MODULATION OF SOMATOSENSORY EVOKED POTENTIALS BY VARIOUS SPINAL CORD STIMULATION MODALITIES IN A PORCINE MODEL

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By

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Abstract

Tonic spinal cord stimulation (SCS), an advancement in the treatment of chronic pain, has been found to reduce somatosensory evoked potentials (SSEPs). Current literature lacks information regarding the effect on SSEPs of newer SCS modalities like burst and high-frequency (HF) SCS. My thesis addresses the current lack of understanding regarding various types of SCS and their effects on SSEPs. I used an anesthetized pig model to investigate the effect of tonic SCS, burst SCS, and HF SCS on SSEP waveform amplitude and latency. Additionally, I studied the effect of a novel type of SCS, ultra-low frequency (ULF), on SSEP amplitude and latency. SSEP waveform amplitudes and latencies were collected during each SCS modality and during washout periods between each SCS modality. SSEP amplitudes were significantly reduced for all 4 studied SCS modalities. The degree of this SSEP amplitude reduction was significantly larger between tonic SCS when compared to burst and ULF SCS. The majority of SSEP amplitude baselines were significantly different from each other. No significant changes were found to SSEP latencies during any of the SCS modalities. Most latency baselines between subjects were significantly different from each other. This project demonstrated the ability of a porcine model to act as an efficacious model for SCS research, due to its similar neural anatomy and compatibility with human stimulation devices. The results outlined above demonstrate that all four types of SCS significantly reduced SSEP waveform amplitudes in a porcine model, which typically signals dysfunction in the sensory pathway, but in the case of SCS application is suggested to be due to sensory transmission interference localized at the site of stimulation (Larson et al. 1974). The similar reductive effect on SSEP amplitudes observed across all four modalities suggests that they may have some mechanistic similarities in the way they affect somatosensory processing at the spinal, thalamic and cortical levels. The presence of outliers in the data is in line with clinical findings in which some patients are deemed non-responders to certain types of SCS. It remains to be seen if the effects on SSEPs by the four types of SCS in this study are correlated with successful pain relief. Future research should focus on mapping the inter- and intra-cellular communication that provides the mechanism for the pain relief that is observed during application of various SCS modalities.

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Abbreviations

Spinal cord stimulation (SCS)

Somatosensory evoked potentials (SSEPs)

High frequency (HF)

Dorsal root ganglion (DRG)

γ-Aminobutyric acid (GABA)

Central nervous system (CNS)

Wide dynamic range (WDR)

High-frequency alternating current (HFAC)

Electroencephalogram (EEG)

Functional magnetic resonance imaging (fMRI)

Conventional medical management (CMM)

Visual analog scale for pain (VAS Pain)

Ultra-low frequency (ULF)

Digital to analog converter (DAC)

Analog to digital converter (ADC)

Chapter 1: Introduction

1.1 Background

Pain is a complex, subjective, and abstract concept. As such, any research work relating to pain is a challenging task. Neuropathic pain is a widespread medical concern, with only a few moderately effective treatment options currently available. Neuropathic pain is defined as pain caused by a lesion in, or injury to the nervous system, and can be dynamic or chronic (Bridges et al. 2001). It is often described by patients as a knife-like or burning pain and is sometimes accompanied by allodynia[1] or hyperalgesia[2]. Neuropathic pain is prevalent to various extents in 1.5-8% of the general population with back and leg pain being the most common (Kumar et al. 2007). The peripheral nociceptive system contains C-fibers, whose cell bodies converge in the dorsal root ganglion (DRG), which terminate on interneurons or projection neurons in the various superficial laminae of the dorsal horn of the spinal cord (Urch 2007). Neuropathic pain research is mainly performed using peripheral nerve or spinal cord injury models, which has influenced the current understanding of the mechanisms underlying this type of pain (Chakravarty and Sen 2010). A review by Chakravarthy and Sen notes that there is an increase in spontaneous firing of afferent neurons at the site of injury following nerve damage. This spontaneous firing in these neurons, also referred to as DRG neurons, is a phenomenon referred to as peripheral sensitization. The same review notes that significant crosstalk occurs between C-fibers and their neighbouring intact sensory neurons, further amplifying the peripheral sensitization. Since the nervous system reacts to location, pattern, and frequency of stimulation (Chakravarthy et al. 2018), the changes in neuronal firing caused by peripheral sensitization can lead to central sensitization where spinal cord reorganization occurs, such as neuronal sprouting[3]

[1] Pain in response to a normally innocuous stimulus
[2] Increased response to a painful stimulus
[3] Formation of functional synapse-like structures that contribute to establishing and sustaining abnormal excitation of DRG, WDR, and nociceptive specific neurons

and channel up/down-regulation[4], which plays a role in the development of the classical symptomology of neuropathic pain (Chakravarty and Sen 2010). Central sensitization refers to the hyperexcitability of pain-transmitting, nociceptive-specific or wide dynamic range (WDR) neurons in the dorsal horn of the spinal cord. Pain is processed in distinct systems: the lateral discriminatory system, and the medial affective/attentional system (De Ridder et al. 2013). De Ridder *et al.* explain that the medial system[5] is triggered by nociceptive-specific neurons relayed through the dorsal horn of the spinal cord to the thalamus and from there to the anterior cingulate cortex, anterior insula, and amygdala. The same study mentions that the lateral system[6] is triggered predominantly by WDR neurons, also relayed in the dorsal horn to different thalamic nuclei and from there to the primary and secondary somatosensory cortex, and the posterior parietal area. It is important to mention that neuropathic pain is a complex concept, with many additional pathways, neurons, channels, and molecules involved than those detailed above. Such information, however, is beyond the scope of this thesis, and I will only focus on the aspects of neuropathic pain that directly pertain to SCS.

Every year, more than 30 000 people are implanted with spinal cord stimulators, a recent advancement in the treatment of neuropathic pain (Zhang et al. 2014). Although spinal cord stimulation (SCS) is an effective method of treating chronic, neuropathic pain, the underlying mechanism by which it functions is currently not well understood. Three modalities of spinal cord stimulation are currently in use, and each modality has a distinct set of stimulation parameters. The oldest modality, tonic SCS was first developed in the late 1960s. This type of SCS is accompanied by uncomfortable paresthesia, or a feeling of pins and needles, over the targeted, painful area (Kapural et al. 2016). Owing to the presence of paresthesia during tonic SCS and with the aim of providing superior pain relief, two new modalities of SCS have recently been developed, namely high-frequency (HF) and burst SCS (Tiede et al. 2013). The difference between tonic, burst, and HF SCS lies in the frequency, pulse width, and current parameters used to create each SCS modality. Both of these newer SCS devices are promoted as paresthesia-free modalities. Tonic spinal cord stimulation (SCS) has been found to reduce and even eliminate the

[4] Change in level and anatomical location of various Na^+ and Ca^+ channel types in DRG, WDR, and nociceptive specific neurons
[5] Responsible for the attentional and emotional characteristics of pain
[6] Responsible for the sensory distinctions of location, severity, and quality of pain

amplitude of somatosensory evoked potentials (SSEPs), although the mechanism underlying this effect is not well understood (Polacek et al. 2007). At the moment, it is unclear if this reduction in SSEPs is directly caused by the pain-relieving mechanism of SCS, or whether it is an unrelated side effect. In one case study, HF SCS has been found to similarly reduce the amplitude of SSEPs (Buonocore and Demartini 2016). This finding has not been substantiated by additional publications. At this time, data regarding the effect of burst SCS on SSEPs have not been published. Preliminary findings, collected in the Norton laboratory, indicate that burst SCS also reduces the amplitude of SSEPs. However, further studies are required.

1.1.1 Gate control theory of pain

In 1967, Melzack and Wall published a paper outlining a new theory concerning pain and its transmission in the nervous system, which subsequently became the most widely accepted pain theory, and has led to several current applications and treatments (Polacek et al. 2007). The gate control theory of pain describes three spinal cord systems including the cells of the substantia gelatinosa in the dorsal horn, the afferent fibers that project toward the spine, and the first central transmission (T) cells in the dorsal horn (Melzack and Wall 1965). The authors proposed that the substantia gelatinosa functions as a gate control on the afferent fibers and modulates their activity before it reaches the T cells. In a healthy person, the afferent nerve fibers activate the central T cells very effectively. The substantia gelatinosa feeds back negatively or positively, depending on which afferent fibers are stimulated originally, onto the afferent terminals to diminish or increase their activation of the T cells (Figure 1-1) (Melzack and Wall 1965). Large afferent fibers activate the negative feedback mechanism, while small afferent the fibers activate positive feedback mechanism. Therefore, increasing large fiber (Aβ) activity and/or decreasing small fiber (Aδ and C) activity should be a useful way to diminish pain transmission or close the gate. The same paper also hypothesized that the afferent signals passed on by the T cells activate specific brain areas that in turn influence the substantia gelatinosa. Finally, the T cells also modulate brain activity to respond to and perceive the stimulus. The therapeutic implications of this hypothesis were great at the time, suggesting a potentially new, unprecedented way of treating/controlling pain.

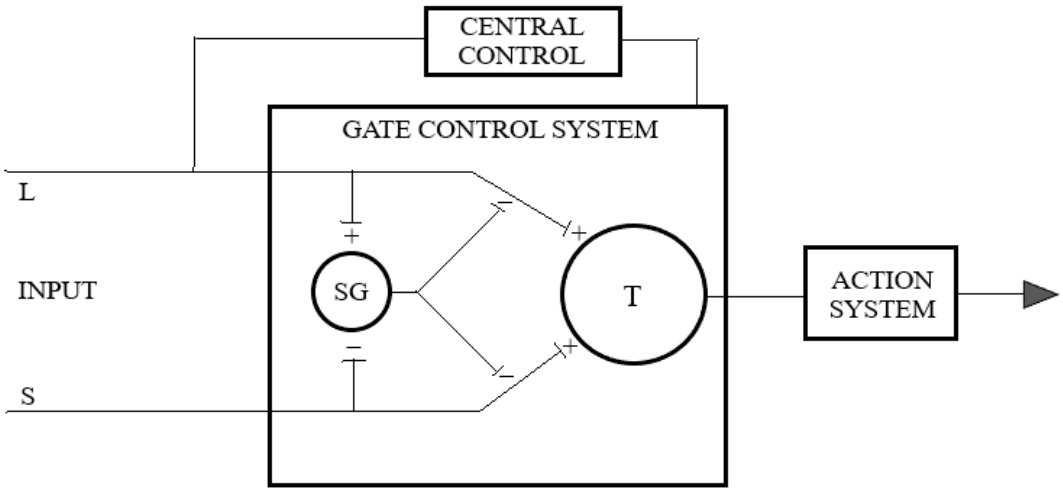

Figure 1-1. Schematic representation of the gate control theory of pain as proposed by Melzack and Wall. The substantia gelatinosa (SG) acts as a gate control on the dorsal column fibers. Large diameter fibers (L) disinhibit the SG, whereas small diameter fibers (S) inhibit the SG. When active, the SG feeds back unto L and S to inhibit their respective activation of the T cells (T). L also affect the central control which oversees the actions of the SG. T project into the action system which allows for effective perception of and response to the stimulus. (Melzack and Wall 1965)

1.1.2 Somatosensory evoked potentials, GABA and the central nervous system

Somatosensory evoked potentials (SSEPs) occur when large-diameter fibers in the nervous system are stimulated (Gugino and Chabot 1990). They carry sensory information to the cortex via the medial lemniscus and thalamus. SSEPs can be created by almost any sensory nerve in the body at the peripheral, spinal, cortical and subcortical levels (Mauguiere 1999). γ-Aminobutyric acid (GABA) is a critical inhibitory signaling molecule in the adult central nervous system (CNS) (Bremner et al. 2006). In the case of the pain transmission, GABA is released from the lamina II of the dorsal horn, also known as the substantia gelatinosa (Yang and Ma 2011), an area previously mentioned as it relates to the Melzack and Wall gate control theory of pain. The substantia gelatinosa houses the many interneurons that inhibit dorsal horn neuronal firing via the release of GABA. Electrophysiological studies have shown that GABA release on the dorsal horn of the spinal cord causes both pre- and post-synaptic inhibition (Gangadharan et al. 2009). This finding is in line with previous research that has outlined that GABA receptors converge on both pre-synaptic primary afferent fiber terminals and post-synaptic neuronal cells (K.J. Charles 2001; S. Towers 2000). Nociceptive signaling in the spinal cord is largely affected by the presence of GABAergic interneurons in the substantia gelatinosa (Bremner et al. 2006). Bremner *et al.* found that the application of a GABA antagonist to the dorsal horn of the spinal cord causes strong disinhibition of dorsal horn neurons, enhancing the transmission of pain. Thus, typical inhibition of dorsal horn neuronal firing is mediated by GABA receptors (Christensen et al. 2018).

1.1.3 Traditional, tonic spinal cord stimulation

In 1967, Shealy and colleagues were the first to publish a report outlining their attempt at dorsal column stimulation (Shealy et al. 1967). Their patient, a 70-year-old man, presented with severe, diffuse pain in his lower chest and upper abdomen. The subject was terminally ill with inoperable bronchogenic carcinoma[7] (Abbas 2018; Goldstraw et al. 2011). An electrode was implanted and sutured to the dura in the upper thoracic region of the spinal canal. The dorsal column of the subject was stimulated with 400ms pulses ranging in frequency from 10-50Hz. The patient immediately reported that his incisional and chest/abdominal pain was no longer present. He did report paresthesia over the previously painful area. Parameters were adjusted

[7] Any type or subtype of lung cancer that originated in the bronchi and bronchioles

whenever the pain recurred over a day and a half. Before the collection of additional data, the subject passed away. Nevertheless, the method outlined in the above case report was a breakthrough in pain treatment and has now become known as tonic or traditional SCS. Today, tonic SCS is characterized by pulses of 50-60Hz, 300-400μs pulse widths, and 4-9mA amplitudes (Kapural et al. 2016). Additionally, it is always accompanied by paresthesia, a prickling feeling of pins and needles, over the chronically painful dermatome (Verrills et al. 2016). Although tonic SCS has been widely accepted in the healthcare field as an effective method of managing various chronic pain conditions, its mechanism of action is not fully understood (Zhang et al. 2014). Based on the evidence to date, the mechanism of tonic SCS is believed to be comprised of a combination of spinal and supraspinal mechanisms (Chakravarthy et al. 2018). Neurophysiological research suggests that tonic SCS functions by exploiting the principles laid out in the gate control theory of pain (Melzack and Wall 1965). Tonic SCS pulses have been found to cause antidromic[8] activation (Valls-Sole et al. 2016) of Aβ, large diameter, peripheral nerve fibers (Linderoth and Foreman 2006). It is theorized that these large diameter fibers activate inhibitory 'gating' interneurons in the fiber collaterals (Chakravarthy et al. 2018). Further research has demonstrated that during SCS, there is an increase in the release of GABA unto the dorsal horn of the spinal cord by inhibitory interneurons (Zhang et al. 2014). It has also been found that this increase in GABA inhibits the activation of wide dynamic range (WDR) neurons (Chakravarthy et al. 2018). Since WDR neurons are partially responsible for the transmission of pain, it is believed that tonic SCS mitigates the C-fiber-mediated wind-up of WDR neurons, and that this occurs via the gate control pathway described above. It has been theorized that during tonic SCS, WDR neurons are not only inhibited pre-synaptically, but also post-synaptically by descending pathways (Foreman and Linderoth 2012). To expand on this concept, various other mediators such as substance P, noradrenaline, and serotonin have also been found to play a role in peripheral sensitization by amplifying the action of descending fiber tracts (Chakravarthy et al. 2018). Although still unconfirmed, these pathways and mediators may be affected by tonic SCS.

[8] Travelling in the opposite direction to that normal in a nerve fiber

1.1.4 High-frequency spinal cord stimulation

In the case of high frequency (HF-10) SCS, the spinal cord is stimulated with pulses of 10kHz delivered for a short pulse width of 30μs (Buonocore and Demartini 2016). With experiments commencing in the 1960s, using various animal models, it was found that the application of a high-frequency (>1000Hz) alternating current (HFAC) could selectively and reversibly block peripheral nerve conduction (Bicket et al. 2016). Tanner and colleagues used a frog model to demonstrate that nerves of different diameters were selectively blocked by varying the amplitude of the applied HFAC (Tanner 1962). This blockade ended immediately upon the removal of the current. Alternatively, the application of direct current was found to cause nerve damage and is therefore inappropriate for clinical use (Whitwam and Kidd 1975). Another experiment looked at the HFAC blockade of descending motor neurons (Kilgore and Bhadra 2004). Interestingly, conduction was not blocked along the entire nerve, but only in the area directly surrounding the HFAC application site. For example, stimulating the motor nerve distal to the HFAC electrode caused a maximum muscle response, indicating that the HFAC only blocked signals originating from an area proximal to the nerve block. Additional research performed by Bicket *et al.* discovered that myelinated and unmyelinated nerves required different parameters[9] to block nerve conduction (Bicket et al. 2016). The same study demonstrated HFAC's ability to selectively block A- (myelinated) and not C- (unmyelinated) fibers, and vice versa, by varying the current's amplitude/frequency relationship. Since C-fibers are often partially responsible for chronic neuropathic pain, this finding regarding the ability to selectively block these fibers was of major importance (Jonas et al. 2018). It is critical to note that the exact mechanism whereby HFAC blocks nerve conduction is still unknown (Bicket et al. 2016). Theories explaining the mechanism responsible for HFAC blocks include hyperpolarization, depolarization, or a combination of the two. Animal model studies referenced by Bicket *et al*. suggest a combination, as nerve membranes were found to be in a state of overall depolarization during application of HFAC, owing to competing depolarizing and hyperpolarizing forces. The theory attempting to explain the mechanism of HFAC blocks suggests that the depolarizing inward sodium/outward potassium currents overtake the smaller hyperpolarizing outward sodium current. Thus, when comparing the pain-relieving mechanisms

[9] Amplitude, frequency, and pulse width

of tonic and HF SCS, it is likely that HF-10 SCS administered epidurally unto the dorsal horns of the spinal cord may block the nerve conduction of noxious stimuli, a pain-relieving mechanism different from that of tonic SCS. As previously mentioned, tonic SCS has been found to decrease firing of the WDR neurons in the dorsal horn (Chakravarthy et al. 2018). HF SCS however, does not inhibit the wind-up[10] phenomenon in these neurons and has not been found to decrease WDR neuron firing (Shechter et al. 2013). Finally, tonic SCS has been found to activate the gracile nucleus, an area of the medulla responsible for organizing neural inputs from the lower extremities, whereas HF-10 SCS does not produce this activation (Song et al. 2014). Together these findings validate the idea that tonic and HF-10 SCS have different pain-relieving mechanisms. Further investigation of HFAC and its application is necessary to understand its implications for HF-10 SCS. The major questions regarding the mechanism of HF SCS in pain modulation involve 1) the effect of HF-10 SCS on the release of neurotransmitters in the nervous system; 2) the mechanism of pain relief exhibited during HF-10 SCS; 3) the relationship between the pain-relieving effect of HF-10 SCS and the inhibition of SSEPs that seems to occur under the same condition.

1.1.5 Burst spinal cord stimulation

Burst SCS is characterized by stimulation bursts of five 1ms pulses at a 500Hz frequency (De Ridder et al. 2013). The burst of pulses lasts 10ms. The bursts themselves occur at a frequency of 40Hz and the inter-pulse interval is therefore, 15ms (Figure 1-2) (Kirketeig et al.

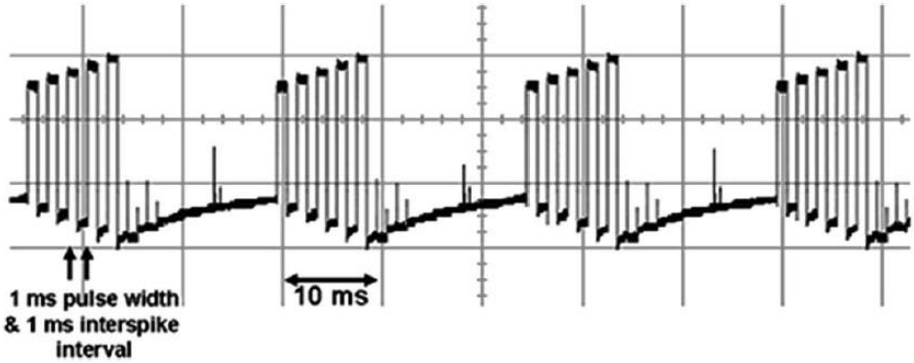

Figure 1-2. Burst SCS waveform at constant current (5mA). Five pulses of 1ms each at a 500Hz frequency. 40Hz inter-burst frequency creates a 15ms interval between bursts (De Ridder et al. 2013).

2019). One of the basic principles of the nervous system is that it will respond not only to the location of stimulation, but also to the frequency, and pattern of this stimulation (Chakravarthy et

[10] Central sensitization leading to nociceptive-specific or WDR neuron hyperexcitability

al. 2018). Thus, temporal differences in burst firing, when compared to tonic firing may have different effects on downstream areas. For example, burst firing in the thalamus has been found to cause increased postsynaptic activation of cortical neurons when compared with tonic firing (Swadlow and Gusev 2001). In burst firing, not only does the first spike of the burst have a very high synaptic efficacy, but the efficacy of subsequent spikes is also high. Ultimately, as stated by Swadlow and Gusev, this increases the likelihood of postsynaptic firing when compared with tonic thalamic firing. In 2013, De Ridder and colleagues performed electroencephalogram (EEG) recordings to localize cortical areas of high synchronicity during tonic, burst, and sham/placebo SCS (De Ridder et al. 2013). They reported that burst SCS was associated with higher neural synchronicity when compared to tonic SCS in the dorsal anterior cingulate cortex and left dorsal lateral prefrontal cortex. As previously mentioned, pain is processed by two separate systems: the lateral discriminatory system, made up of the primary sensorimotor area, secondary somatosensory cortex, and posterior insula, and the medial affective/attentional system. Using functional magnetic resonance imaging (fMRI) techniques, tonic SCS has been found to primarily modulate the lateral discriminatory system of pain (Stancak et al. 2008). As the anterior cingulate cortex is responsible for the mediation of the affective/attentional system of pain, the data collected by De Ridder and colleagues suggests that burst SCS modulates activity in the medial system *in addition to* the lateral discriminatory system (De Ridder et al. 2013). When a 2007 publication found that burst stimulation of the Schaffer collateral pathway in the hippocampus, a pathway unrelated to pain, led to long-term potentiation between the Schaffer collaterals and the postsynaptic CA1 neurons, pain researchers theorized that burst SCS could also be used to cause long-term synaptic changes in the brain's pain pathways (Chakravarthy et al. 2018). In an animal model of chronic pain, burst SCS has been found to reduce hyperalgesia and reestablish physical activity more effectively than tonic SCS (Gong et al. 2016). Similarly to tonic SCS, burst SCS has been demonstrated to cause decreased firing in WDR neurons and the high-threshold neurons of the dorsal horn and this decrease correlates with an increase in pulses per burst, pulse width or stimulation amplitude (Chakravarthy et al. 2018). An important note, however, is that GABA antagonists do not change the decrease in WDR firing during burst SCS but rather do during tonic SCS. To reiterate, GABA antagonists *have* been found to eliminate WDR inhibition during tonic SCS, which is in line with our previous discussion of the GABA released unto the dorsal horn during tonic SCS. This finding suggests that burst SCS does not act

via GABA-ergic signaling. This raises an important question, in that if GABA is not the transmitter inhibiting WDR neuron firing, then another molecule, pathway or transmitter must be responsible. It is therefore important to discover what this might be. As with tonic SCS and HF SCS, many questions remain pertaining to the mechanism of burst SCS and its role in the modulation of neuropathic pain. Consequently, more research is still required to fully unravel the exact mechanism of action of burst SCS.

1.1.6 Somatosensory evoked potentials and spinal cord stimulation

In the past 50 years, research into the relationship between SSEPs and the pain-relieving mechanism of SCS to treat neuropathic pain has drawn the attention of the medical community. In the 1970s, several studies found that tonic SCS reduced the amplitude of SSEPs, with lower intensity SCS, and completely blocked SSEPs with higher intensity SCS than was needed to relieve pain (Bantli et al. 1975; Doerr et al. 1978; Larson et al. 1974). These findings were corroborated in a recent comprehensive review which considered 24 published studies (Bentley et al. 2016). Fifteen of these studies were deemed neuro-electrical in scope. Most of these considered the effect of tonic SCS on SSEPs responding to innocuous or painful peripheral stimulation. The authors mention that seven of those 15 studies used innocuous tibial nerve stimulation prior to measuring the subsequent SSEPs. The results of those seven studies strongly point to traditional, tonic SCS having an inhibitory effect on the amplitude of recorded SSEPs. Somatosensory evoked potential latency represents the time it takes for an SSEP to travel from the area of stimulation to the recording electrode. Latency is dependent on several factors including but not limited to height, skin and core temperature, and state of consciousness (Chu 1986; Mauguiere 1999). The 2016 review by Bentley and colleagues reported that SSEP latency is shown to be lengthened by traditional SCS, but the extent of this tendency differed from study to study (Bentley et al. 2016). It is unclear whether these studies considered the confounding factors (height, etc.) mentioned above, when analyzing their latency data. If confounding factors were not considered, an analysis that controlled for these variables may have normalized the latency results. Lastly, the review also mentioned two studies whose results demonstrate that SCS likely modifies SSEPs at both the spinal and thalamic level.

In comparison to tonic SCS, much less is known regarding the relationship between newer-type SCS modalities and SSEPs. One case report published in 2016, compared the effect of traditional SCS and HF-10 SCS on SSEPs (Buonocore and Demartini 2016). Multiple surgical procedures failed to adequately relieve the subject of back and left leg pain. Subsequently, the subject was implanted with a traditional SCS lead and pulse generator and she reported good pain relief for one year following the implantation. Buonocore and Demartini implanted a new lead that was implanted epidurally into the patient's spinal canal at thoracic vertebra 8 (T8), with a slight overlap of the old lead. SSEPs were collected from recording electrodes placed on the scalp and a reference electrode was also placed on the forehead of the subject. The tibial nerve of the patient was stimulated with pulses of a high enough intensity to produce a muscle twitch (5Hz; pulse-width of 0.1ms). SSEPs were collected during three separate program types as follows: no SCS, traditional SCS, no SCS, HF SCS. For each of these programs, two sets of 500 pulses were averaged. The washout period between programs was fifteen minutes long. The authors found that both traditional SCS and HF SCS reduced the amplitude of SSEPs (Figure 1-3) (Buonocore and Demartini 2016). This research demonstrated for the first time, that HF SCS

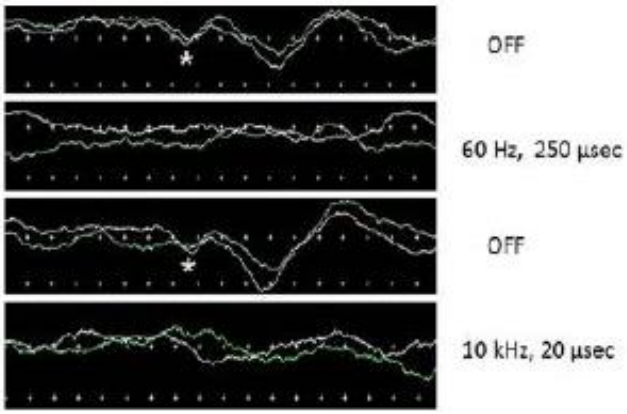

Figure 1-3. Inhibition of averaged SSEPs in tonic SCS and HF SCS. An SSEP is demonstrated by the presence of an asterisk (*). The second row represents the SSEP response to traditional SCS, while the fourth row represents the SSEP response to HF SCS (Buonocore and Demartini 2016).

inhibits SSEPs. The findings have yet to be reproduced by other publications. Moreover, the case report did not look at the effect of HF SCS on SSEP latency. Further research should also include SSEP latency data analysis. Interestingly, the patient reported no paresthesia during the HF SCS period.

The effect of burst SCS on SSEPs has not been explored to date in a peer-reviewed publication, although a recent study found a decrease in laser-evoked potential amplitude due to burst SCS (Bocci et al. 2018). Preliminary data collected from ten patients in the Norton laboratory was presented at the International Neuromodulation Society's 13th Annual World Congress (2017). The data failed to yield significant results when comparing SSEP amplitude and latency across 12 SCS levels (0-11) for all ten patients. However, a comparison between no stimulation and therapeutic stimulation levels in all patients demonstrated a significant decrease in the amplitude of SSEPs. Additionally, when comparing zero stimulation to therapeutic stimulation levels in all patients, there was a significant increase in the latency of SSEPs. This is perhaps more clinically relevant as it relates to the therapeutic SCS level that the subjects use daily to treat their pain. These findings have yet to be confirmed by additional research or publications.

1.1.7 Effectiveness of various SCS modalities for chronic pain treatment

Following the gate control theory of pain, the new and promising technique of spinal cord stimulation (SCS) was developed and implemented in treatments for various chronic pain syndromes, syndromes which typically include some form of neuropathic pain. In 2007, Kumar *et al.* published a multicenter, randomized, and controlled trial that compared conventional medical management (CMM) and tonic SCS in terms of their effect on neuropathic pain owing to primary nerve injury (Kumar et al. 2007). In this study, 100 patients with failed back surgery syndrome were followed for 12 months after having been randomized in either strictly CMM group or SCS group. In this study, CMM included oral analgesics, nerve blocks, epidural drugs, physical or chiropractic therapy. Four days before each study checkup, patients were asked to rate their back and leg pain on the visual analog scale (VAS) for pain three times a day, every day, in a 'pain diary'. The VAS for pain is likely the most common way of measuring pain in modern studies, as it is meant to help determine measurements across a range of values that are difficult to measure directly (Hawker et al. 2011). The scale is a line of 100mm with its outer limits defined as two opposite extremes: no pain, and maximal pain. The downfall of this scale is its obvious subjectivity. A modified version of the VAS pain is commonly used by owners to assess their animal's pain in veterinary research (Anna K. Hielm-Björkman 2011). Evaluations for pain, mood, behavior, and lameness in a VAS format have been used by dog owners. At least one of these questionnaires has been tested for reliability and validity and was found to be

psychometrically sound. Primary outcome data for the 2007 study was available for 93 patients (Kumar et al. 2007). At six months, 48% of SCS patients achieved over 50% leg pain relief, compared to the 9% of patients who experienced relief in the CMM group. SCS group patients reported a higher quality of life, function and treatment satisfaction than their CMM counterparts. Similar results were reported at 12 months. A study published in 2014 demonstrated the success of tonic SCS in treating painful diabetic neuropathy (PDN) with tonic SCS (de Vos et al. 2014). Six months following implantation, 65% of patients reported significant pain reduction. Ninety-five percent of patients who were implanted with SCS devices said they would recommend the therapy to other patients with PDN. Patients with PDN undergoing SCS were also found to significantly reduce their analgesic intake, when intake before implantation was compared with intake at their six-month follow-up appointment. Tonic SCS has also been found to be successful in the treatment of complex regional pain syndrome when accompanied by physical therapy as opposed to exclusive physical therapy (Atkinson et al. 2011). The same study reported that at three and five year follow up appointments, complex regional pain syndrome patients described a reduction in SCS treatment effectiveness. Despite this, most patients were still satisfied with their treatment. Atkinson *et al.* asserted that tonic SCS has also reportedly been used for refractory angina pectoris and peripheral ischemic limb pain.

In terms of chronic back and leg pain, burst SCS has been found to be a more effective treatment option than tonic SCS. In one study, 91% of patients reported a preference for burst SCS compared to traditional SCS (Courtney et al. 2015). Similar results were found in a 2013 study by De Ridder and colleagues (De Ridder et al. 2013). Additionally, the researchers found that burst SCS reduced VAS for pain scores better than tonic SCS and placebo stimulation. HF-10 SCS is safe and effective in the long-term treatment of chronic low back and leg pain (Al-Kaisy et al. 2014). Furthermore, HF-10 SCS reduces low back VAS for pain scores to a greater degree than tonic SCS (Bicket et al. 2016). Similar results have been found for leg VAS for pain, although the difference in pain reduction between HF-10 and tonic SCS is smaller. It has been demonstrated that in terms of HF SCS, frequencies of 10kHz and 1kHz are equally effective in pain relief, with 1kHz stimulation requiring less charge and ultimately a reduced charging burden on patients (Thomson et al. 2018). The effectiveness of burst and HF-10 SCS for other chronic pain conditions, such as those outlined above, has not been researched extensively.

The human nervous system is incredibly complex. Dissecting the elaborate interplay between sensory functioning, nociceptive signaling, mediating neurotransmitters, pain relief due to SCS, and the reduction in SSEPs during SCS will be difficult. Further research is needed to supplement current knowledge regarding the pain-relieving mechanisms of all three SCS modalities, their effects on SSEPs, and the neurochemical events that may link these processes together.

1.1.8 Porcine model for the study of human pathologies

Animals models are valuable for understanding disease progression and testing disease therapies (Bassols et al. 2014). Bassols *et al.* advise that animal models must enable research to meet several guidelines: standardization, reproducibility, and the ability to translate research among species and research environments. The porcine model is a suitable medical model for humans due to many features. Among these are their similar size, physiology, and disease progression, presence of stable cell lines, and effective transgenic, proteomic, genomic, and cloning technologies. Although the use of pigs for research can often be accompanied by increased cost, husbandry requirements, and smaller sample sizes (Eric M. Walters 2013), porcine models allow for interdisciplinary research involving several organs and tissues, as well as collection of multiple samples, when compared to rodent models (Bassols et al. 2014). The development of the central nervous system (CNS), as well as the cortical surface area, weight, volume, myelination, and electrical activity of the pig brain are more similar to humans, when compared to other non-primate models (Lind et al. 2007). This makes the porcine model advantageous for neuroscience research.

In terms of pain-related SCS, research to date has favoured rodent models over large-animal models. Recently, however, swine models have begun to be used extensively in neuroscience research (Lind et al. 2007). A review by Lind *et al*. for example, states that the porcine model appeared four times more often between 1996 and 2005 than the decade before. In 2013, a project used a porcine model to study the effect of spinal cord stimulation on coordinated stepping and weight bearing following spinal cord injury (Hachmann et al. 2013). Another study, in a porcine model of cardiac failure, applied thoracic SCS to observe its effects on the cardiac sympathetic system and ventricular function (Liao et al. 2015). Another project in the cardiology field looked at the effect of SCS on ventricular arrythmias in a porcine model of acute ischemia

(Howard-Quijano et al. 2017). Current literature related to pain, SCS, and SSEPs in a porcine model is non-existent.

1.2 Objectives and hypotheses

Objective 1. To study the relationship between somatosensory evoked potential (SSEP) amplitude and tonic, burst, and HF spinal cord stimulation (SCS) in a porcine model.

Hypothesis 1A. In agreement with previously published findings, I posit that tonic SCS will reduce the amplitude of SSEPs (Bantli et al. 1975; Doerr et al. 1978; Larson et al. 1974).

Hypothesis 1B. Both burst and HF SCS will have a similar reductive effect on the amplitude of SSEPs. The HF portion of this hypothesis is corroborated by a single recent publication (Buonocore and Demartini 2016), and the burst portion of this hypothesis is based on preliminary data drawn from my honors project (Norton 2017).

Objective 2. To study the relationship between somatosensory evoked potential (SSEP) amplitude and ultra low-frequency (ULF) spinal cord stimulation (SCS), a novel SCS modality, see sections 1.3, 2.2.1.3, and 2.4.1.3.

Hypothesis 2. ULF SCS will also have a reductive effect on SSEP waveform amplitude.

Objective 3. To study the effect of tonic, burst, HF, and ULF spinal cord stimulation (SCS) on somatosensory evoked potential (SSEP) latency.

Hypothesis 3A. Tonic, burst and HF SCS will increase SSEP latency.

Hypothesis 3B. ULF SCS will have a lengthening effect on SSEP latency.

1.3 Significance

The significance of spinal cord stimulation (SCS) as a pain treatment is undeniable. It is important that the mechanisms whereby SCS relieves pain are fully understood. The reduction in SSEPs that has been observed during some SCS modalities may or may not play a role in the pain-relieving mechanism of SCS treatments. For this reason, further investigation into the effect of tonic, burst, and HF SCS on SSEP amplitude and latency is necessary. My project aimed to investigate this effect, to provide a more robust understanding of the role of SSEPs in the functional mechanisms of SCS modalities. A secondary goal of my project was to study a potential new SCS modality, ULF SCS, whose exact stimulation parameters are described in chapter 2. Since HF SCS occurs at a frequency that is beyond detection by the cells, it has been hypothesized that the reason it is effective is that it leads to the depolarization of the cells and

therefore deactivation of the ion channels themselves (Van Buyten et al. 2013). Therefore, ULF SCS may be another way of achieving the same effect as HF SCS, with lower energy requirements. The lower energy requirements of ULF SCS could provide superiority of implants in terms of battery life, thereby conferring greater convenience to its users. ULF SCS (or any similar SCS modality) has not been explored to date in a peer-reviewed publication, and therefore its potential effects on the nervous system are unknown. The development of new pain-relieving therapies is always in demand to successfully treat patients experiencing pain who are non-responsive to other treatment options. ULF SCS demonstrating a similar effect on SSEPs as the three SCS modalities currently in use would be promising and could indicate ULF SCS's suitability for future research into its potential pain-relieving effects.

Chapter 2: Methods

2.1 Ethics statement

All animals were treated according to guidelines of the Canadian Council for Animal Care (CCAC) under the supervision of the University of Saskatchewan Committee on Animal Care and Supply under the animal protocol approval number 20190093. Animals were physically inspected for any signs of disease or injury. Food (not water) was withdrawn for 8 hours before anesthesia to reduce the risk of aspiration of stomach contents. Animals were transported to the surgical suite following the University Animal Care Committee (UACC) approved SOP Z101.

2.2 Pilot methodology

A preliminary methodology was performed on two 10-week old Camborough/PIC Boar 327 pigs sourced from the herd at the University of Saskatchewan. Spinal cord stimulation modalities (1) – (3) were randomized for each animal, with (4) always being collected last, as this novel stimulation parameter could potentially have caused nerve/tissue damage.

Table 2-1. Pilot subject information

Subject Number	Subject Sex	Subject Weight (Kg)	1st Modality Recorded	2nd Modality Recorded	3rd Modality Recorded
1	Male	20	Burst	Tonic	HF
2	Male	20	HF	Burst	Tonic

2.2.1 Experimental procedure

2.2.1.1 Induction of anesthesia

The pigs were initially sedated with intramuscular ketamine (20mg/kg) and xylazine (2mg/kg). Once sedation was achieved, intravenous access was established. When anesthesia was induced, the pigs' tracheas were intubated with an appropriately sized endotracheal tube. Muscle relaxants were not used to facilitate nerve stimulation. Anesthesia was maintained with

isoflurane (end-tidal concentration 1.5-3%) in 100% oxygen. A multichannel physiological monitor was used to monitor electrocardiography, arterial oxygenation, heart rate, blood pressure (systolic, mean, and diastolic), respiratory rate, tidal volume, minute volume, end-tidal CO2, and expired isoflurane concentration to monitor the depth of anesthesia. Throughout the procedure, animal care technicians administered isotonic saline (0.9% saline) at a rate of 10ml/kg/hour to ensure the animals did not become dehydrated.

2.2.1.2 Surgical procedure

A midline incision was made at the thoracolumbar junction of each animal (Swindle 2007). The junction was found by counting the ribs. Blunt dissection was used to remove muscle and other soft tissues to expose the spinous processes and lamina (Figure 2-1) (Federici et al. 2012). These were removed using rongeurs and bleeding was controlled using a heat cautery (Fine Science Tools), Surgicel, and bone wax as appropriate. When the dura was fully exposed, an epidural stimulating electrode was slid onto the dura and connected to the stimulator set-up.

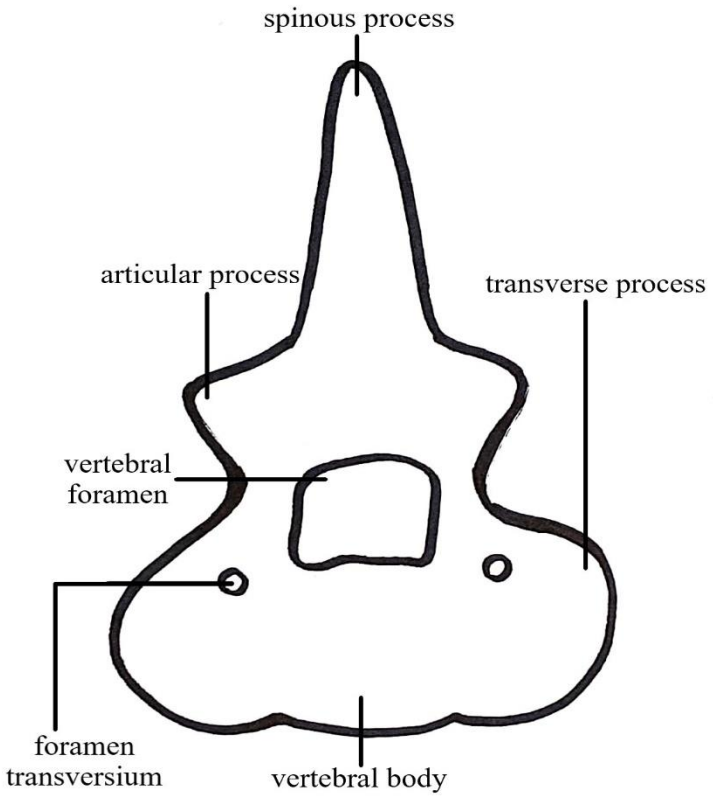

Figure 2-1. Labelled drawing of the structure of a single vertebra. The spinal cord runs through the vertebral foramen. The epidural space in the vertebral foramen contains fat.

2.2.1.3 Spinal cord stimulation (SCS) modality parameters

(1) Traditional, Tonic SCS – 50Hz, 200μs pulse width, and 5mA amplitude

(2) Burst SCS – 500Hz, 40Hz burst frequency, 1ms pulse width, 15ms inter-burst interval, and 5mA amplitude

(3) High-Frequency SCS – 1000Hz, 50μs pulse width, and 5mA amplitude

(4) Ultra Low-Frequency SCS – 1 Hz, 2ms pulse width, and 1mA amplitude

For traditional, high-frequency, and ultra low-frequency SCS, the frequency was set using a Digitimer pulse generator. This signal was channeled into a Digitimer DS7A which was used to set the pulse width and amplitude. The Digitimer DS7A was directly connected to the epidural stimulating electrode. For burst SCS, the burst pattern (frequency and pulse width) was coded for using the graphical sequence editor on CED Spike2 software. The sequence was then run from Spike to the CED Micro 1401-3 which converts the digital code to an analog signal recognized by the Digitimer DS7A. The amplitude dial on the Digitimer device was used to set the amplitude of the analog output to the epidural stimulating electrode. All modality parameters were tested prior to epidural stimulation using an oscilloscope (Figure 2-2). The parameters for

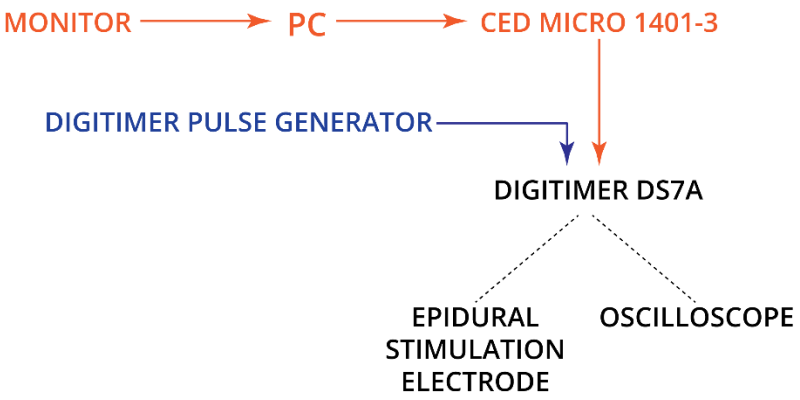

Figure 2-2. Flow chart of instrumentation used to generate spinal cord stimulation modalities. Traditional, HF, and ULF SCS (blue), and burst SCS (red). All modalities share instrumentation from the Digitimer DS7A onward.

ULF SCS were chosen based on the following observations: 1) currents with magnitudes lower than those required for creating action potentials can still lead to alterations in neural excitability and 2) stimulating certain neuronal areas with transcranial alternating current stimulation (tACS),

at frequencies below intrinsic oscillation frequencies for any given area, can slow down subsequent oscillations in that region (Zago 2016).

Based on current literature, it is likely that different SCS modalities successfully treat chronic neuropathic pain via different mechanisms (Miller et al. 2016). A review by Miller *et al.* maintains that the specific parameters of each SCS modality ensure that each modality administers different amounts of charge per second to the dura of the spinal cord. These differences in charge administered per second are likely why each modality activates different neurons, and to different ends. My primary interest in this experiment was the investigation of the clinical paradigms of SCS used to provide patients with adequate pain relief. I was not attempting to demonstrate that changes in any one parameter specifically caused changes in SSEP amplitude and latency, but rather that the clinical stimulation modalities as a whole were responsible for these changes. To this end, I chose not to equalize charge across the SCS parameters.

2.2.1.4 Collection of somatosensory evoked potentials (SSEPs)

The somatosensory evoked potential signals were recorded by electrodes and amplified through a Digitimer pre-amplifier, which in turn was connected to a Digitimer isolator. The signal was then passed through a Digitimer low-pass (30Hz) filter, then a Digitimer high-pass (3000Hz) filter (Nuwer 1994) before reaching the second CED micro 1401-3 and being subsequently collected and stored in CED Spike2 software for PC (Figure 2-3).

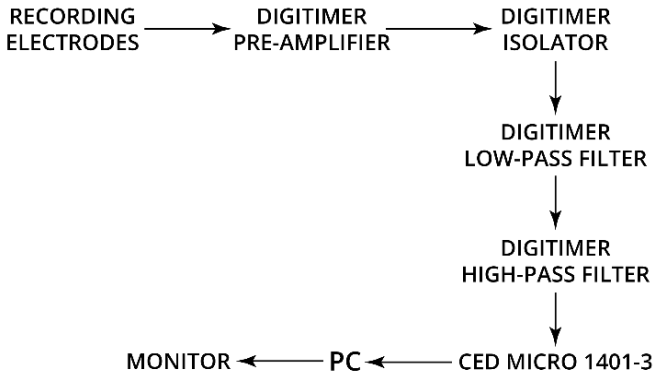

Figure 2-3. Flow chart of instrumentation used to collect somatosensory evoked potentials (SSEPs)

One ground electrode was placed into the tissue above the tail bone of each animal, since it is traditional to designate a boney prominence as an electrically neutral zone (Yves Blanc

2010). The ground electrode ultimately allowed for the cancellation of any electrical ‘noise’ so that electrical potentials in the areas of interest could be accurately measured (Moller 2006). An epidermal stimulation electrode was placed close to the right posterior tibial nerve and connected to a constant current stimulator (Digitimer multi-pulse stimulator D185). The nerve was stimulated with 500μs pulse widths, a maximum 40mA current, and a 400V voltage maximum at a frequency of 4Hz. A recording electrode was placed in the muscle of the leg (hindlimb) proximal to the stimulation electrode. Two additional recording electrodes were placed over the sensory cortex in both hemispheres. Recording electrodes placed in two locations allowed for analysis of SSEP amplitude and latency peripherally and supraspinally. All electrodes used for this portion, save the tibial nerve stimulating electrode, were Medtronic Xomed Inc. paired subdermal electrodes. A subdermal needle electrode was not compatible with the Digitimer D185 and was therefore replaced by the epidermal stimulation electrode solely for the tibial nerve stimulation portion.

While the posterior tibial nerve was being stimulated, SSEP amplitudes and latencies were recorded from a two-minute baseline control run where there was no SCS. Then in random order, SSEP amplitudes and latencies were recorded for two minutes each for modalities (1) – (3) by producing the different SCS modalities (refer to 2.2.1.3) and stimulating the posterior tibial nerve as outlined above. There was a 2-minute washout period between each modality, in which no SCS modality was used. During these washout periods, while stimulating the posterior tibial nerve, SSEP data was collected from additional two-minute control runs to observe if there were any lasting effects from the previous modality. After another two-minute washout and baseline recording period, the posterior tibial nerves were stimulated once again and the resulting SSEP data was recorded under the final modality (4) for two minutes. Lastly, a final two-minute baseline SSEP measurement was performed.

2.2.1.5 Euthanasia and disposal of animals

Animals were euthanized via general anesthetic overdose (pentobarbital 100 mg/kg) after the experiment. Animals were used for additional research by other research groups following euthanasia. These groups were responsible for the proper disposal of the animals.

2.3 Pilot results

During our pilot experiments, we noted an increase in natural vocalization in both subjects before initial sedation, which is a sign of acute stress in pigs (Marko A.W.Ruis 2001). In

pigs, avoiding /hiding from a threat or facing up to it is considered normal behavior, whereas repetitive behaviors with no apparent purpose are considered abnormal (Silvia Martinez-Miro 2016). We did not observe any abnormal behaviors in either subject. We did not perform an evaluation to assess the subjects' stress levels before sedating the animals.

Table 2-2. SSEP amplitudes (μV) for various SCS modalities

	SSEP Amplitude (μV)			
	Subject 1		Subject 2	
Initial Baseline		1.4		1.6
1st Randomized Modality	Modality: Burst	0.6	Modality: HF	0.4
Washout 1		1.3		1.5
2nd Randomized Modality	Modality: Tonic	0.52	Modality: Burst	1.5
Washout 2		1.3		1.6
3rd Randomized Modality	Modality: HF	0.8	Modality: Tonic	0.6
Washout 3		1.3		1.56
ULF SCS		0.65		0.5
Final Baseline		1.6		1.5

In subject 1, the SSEP amplitude was reduced from the averaged baseline by 62% during tonic SCS, 57% during burst SCS, 42% during HF SCS, and 53% during ULF SCS. In subject 2, we observed SSEP amplitude reductions of 61% during tonic, 74% during HF, and 68% during ULF SCS. Interestingly, SSEP amplitude was only reduced by 4% from the averaged baseline during burst SCS in subject 2. A paired t-test with a significance level of 0.05 found that baseline amplitudes between subjects were not significantly different ($p=0.071$). These pilot results from the first two pigs are used for further analysis in *Chapter 3*, in combination with data from eight additional subjects that underwent a definitive methodology outlined below.

2.4 Definitive methodology

The following definitive methodology was performed on an additional eight, 10-week old Camborough/PIC Boar 327 pigs. An SCS modality (1) – (3) was randomly assigned to each subject animal.

Table 2-3. Subject information

Subject Number	Subject Sex	Subject Weight (Kg)	1st Modality Recorded	2nd Modality Recorded	3rd Modality Recorded
3	Female	28	Tonic	Burst	HF
4	Male	31	Tonic	HF	Burst
5	Male	35	Burst	HF	Tonic
6	Male	35	HF	Tonic	Burst
7	Male	35	Burst	Tonic	HF
8	Male	21	HF	Burst	Tonic
9	Male	21	Tonic	Burst	HF
10	Male	18	Tonic	HF	Burst

2.4.1 Experimental procedure

2.4.1.1 Induction of anesthesia

No changes were made to the anesthesia procedure outlined in 2.2.1.1.

2.4.1.2 Surgical procedure

No changes were made to the surgical procedure outlined in 2.2.1.2. An image representation of the dura exposure following the surgical procedure can be found below (Figure 2-4).

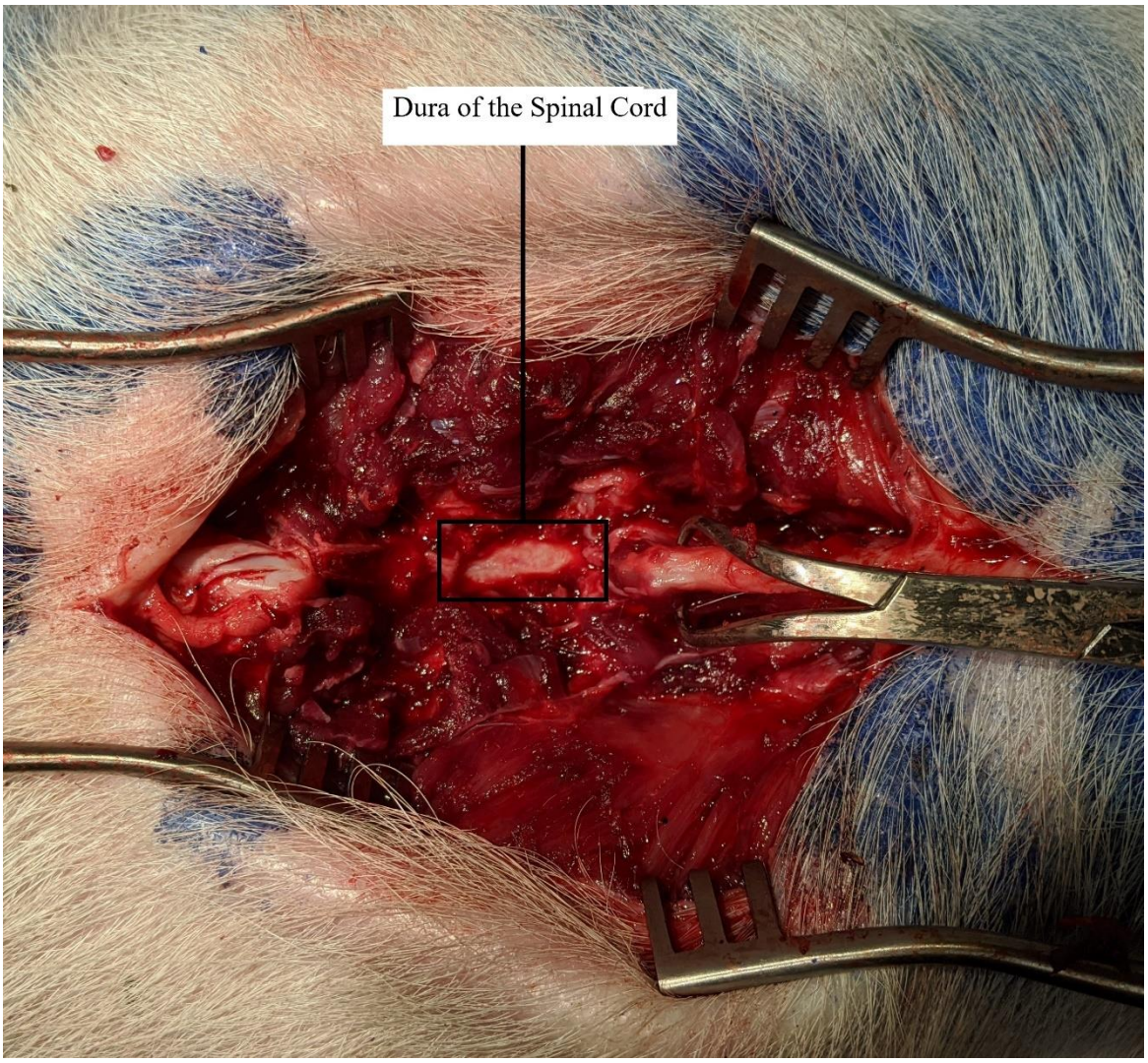

Figure 2-4. Image of surgical field. Image of subject #10 demonstrating the surgical field and exposure of the dura of the spinal cord during the experimental procedure.

2.4.1.3 Spinal cord stimulation (SCS) modality parameters (Figures 2-5, 2-6)

(1) Traditional, Tonic SCS – 50Hz, 300µs pulse width, and 5mA amplitude

(2) Burst SCS – 500Hz, 40Hz burst frequency, 1ms pulse width, 15ms inter-burst interval, and 5mA amplitude

(3) High-Frequency SCS – 1000Hz, 30µs pulse width, and 5mA amplitude

(4) Ultra Low-Frequency SCS – 0.5 Hz, 100ms pulse width, and 1mA amplitude

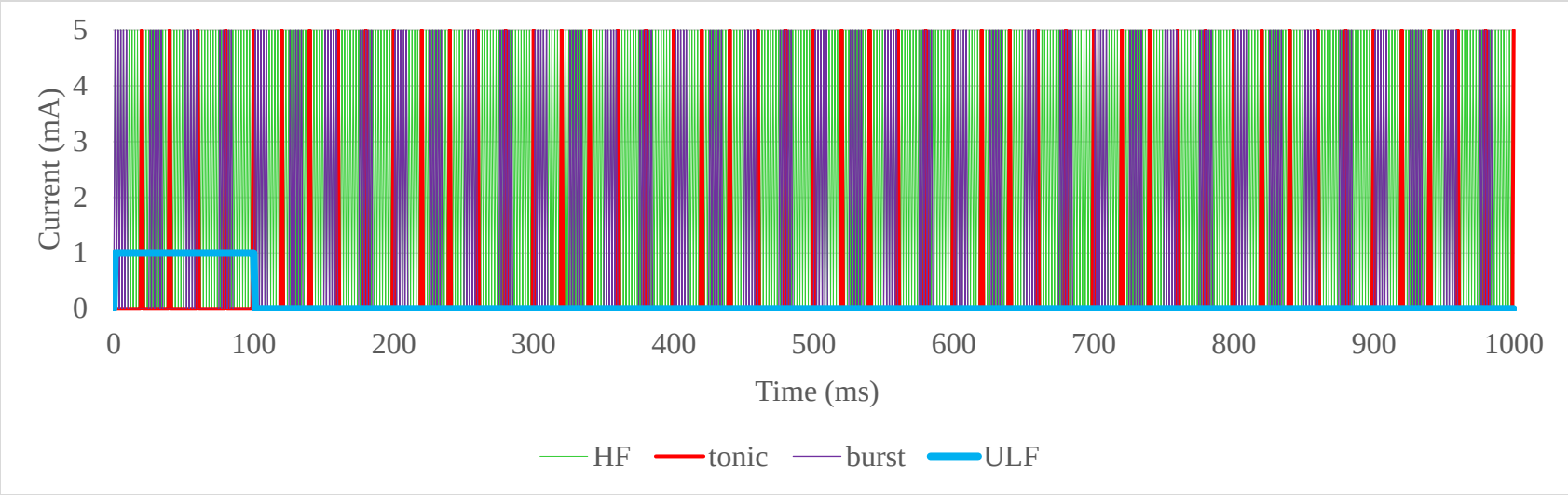

Figure 2-5. Comparison of HF, tonic, burst, and ULF SCS waveforms over a 1 second period. The current (mA) is displayed as a function of time (ms). The graph demonstrates the significant differences in parameters among the four types of SCS: HF (green), tonic (red), burst (purple), and ULF (blue).

Figure 2-6. Focused, to-scale view of the characteristic waveforms for the four types of SCS studied, demonstrating the significant variability between the modalities. Tonic SCS (red), burst SCS (purple), HF SCS (yellow), ULF SCS (blue). Written values for these parameters can be referenced at the start of 2.4.1.3.

The reader will note some underlined changes in SCS parameters (1), (3), and (4) from the pilot methodology. The pilot research demonstrated some clear obstacles that needed to be corrected in future experiments. Among these was the large amount[11] of electronic equipment required to create the SCS modalities, as well as the parameter limitations presented by the equipment interface designs. For example, the minimum frequency setting on the Digitimer Pulse Generator was 1Hz and the Digitimer DS7A had limited pulse width options (50µs, 100µs, 200µs, 500µs, 1000µs, and 2000µs). This meant that the parameters used for all modalities excluding burst (2) were shifted slightly from ideal clinical values to account for these equipment limitations. To remedy this, the same graphical sequence editor on CED Spike2 software that had been used for the burst modality (2) in the pilot research was used. Graphical sequences were created in CED Spike2 for all four SCS modalities which were then converted to text sequences. These text sequences were then run from the same CED Spike2 software to the CED Micro 1401-3 which converts the digital code to an analog signal recognized by the Digitimer DS7A. The amplitude dial on the Digitimer device was used to set the amplitude of the output to the epidural stimulating electrode.

The methodology was further optimized by reducing the number of electronic devices required. Using modality-respective text sequences generated by CED Spike2, while collecting SSEP data using the same software, the use of eight electronic devices (two PCs, two monitors, two mice, two keyboards) was reduced to the use of a single laptop. The use of a single laptop also meant that only a single CED Micro 1401-3 was required, using DAC outputs to create the SCS parameters and the ADC inputs to collect the SSEP data.

2.4.1.4 Collection of somatosensory evoked potentials (SSEPs)

The somatosensory evoked potential signals were collected via electrodes connected to Digitimer pre-amplifier, after which the amplified signal was passed through a Digitimer isolator. The signal was then passed through a Digitimer low-pass (30Hz) filter, then a Digitimer high-pass (3000Hz) filter (Nuwer 1994) before reaching the ADC inputs on the CED micro 1401-3 and being subsequently recorded and stored in CED Spike2 software for PC.

[11] More than 20 pieces of electrical equipment not including power bars, connecting cables, and electrodes

To further simplify the pilot methodology, a constant current nerve stimulator was engineered for our purposes and, when connected to an electrode, was used to stimulate the tibial nerve. Stimulation of the tibial nerve using this stimulator occurred at a frequency of 4Hz, with 300μs pulse widths, a 30mA current, and a 400V voltage maximum. By engineering a device specific to the experimental procedure, the need for the use of the Digitimer Pulse Generator and the Digitimer multi-pulse stimulator D185 was eliminated. The location and number of ground and recording electrodes were not changed from the pilot methodology. All electrodes used for this portion were Medtronic Xomed Inc. paired subdermal electrodes.

The rest of the procedure is identical to that outlined in the pilot methodology. The experimental timeline for SSEP collection is demonstrated below (Figure 2-7). Additionally, an instrumentation flowchart of the definitive methodology is presented below (Figure 2-8).

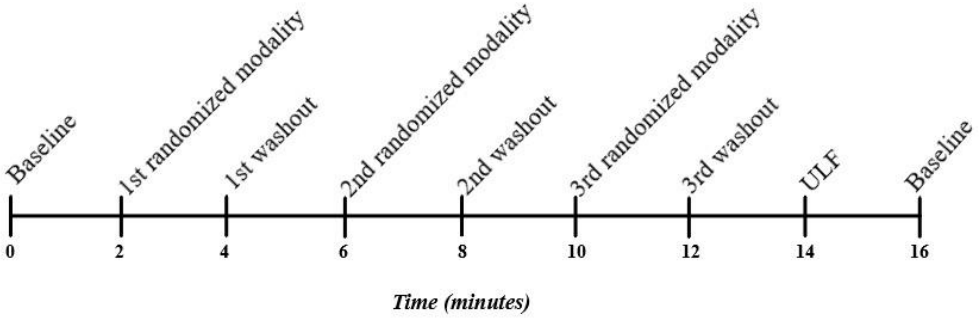

Figure 2-7. Experimental SSEP collection timeline. The tibial nerve was stimulated throughout the entire collection period. SCS parameters were turned on and off based on the order in the randomization protocol.

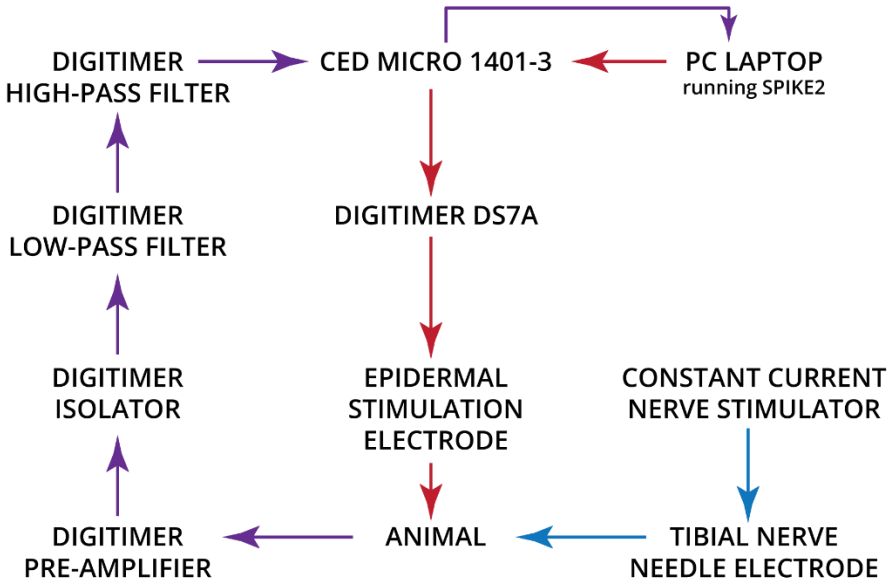

Figure 2-8. Instrumentation set-up used in the definitive methodology. Instruments for tibial nerve stimulation (blue), instruments used to create and apply spinal cord stimulation (SCS) modalities (red), and instruments required for the collection of somatosensory evoked potentials (SSEPs) (purple).

2.4.1.5 Euthanasia and disposal of animals

No changes were made to the euthanasia and disposal procedures outlined in 2.2.1.5.

2.4.1.6 Data analysis and handling

Data analysis was performed on ten subjects; two pigs underwent the pilot methodology, while the remaining eight underwent the definitive methodology. Raw SSEP data files were stored on a passcode-locked laptop PC stored in a locked safety cabinet in a locked laboratory.

Data was processed using the spike-sorting feature in CED Spike software which averaged all SSEPs collected over each two-minute run. This process ended in the output of a single SSEP waveform amplitude reading that represents this average. All waveform amplitude readings discussed hereafter refer to this spike-sorted output. Additionally, a single SSEP latency reading was also output through a similar averaging process. The initial 10µs time resolution for sampling at 100kHz was averaged by the CED Spike software during data collection to create an effective sampling rate of 1kHz and a time resolution of 1ms. At the time of data processing, the

120000 single millisecond samples from each two-minute collection were averaged once again to provide the final SSEP latency value. The latency values displayed henceforth are representative of this average.

SSEP amplitude and latency outputs from the spike sorting process were exported from CED Spike2 to Microsoft Excel 16. These amplitude and latency values were then stored and analyzed in either Microsoft Excel 16 or IBM SPSS 25 files on a secondary passcode-locked laptop. Graphing was done using either Microsoft Excel 16 of IBM SPSS 25. Raw data, Excel, and SPSS files will be appropriately archived following the successful defense of this thesis.

2.4.1.6.1 SSEP amplitude data analysis

In SPSS, I performed a descriptive analysis of the somatosensory evoked potential (SSEP) amplitudes from Table 3-3 to detect any potential outliers, the results of which can be found in Figure 3-1. The descriptive analysis also tested the homogeneity of variance and distribution of the SSEP amplitude data.

Statistical tests for SSEP amplitude analysis were performed on data from three categories: *responders*, *non-responders*, and *all.* SSEP amplitudes above 80%, meaning a reduction from the baseline average of less than 20%, were placed in the *non-responders* category. The rest of the data points were placed in the *responders* category. The *all* category was made up of all data points.

To compare SSEP amplitudes across all four conditions (SCS modalities) and all ten subjects, a repeated measures analysis of variance was performed in SPSS. This allowed for the comparison of the amplitudes both within and between the subjects. Additionally, for more specificity, paired t-tests were performed on all possible SCS modality combinations to provide pairwise comparisons. Results from this analysis can be seen in Table 3-6.

To determine if any of the four SCS conditions caused a significant change in amplitude, paired t-tests were performed comparing SSEP amplitudes to baselines for each SCS condition.

Baseline values were not associated with the SCS modalities, and as such, baseline statistical analyses were not broken down into *responders*, *non-responders*, and *all* categories. A repeated measures analysis of variance was performed on the five baseline/washout amplitude readings across all ten subjects.

2.4.1.6.2 SSEP latency data analysis

Using SPSS, a descriptive analysis was performed on the SSEP latencies represented as percentages of averaged baselines found in Table 3-5.

To compare the SSEP latencies across all four conditions (SCS modalities) and all ten subjects, I performed a repeated measures analysis of variance using SPSS. This allowed for a comparison both within and between the subjects.

Paired sample t-tests were performed using excel to compare the SSEP latency changes to baseline SSEP latencies for each subject and all four SCS conditions.

A repeated measures analysis of variance was performed on the baseline SSEP latency values for all ten subjects. For added specificity, paired sample t-tests were performed on baseline latency values between all combinations of subjects.

Chapter 3: Results

3.1 Data

Similarly to the pilot results, all pigs demonstrated an increase in natural vocalization immediately prior to and in the early stages of sedation, which is not abnormal, and is associated with a short and finite period of acute minor stress, of no more than 5 minutes (Marko A.W.Ruis 2001). Furthermore, no other stress-induced behavioural indicators were noted in any of the remaining 8 animals (Silvia Martinez-Miro 2016).

SSEP amplitudes in microvolts can be observed in Table 3-1. Table 3-2 displays processed SSEP amplitudes in the form of baseline averages and standard deviations, as well as SCS modality-specific SSEP amplitude values per subject. Table 3-3 presents modality-specific SSEP amplitude values expressed as percentages of averaged baselines.

Table 3-4 displays processed SSEP latencies in the form of baseline averages and standard deviations, as well as SCS modality-specific SSEP latency values per subject. Table 3-5 presents modality-specific SSEP latency values that have been expressed as percentages of averaged baselines.

Table 3-1. SSEP amplitudes (μV) for randomized SCS modalities for subjects 1-10

Subject #	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
SSEP Amplitude (μV)										
Initial Baseline	1.4	1.6	5.6	9.7	8.7	10.2	6.5	7.7	6.8	9.8
1st Randomized Modality	0.6	0.4	3.2	2.8	8.1	2.3	3.1	4.3	2.5	3
Washout 1	1.3	1.5	5.5	9.7	8.7	10.1	6.6	7.7	6.8	9.8
2nd Randomized Modality	0.52	1.5	4.2	3.2	8.8	2.5	2.6	3.6	3.9	3.2
Washout 2	1.3	1.6	5.6	9.6	8.7	10.2	6.5	7.7	6.8	9.8
3rd Randomized Modality	0.8	0.6	3.8	3.6	5.3	2.9	2.8	1.5	2.7	4.6
Washout 3	1.3	1.56	5.5	9.5	8.8	10.1	6.4	7.7	6.7	9.8
ULF SCS	0.65	0.5	4.5	3.7	4.6	2.6	3.2	2.9	3.1	3.6
Final Baseline	1.6	1.5	5.5	9.5	8.6	10.1	6.4	7.6	6.7	9.8

Table 3-2. SSEP amplitudes (μV) for various SCS modalities for subjects 1-10

Subject #	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
SSEP Amplitude (μV)										
Averaged Baseline	1.38	1.55	5.54	9.60	8.70	10.14	6.48	7.68	6.76	9.80
Baseline Standard Deviation	0.13	0.05	0.05	0.10	0.07	0.05	0.08	0.04	0.05	0.00
Tonic	0.52	0.6	3.2	2.8	5.3	2.5	2.6	1.5	2.5	3
Burst	0.6	1.5	4.2	3.6	8.1	2.9	3.1	3.6	3.9	4.6
HF	0.8	0.4	3.8	3.2	8.8	2.3	2.8	4.3	2.7	3.2
ULF	0.65	0.5	4.5	3.7	4.6	2.6	3.2	2.9	3.1	3.6

Table 3-3. SSEP amplitudes expressed as percentages of subject-specific averaged baselines. Representing subjects 1-10 and tonic, burst, HF, and ULF SCS.

Subject #	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
	Percentage of Average Baseline (%)									
Tonic	37.68	38.66	57.76	29.17	60.92	24.65	40.12	19.53	36.98	30.61
Burst	43.48	96.65	75.81	37.50	93.10	28.60	47.84	46.88	57.69	46.94
HF	57.97	25.77	68.59	33.33	101.15	22.68	43.21	55.99	39.94	32.65
ULF	47.10	32.22	81.23	38.54	52.87	25.64	49.38	37.76	45.86	36.73

Table 3-4. SSEP latencies (ms) for various SCS modalities for subjects 1-10

Subject #	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
SSEP Latency (ms)										
Averaged Baseline	23.06	22.88	23.54	23.32	23.76	23.96	23.66	22.76	22.82	22.58
Baseline Standard Deviation	0.06	0.08	0.11	0.11	0.06	0.06	0.06	0.06	0.05	0.05
Tonic	23.1	22.9	23.4	23.2	23.8	23.8	23.7	22.7	22.8	22.5
Burst	23.2	23.0	23.4	23.2	23.8	23.9	23.6	22.7	22.9	22.5
HF	23.1	22.9	23.5	23.3	23.7	23.9	23.7	22.7	22.7	22.7
ULF	23.2	22.9	23.6	23.4	23.8	24.0	23.6	22.8	22.8	22.6

Table 3-5. SSEP latencies expressed as percentages of subject-specific averaged baselines. Represented for subjects 1-10 and tonic, burst, HF, and ULF SCS.

Subject #	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Percentage of Average Baseline (%)										
Tonic	100.17	100.09	99.41	99.49	100.17	99.33	100.17	99.74	99.91	99.65
Burst	100.61	100.52	99.41	99.49	100.17	99.75	99.75	99.74	100.35	99.65
HF	100.17	100.09	99.83	99.91	99.75	99.75	100.17	99.75	99.47	99.53
ULF	100.61	100.09	100.25	100.34	100.17	100.17	99.75	100.18	99.91	100.09

3.2 SSEP amplitude results

3.2.1 Descriptive analysis

Three outliers were detected: cases 6, 18, and 19 (Figure 3-1). Case 6 represents the burst modality in pig #2, case 18 represents the burst modality in pig #5, and case 19 represents the high frequency (HF) modality in pig #5. The burst modality in pig #2 was the 2nd randomized modality. The burst modality in pig #5 was the 1st randomized modality, while the HF modality in pig #5 was the 2nd randomized modality. There are were not enough outlier points or a large enough sample size to determine if the randomization order of either pig #2 or pig #5 was responsible for the presence of outliers. However, pig #8 had the same randomization protocol as pig #2 and did not demonstrate any outlier amplitude values, suggesting that a randomization order of HF, burst tonic is not responsible for the outlier value. Unfortunately, no other subject received the same randomization protocol as pig #5, so a similar comparison was not possible. Due to the outlier amplitude values in pig #5, having another experimental subject undergo the same randomization protocol would have been warranted. Due to university and laboratory closures related to the COVID-19 pandemic however, another experiment was not possible. The

presence of these outliers will be further discussed in chapter 4. A Levene's test of the data set demonstrated homogeneity of variance ($\alpha = 0.05$; $W(3,36) = 1.79$; $p = 0.167$). A Shapiro-Wilk's test demonstrated that the data set was normally distributed [$\alpha = 0.05$, tonic ($W(10) = 0.92$; $p = 0.363$), burst ($W(10) = 0.88$; $p = 0.136$), HF ($W(10) = 0.89$; $p = 0.190$), ULF ($W(10) = 0.87$; $p = 0.112$)].

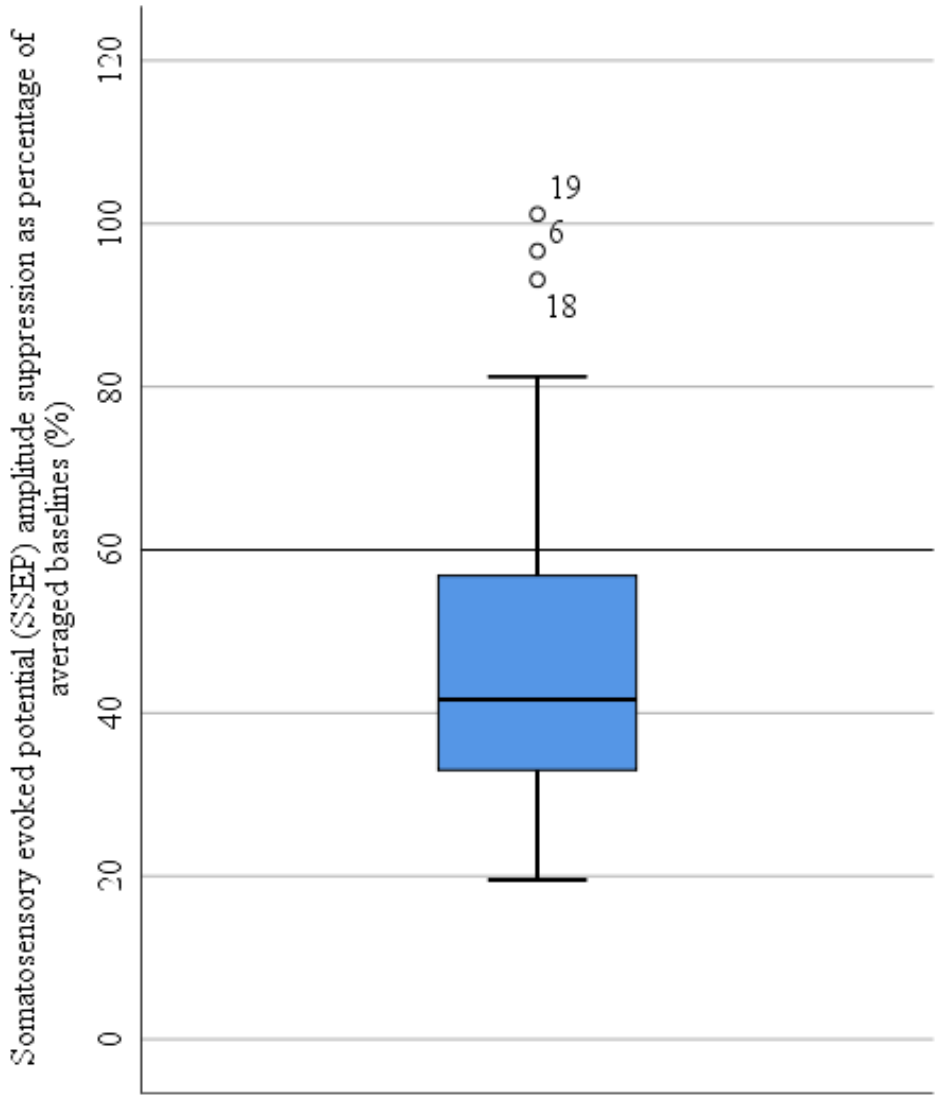

Figure 3-1. Box and whiskers plot of somatosensory evoked potential (SSEP) amplitudes expressed as percentages of averaged baselines. The horizontal line within the box indicates the median, the boundaries of the box indicate the 25th- and 75th -percentiles, and the whiskers indicate the highest and lowest values of the results. The outliers are indicated as open circles with corresponding case numbers located next to each open circle.

3.2.2 Repeated measures analysis of variance – SSEP amplitude means

In the *all* category, with outliers included, the repeated measures analysis of variance indicated that at least two of the SSEP amplitude means between the four SCS modalities were significantly different from each other ($\alpha = 0.05$; $F(3) = 3.96$; $p = 0.018$) (Figure 3-2a). The same analysis for the *responders* category yielded similar results, once again showing that at least two of the SSEP amplitude means between the four conditions were significantly different ($\alpha = 0.05$; $F(3) = 6.06$; $p = 0.004$) (Figure 3-2b). The same analysis for the *non-responders* category was not possible due to the limited nature of non-responder data points. No comparison between the two non-responder conditions, burst and HF, was possible due to the presence of only a single data point in the HF condition.

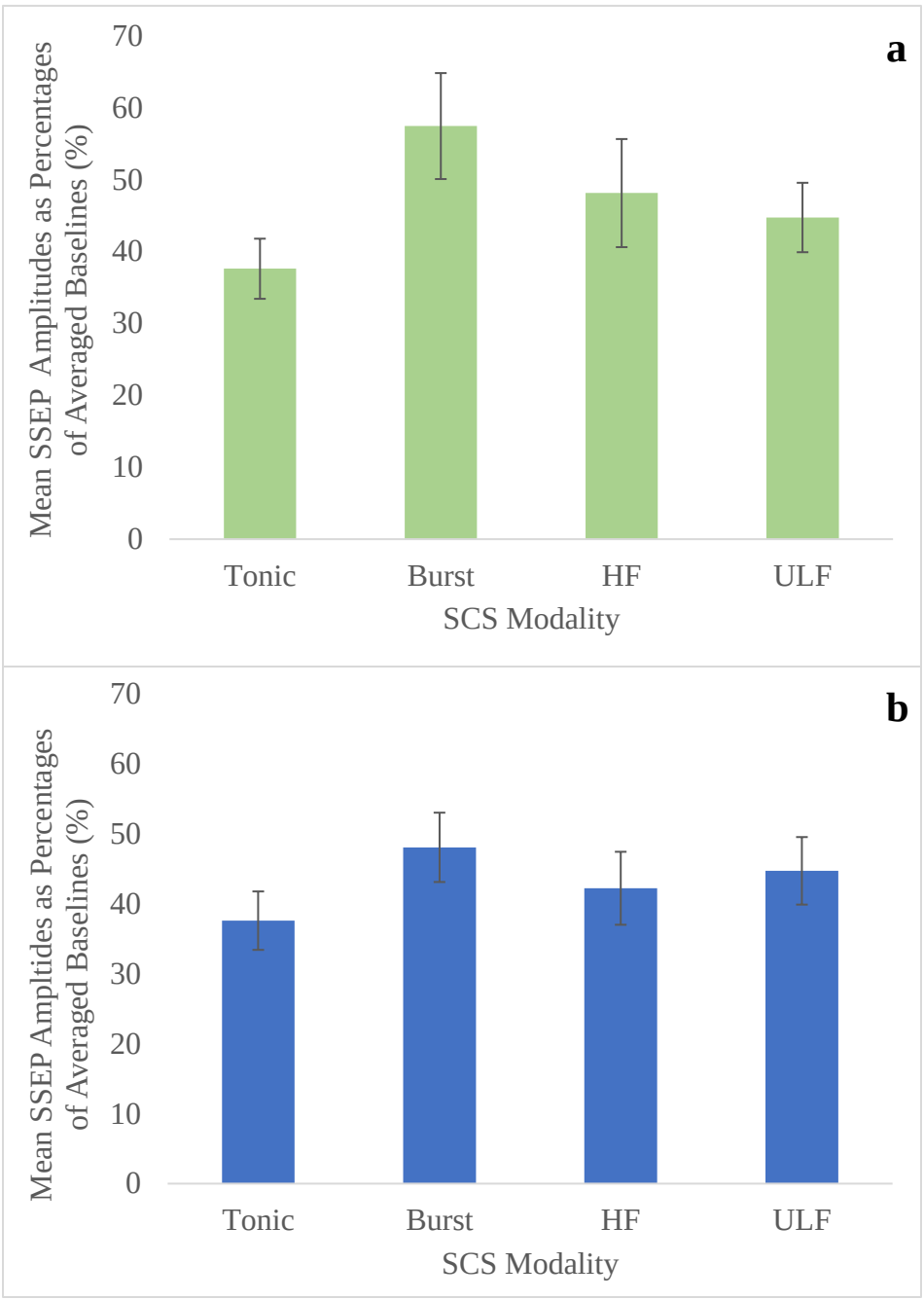

Figure 3-2. Bar charts of SSEP amplitude means. Bar charts demonstrating mean SSEP amplitudes as percentages of averaged baselines for tonic, burst, HF, and ULF SCS for the *all* category (a) and the *responders* category (b). Error bars represent the standard error of the mean

3.2.3 Paired sample t-tests – SSEP amplitudes

SSEP amplitude means between tonic and burst modalities were significantly different in both the *all* ($\alpha = 0.05$; $t(9) = -3.83$; $p = 0.004$) and *responders* ($\alpha = 0.05$; $t(7) = -4.61$; $p = 0.002$) categories (Table 3-6).

Table 3-6. SSEP amplitude pairwise comparisons. Paired t-test results comparing SSEP amplitudes for all SCS modality combinations for *all* and *responders* categories. Highlighted SCS modality combinations represent significant interactions.

SCS modality combination	Paired t-test significance for *all*	Paired t-test significance for *responders*
Tonic and Burst	0.004	0.002
Tonic and HF	0.081	0.164
Tonic and ULF	0.047	0.047
Burst and HF	0.250	0.355
Burst and ULF	0.107	0.277
HF and ULF	0.570	0.636

Similarly, the SSEP amplitude means between tonic and ULF modalities are significantly different in both *all* and *responder* categories ($\alpha = 0.05$; $t(9) = -2.30$; $p = 0.047$). The remaining interactions demonstrate that the means for the rest of the modality combinations do not differ significantly from each other in either category ($\alpha = 0.05$, $p > \alpha$). As stated previously, no t-test for the *non-responders* category was possible.

3.2.4 Paired sample t-tests – change from baseline

For the *all* category, the data demonstrates that all four SCS modalities significantly reduced SSEP amplitude from each subject's average baseline [$\alpha = 0.05$, tonic ($t(9) = -14.93$; $p =$ 1.18E-7), burst ($t(9) = -5.77$; $p =$ 2.00E-4), HF ($t(9) = -6.90$; $p =$ 7.06E-5), ULF ($t(9) = -11.44$; $p =$ 1.15E-6)]. For the *responders* category, the data demonstrates that all 4 SCS modalities significantly reduced SSEP amplitude from each subject's average baseline [$\alpha = 0.05$, tonic ($t(9) = -5.77$; $p =$ 1.18E-7), burst ($t(7) = -10.47$; $p =$ 1.58E-5), HF ($t(8) = -11.06$; $p =$ 3.97E-6), ULF ($t(9) = -11.44$; $p =$ 1.15E-6)]. For the *non-responders* category, the data demonstrated no significant decrease in SSEP amplitude from average baseline for the burst modality ($\alpha = 0.05$, $t(1) = -2.89$; $p = 0.212$). The same analysis was not possible for the HF modality, with only one

data point, but the 1.15% difference observed from baseline during HF SCS for the single non-responder does not appear to be significant.

3.2.5 Repeated measures analysis of variance – baselines

Only the baseline readings from Pig #1 and Pig #2 were not significantly different from each other ($\alpha = 0.05$; $t(4) = -2.44$; $p = 0.071$). The rest of the baselines were significantly different from each other ($\alpha = 0.05$, $p < \alpha$).

3.3 SSEP latency results

3.3.1 Descriptive analysis

The analysis did not detect any outliers. A Shapiro-Wilk's test demonstrated that the data was normally distributed [$\alpha = 0.05$, tonic ($W(10) = 0.88$; $p = 0.133$), burst ($W(10) = 0.90$; $p = 0.204$), HF ($W(10) = 0.95$; $p = 0.703$), ULF ($W(10) = 0.96$; $p = 0.772$)]. A Levene's test of the data demonstrated homogeneity of variance based on the median ($\alpha = 0.05$; $W(3,36) = 1.34$; $p = 0.276$), but a lack of homogeneity based on the mean ($\alpha = 0.05$; $W(3,36) = 3.26$; $p = 0.033$). Two more robust equality of means tests were also performed. The Welsh's test demonstrated equality of means ($\alpha = 0.05$; $W(3,19.6) = 2.55$; $p = 0.085$), as did the Brown-Forsyth's test ($\alpha = 0.05$; $W(3,30.1) = 1.82$; $p = 0.166$). Despite the failure of the data to demonstrate homogeneity of variance based on the mean, I chose to move forward with the repeated measures analysis of variance. With such a small sample size, one point can easily sway the homogeneity of variance results, and this may have been the reason for the failure of the data to pass the Levene's test based on mean distribution.

3.3.2 Repeated measures analysis of variance – latency means

The test indicated that the SSEP latency means between the four SCS modalities were not significantly different from each other ($\alpha = 0.05$; $F(3) = 2.23$; $p = 0.108$).

3.3.3 Paired sample t-tests – change from baseline

The data demonstrated that none of the four SCS modalities significantly changed SSEP latencies from each subject's average baseline [$\alpha = 0.05$, tonic ($t(9) = -1.78$; $p = 0.108$), burst ($t(9) = -0.42$; $p = 0.683$), HF ($t(9) = -0.61$; $p = 0.555$), ULF ($t(9) = 2.11$; $p = 0.064$)].

3.3.4 Repeated measures analysis of variance – baselines

Baseline SSEP latencies were significantly different between at least two subjects ($\alpha = 0.05$; $F(9) = 226$; $p = 0.0001$).

3.3.5 Paired t-tests – baselines

The majority of pairwise combinations yielded p-values below 0.05, indicating that those subject combinations had baseline latency values that were significantly different from each other ($\alpha = 0.05$). The following pairwise combinations had baseline latency values that were not significantly different from each other ($\alpha = 0.05$): pig #2 and pig #8 ($t(4) = 2.45$; $p = 0.070$), pig #2 and pig #9 ($t(4) = 1.50$; $p = 0.208$), pig #3 and pig #7 ($t(4) = -2.45$; $p = 0.070$), pig #5 and pig #7 ($t(4) = 2.24$; $p = 0.089$), and pig #8 and pig #9 ($t(4) = 2.45$; $p = 0.070$).

Chapter 4: Discussion

4.1 Major findings

The results of this project demonstrate that SSEP amplitudes were reduced from baseline for all four SCS modalities and that SSEP latencies were unchanged from baseline values for all four SCS modalities. Thirty-seven of the 40 SSEP amplitude data points were placed in the *responders* category, compared with three outliers that were placed in the *non-responders* category. The data from this project demonstrated that there was a significant decrease in SSEP amplitude from baseline averages for all four SCS modalities [$\alpha = 0.05$, tonic ($t(9) = -14.93$; $p = 1.18E-7$), burst ($t(9) = -5.77$; $p = 2.00E-4$), HF ($t(9) = -6.90$; $p = 7.06E-5$), ULF ($t(9) = -11.44$; $p = 1.15E-6$)]. SSEP amplitude baselines were significantly different from each other ($\alpha = 0.05$; d.f. 4; $p < 0.05$), except between pig #1 and pig #2 ($\alpha = 0.05$; $t(4) = -2.44$; $p = 0.071$). SSEP latencies were not significantly changed from baseline for any of our studied modalities [$\alpha = 0.05$, tonic ($t(9) = -1.78$; $p = 0.108$), burst ($t(9) = -0.42$; $p = 0.683$), HF ($t(9) = -0.61$; $p = 0.555$), ULF ($t(9) = 2.11$; $p = 0.064$)]. No SSEP latency outliers were detected. Latency baselines between subjects were significantly different from each other for the most part ($\alpha = 0.05$; d.f. 4; $p < 0.05$), except for a the following pairwise combinations ($\alpha = 0.05$): pig #2 and pig #8 ($t(4) = 2.45$; $p = 0.070$), pig #2 and pig #9 ($t(4) = 1.50$; $p = 0.208$), pig #3 and pig #7 ($t(4) = -2.45$; $p = 0.070$), pig #5 and pig #7 ($t(4) = 2.24$; $p = 0.089$), and pig #8 and pig #9 ($t(4) = 2.45$; $p = 0.070$). This project was the first to introduce ULF as a new SCS parameter. ULF SCS had a comparable effect on SSEPs as the other three SCS modalities, suggesting that it may also have similar pain-relieving effects. ULF SCS should be further studied in this capacity, first in animal models, to provide a new, effective SCS treatment option to patients suffering from chronic pain. In line with clinical occurrence in human patients implanted with SCS devices of various modalities, some of the subjects did not respond to specific types of SCS. Additionally, the majority of baseline SSEP amplitude and latency readings were significantly different between the subjects. Those two most recent points will be further discussed in 4.2.4 Clinical considerations. The porcine model used in this project proved to be valuable for SCS research. The size and anatomy

of the animals meant they were ideal for studying SCS using the same devices and electrode leads that would be used clinically in humans.

4.2 Effect of spinal cord stimulation (SCS) on somatosensory evoked potentials (SSEPs)

A recent study found that tonic, burst, and HF SCS caused a decrease in the amplitude of laser evoked potentials in human patients, with burst causing a significantly larger reduction (Bocci et al. 2018). The same study found that burst SCS also caused a significantly longer latency that the other two SCS modalities, while tonic and HF SCS did not significantly change latency from baseline.

4.2.1 SSEP amplitude

The results of my investigation demonstrate that in the *all* category, tonic SCS reduced the waveform amplitudes of SSEPs to an average 37.6±13.2% of subject-specific averaged baselines in the porcine model. This reduction value was maintained in the *responders* category. There were no non-responders to tonic SCS in this project. These results are in agreement with previous research in humans (Bentley et al. 2016). Also, in line with a previously published case study on a human female (Buonocore and Demartini 2016), the porcine results corroborate that in the *all* category, HF SCS also causes a significant reduction in the waveform amplitudes of SSEPs to an average of 57.5±23.3% of each subject's averaged baseline. This percentage became 48.1±14.0% in the *responders* category. Pig #5 was a HF non-responder and demonstrated an increase in SSEP amplitude of 1.15% during HF SCS. Furthermore, my results demonstrate that in the *all* category, burst SCS also reduced the waveform amplitudes of SSEPs in pigs to an average of 48.1±23.8% of subject-specific averaged baselines. The SSEP amplitude reduction percentage was 42.2±15.7% in the *responders* category. The burst modality contained two *non-responders*, pig #2 and #5, whose SSEP amplitudes were reduced to 94.9±2.5% of subject-specific averaged baselines. Lastly, the results in the porcine model show a significant decrease in SSEP amplitude during the novel ULF SCS to an average 44.7±15.3% of each subject's averaged baseline in the *all* category. The *responders* category shared the same percentage. There were no non-responders to the ULF SCS parameter. The relevance of the three outliers discussed in this section will be further explored in 4.2.4 Clinical considerations.

The SSEP amplitudes (expressed as percentages of each subject's averaged baseline) were significantly different between two pairs in both the *responders* and *all* categories: tonic and burst SCS [*all* ($\alpha = 0.05$; $t(9) = -3.83$; $p = 0.004$), *responders* ($\alpha = 0.05$; $t(7) = -4.61$; $p =$

0.002)] and tonic and ULF SCS ($\alpha = 0.05$; $t(9) = -2.30$; $p = 0.047$). The rest of the possible pairs were not significantly different from each other ($\alpha = 0.05$; $p > 0.05$). What this ultimately means is that the difference in SSEP reduction between the four modalities was negligible, except for the difference in reduction between tonic and burst, and tonic and ULF SCS. Additionally, this project found that baseline amplitude readings from every single subject were significantly different from each other ($\alpha = 0.05$; d.f. 4; $p < 0.05$), except for the baseline SSEP readings from pig #1 and #2, whose baselines were not significantly different from each other ($\alpha = 0.05$; $t(4) = -2.44$; $p = 0.071$). The impact of this will also be discussed in 4.2.4 Clinical considerations.

4.2.2 SSEP latency

No SSEP latency outliers were detected in this experiment. This project found that no significant changes occurred in SSEP latency in any of the four studied modalities. SSEP latencies were not significantly changed from baseline values [$\alpha = 0.05$, tonic ($t(9) = -1.78$; $p = 0.108$), burst ($t(9) = -0.42$; $p = 0.683$), HF ($t(9) = -0.61$; $p = 0.555$), ULF ($t(9) = 2.11$; $p = 0.064$)]. Interestingly, most baseline latency values were found to be significantly different from each other in a between-subjects analysis ($\alpha = 0.05$; d.f. 4; $p < 0.05$) except for the following pairwise comparisons ($\alpha = 0.05$): pig #2 and pig #8 ($t(4) = 2.45$; $p = 0.070$), pig #2 and pig #9 ($t(4) = 1.50$; $p = 0.208$), pig #3 and pig #7 ($t(4) = -2.45$; $p = 0.070$), pig #5 and pig #7 ($t(4) = 2.24$; $p = 0.089$), and pig #8 and pig #9 ($t(4) = 2.45$; $p = 0.070$). The implications of these latter two points will be further discussed below.

4.2.3 Limitations

It is important to note that SSEP amplitudes and latencies can be affected by numerous variables, including but not limited to the quality of the nerve stimulation (Spiess et al. 2008), the placement of stimulating and recording electrodes in relation to each subject's anatomy, electrical interference, temperature, blood pressure, oxygenation levels, height , and types of anesthesia (Chu 1986; John P. Lubicky 1989; Mauguiere 1999). Ketamine, xylazine, and isoflurane (the anesthetic agents used in this project) have been found to affect SSEPs to various extents in different studies (Geoffrey Truchetti 2015; S.M. Hayton 1999; Usha Devadoss 2010). Since most anesthetics do have an effect on SSEPs, and the use of ketamine, xylazine and isoflurane do not impede the recording of reproducible SSEPs, the experiments performed for this thesis did use these agents. They were more readily available, the animal care technicians were familiar with their use, and their dosage was more easily titrated to effect than alternative anesthetics. All subjects received

the same dosage per weight of ketamine and xylazine, and the same % isoflurane in 100% oxygen throughout the procedures. Additionally, the height/length of the animals, blood pressure, oxygenation levels, and electrode placement varied, but were controlled for in the data analysis by calculating the percent change from baseline for amplitude and latency for each animal. SSEP amplitudes and latencies have also been found to be affected to varying degrees in different studies, by interference due to motor activity, external vibrations, or unintentional activation of sensory-proprio-receptors (Jones 1981; Phanor L. Perot Jr. 1983; V. Ibanez 1989). Once again, these variables were controlled for by calculating the percent change from baseline for amplitude and latency for each animal. Additionally, their effects were minimized by avoiding physical contact between researchers and the subjects during SSEP collection, lack of motor interference due to sedation, and environment standardization to reduce differences in vibrational interference between procedures. There is tremendous benefit to using baselines to standardize external factors, like those mentioned above, by using each animal as their own control, especially in smaller sample sizes. The only anatomical limitation observed during the experiments was a smaller epidural space in the spinal canal of the pigs as compared to humans. Sample size limitations were also present due to increased cost, increased husbandry requirements, transportation logistics due to size, and animal sentience. (Eric M. Walters 2013; Faragli et al. 2020) Finally, one of the ten subjects was a female subject (pig #3). This was due to an error in the transfer of the subjects to the surgical facility. Unfortunately, due to the presence of only one female subject, a comparison in results between female and male subjects was not possible.

4.2.4 Clinical considerations

All four studied types of SCS were found to reduce SSEP amplitudes in the porcine model. Clinically, this has been observed in patients exposed to tonic and HF SCS. Unpublished data from the Norton laboratory has also demonstrated that burst SCS also reduces wavelength amplitudes of SSEPs in human subjects (Norton 2017). This is the first time ULF SCS has been studied. It is a novel type of stimulation that, if used in humans in the future, could provide potential pain relief to patients with chronic pain who do not respond to other types of SCS.

Outlier data points found in the descriptive statistical analysis were occurrences where certain SCS modalities in certain subjects caused little to no SSEP amplitude reduction, which is contrary to the rest of the data. Although outliers would normally be a point of concern in small

sample sizes, the presence of outliers in this data is in line with clinical occurrences (Buonocore and Demartini 2016; Janssen et al. 2012; Larson et al. 1974; Song et al. 2014; Urasaki et al. 2014), where certain subjects do not respond to certain types of SCS for unknown reasons. A recent study has even suggested specifically using the effect of SCS on SSEPs as a screening tool for the probability of SCS implantation leading to successful pain relief in human patients (Urasaki et al. 2014). It is therefore unsurprising that the same phenomenon is also demonstrated in the chosen porcine model.

In clinical practice, it is common for different individuals to have different baseline SSEP amplitude and latency values, especially while under anesthesia (John P. Lubicky 1989). The data collected in this project in a porcine model demonstrated similar results, wherein the majority of SSEP baseline amplitude and latency readings between subjects were significantly different from each other. Only one of 45 baseline amplitude interactions was deemed not significant. Only five baseline latency interactions of 45 were deemed not significant. This inter-subject amplitude and latency baseline variability is unlikely to be due to anesthesia, temperature, blood pressure, oxygenation levels, or quality of the tibial nerve stimulation, as these variables were very similar if not identical across all subjects. More likely, it was owing to the location of the recording needle electrodes in relation to each subject's anatomy, and the length/height of each porcine subject. It is also important to mention that in terms of SCS' effect on SSEP latency, current literature suggests that tonic SCS at least, leads to an increase in SSEP latency. My findings contradict this, in that no changes in SSEP latencies were observed during any of the four SCS modalities. It is also worth noting, however, that the extent to which latencies are increased, if at all, varies from study to study (Bentley et al. 2016), indicating that the results from this project were not entirely unexpected.

4.3 Porcine model for spinal cord stimulation (SCS) research

To my knowledge, this research is the first to use a porcine model for SSEP-related SCS research. Owing to the similar size and anatomy of the model to human subjects, the SCS equipment did not need to be miniaturized, allowing for a more direct comparison of SCS effects in pigs to potential effects in humans. I was able to use a typical clinical epidural stimulation electrode and SCS parameters that directly mimic the parameters used clinically in humans. Initial sedation was achieved easily with ketamine and xylazine and anesthesia was maintained

throughout surgery with isoflurane. The standard operating procedures used eliminated undue stress and pain to the animals. It must be noted that smaller pigs facilitated the surgical procedure. This was mostly owing to less tissue blocking access to the spinal cord in smaller subjects, thereby leading to a shallower surgical field. Additionally, the spinous processes and laminae of the vertebrae were more easily removed on the smaller subjects. Also note that the porcine model had significantly less fat covering the dura of the spinal cord (Prats-Galino et al. 2015) and a smaller spinal canal than humans(Bozkus et al. 2005; Busscher et al. 2010), which meant that sliding the epidural stimulating electrode rostrally into the vertebral foramena, a common practice in human SCS device implantation surgery (Richter et al. 2011; van Helmond et al. 2017), was made impractical. Porcine models are often used to study wound healing due to their similarity to humans, suggesting that the model would also be suitable for longer-term recovery based SCS studies (Philandrianos et al. 2012). No complications in SSEP collection were noted that were attributable to the use of this model. A pertinent limitation is that owing to the ethical (animal sentience) and logistical (increased cost, husbandry requirements, size) complexities of the porcine model accepted by the research community (Faragli et al. 2020), projects utilizing the model suffer from smaller sample sizes when compared with more traditional rodent research (Eric M. Walters 2013). By using the same specimen for two additional experiments following SSEP data collection, this project abided by ethical guidelines aimed at reducing, replacing, and refining the use of animals in research. It is important to remember that, with the use of any animal model, direct comparisons to humans cannot be made (Nestler and Hyman 2010). Model-based findings, however, can corroborate clinical evidence observed in human populations or suggest appropriate steps forward in human experiments and trials. This project has further solidified the role of the porcine model as a suitable and advantageous model for neurophysiology research.

4.4 Future inquiries

Unfortunately, owing to the non-recovery and animal-model nature of my project, I was not able to collect or analyze data on any chronic pain-related measures. Going forward, the four SCS modalities observed in my project should be compared as directly as is possible with regards to actual pain management. Comparisons have been made between tonic and burst SCS (Courtney et al. 2015; De Ridder et al. 2013), indicating that burst seems more effective in chronic pain relief. HF SCS has also been found to be more effective than tonic SCS in the

management of certain chronic pain disorders (Bicket et al. 2016). This is the first time ULF stimulation has been studied as a modality of SCS, and future experiments should look at its effect in human populations. Further research is also required into all four types of SCS to determine their effectiveness in a wider range of pain disorders. This could ultimately become a screening tool if and when certain pathologies do not respond effectively or consistently to SCS.

As was stated earlier, the exact mechanism of pain relief of each SCS modality is not yet clear. SCS experiments on animal models with collection of dorsal root ganglion neurons, the neurons primarily responsible for the transmission of pain and mechanical stimuli to the dorsal horn of the spinal cord (Carolyn M. Sawyer 2009), post-stimulation, would allow for the analysis of early gene products created as a result of the stimulation parameter (H.L Jameson 2001; Hong et al. 2009). A recent study looked at early gene products related to tonic SCS in a rodent model, but additional and similar studies are required looking at early gene products related to burst, HF, and ULF SCS in various animal models (Tilley et al. 2017). This type of analysis could help elucidate the precise mechanisms occurring intra-cellularly that enable the pain-relief observed with the various SCS modalities. Future research should seek to confirm antidromic A-β fiber and subsequent inhibitory 'gating' interneuron activation during tonic SCS, the decreased wind-up in the WDR pain-transmitting neurons, and the precise signaling molecules, in addition to GABA, that play a role in these processes. Continued work must be done to connect these neuronal activation patterns and the pain-relief observed in patients deemed responsive to tonic SCS. It is also important that future study elucidates the signaling pathways that cause pain relief in the HF and burst SCS conditions. In the HF SCS condition, questions remain around its similarity to HFAC, and the mechanism by which its application potentially blocks nerve conduction and relieves pain. In the burst SCS condition, research has shown a decrease in WDR firing, a manifestation shared with tonic SCS. It appears, however, that the two SCS conditions may not act via the same signaling molecules. Future research should clarify if and where there is a convergence in the mechanisms of pain relief between tonic and burst SCS. Henceforth, ULF SCS and its potential effect on pain must also be studied. Lastly, the relationship between the pain relief mechanisms and SSEP amplitude reductions and varying latency changes of each SCS modality needs to be explained to understand if either the SSEP waveform amplitude reduction plays a role in pain relief, or if it is an unrelated additional effect of the stimulation.

Chapter 5: Conclusion

The complex neurological phenomena, from molecular to electrical signaling, occurring during all four types of SCS studied in this project have yet to be fully understood, despite the extensive work being done in the field. This important research must continue if we are ever to fully understand the physiological reasons SCS provides pain-relief to certain patients, and the ways whereby the resulting reductions in SSEP amplitudes and variable changes in SSEP latencies are involved. This non-recovery study demonstrated the efficacy of a porcine model for SCS and SSEP research, which could lead to exciting new SCS research opportunities. This project has also established a safe, ethical, and effective methodology for SCS research in pigs, which could be modified for various recovery, early gene product, neurophysiological, or signaling molecule studies in the future. SCS responders demonstrated that all four SCS modalities caused a significant decrease in SSEP amplitude from averaged baseline for each subject [$\alpha = 0.05$, tonic ($t(9) = -14.93$; $p = 1.18E\text{-}7$), burst ($t(9) = -5.77$; $p = 2.00E\text{-}4$), HF ($t(9) = -6.90$; $p = 7.06E\text{-}5$), ULF ($t(9) = -11.44$; $p = 1.15E\text{-}6$)]. Two pigs in our study were deemed non-responsive to certain types of SCS. One subject was unresponsive to burst SCS, while another was unresponsive to both burst and HF SCS. These findings are in line with current observations in clinical practice settings where certain patients do not respond to certain types of SCS (Buonocore and Demartini 2016; Janssen et al. 2012; Larson et al. 1974; Song et al. 2014; Urasaki et al. 2014). Furthermore, baseline SSEP amplitude readings were significantly different between most subjects ($\alpha = 0.05$; d.f.4; $p < 0.05$), which also corroborates clinical observations where patients demonstrate different baseline SSEP amplitude readings (John P. Lubicky 1989; Spiess et al. 2008). SSEP latencies did not vary significantly from baseline in any modality [$\alpha = 0.05$, tonic ($t(9) = -1.78$; $p = 0.108$), burst ($t(9) = -0.42$; $p = 0.683$), HF ($t(9) = -0.61$; $p = 0.555$), ULF ($t(9) = 2.11$; $p = 0.064$)]. There were no SSEP latency outliers. Latency baselines between most subjects were significantly different from each other ($p < \alpha$). This project was the first to study the effect of ULF SCS, a novel parameter, on SSEPs. Results demonstrated that ULF SCS had a similar effect on SSEPs as the other three modalities, suggesting that it may also share their

pain-relieving effects. This remains to be studied. ULF could eventually provide an additional SCS option to patients seeking relief from chronic neuropathic pain syndromes.

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