using the Intelligent Hearing Systems SmartEP. Number of peaks and latencies of the peaks were looked at. Prior to the onset of sepsis 4 peaks were recorded. After sepsis 5 to 6 peaks (4 of 7 rats), (3 of 7 rats) were recorded. The only significant change in latency was that of peak 3. Before sepsis the average latency was 2.89 +/- 0.111, and after 2.50 +/- 0.096 this gave a p-value equal to 0.04.

Figure 3

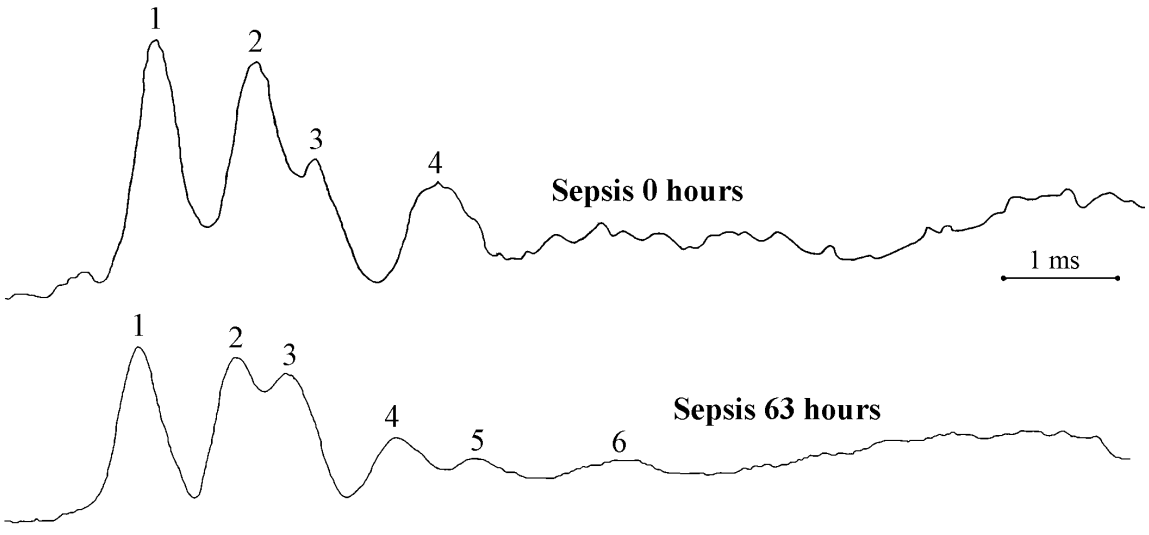
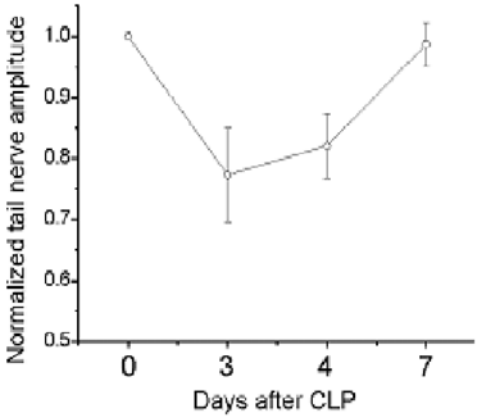


Figure 3 shows two ABR traces. One taken before the cecal ligation and puncture surgery and the other was taken 63 hours after the surgery. The first trace, time zero, shows only 4 peaks. The second trace, 63 hours later, shows 6 peaks. The addition of peaks 5 and 6 are apparent in the bottom trace.

Seven Day Study

Figure 4

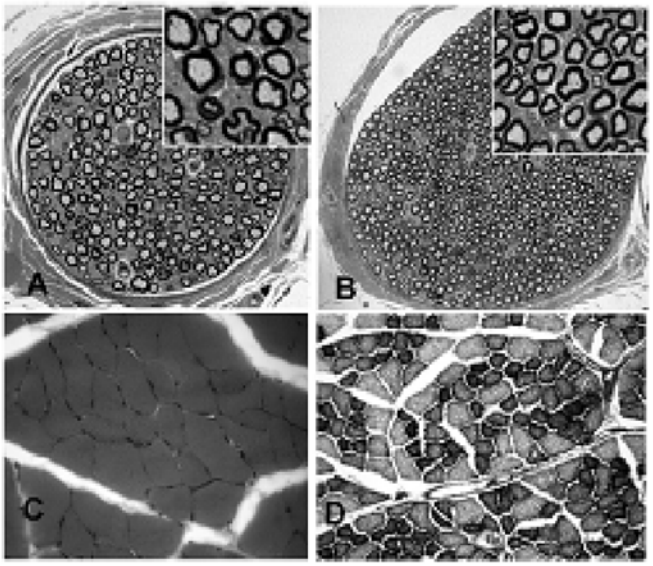


The six rats that participated in the seven day study showed the same motor and sensory nerve dysfunction 72 hours after cecal ligation and puncture that were seen in the rats that did not; increases in CMAP duration, increases in SNAP duration and latency, and decrease in SNAP amplitude. However, these six rats experienced significantly rapid recovery of their sensory nerve function 7 days after the CLP. Figure 4 illustrates the reversible changes in sensory nerve amplitudes that occurred to the six rats that were part of the seven day study. After 72 hours the sensory nerve amplitude decreased 30%, and after 168 hours the sensory nerve amplitudes returned back to the original value. The latency and duration both increased by 25%, and did not return to their original valves by day seven.

Nerve and Muscle Pathology

Nerve and muscle biopsies taken from the rats in the seven day study are shown in figure 5. These images show no significant demyelination or degeneration of axons despite abnormal finds of both sensory and motor nerve conduction 72 hours after

Figure 5



inducing sepsis. Figure 5 block A shows the pathology of the sural nerve (a pure sensory nerve) and figure 5 block B shows the pathology of the peroneal nerve (sensory and motor axons). Cross sections from both of these nerves do not indicate any loss of axons or myelin. Sections taken from the tibialis anterior muscle, the muscle that was studied using nerve conductions, are also shown in figure 5. Figure 5 block C shows staining using hematoxylin and eosin and block D shows NADH staining. In both of these muscle stains there are no target or angular fibers to suggest muscle denervation.

Electrolytes

Table 1 shows the mean +/- SEM of electrolyte values for 5 control and 5 septic rats. None of these differences are statistically significant.

Table 1

	Sodium	Potassium	Calcium	Glucose	Creatinine
Control	146.4 ± 2.1	6.3 ± 0.6	12.0 ± 0.4	198.4 ± 53.2	0.28 ± 0.05
Septic	150.6 ± 1.5	6.7 ± 0.5	11.3 ± 0.2	105.4 ± 7.0	0.28 ± 0.05

IV. Discussion

Auditory Brainstem Response (ABR)

The results obtained from the ABR where used to determine if changes occurred in the central nervous system. The significant decrease in latency of peak 3 and the emergence of peaks 5 and 6 in the septic animal are difficult to interpret. Each peak in the ABR represents one component of the auditory pathway. These peaks represent different elements in rats and humans. In the rat, the acoustic nerve (CN VIII) produces peak 1, the cochlear nucleus generates peak 2, the superior olivary nucleus peak 3, the lateral lemniscus peak 4, and the inferior colliculus peak 5 (Strata, deIpolyi et al. 2005). An increase in the latency between any two peaks may indicate abnormal myelination of the axon, abnormal nerve conductions in the central nervous system, or deficits in the brainstem, thalamic and/or cortical regions of the brain (Strata, deIpolyi et al. 2005). An increase in the latency of peak 3 would point to deficits in the cochlear nucleus and/or the superior olivary nuclei or slowing of nerve conduction through these areas of the brainstem. An explanation for a decreased latency or the appearance of new peaks could not be found in the literature. Decreased amplitude of peaks however, has been linked to the dysfunction of that component (Strata, deIpolyi et al. 2005; Rice and Shapiro 2006).

I believe the changes mentioned above could be due to alterations in the inhibitory and excitatory connections of these brain areas. Changes in the inhibitory and excitatory synapses could also result in the recruitment of new brain areas. Decreased inhibition to an area of the brain would cause that area to become active, allowing it to appear in the ABR recording. Recruitment of new brain areas would support the

appearance of peaks 5 and 6. Increases in excitatory and decreases in inhibitory neuron influences could be responsible for the decreased latency of peak 3, causing an increase in the speed of signal transmission. More data obtained from new experiments would be necessary to fully answer this question.

Electrocardiogram (ECG)

No significant data was collected involving ECG. The apparatus that was used to perform the recordings was never completely functional. Due to its inconsistency that data was not considered legitimate. Future studies involving the effects of sepsis on ECG recordings are planned now that a new, completely functional unit is available.

Nerve Conductions

As stated earlier, the classic case of critical illness polyneuropathy (CIP) presents with decreased sensory nerve action potential (SNAP) and compound muscle action potential (CMAP) amplitudes, while latency and duration of both remains unaffected. These findings are commonly thought to be the result of a primary axonal degeneration (Kimura 2001). Death of axons would explain these decreased amplitudes since fewer fibers are available to transmit action potentials. The action potentials that are being transmitted are not affected by the death of neighboring axons so the latency, duration, and amplitude of these would not be expected to change. When summing action potentials, as done in SNAP and CMAP recordings, fewer action potentials will decrease the amplitude of the trace, but not affect the latency or duration of the trace. Our results for SNAP and CMAP were not consistent with an axonal neuropathy. We did see a decrease in SNAP amplitude but the CMAP amplitude was unchanged. However in our

experiment SNAP latency and duration as well as CMAP duration increased, which is not consistent with the classic presentation of an axonal neuropathy. These results, however, do not eliminate the possibility of axonal degeneration. Our data may be the additive effect of axonal degeneration and some other problem.

Other neuropathies, known as demyelinating neuropathies, have also been reported (Kimura 2001). However, demyelinating neuropathies do not present in the same fashion as the classic axonal degeneration. A demyelinating neuropathy presents with increased latency and duration of SNAP recordings. This may or may not include a decrease in the amplitude of the SNAP. The loss of myelin would cause the signal to take longer to travel the length of the axon, accounting for increased latency and duration of SNAP and CMAP recordings. If excessive myelin has been lost then the signal may not be conducted at all causing the action potential to be lost. This would cause a decrease and/or loss of SNAP and CMAP amplitude. The changes seen in our nerve conduction studies could be the result of both an axonal degeneration and demyelinating neuropathy.

The data collected from the seven day study of the septic rats shows that the changes in the SNAP recordings are rapidly reversible. The rats that took part in the seven day study experienced a significant decrease in SNAP amplitude 3 days after CLP. On average there was a 30% drop in amplitude, and a 25% increase in the latency and duration. However, the amplitude recovered rapidly back to normal between days 3 and 7. This recovery is too rapid to be the result of axon regeneration. The biopsies sent for pathology also show no indications of axonal degeneration or demyelination of axons. These two pieces of evidence point away from structural problems with the nerve and toward external or intrinsic malfunction of neurons.

Changes in the electrolyte levels in the external environment could have a detrimental effect on the production of action potentials. Increases in potassium lead to a depolarized resting potential which causes a greater number of sodium channels to be inactivated. The more sodium channels that are inactivated the smaller the action potential will be. If all the axons in the nerve are producing small action potentials, when they are summed together the result will have a decreased amplitude. After checking blood levels of potassium, sodium, calcium, glucose, and creatinine, no changes were significant.

The intrinsic functions of the axon were investigated through collaborative efforts with members of Timothy Cope's lab. They performed neurophysiologic studies of individual axons in the dorsal root, and were able to record resting membrane potential, leakiness of the axolemma, and action potential generation through depolarization and hyperpolarization. After examination of dorsal root axons in control and septic animals it was determined that resting membrane potential and leakiness of the axolemma were not significantly different between the two groups. However the amplitudes of action potentials from individual axons were significantly smaller in the septic rats. The reason for this decrease in amplitude could be due to two things. One, fewer sodium channels are present or two, the normal number of sodium channels are present but they are activated at lower potentials. To test this, axons septic and control rats were given a hyperpolarizing potential. The amplitudes of action potentials generated by the hyperpolarizing pulses were significantly larger than those of action potentials triggered by depolarizing pulses in the septic rats. In the control rats the hyperpolarizing pulses did not change the amplitude of the action potentials. We believe that the hyperpolarizing

current delivered to the septic rats is relieving the inactivation of sodium channels and allowing for more sodium channels to participate in action potential production. Having more sodium channels activated will lead to larger amplitude action potentials. In this experiment septic rats were able to produce action potentials with amplitudes equal to those of control rats when lower membrane potentials were created by a hyperpolarizing pulse. If the number of sodium channels had decreased then the amplitude of the action potential would not have improved.

The changes in excitability of dorsal root axons in our septic rat model are very similar to the changes seen in sodium channels of skeletal muscle (Bird and Rich 2002; Teener and Rich 2006). Previous experiments performed by Mark Rich and company have shown decreases in CMAP amplitudes in rats treated with corticosteroids and muscle denervation(Rich, Pinter et al. 1998; Rich and Pinter 2001). The extensor digitorum longus muscle was harvested from the rats and intracellular recordings were performed on individual muscle fibers. They noticed a significant decrease in the size of muscle fiber action potential amplitude across a variety of resting membrane potentials. This led them to believe that resting membrane potential was not involved in decreased amplitude. They also induced changes in the membrane permeability by using chloride channel toxin(Rich, Pinter et al. 1998). This resulted in no change of action potential amplitude. After ruling out resting membrane potential and sacrolemma permeability they turned to sodium channel changes. They concluded that some defect in sodium current density was the likely reason for the inexcitability of the muscle(Rich and Pinter 2001). The reason for the reduced sodium current could be due to many things. A decrease in the density of sodium channels, decrease in driving force of sodium current,

or the voltage dependence of sodium channel activation and/or inactivation might be altered. Intracellular recording and loose patch voltage clamp studies where performed on rats treated with corticosteroids and denervated muscles(Rich and Pinter 2001). It was found that inactivation of sodium channels was due to a shift in the voltage dependence of channel inactivation to more negative potentials. This meant that at a given resting membrane potential more sodium channels were inactivated and unable to contribute to action potential production in the steroid denervated rats(Rich and Pinter 2001). Muscle that was denervated and treated with corticosteroids produced smaller action potentials than control muscles at the same membrane potential. When compared to control, the sodium channels in the steroid denervated rat muscle fibers became inactivated at lower membrane potentials(Rich and Pinter 2001).

These findings are similar to those obtained from the sodium channels of dorsal root axons. Since loose patch voltage clamp studies cannot be performed on nerve, we cannot say that there is a shift in voltage dependence of sodium channel activation. We do believe that the mechanism is similar to what is occurring in skeletal muscle.

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