THE EFFECTS OF TRANSCUTANEOUS ELECTRICAL NEUROSTIMULATION ON ANALGESIA AND PERIPHERAL PERFUSION

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ABSTRACT

The Effects of Transcutaneous Electrical Neurostimulation on Analgesia and Peripheral Perfusion

Leah Schafer

Peripheral arterial occlusive disease (PAOD) affects 8 to 12 million Americans over the age of 50. As the disease progresses, arterial occlusions arising from atherosclerotic lesions inhibit normal metabolic vasodilation in the peripheries, resulting in limb ischemia and claudication. Pharmacological and surgical treatments currently used to treat both the hemodynamic and pain symptoms associated with PAOD can involve adverse and potentially life-threatening side effects. Thus, there is a need for additional innovative therapies for PAOD.

Neurostimulation has a known analgesic effect on both acute and chronic pain. Although the exact mechanisms remain under investigation, local vascular tone may be modulated by neurostimulation in addition to pain modulation. The Gate Control Theory proposes that electrical activation of mechanoreceptive afferent somatosensory nerves, specifically Aβ fibers, inhibits pain signaling to the brain by activating an inhibitory interneuron in the dorsal horn of the spinal cord which dampens signaling from afferent, C type peripheral nociceptor nerves. Interestingly, Aβ fiber activation may also inhibit norepinephrine release from sympathetic nerve terminals on efferent neurons by activating α-2 adrenergic receptors along the same dermatome, resulting in localized vasodilation in both limbs. Ultimately, electrical stimulation may decrease mean blood pressure and increase local blood flow.

The focus of this study was to optimize protocols and perform a small scale clinical study to investigate hemodynamic and analgesic responses to neurostimulation during acute ischemia. We hypothesized that ganglial transcutaneous electrical neurostimulation (TENS) and interferential current (IFC) treatments would decrease pain perception and vascular resistance in the periphery in young, healthy subjects. We further hypothesized that IFC may have a greater hyperemic and analgesic effect on acute ischemia than TENS as its current waveform may be more efficient at overcoming skin impedance. Interestingly, we found trends suggesting that TENS and IFC may increase vascular resistance (VR) and have no noticeable analgesic effect, though TENS may have a slightly lower increase in VR associated with an increase in pain. Further work characterizing the hemodynamic effects of different stimulus waveforms is needed to inform future research into possible neuromodulation therapies for ischemic disease.

Keywords: Neurostimulation, ischemia, blood flow, hyperemia, vascular resistance, analgesia, peripheral artery occlusive disease

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> "We can't have full knowledge all at once. We must start by believing; then afterwards we may be led on to master the evidence for ourselves."
>
> – Thomas Aquinas

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CHAPTER 1: INTRODUCTION

1.1 PERIPHERAL ARTERY OCCLUSIVE DISEASE

1.1.1 Prevalence and Etiology

Peripheral arterial occlusive disease (PAOD) affects 10% of the American population, rising to 20% in persons over 70 years of age [1]. PAOD is more prevalent in men than in women, though non-fatal events are more frequent in women with PAOD than men [2]. Risk factors associated with PAOD also include diabetes, smoking, hypertension, and dyslipidemia [3], **Figure 1.1**.

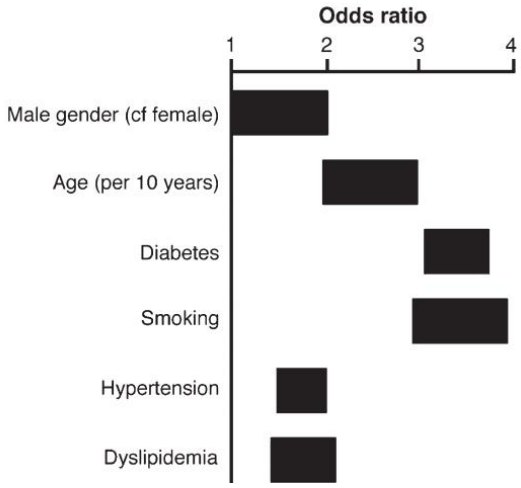

Figure 1.1: Risk Factors for PAOD. Gender, age, smoking, and diabetes effect the risk of developing PAOD [3]. Males have 10-20% greater risk than females. Increased age raises risk by 20-30% for each 10 year age bracket. Diabetes and smoking increase risk by 30-40%, while hypertension and dyslipidemia increase risk by 10-20%.

PAOD is caused by atherosclerosis that leads to arterial stenosis in peripheral conduit arteries, **Figure 1.2**. Although resting blood flow in PAOD patients is similar to

that in a healthy person, arterial occlusions inhibit metabolic vasodilation in the peripheries, resulting in limb ischemia [4]. Once metabolic demands rise above tissue perfusion levels, muscle fatigue and acute ischemic pain result. The pain, also known as intermittent claudication (IC), and fatigue often subside after the cessation of muscle contraction and a return to resting metabolic demand. Although symptomatic stabilization may occur due to the development of collaterals, pain and fatigue can become chronic as arterial stenosis progresses [3].

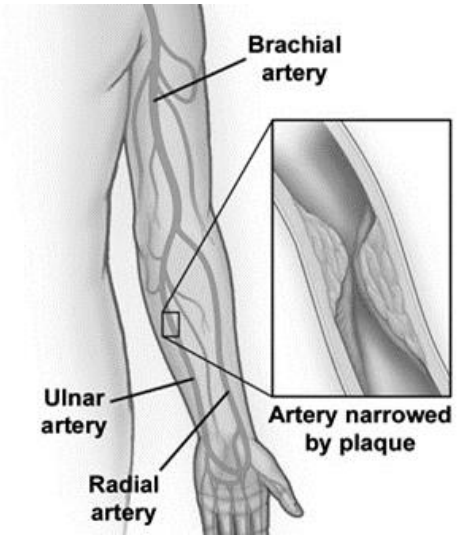

Figure 1.2: Atherosclerotic Arterial Stenosis: The narrowing and hardening of peripheral arteries in PAOD causes decreased blood flow and vascular tone [5].

1.1.2 Diagnosis

When claudication and fatigue symptoms occur, several tests are used to screen for PAOD. For artery disease in the legs, the most widely used test is the ankle-brachial systolic pressure index (ABI) which compares ankle blood pressure to arm pressure at rest. A resting ABI of ≤0.90 used as a hemodynamic definition of leg PAOD [6]. A

similar comparative blood pressure reading is used for PAOD screening in the arms, where a reduced blood pressure in one arm as compared to the other, as well as reduced pressure distal to the suspected blockage, is indicative of peripheral arterial stenosis.

Diagnosing PAOD in asymptomatic patients requires advance screening. For this reason, coronary artery disease (CAD) can be indicative of PAOD in asymptomatic patients as PAOD and CAD are both manifestations of atherosclerosis. In the primary care setting, approximately half of patients diagnosed with PAOD also have CAD, and PAOD patients are at a higher risk for heart attacks and strokes [3]. Other hemodynamic imaging studies used to diagnose or characterize PAOD include Doppler ultrasound, magnetic resonance angiogram (MRA), and x-ray arteriogram.

1.1.3 Current Treatment Options

Following diagnosis, current treatment options for PAOD include lifestyle changes, pharmacologic interventions, and/or surgery. Diet modification is directed toward lowering low density lipoprotein (LDL) consumption, as LDL cholesterol plays a major role in endothelial activation associated with atherosclerotic plaque formation [7]. Increasing exercise and smoking cessation are also important lifestyle changes known to decrease LDL concentration and improve overall cardiovascular health [8]. However, diet and exercise alone are often not sufficient to achieve recommended lipid levels; therefore, pharmacological treatments are often necessary.

Statins are prescribed to lower LDL cholesterol levels in PAOD patients and are associated with a 20% reduction in major adverse cardiovascular events such as myocardial infarction and stroke [9, 10]. Furthermore, the antiinflammatory, antiproliferative, and antithrombogenic properties of statins improve claudication and

atherosclerosis associated with PAOD [11]. Antihypertensive drugs such as diuretics, β-adrenergic inhibitors (e.g. β-blockers), angiotensin-converting-enzyme (ACE) inhibitors/angiotensin receptor blockers, and calcium channel blockers are also commonly prescribed to reduce blood pressure (BP), which in turn slows the progression of atherosclerosis by reducing shear and oxidative stress in the blood vessel lumens. Thiazide diuretics are safe and effective for reducing BP in the general patient population, while ACE inhibitors are often used in patients with diabetic renal disease or congestive heart failure [12]. Calcium channel blockers are used in cases in which hypertension is more difficult to control, while adrenergic inhibitors are selectively used for cardioprotection in PAOD patients who also have concomitant coronary disease [3].

If drug therapies are insufficient, surgical intervention is also used to improve blood flow in PAOD patients. Percutaneous transluminal angioplasty (PTA) is a minimally invasive procedure used to compress atherosclerotic plaque inside the arterial wall, **Figure 1.3**. Long-term success rates for aortoiliac and femoropopliteal PTA are between 50-70% after 5 years [13]. However, hyperplasic restenosis due to a combination of localized inflammation, atherosclerosis, thrombosis, scar tissue formation, and proliferation [14, 15] occurs in up to 25–30% of PAOD patients and is a major problem limiting its long-term efficiency [11, 16]. Thus, angioplasty is often followed by stenting to preserve the structure of the vessel wall and reduce restenosis.

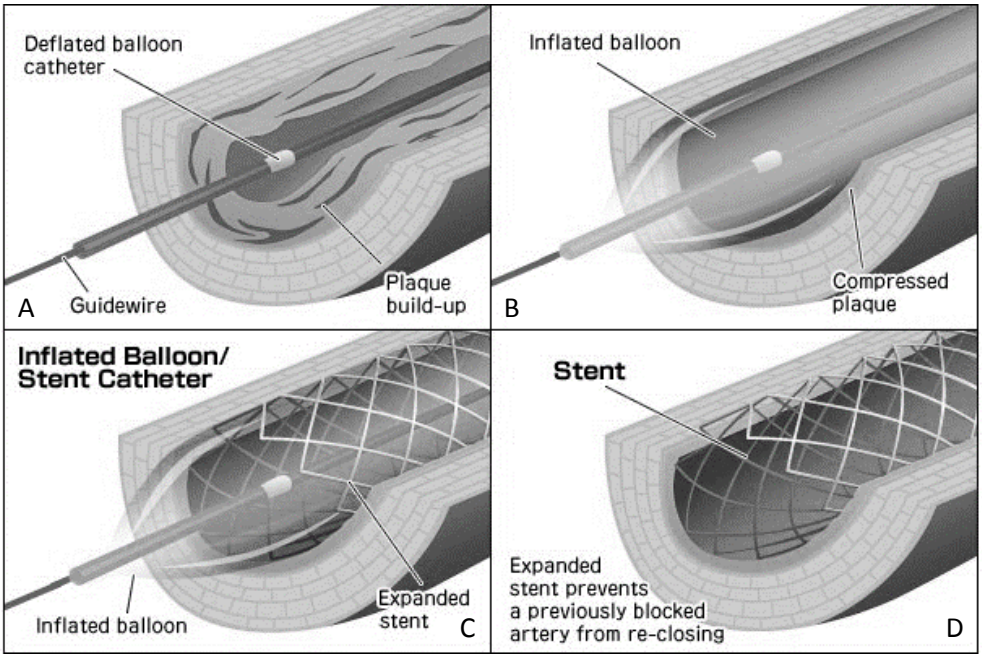

Figure 1.3: Percutaneous Angioplasty and Stenting. A. Intravascular deflated balloon catheter guidewire inserted into stenosed region. B. Non-stented balloon inflated; plaque compressed against arterial wall. C. Stented balloon inflated; plaque compressed and stent expanded. D. Stent preserves vessel shape and delays restenosis [17].

Other intervention options for PAOD include atherectomy and bypass grafting. Rather than being compressed, plaque is removed by cutting, pulverizing, and shaving via a catheterized endarterectomy device. Although initial success is greater than PTA, restenosis and patency constraints occur in almost half of the patients at 12 months post-atherectomy [18]. Arterial bypass grafting is a more invasive surgical intervention used as a last line of treatment for cases in which pharmacological or percutaneous interventions are not effective. This procedure involves redirecting blood flow around the stenosed section by attaching a healthy autologous or synthetic blood vessel at either end

of the blockage, **Figure 1.4**. However, over the past 20 years, the use of bypass surgery to treat PAOD has decreased by 42% in clinical settings [19].

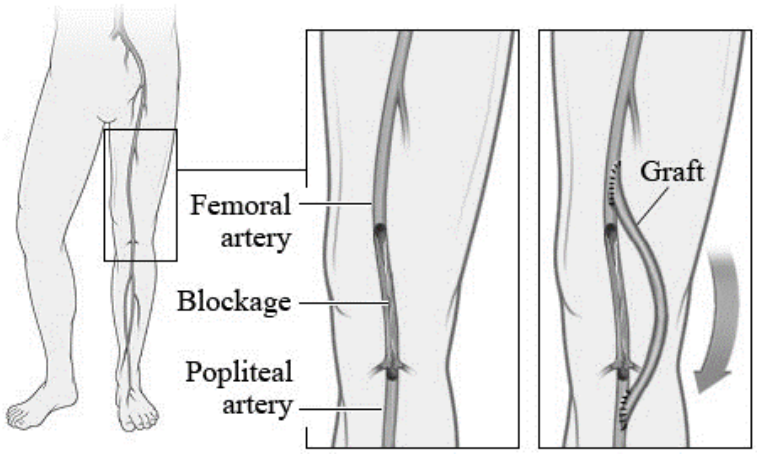

Figure 1.4: Arterial Bypass Graft. Blood flow is redirected around the stenosed region by grafting a new vessel around the blockage [20].

Cell-based therapies for PAOD are currently under investigation. An ongoing Stage 3 trial is investigating the safety and efficacy of autologous bone marrow aspirate concentrate (BMAC) for treating critical limb ischemia due to peripheral arterial disease [21]. It is postulated that intramuscular injections of BMAC into ischemic tissues will result in improved angiogenesis and blood flow. If successful, this treatment could improve blood flow and reduce ischemic pain.

Although treatment options do exist for PAOD and its symptoms, long-term efficacy is limited. Lifestyle changes may slow the progression of the disease, but may not be sufficient for disease management. Pharmacological and surgical complications are also prevalent. Statins impair memory, damage the liver, and raise blood sugar [22], while diuretics and beta-blockers may also cause insulin resistance [23]. Angioplasty and stenting have high restenosis rates and increase the thrombogenicity of the vessel wall,

while arterial grafts are very invasive and expensive and have a higher risk of major adverse cardiac events [24]. To more safely and effectively address PAOD and its symptoms, additional approaches are needed. Electrical stimulation is one such alternative to drug treatments for painful conditions and possibly ischemia.

1.2 NEUROSTIMULATION

1.2.1 Modalities and Functions

Several modalities of neurostimulation exist, including transcutaneous stimulation such as TENS and interferential current (IFC) as well as implanted technologies such as spinal cord stimulation (SCS) and deep brain stimulation. Implanted devices tend to be more effective at alleviating pain but carry a risk of device failure or surgical complication and are therefore reserved for more severe cases, while transcutaneous modalities have been proven to be safe and effective for the general patient population with more moderate pain and are available both clinically and commercially [25].

Both implanted and transcutaneous forms of neurostimulation have a known analgesic effect on patients suffering from acute [26, 27, 28] and chronic [29, 30, 31] pain, and on healthy subjects in whom acute pain has been induced experimentally [32, 33, 34, 35]. Although clinical and experimental pain are not directly comparable, experimental pain is used to investigate pain pathophysiology and to evaluate analgesic effects under controlled conditions [36]. The onset and duration of analgesia may vary considerably between patients [37], and the same protocol may have different degrees of antinociception in acute experimental pain compared with chronic clinical pain [38]. Neurostimulation may also have a hyperemic effect [32, 39]. While the exact molecular

pathways for how neurostimulation achieves these effects remain under investigation, there is likely more than one mechanism of action.

1.2.2 Mechanisms of Action

1.2.2.1 Modulating Pain: Gate Control Theory and Endogenous Signaling

The most prevalent model for electrically-induced analgesia is the gate control theory (GCT). The GCT postulates that analgesia is achieved by electrical activation of afferent Aβ (large, cutaneous, myelinated) fibers which synapse onto ascending neurons in the central nervous system (CNS) on the same level as afferent C (small, cutaneous, unmyelinated) nociceptive fibers, **Figure 1.5**. Nociceptive signals traveling through C fibers from peripheral nociceptors activate second-order neurons in the *substantia gelatinosa* on dorsal horns along the spinothalamic tract (STT). STT neurons are responsible for carrying the signal to the thalamus for pain cognition.

Neuropeptide substance P is involved with modulating ascending nociceptive information in the STT, as is nitric oxide (NO). NO activates a guanyl cyclase protein signaling cascade, which in turn elevates intracellular cyclic guanosine monophosphate (cGMP) levels, further activating a protein kinase G cascade and ultimately amplifying the pain signal in the STT neuron. NO may also react with superoxide and increase central pain sensitization and hyperalgesia [40].

When an electrical stimulus is applied, mechanoreceptive Aβ neurons are activated and accompanied by a localized tingling, "buzzing" sensation known as paresthesia. As Aβ signaling increases, the ratio of large-fiber to small-fiber activity increases, activating an inhibitory interneuron synapsing to the ascending ST neuron and ultimately weakening the pain signal to the brain [41].

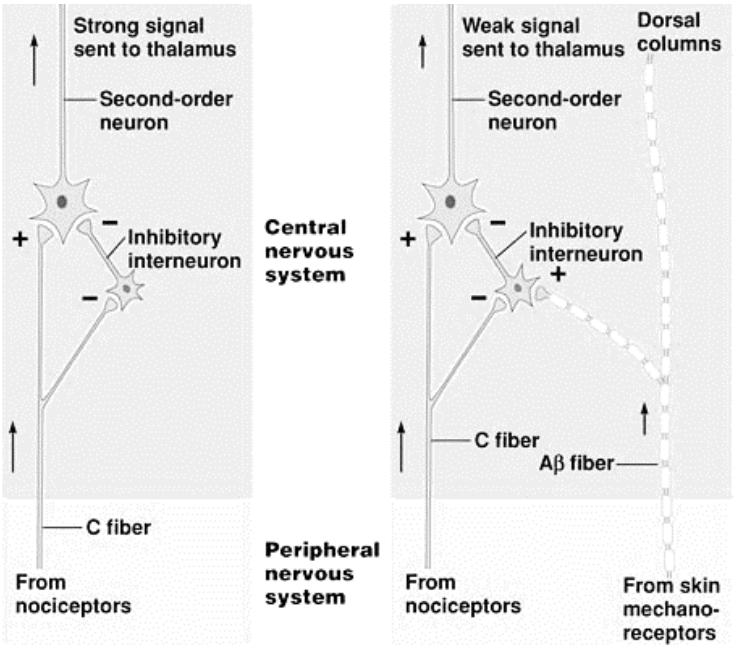

Figure 1.5: Gate Control Theory for Modulating Pain. A. Unmodulated (normal) pain: Peripheral pain signals travel up afferent C fibers to the CNS where they stimulate a second-order ST neuron and inhibit suppression by the inhibitory interneuron. B. Modulated pain: Neurostimulation stimulates afferent Aβ fibers parallel to afferent pain fibers in the CNS, resulting in the activation of an inhibitory interneuron and a suppressed pain signal to the thalamus [42].

Simultaneous to the reduction in pain sensation, the effect of the metaboreflex may be reduced. Normally, the metaboreflex is triggered by ischemic by-products such as adenosine and potassium which stimulate intramuscular chemoreceptors that send signals to type C fibers. Inhibition of type C small-fiber afferent signals by simultaneous Aβ activation would decrease the strength of the metaboreflex, resulting in a systemic decrease in vascular resistance [43]. Interestingly, the vasodilatory effect of

neurostimulation is likely stronger in PAOD patients than in healthy individuals. PAOD increases sympathetic activation as evidenced by increased concentrations of ischemic by-products and mean blood pressure (MBP) in response to exercise [44].

Endogenous opiate release may also be effected by neurostimulation. B-endorphin levels increase in the lumbar cerebrospinal fluid (CSF) with low-frequency stimuli, resulting in an antinociceptive effect [45]. These effects were reversed by naloxone, indicating that low-frequency analgesia is mediated by micro-opioid receptor activity [46]. Interestingly, high-frequency TENS results in increased dynorphin A levels in the CSF with analgesic effects that are not reversed by naloxone, implicating dynorphin-binding receptor activity [45]. These results indicate a frequency-dependent endogenous response to neurostimulation.

1.2.2.2 Modulating Blood Flow and Ischemic Pain: α-2A Receptor Activation

It is also postulated that neurostimulation increases blood flow and decreases pain in the periphery via a second Aβ fiber pathway. Although the mechanism is unclear, ganglial stimulation of Aβ fibers initiates an efferent action potential that propagates down to α-2 adrenergic receptors (α-2A-Rs) in vascular sympathetic neuron terminals. These receptors are responsible for presynaptic inhibition of smooth muscle contraction by inhibiting norepinephrine (NE) release from sympathetic nerve terminals, **Figure 1.6**. α-2A-Rs are coupled to N-type calcium (Ca^{2+}) channels in SNS neuron terminals, and activation reduces Ca^{2+} influx and subsequently decreased SNARE complex activity. Less norepinephrine (NE) is released into the synaptic cleft, and the interrupted sympathetic neuron signaling decreases vasoconstriction in the affected tissues and ultimately increases blood flow and reduces ischemic pain [47].

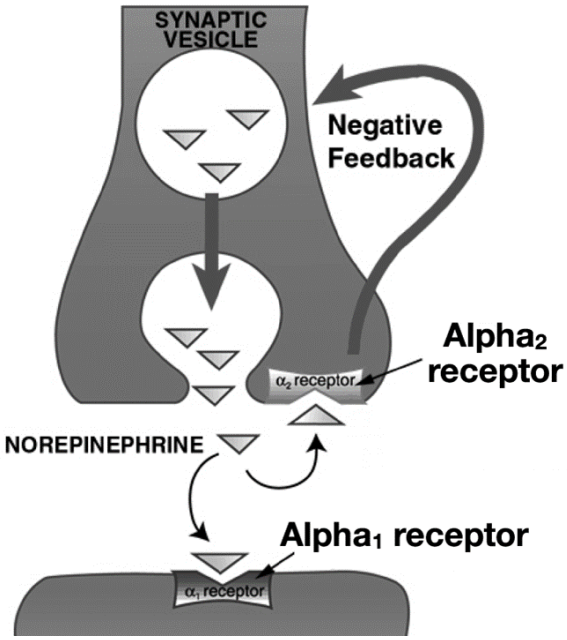

Figure 1.6: α-2A Receptor Activation. Activation of α-2 adrenergic receptors causes presynaptic inhibition of signal transmission due to suppressed neurotransmitter (i.e. norepinephrine, NE) release [47].

Interestingly, neurostimulation may have a time-sensitive effect that does not immediately present but extends beyond the period of stimulation itself, termed the "carry-over" effect. Evidence suggests that while TENS does not improve time to onset of ischemic pain, pre-treatment with TENS increases local blood flow and improves exercise tolerance at later time points [48]. Although the mechanism is unclear, it is possible that the carry-over effect may be associated with latencies in cellular activation.

In the context of PAOD, increased blood flow to ischemic peripheral tissues resulting from α-2A receptor activation would also reduce ischemic pain. In this way,

neurostimulation may have an additive analgesic effect in occluded tissues by simultaneously closing the pain gate and alleviating peripheral ischemia, **Figure 1.7**.

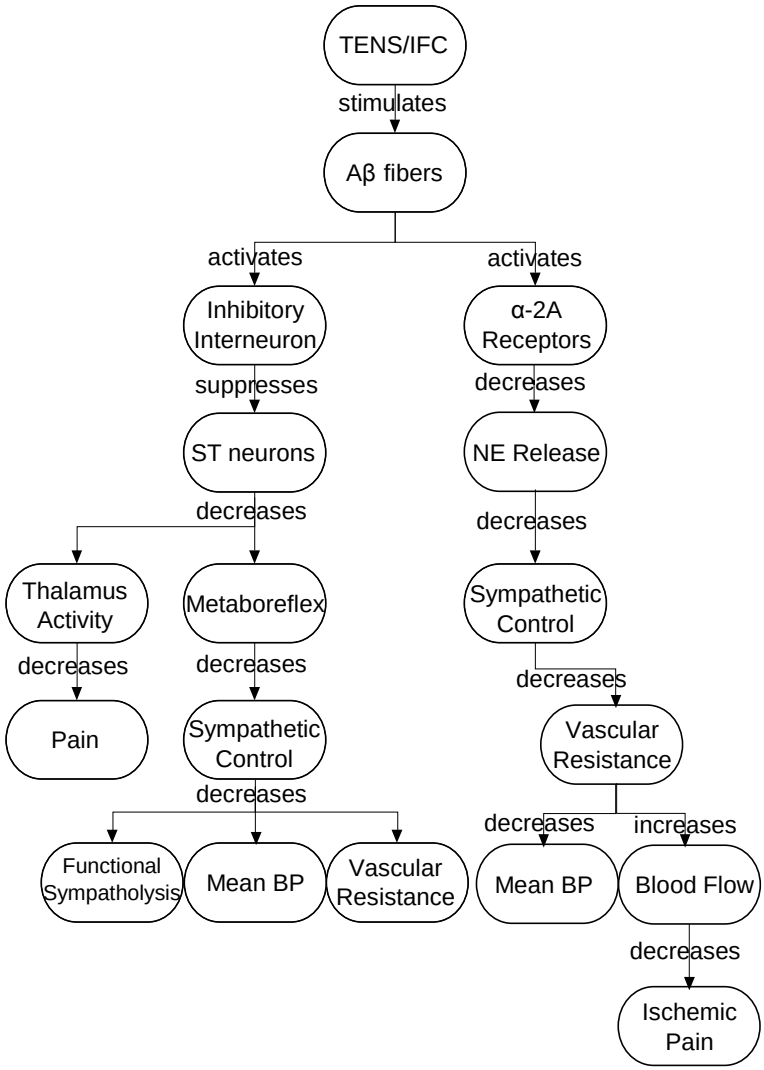

Figure 1.7: Effects of Neurostimulation on Pain and Blood Flow. Stimulation of Aβ fibers has two effects: closing the pain gate in the central afferent pathway and activating α-2A receptors in the peripheral efferent pathway. Both pathways result in decreased pain and sympathetic control and ultimately increased blood flow.

1.2.3 Justification for the Use of TENS and IFC

A combination TENS/IFC transcutaneous neurostimulation device was chosen for the study because of its low cost and non-invasiveness, though the methodologies for investigating changes in peripheral perfusion associated with neurostimulation proposed by our study may translate to future research associated with implantable technology such as SCS. To our knowledge, there have been no previous studies directly comparing the hemodynamic effects of TENS and IFC, although studies with similar protocols have investigated each individually [34, 49, 50, 32, 51, 52]. Although both types of stimulation are known to effect pain and blood flow, the waveform and frequency settings have not yet been optimized for all possible indications.

1.2.4 Waveform Characteristics

The two current waveforms most often used to study the analgesic effects of transcutaneous neurostimulation are biphasic pulsed currents characteristic of transcutaneous electrical nerve stimulation (TENS) and burst-modulated, sinusoidal alternating currents characteristic of interferential current (IFC) [53, 54]. More specifically, two out-of-phase sine waves combine to produce an IFC, **Figure 1.8** [55]. These two waveforms are also used in implantable SCS therapies, with conventional SCS utilizing a symmetric pulsatile current similar to TENS while more contemporary therapies utilize burst-mode currents similar to IFC [56].

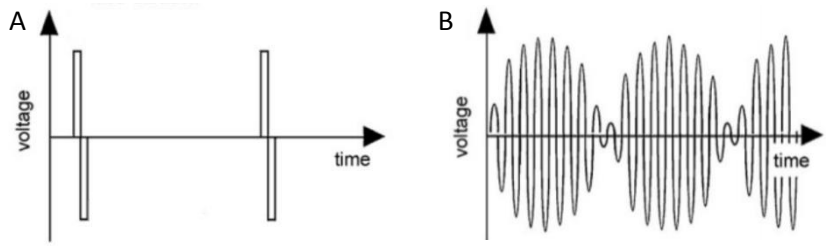

Figure 1.8: TENS and IFC Stimulus Waveforms. A. Biphasic pulsed current characteristic of conventional TENS. B. Sinusoidal burst-modulated alternating current characteristic of IFC [29].

Since membrane properties such as voltage-gated ion channel density, input resistance, capacitance, and synaptic contacts vary considerably between different neuron types and substructures (e.g. C fiber vs. Aβ fiber, axon vs. soma), it is likely that a waveform-dependent response exists [57, 58]. Conventional pulsatile current, such as in TENS, contains broad spectral energy that may limit the ability to preferentially activate neuronal targets, while narrow band sinusoidal waveforms, such as in IFC, may provide greater selective control [59]. Indeed, symmetrical charge-balancing stimuli greatly diminish selectivity in stimulating targeted neurons within the CNS, while asymmetrical biphasic stimuli enable selective activation of cells [60]. What is more, sinusoidal IFC waveforms may more readily overcome skin impedance and stimulate deeper Aβ fibers than pulsed TENS and therefore have greater analgesic and hyperemic effects [49, 61, 62]. It is also possible that burst-modulated currents have a different effect than symmetrically pulsed currents, as well as high versus low frequencies [63]. Indeed, different endogenous signaling mechanisms occur during SCS with burst mode versus tonic mode stimuli [56] as well as with high (100 Hz) versus low (20 Hz) stimulus frequencies [46].

Although there is significant evidence that both TENS [32, 62, 64, 65, 66, 67] and IFC [68, 69, 49] effectively reduce experimentally induced pain, there is limited research comparing high and low frequency TENS and IFC treatments in their efficacy in increasing blood flow. However, there is little consensus in studies attempting to characterize changes in pain or blood flow by stimulus frequency or waveform [50]. Rather, optimal settings of stimulus parameters are subjective and are determined by trial and error [70].

1.3 OVERVIEW AND SPECIFIC AIMS

1.3.1 Overview

Neurostimulation may offer an innovative treatment option for patients suffering from PAOD. To date, there is no consensus on the effectiveness of different types of neurostimulation on modulating blood flow and pain in ischemic tissues, though it is believed that electrical stimulation decreases thalamus activity and sympathetic control of vascular tone by activating Aβ fibers. The focus of our study is to investigate hemodynamic and analgesic responses to transcutaneous neurostimulation during ischemia by performing a small scale clinical study and optimizing methodologies and protocols.

1.3.2 Specific Aims

The specific aims of this thesis are as follows:

- Aim 1: Develop and optimize a protocol for investigating hemodynamic and analgesic responses to transcutaneous neurostimulation during acute ischemia in young, healthy Cal Poly students through exploring stimulus waveforms and frequencies during pilot studies.

- Aim 2: Test the hypothesis that transcutaneous electrical neurostimulation (TENS) and interferential current (IFC) treatments at the ganglia would result in decreased pain and vascular resistance in the periphery in young, healthy subjects.
- Aim 3: Test the hypothesis that IFC has a greater hyperemic and analgesic effect on acute ischemia than TENS due to differences in stimulus current waveforms.

CHAPTER 2: PILOT WORK

2.1 INTRODUCTION

The overall goal of the pilot work was to develop and optimize a protocol for investigating hemodynamic and analgesic responses to transcutaneous neurostimulation during acute ischemia in young, healthy subjects. Therefore, the goals of the first pilot study were to ensure that our blood flow measurement instrumentation was functioning as expected, i.e. reporting zero perfusion during occlusion and hyperemia during recovery, and to optimize the neurostimulation frequency to elicit elevated perfusion and decreased pain during occlusion. Endogenous pain control mechanisms may be affected differently by high versus low stimulus frequencies [45, 46] and therefore we hypothesized that high (100 Hz) TENS and IFC stimulation frequencies would increase blood flow and analgesia during acute experimental pain in healthy subjects compared to low frequencies (20 Hz).

After determining optimal instrumentation settings and neurostimulation frequency parameters in pilot study I, pilot studies II, III, and IV tested the hypothesis that neurostimulation has analgesic and hyperemic effects, possibly elevated with IFC as compared to TENS due to different effects of biphasic and sinusoidal stimulus waveforms on Aβ fibers [56]. After observing no noticeable differences in analgesic trends associated with TENS and IFC during pilot study II, pilot studies III and IV utilized multiple pain scales to better quantify sensations of pain experienced as a result of arterial occlusion. The additional pain scales gave insight that neurostimulation paresthesia was being perceived as a painful stimulus by the otherwise healthy subjects.

For this reason, pilot study IV accounted for sensations of paresthesia by including paresthesia descriptors in the general pain assessments. In this way, each consecutive pilot study served to refine our hypotheses and methodologies for the main investigative study.

All participants completed an Informed Consent form and a confidential Medical History Questionnaire that was reviewed by the primary researcher prior to treatment. Any contraindications for transcutaneous neurostimulation, i.e. pregnancy or history of epilepsy, cardiovascular disease, dermatitis, syncope, or chronic pain, were grounds for exclusion, though no participants were excluded during any pilot work. All recruitment and experiments were performed in accordance with protocols approved by Cal Poly's Human Subjects Committee.

2.2 PILOT STUDY I

2.2.1 Methods

Pilot study I was performed on 12 healthy Cal Poly students aged 18 to 23 years assigned to one of two treatment groups: TENS (n=6) and IFC (n=6). Each group received three treatments: high frequency (100 Hz), low frequency (20 Hz), and sham (0 Hz) neurostimulation, all involving 50 μs pulses at 8 mA pulse amplitude. Neurostimulation leads were always applied to the participant's back regardless of treatment to maintain a single-blinded study. The participant was never notified of the treatment that was being applied, and all sensors and cuffs were applied in the same manner for every treatment. Treatment order was randomized and treatments were performed consecutively with a 10-minute rest period allotted between trials to minimize fatigue.

The cell bodies of Aβ fibers that innervate the arms and hands form ganglion parallel to the 7th cervical and 4th thoracic vertebrae (C7 and T4, respectively). The modulatory effects of TENS and IFC on pain and blood flow are substantiated when the electrodes are placed over the C7 and T4 ganglion rather than over the active muscles of the hand and forearm [unpublished observations]. Therefore, two pairs of neurostimulation electrodes (InTENSity TENS/IFC Combination Stimulator, Current Solutions LLC, Austin, TX, USA) were aligned with the C7 and T4 vertebrae on either side of the spinal column in a quadripolar formation using re-usable carbon electrode pads (Tyco Gel Pads, Santamedical, Tustin, CA, USA)**.** Participants wore a loose shirt or tank top to allow access to the upper back, **Figure 2.1**.

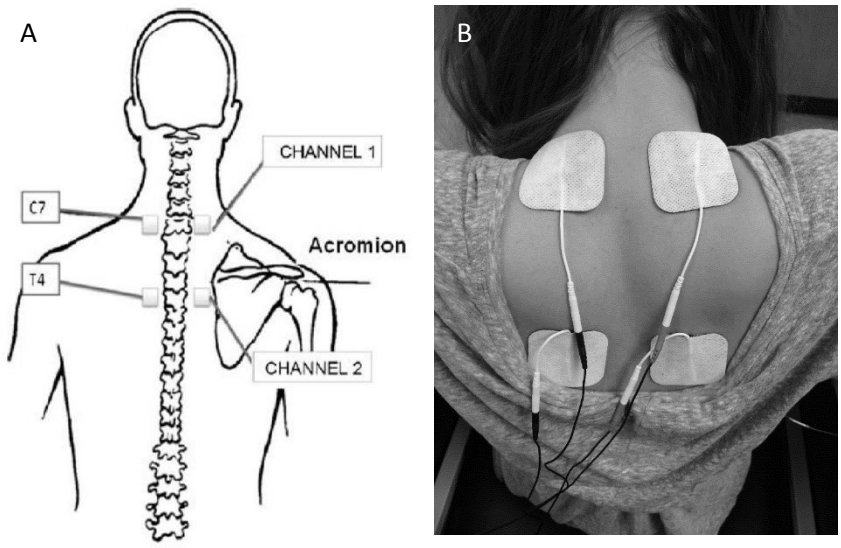

Figure 2.1: Electrode Placement. A. Topical electrodes were aligned with the C7 and T4 vertebrae for ganglial stimulation [32]. B. Participants wore loose clothing to allow access for electrode placement in a quadripolar formation.

Two of the most prevalent methods for experimentally inducing pain are the submaximal tourniquet technique and the cold pressor test, both of which cause decreased blood flow to the effected tissues. We chose to use the tourniquet technique as it takes effect quicker and had a more rapid reperfusion rate after releasing the occlusion [71, 65, 68, 69], allowing for a more efficient protocol. Therefore, ischemic conditions similar to PAOD were modeled in otherwise healthy subjects using a submaximal tourniquet technique whereby a manual blood pressure cuff was inflated to 180 mmHg for 3 minutes on the dominant forearm. To test the hypothesis that neurostimulation increases perfusion associated with acute ischemia, we measured changes in local blood flow (BF) distal to the occlusion and mean arterial pressure (MBP) on the contralateral arm.

At the start of each treatment session, participants sat in a relaxed position with their arms resting on a tray. An automated blood pressure cuff (Omron 7 Series Wireless Upper Arm Blood Pressure Monitor, BP761, Hoffman Estates, IL, USA) was applied to the contralateral upper arm to measure MBP and HR every 3 minutes as specified in the monitor's instructions for use on timing. After attaching the electrodes to the upper back, an optic Laser Doppler Flowmetry (LDF) skin probe (VP1 probe, Moor Instruments, Wilmington, DE, USA) was adhered to each palm using double sided adhesive (PADs, Moor Instruments). A hand grip dynamometer (ADInstruments, Colorado Springs, CO, USA) was gripped in the dominant hand. The probe cables coupled to a LDF data acquisition unit (moorVMS-LDF, Moor Instruments), which output to a PowerLab DAQ (PowerLab, ADInstruments) and digital chart recording software (LabChart 8.0, ADInstruments) **Figure 2.2**.

The hyperemic and analgesic effects of each treatment type were evaluated during the pressor response to static handgrip exercise at 30% maximal voluntary contraction for 3 minutes followed by a 3 minute occlusion. Change in distal blood flow and pain from resting baseline values were evaluated before, during, and after exercise and occlusion. This temporary circulatory occlusion in young healthy subjects was an imperfect approximation to PAOD as chronically ischemic tissues have depleted adenosine triphosphate (ATP) and glycogen stores, as well as elevated levels of metabolic byproducts such as lactate, which hinder rapid reperfusion (i.e. reactive hyperemia) once the occlusion is removed [72]. PAOD patients will also have tremendous endothelial dysfunction as compared to healthy young subjects, hindering their vasculature's capability to respond to stimuli. Therefore, we would expect the reperfusion rates observed in response to our experimentally induced ischemia to be faster than in PAOD patients.

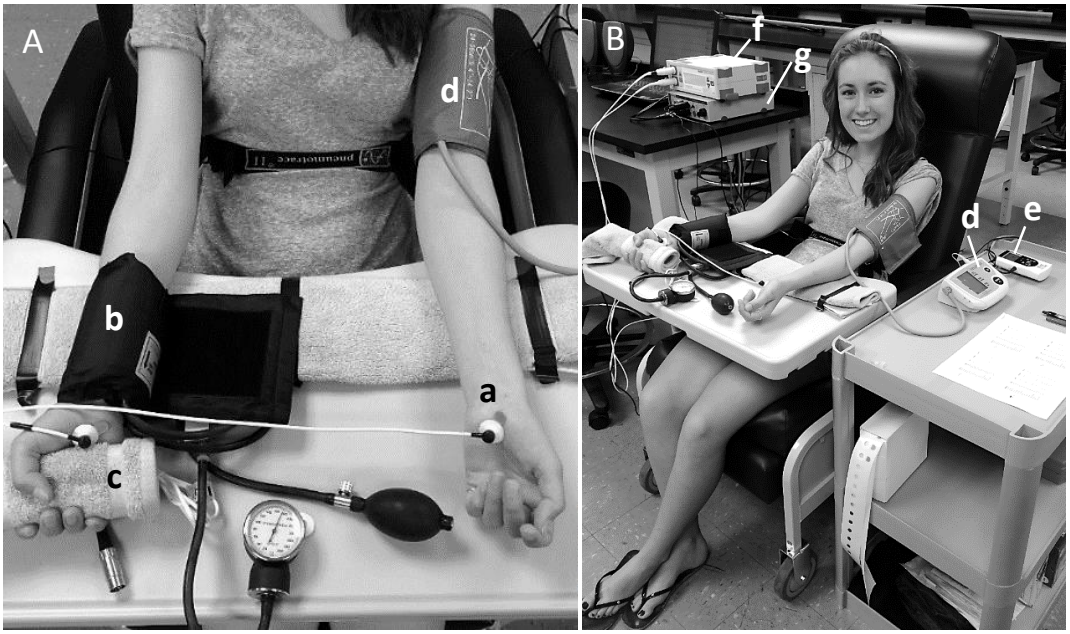

Figure 2.2: Experimental Setup. A. Participants sat in a relaxed position with arms resting on the tray in a prone position. Optic Laser Doppler Flowmetry (LDF) skin probes (a) were adhered to each palm using double sided adhesive. A manual BP cuff (b) was affixed to the participant's dominant forearm to occlude the treatment hand. A hand grip dynamometer (c) was gripped in the dominant hand. An automated BP monitor was affixed to the upper contralateral arm (d) and the TENS/IFC unit (e) electrodes were placed on the upper back. B. The probe cables coupled to a moorVMS-LDF data acquisition unit (f) which connected to a PowerLab DAQ (g) via two analog inputs. The LDF signals were transmitted to a laptop via USB cable and recorded in real time using LabChart v.8 software.

The Numeric Rating Scale (NRS) was used to assess pain on a scale of 0 – 10 every 60 seconds, 0 being no pain and 10 being the worst pain imaginable. The maximum pain was reported for each 60 second interval and raw hemodynamic data was averaged

for 60 second intervals during each phase. Although both absolute change and percent change models were run for both responses, absolute change had more statistical power (higher R^2) for analyzing this pain and blood flow dataset and therefore all results are reported in terms of absolute change from baseline. Blood flow and pain responses were compared to phase, ischemic conditions, and treatment type by two-way ANOVA for repeated measures using Minitab statistical software. Post hoc comparisons were made using Tukey-Kramer's intervals.

2.2.2 Results

2.2.2.1 Pain

As expected, pain trended to increase during occlusion. However, neurostimulation did not appear to have an analgesic effect as predicted; to the contrary, pain trended to be greater with both high and low frequency TENS and IFC treatments at each phase than the sham treatment, **Figure 2.3**.

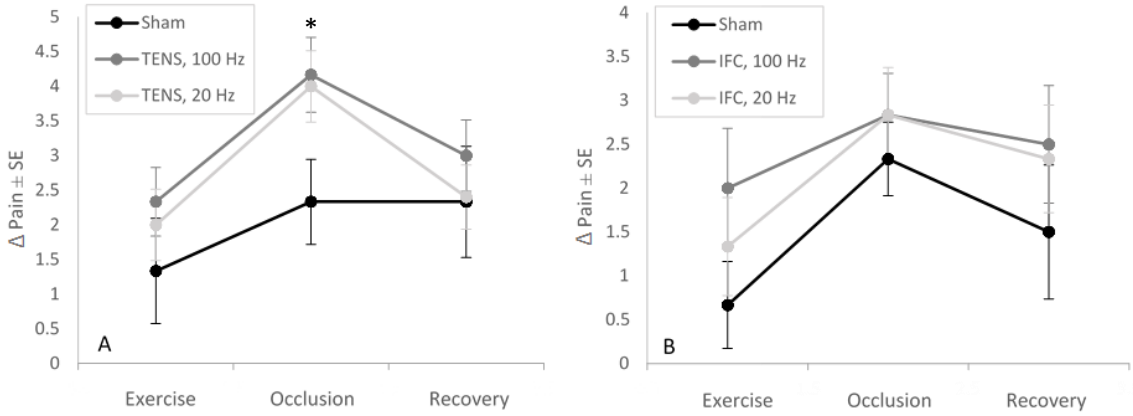

Figure 2.3: Change in Pain for Pilot Study I. Change in pain from baseline during A. High (100 Hz) and low (20 Hz) frequency TENS and B. High (100 Hz) and low (20 Hz) frequency IFC (n=6). Values are shown as mean ± SE. *p≤0.05 for Δpain vs. phase.

2.2.2.2 Blood Flow

As expected, blood flow increased in the palm during exercise and during the recovery phase following an acute forearm occlusion. There were no differences in blood flow between high and low frequency TENS treatments, though perfusion was lower during the recovery phase of the high frequency IFC treatment, **Figure 2.4.**

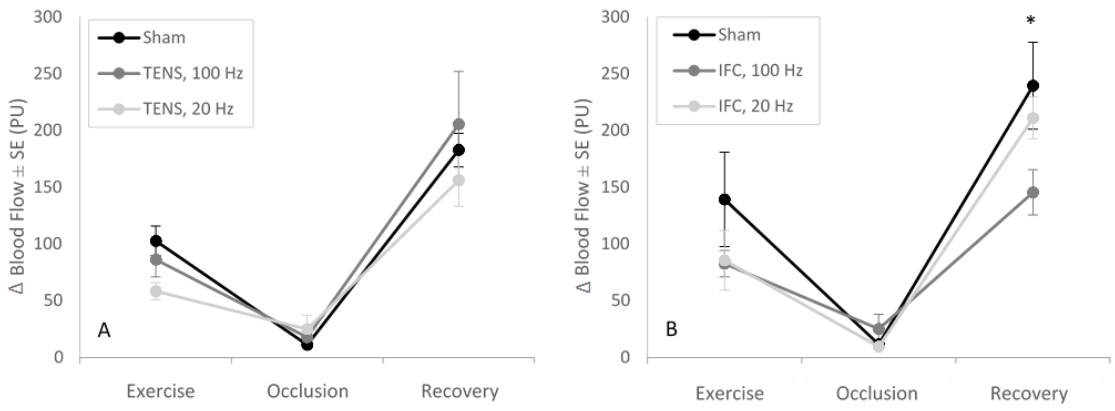

Figure 2.4: Change in Blood Flow for Pilot Study I. Change in blood flow from baseline during A. High (100 Hz) and low (20 Hz) frequency TENS and B. High (100 Hz) and low (20 Hz) frequency IFC (n=6). Values are shown as mean ± SE. *$p \leq 0.05$ for Δblood flow vs. phase.

2.2.3 Discussion

To test the hypothesis that high (100 Hz) TENS and IFC stimulation frequencies increase blood flow and analgesia more so than low frequencies (20 Hz), pilot study I compared changes in blood flow and pain elicited by both modalities before, during, and after acute ischemia. Both TENS and IFC had a hyperalgesic effect during exercise, occlusion, and recovery, **Figure 2.3**. This result is not substantiated by the main body of

research emphasizing the analgesic effects of transcutaneous neurostimulation. It is possible that paresthesia associated with the vibrational mechanoreception of neurostimulation near the ganglia was interpreted by first-time neurostimulation users as "pain," creating arbitrarily high pain measurements with TENS and IFC treatments. In subsequent studies, participants will be instructed to concentrate on pain originating exclusively in their treatment arm to promote specificity.

The increase in local blood flow during exercise and immediately following the release of an upstream occlusion, **Figure 2.4**, may be explained by metabolic vasodilation and reactive hyperemia, respectively. Metabolic byproducts released during exercise cause vascular smooth muscle cells to relax, resulting in vasodilation and increased blood flow. These byproducts also activate the metaboreflex, which in turn selectively inhibits sympathetic vasoconstriction in active tissues in a process known as functional sympatholysis. Reactive hyperemia, or the rapid increase in perfusion following ischemia, is attributed to the release of local vasodilator metabolites in hypoxic tissues.

We hypothesized that neurostimulation activates peripheral α-2 adrenergic receptors, inhibiting norepinephrine release and decreasing local sympathetic tone [73]. This results in an increase in blood flow independent of functional sympatholysis or reactive hyperemia. However, at this sample size (n=6), we did not see sufficient evidence that neurostimulation has a hyperemic or an analgesic effect. Moving forward, a larger sample size would allow us to improve our predictive power. We must also control for vasodilation mediated by local metabolites following ischemia. To isolate TENS or

IFC-induced hyperemia from metabolically-induced hyperemia, pilot study II will incorporate a control treatment without post-exercise occlusion (PECO-).

Since there were no significant differences in pain or blood flow between 100 Hz and 20 Hz frequencies for either TENS or IFC treatment, future work will use a standard 100 Hz frequency to control for possible effects of frequency on Aβ fiber activation similar to frequency settings used in comparator studies [51, 32].

2.3 PILOT STUDY II

2.3.1 Methods

Pilot study II was conducted on 9 healthy Cal Poly students age 18-23. Treatments were blinded, randomized, and followed by 10-minute rest periods. TENS and IFC settings were standardized for every treatment at 100 Hz frequencies, though the main protocol for pilot study II closely followed pilot study I.

Study II controlled for the metaboreflex by selectively applying the occlusion and comparing trends in blood flow with (PECO+) and without (PECO-) ischemia. A blocked experimental design was used to evaluate both TENS and IFC treatments in relation to a placebo (sham) treatment. Each participant received a total of six treatment combinations: TENS, PECO+; TENS, PECO-; IFC, PECO+; IFC, PECO-; placebo, PECO+; and placebo, PECO-. Completing all six treatment types on the same individual allowed us to control for differences in neural and cardiovascular physiology between subjects.

Furthermore, pilot study II individualized the intensity of the neurostimulation for every treatment session to account for differences in pain tolerances between participants. At the beginning of each treatment, the stimulus amperage was increased from 0 mA to the subject's personal pain tolerance threshold, then dropped 1 mA and held constant

throughout the rest of the session. If the motor threshold was reached before the pain threshold such that involuntary muscle twitching occurred, as seen in 2 of the 9 subjects, the intensity was dropped to 1 mA below motor threshold.

Hemodynamic and pain responses were measured and analyzed similarly to pilot study I. Absolute change and percent change models were run for both responses and percent change had more statistical power (higher R^2) for change in blood flow with the pilot study II dataset. Therefore, pain data was analyzed in terms of absolute change while blood flow data was analyzed in terms of percent change. Two-way ANOVA for repeated measures and Tukey-Kramer post hoc analysis were completed in Minitab.

2.3.2 Results

2.3.2.1 Pain

Similar to the trends in pilot study I, NRS pain trended to increase during exercise and when occlusion was applied (Placebo+, TENS+, IFC+). Indeed, pain increased each successive minute during occlusion (t=6-9 min), **Figure 2.5**. In contrast to pilot study I, both TENS and IFC treatments trended to lower ischemic pain during occlusion in pilot study II, **Figure 2.6**.

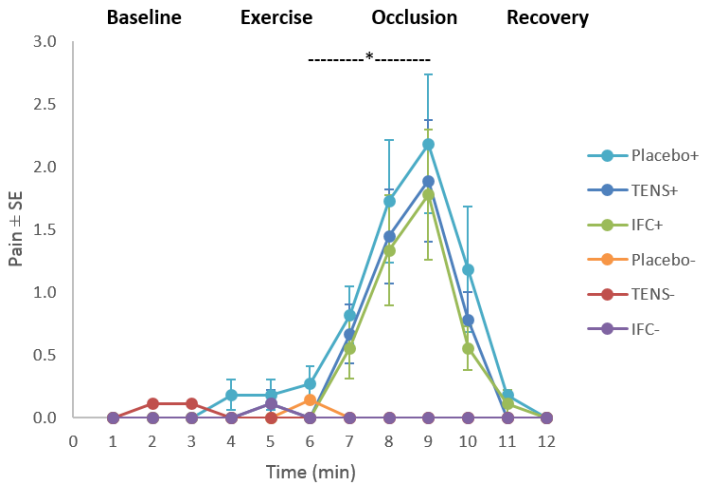

Figure 2.5: Absolute Pain across Time during Pilot Study II. Absolute pain every minute during baseline, exercise, occlusion, and recovery phases for each treatment combination (n=9). Values are shown as mean ± SE. *p≤0.05 for Δpain vs. time.

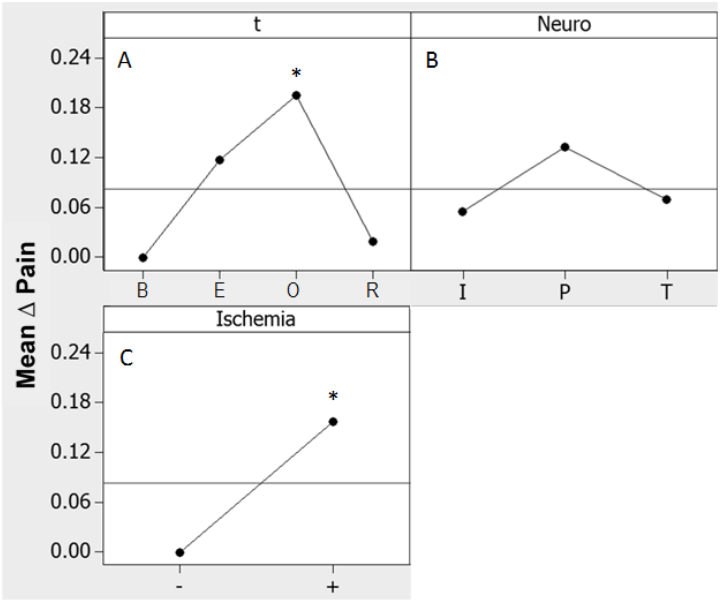

Figure 2.6: Change in Pain during Pilot Study II. Change in mean pain (n=9) from baseline over A. Time (Baseline, Exercise, Occlusion, and Recovery phases), B. Neurostimulation type (IFC, Placebo, TENS), and C. Ischemia (PECO-, PECO+). *p≤0.05 for Δpain vs. time, neurostimulation treatment, & ischemia.

2.3.2.2 Blood Flow

As expected, blood flow increased during exercise, decreased during occlusion (+), and increased during recovery following occlusion, **Figure 2.7A**. When occlusion is not applied, blood flow increases during exercise and remains above baseline for the following 9 minutes, **Figure 2.7C**. Interestingly, blood flow increased in the contralateral arm with IFC treatment during occlusion and remained elevated during recovery, **Figure 2.7B**, while without occlusion there was no difference in blood flow with IFC treatment, **Figure 2.7D**. Another interesting trend was seen in contralateral blood flow with TENS treatment, as TENS increased blood flow during exercise while IFC and placebo treatments did not (confidence interval included 0 %Δ), **Figure 2.7B,D**.

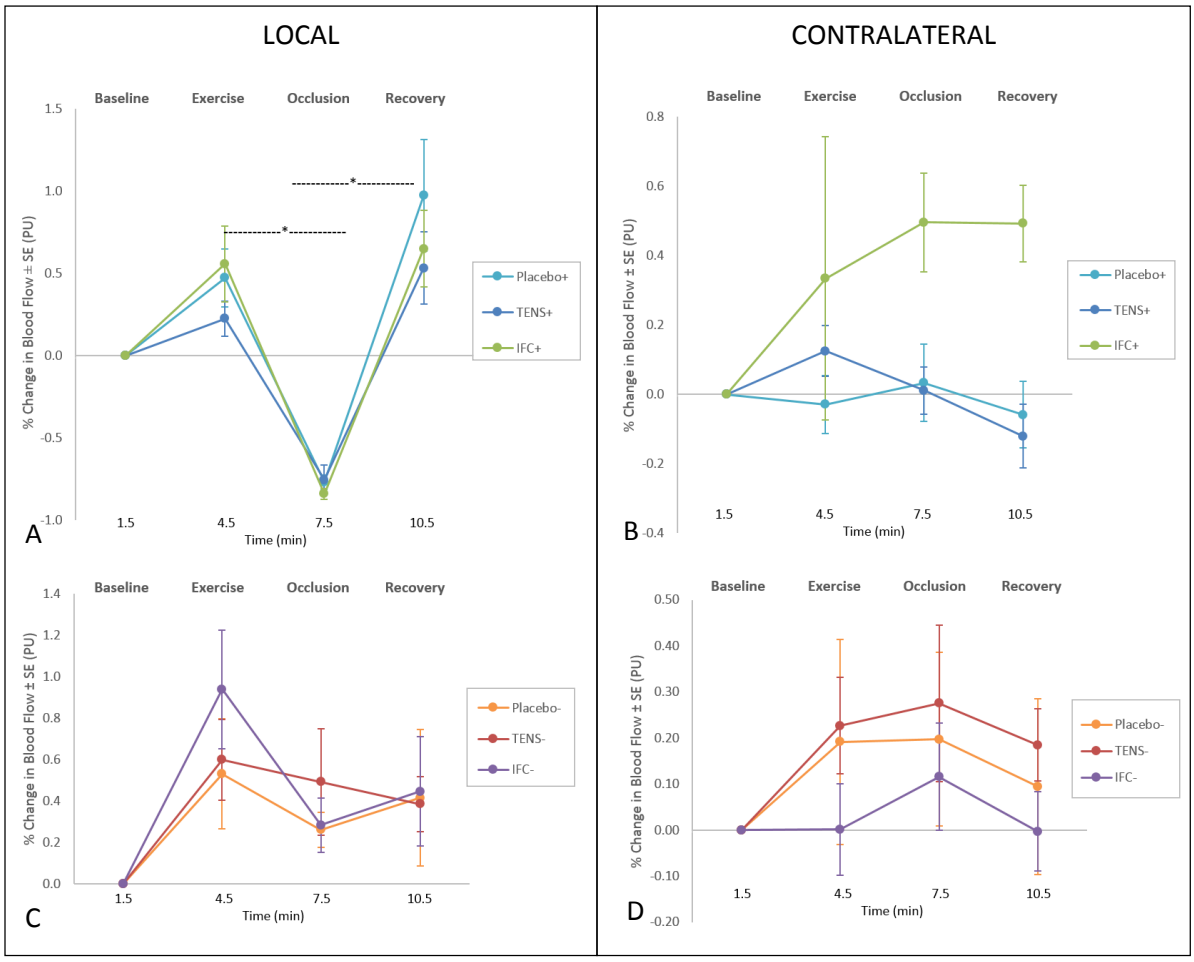

Figure 2.7: %Change in Blood Flow during Pilot Study II. Percent change in blood flow from baseline during exercise, occlusion, and recovery phases with TENS (n=9) and IFC (n=9) with occlusion (PECO+) in the A. treatment (dominant) and B. contralateral hands, and without occlusion (PECO-) in the C. treatment and D. contralateral hands. Values are shown as mean ± SE. *p≤0.05 for %Δblood flow vs. phase.

2.3.3 Discussion

To test the hypothesis that TENS and IFC stimulation have different effects on perfusion and analgesia, pilot study II compared changes in blood flow and pain elicited by both modalities before, during, and after acute ischemia. In contrast to hyperanalgesic

trends seen in pilot study I, pilot study II showed analgesic trends associated with both TENS and IFC during occlusion as predicted, **Figures 2.5, 2.6**. We also saw that ischemic pain increased in severity in a similar manner for placebo, TENS, and IFC treatments the longer the occlusion was maintained, though the maximal change in pain was relatively low on the NRS pain scale.

It is possible that the analgesic effects of neurostimulation are more pronounced with chronic pain in diseased patients than with acute experimental pain in healthy patients [38]. Instead, future studies will evaluate pain in a quantitative manner using the Numeric Rating Scale (NRS) as well as in a qualitative manner using the Faces and Short Form McGill Pain Questionnaire (SF-MPQ) scales, **Appendices H, I**. This may help to better measure changes in uncomfortable sensations at lower pain stimulus intensities.

The increase in blood flow observed during recovery when occlusion was applied (+ treatments) and static blood flow observed when occlusion was not applied (- treatments), **Figure 2.7**, supports the hypothesis that reactive hyperemia is independent of electrical stimulation. Additionally, the observed differences in blood flow with TENS during exercise and with IFC during occlusion indicate that pulsed biphasic and burst-modulated alternating stimulus waveforms may have different effects on blood flow.

However, there was no evidence that TENS or IFC had an overall analgesic or hyperemic effect independent of metabolic demands. Healthy young subjects (n=11) similar to our study population have shown increased calf blood flow with local TENS treatment at rest, during exercise, and during occlusion as compared to placebo treatment [32], **Figure 2.8**, supporting the hypothesis that neurostimulation can increase blood flow through a secondary mechanism such as Aβ fiber activation.

It is possible that electrodes were not consistently placed over the C7 and T4 ganglion during each trial, resulting in an arbitrarily high type II error in our pain and blood flow results. To improve consistency, electrode pads will not be removed from the participant's back during the resting period between trials. The accuracy of electrode placement will also be improved by receiving instruction from a licensed physical therapist on how to palpate for the C7 and T4 vertebrae prior to attaching the electrode pads to the skin.

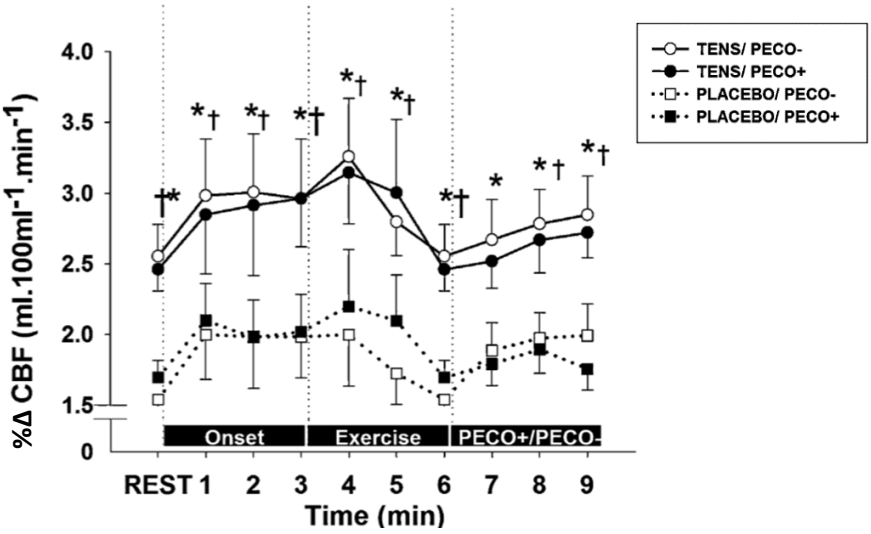

Figure 2.8: %Change in Calf Blood Flow in Comparator Study. TENS treatment increased blood flow regardless of exercise or ischemia in healthy young subjects similar to our study population [32]. Values are shown as mean ± SE. *p≤0.05 for %ΔCBF vs. time.

2.4 PILOT STUDY III

2.4.1 Methods

Pilot study III focused on expanding our pain measurement techniques. A protocol very similar to pilot study II was performed on 9 healthy Cal Poly students age

18-23. Treatments were blocked by neurostimulation (TENS/placebo) and occlusion (PECO+/PECO-) and performed in a randomized, single-blinded manner. Pain was assessed at 60 second intervals using both quantitative and qualitative pain scales: the Numeric Rating Scale (NRS) and Faces scale, respectively, **Appendix H**. Participants verbalized their numeric pain on a scale of 0 – 10, and then identified which face (A – G) best described their pain. Lettered scores were assigned weights of 0 – 6, respectively, for quantitative analysis. A SF-MPQ was administered orally halfway through both the exercise phase and the occlusion phase with the participant rating each descriptor as 'none, mild, moderate, or severe,' **Appendix I**. These qualitative scores were assigned weights of 0, 1, 2, and 3, respectively, for quantitative analysis, **Figure 2.9**.

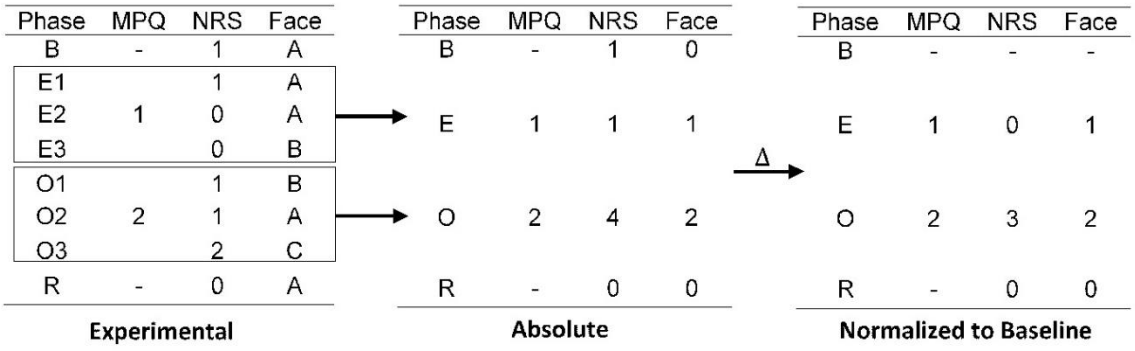

Figure 2.9: Sample Pain Measurement Consolidation and Normalization. NRS and Face pain measurements were consolidated down into one summary value for each phase. The NRS values were summed for both the E and O phases and the sum recorded as the absolute pain measurement for that phase. The Face letters were assigned numerical weights (A=0, B=1, C=2, ect.) and the highest weight recorded for each phase. The absolute values were then normalized to the baseline values by taking the difference

between that phase and baseline. The normalized values were used for quantitative analysis.

2.4.2 Results

All three scales show the same trends in pain for each neurostimulation type, **Figures 2.10, 2.11, 2.12**. As expected, pain increased with occlusion. However, pain trends observed during TENS treatments were no different than trends observed during placebo treatments.

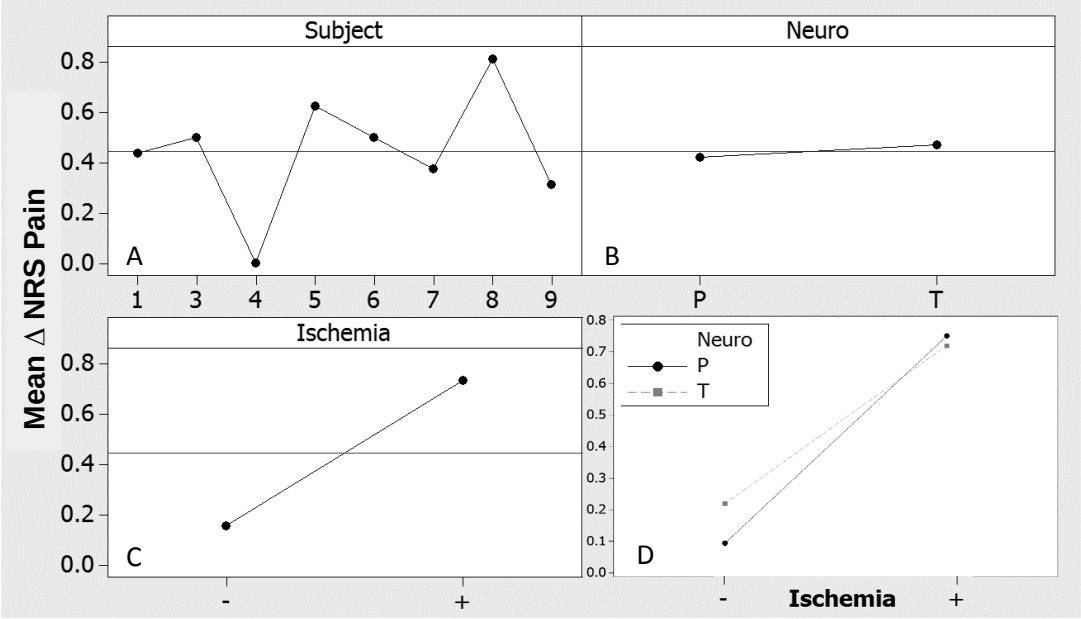

Figure 2.10: Mean Change in Pain during Pilot Study III Using the NRS Pain Scale. Change in average pain compared across A. Subject (n=9), B. Neurostimulation (TENS, Placebo), C. Ischemia (+/-), and D. In relation to neurostimulation with and without ischemia (P-, P+, T-, T+).

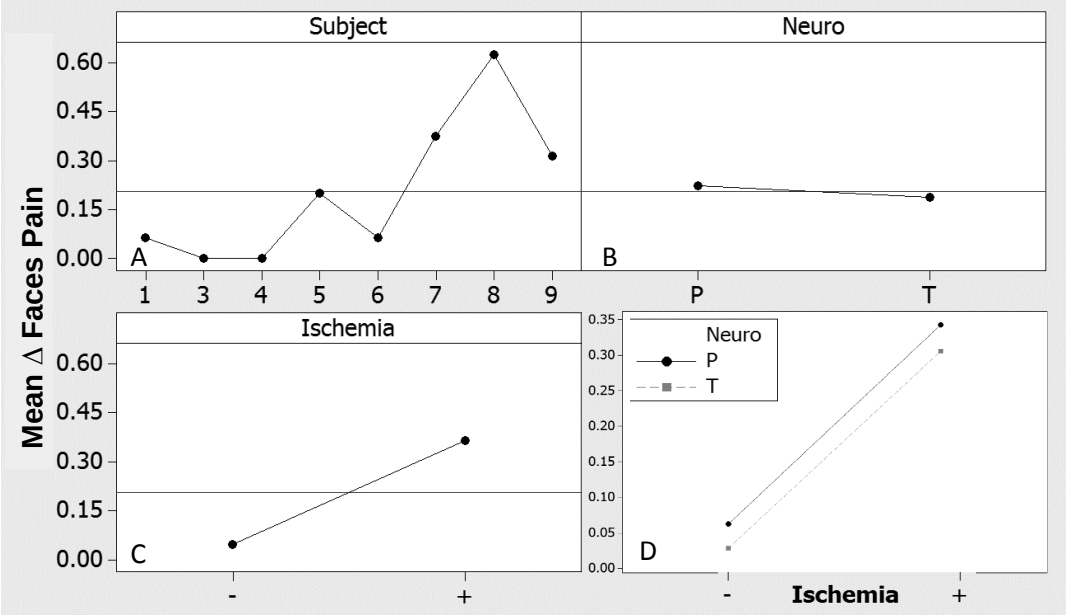

Figure 2.11: Mean Change in Pain during Pilot Study III Using the Faces Pain Scale. Change in average pain compared across A. Subject (n=9), B. Neurostimulation (TENS, Placebo), C. Ischemia (+/-), and D. In relation to neurostimulation with and without ischemia

(P-, P+, T-, T+).

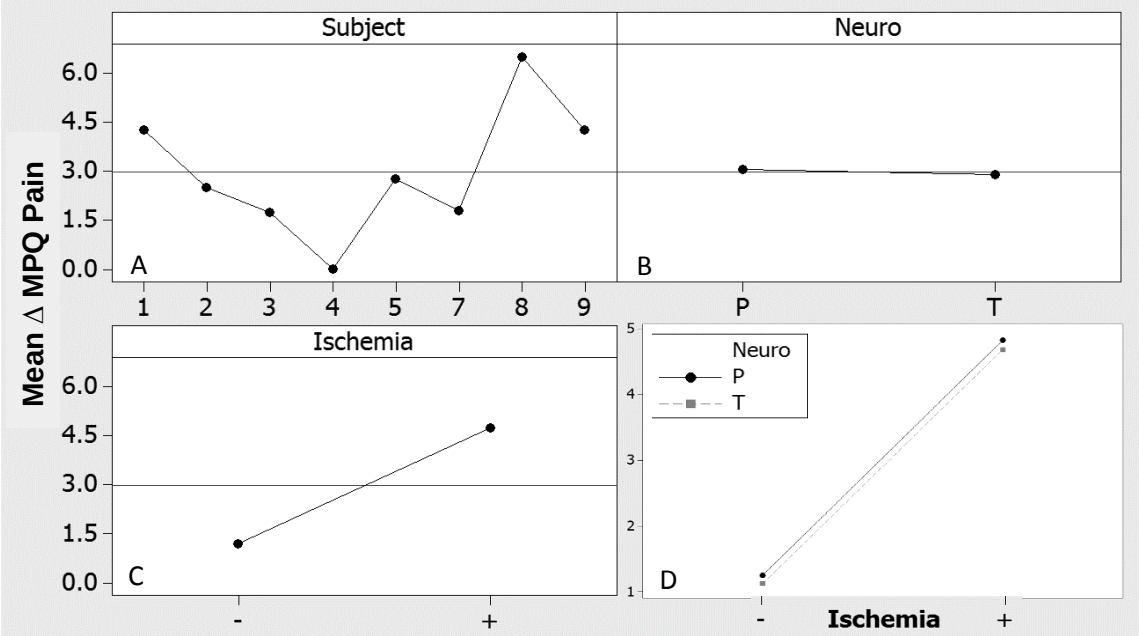

Figure 2.12: Mean Change in Pain during Pilot Study III Using the MPQ Pain Scale. Change in average pain compared across A. Subject (n=9), B. Neurostimulation (TENS, Placebo), C. Ischemia (+/-), and D. In relation to neurostimulation with and without ischemia

(P-, P+, T-, T+).

2.4.3 Discussion

Although the NRS, Faces, and MPQ pain scales used different schemes (numeric, associative, and descriptive, respectively) to quantify the intensity of ischemic pain, all three scales resulted in increased pain trends for TENS treatments. Interestingly, the exact same protocol that resulted in analgesic trends with TENS in pilot study II resulted in hyperalgesia in pilot study III. This inconsistency in results warrants a fourth pilot study to determine our ability to replicate our results before beginning a larger trial with an appropriately powered sample size.

2.5 PILOT STUDY IV

2.5.1 Methods

Pilot study IV was conducted in the same manner as pilot study III on 12 healthy Cal Poly students age 18-23. Treatments were blocked by neurostimulation (TENS/IFC/placebo) and occlusion (PECO+/PECO-) and performed in a randomized, single-blinded manner. Pain was assessed at 60 second intervals using the NRS and Faces scales and an SF-MPQ was administered during each phase. The SF-MPQ was modified to include 5 paresthesia descriptors in addition to the 25 pain descriptors to allow participants to identify both paresthesia and pain sensations associated with ischemia.

2.5.2 Results

2.5.2.1 Pain

As expected, occlusion (ischemia +) was correlated with an increase in pain both during ischemia and immediately following occlusion, **Figure 2.13**. Interestingly, neurostimulation itself was painful without occlusion (ischemia -) in the first few minutes after exercise, **Figure 2.13A**, but not at later time points (ischemia -), **Figure 2.13B**.

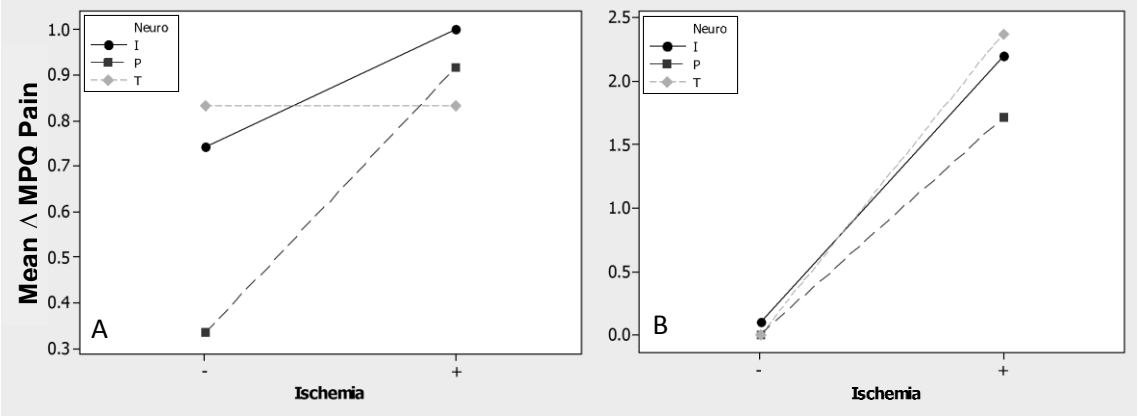

Figure 2.13: Mean Change in Pain during Pilot Study IV Using the MPQ Pain Scale. A. Pain increased during occlusion (ischemia +) for IFC, TENS, and Placebo treatments, though TENS and IFC caused an increased pain without occlusion (ischemia -). B. Pain increased during recovery following occlusion (ischemia +) for IFC, TENS, and Placebo treatments.

2.5.2.2 Blood Flow

Hyperemic trends occurred with the release of an occlusion during the recovery phase, as expected. In pilot study II, both TENS and IFC amplified hyperemic trends compared to placebo treatments, possibly supporting the hypothesis that neurostimulation has a hyperemic effect in ischemic tissues, **Figure 2.14A**. However, TENS and IFC trended similarly to placebo treatments in pilot study IV, **Figure 2.14B**.

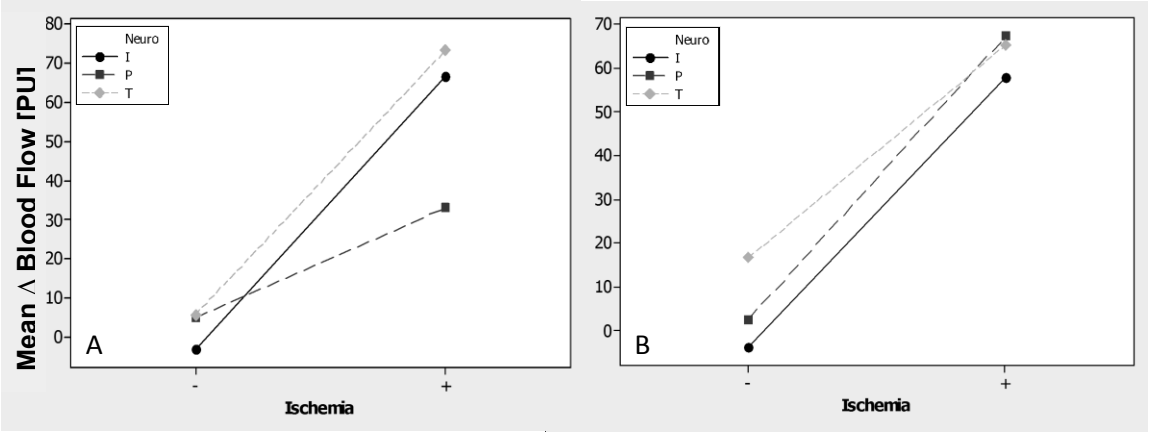

Figure 2.14: Comparison of Mean ΔBlood Flow during Recovery for Pilot Study II and IV. Blood flow increased following occlusion (ischemia +) for IFC, TENS, and Placebo treatments. ΔBlood flow A. During pilot study II showed a greater hyperemic trend with IFC and TENS treatments, while B. During pilot study IV all three treatments trended similarly.

2.5.3 Discussion

Including paresthesia in our pain measurements allowed us to better quantify paresthesias being interpreted as painful stimuli by subjects using TENS and IFC. Paresthesia significantly increased "pain" in resting, non-occluded tissues with neurostimulation treatments as compared to placebo treatments in the 4^{th}-6^{th} minutes of the protocol ("occlusion phase") but not in the 7^{th}-9^{th} minutes ("recovery phase"). It is possible that peripheral nociceptors adapted to paresthesia such that participants felt the sensation at earlier time points but not later time points.

It is also possible that the static handgrip exercise itself was painful, resulting in increased pain sensations in the 3 minutes immediately following exercise that subsided over time [74]. To reduce pain associated with the handgrip exercise, we will procure to a

more ergonomic hand dynamometer (iWorx, San Luis Obispo, CA, USA) for the clinical study.

Although there were no statistically significant differences in blood flow observed with neurostimulation, the exaggerated hyperemic trends associated with TENS and IFC seen in pilot study II were not duplicated in pilot study IV. While these trends are not consistent between the two pilot studies, we must increase our statistical power before confident conclusions can be made. To this effect, our clinical study will use a much larger sample size than the pilot studies with the goal of finding a definitive effect of neurostimulation on perfusion. Sample size will be determined by a power analysis using the pain and blood flow data variability observed between subjects in pilot study IV. Furthermore, instead of looking at only blood flow measurements, we will account for SNS control of systemic perfusion by incorporating blood pressure measurements. Moving forward, whole-limb perfusion will be evaluated in terms of vascular resistance ($\Delta Resistance = \Delta Pressure/\Delta Flow$). We will also control for caffeine, a known vasoconstrictor, to more accurately evaluate sympathetically-mediated changes in vascular tone.

CHAPTER 3: CLINICAL STUDY

3.1 INTRODUCTION

3.1.1 Proposed Hemodynamic Mechanism of TENS and IFC

The main body of research involving TENS and IFC focuses on their analgesic effects on neuromuscular pain, which can be explained by the gate control theory. However, it is also postulated that neurostimulation increases blood flow to the peripheries via a related pathway. In the context of PAOD, increased blood flow to ischemic peripheral tissues should also reduce pain, resulting in an additive analgesic effect. This focus of this study is to investigate hemodynamic responses to neurostimulation during acute ischemia. Although the mechanism is unclear, electrical stimulation may suppress local sympathetic tone and ischemic pain by activating Aβ fibers (large-diameter) parallel to the nociceptive C fibers (small-diameter) in the dorsal horn. Ganglial stimulation of Aβ fibers initiates an efferent action potential that propagates down to α-2 adrenergic receptors (α-2A-Rs) in vascular sympathetic nerve terminals. α-2A-Rs are coupled to voltage-dependent N-type calcium channels via a G protein. α-2A-R activation inhibits calcium influx responsible for presynaptic norepinephrine release, resulting in localized vasodilation and ultimately increased blood flow and reduced ischemic pain in the affected tissues [73].

3.1.2 Study Aims and Hypotheses

To our knowledge, there have been no previous studies directly comparing the hemodynamic effect of TENS and IFC, although studies with similar protocols have investigated each individually [32, 52]. The main goal of the preclinical work was to

develop protocols for investigating changes in vascular resistance and perceived pain elicited by both neurostimulation techniques and to a placebo (control) treatment.

We hypothesized that transcutaneous neurostimulation will increase blood flow and decrease vascular resistance, possibly due to a decrease in sympathetic activity. This may occur both indirectly from a decrease in metaboreflex activation and directly from selective inhibition of norepinephrine release.

Additionally, we hypothesize that TENS and IFC neurostimulation modalities will have differing effects on blood flow and vascular resistance. It is possible that the sinusoidal waveform in IFC may more readily overcome skin impedance than the biphasic pulsed waveform characteristic of TENS and thus have a more significant effect on the Aβ afferent fibers, resulting in a greater inhibitory effect on pain and sympathetic tone. We further hypothesized that IFC may have a greater hyperemic effect than TENS as its current waveform may be more efficient at overcoming skin impedance. Lastly, we hypothesize that vascular resistance will inversely correlate to ischemic pain, **Table 3.1**.

Table 3.1: Expected Trends for Hemodynamic Factors and Pain during Recovery.

Expected Outcome	Placebo		TENS		IFC	
	PECO-	PECO+	PECO-	PECO+	PECO-	PECO+
Heart Rate	-	-	↓	↓↓	↓	↓↓
Skin Temperature	-	↑	↑	↑↑	↑	↑↑
Mean Blood Pressure	-	↓	↓	↓↓	↓	↓↓
Local Blood Flow	-	↑	↑	↑↑	↑	↑↑
Contralateral Blood Flow	-	↑	-	↑↑	-	↑↑
Vascular Resistance	-	↓	↓	↓↓	↓	↓↓
Ischemic Pain	-	↓	↓	↓↓	↓	↓↓

3.2 STUDY PARTICIPANTS

The preclinical study was performed on 45 healthy Cal Poly students aged 18 to 23 years. All participants completed an Informed Consent form (**Appendix A**), a Medical History Questionnaire (**Appendix B**), and a W9 Tax Form (**Appendix C**), prior to treatment, the latter for acquiring participant compensation in the form of a $25 Visa gift card. These forms, along with all hemodynamic and pain data, were kept confidential with the exception of the W9 form which was submitted to the Sponsored Programs department.

The Medical History Questionnaire was reviewed by the primary researcher prior to starting treatment. Any contraindications for transcutaneous neurostimulation, i.e. pregnancy or history of epilepsy, cardiovascular disease, dermatitis, syncope, or chronic pain, were grounds for dismissal. Furthermore, participants fasted from caffeine for at least 12 hours prior to the treatment, as caffeine is a known vasoconstrictor. This information, as well as age and body mass data, was also collected on the Questionnaire. No participants were dismissed as a result of medical contradictions or non-compliance with fasting from caffeine.

All recruitment and experiments were performed in accordance with a protocol approved by the Cal Poly Human Subjects Committee. The petition for approval (**Appendix D**), Overview PowerPoint (**Appendix E)**, and a detailed protocol (**Appendix F**) can be found in the appendices.

3.3 MATERIALS AND METHODS

Similar to the pilot studies, the hypothesis that neurostimulation increases perfusion during acute ischemia was evaluated by measuring mean blood pressure (MBP)

and blood flow (BF) before, during, and after ischemia. These response variables were then combined into terms of vascular resistance (VR) to assess the conductive effect of neurostimulation ($\Delta Resistance = \Delta Pressure/\Delta Flow$). To test the hypothesis that neurostimulation diminishes sympathetic tone, we also measured heart rate (HR) as decreased HR would be indicative of decreased SNS activity. Since the stimulus is applied at the ganglion, we expected to see a similar decrease in SNS activity along the same dermatome in the contralateral limb.

Lead electrodes and their corresponding ground electrodes were aligned with the vertebrae approximately two inches from either side of the spinal column in a quadripolar formation, **Figure 2.1**. To ensure accurate electrode placement, participants wore a loose shirt or tank top to allow access to the upper back and the C7 and T4 vertebrae were identified via manual palpation. Participants sat in a relaxed position with their arms supinated and four neurostimulation leads (InTENSity TENS/IFC Combination Stimulator, Current Solutions LLC) were attached to the participant's upper back with re-usable carbon electrode pads (Tyco Gel Pads, Santamedical). Both the TENS and IFC treatments involved 50 μs pulses at a rate of 100 pps at intensities below the motor threshold (1-15 mA). An optic Laser Doppler Flowmetry (LDF) skin probe (VP1 probe, Moor Instruments) was adhered to each palm using double sided adhesive (PADs, Moor Instruments). The probe cables coupled to a LDF data acquisition unit (moorVMS-LDF, Moor Instruments), which output to a PowerLab DAQ and digital chart recording software (LabChart 8.0).

A skin thermistor (ADI Instruments) was taped to the palm before an ergonomic hand grip dynamometer (iWorx) was gripped in the dominant hand. Post-exercise

circulatory occlusion (PECO) mimicked acute ischemia, which is present in patients with PAOD, in otherwise healthy subjects by inflating a manual blood pressure cuff to 180 mmHg on the forearm of the treatment arm for 3 minutes. An automated blood pressure cuff was applied to the contralateral upper arm to measure MBP and HR every 2.5 minutes as specified in the monitor's instructions for use (Omron 7 Series Wireless Upper Arm Blood Pressure Monitor, BP761), **Figure 2.2**. Data recordings were compiled into a master spreadsheet for later analysis, **Appendix G**.

A crucial part of the experimental design was distinguishing between changes in vascular tone related to neurostimulation versus vasoactive reflexes such as the metaboreflex. To selectively induce acute ischemia and examine the effects of both TENS and IFC in relation to a placebo treatment, each treatment type was applied with (PECO+) and without (PECO-) vascular occlusion. A randomized blocked experimental design ensured that each participant received a total of six treatment combinations: TENS, PECO+; TENS, PECO-; IFC, PECO+; IFC, PECO-; placebo, PECO+; and placebo, PECO-. Neurostimulation leads were always applied to the participant's back regardless of treatment type to help maintain a single-blinded study. The participant was never notified of which treatment type was being applied, and all sensors and cuffs were applied the same way for every treatment. Treatment order was randomized and treatments were performed consecutively. A 10-minute rest period was allotted between trials to minimize fatigue. The intensity of the neurostimulation was individualized for every session and held constant below the motor and pain thresholds.

After familiarizing the participant with the monitoring equipment, pain scales, and protocol, a resting baseline was recorded for one minute, followed by three minutes of

handgrip exercise, **Figure 3.3**. Maximal handgrip force of the dominant hand was determined by the highest output obtained in three trials, each 1 second in duration. A static grip exercise at 30% of maximal grip force was then maintained for the remainder of the 3 minutes. Next, for PECO+ treatment types, the participant released the hand dynamometer and the blood pressure cuff on the treatment forearm was manually inflated to 180 mmHg for 3 minutes to induce ischemia in the treatment hand. For PECO- treatment types, the blood pressure cuff was not inflated and the participant released the dynamometer. At the 7th minute, the blood pressure cuff was released and a one minute recovery period was recorded.

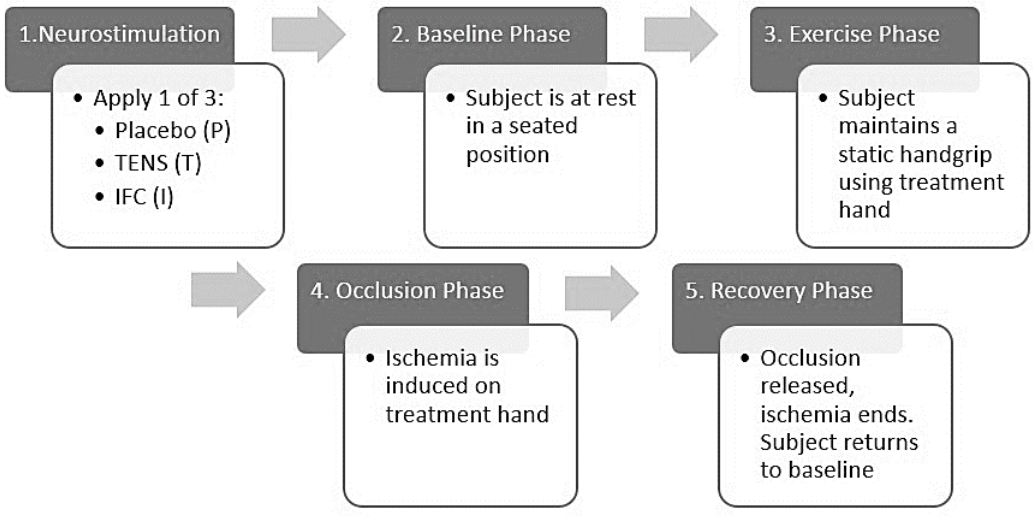

Figure 3.1: Protocol Flowchart. After the neurostimulation treatment type (placebo, TENS, or IFC) was turned on and the intensity adjusted to below the pain and motor thresholds, the 1 minute of baseline was recorded, followed by 3 minutes of static handgrip exercise, 3 minutes of PECO+/PECO-, and 1 minute of recovery.

Pain was assessed at 60 second intervals using the Numeric Rating Scale (NRS) and Faces scale, **Appendix H**. A modified Short-Form McGill Pain Questionnaire (mSF-MPQ), **Appendix I**, was administered halfway through both the exercise phase and the occlusion phase to allow participants to identify both paresthesia and pain sensations associated with ischemia.

Raw data was separated into four phases according to the experimental protocol: baseline, exercise, occlusion, and recovery, **Figure 3.2**. Hemodynamic responses were averaged for 30 second intervals during each phase and then expressed as an absolute change from the baseline value. All experimentally measured hemodynamic factors, including skin temperature (°C), respiratory rate (BPM), and local and contralateral blood flow (PU), were compared by two-way ANOVA for repeated measures while interactions between each factor and treatment phase and ischemia were compared by three-way ANOVA for repeated measures using JMP statistical software. Post hoc comparisons were analyzed using Tukey-Kramer's method, **Appendix M**.

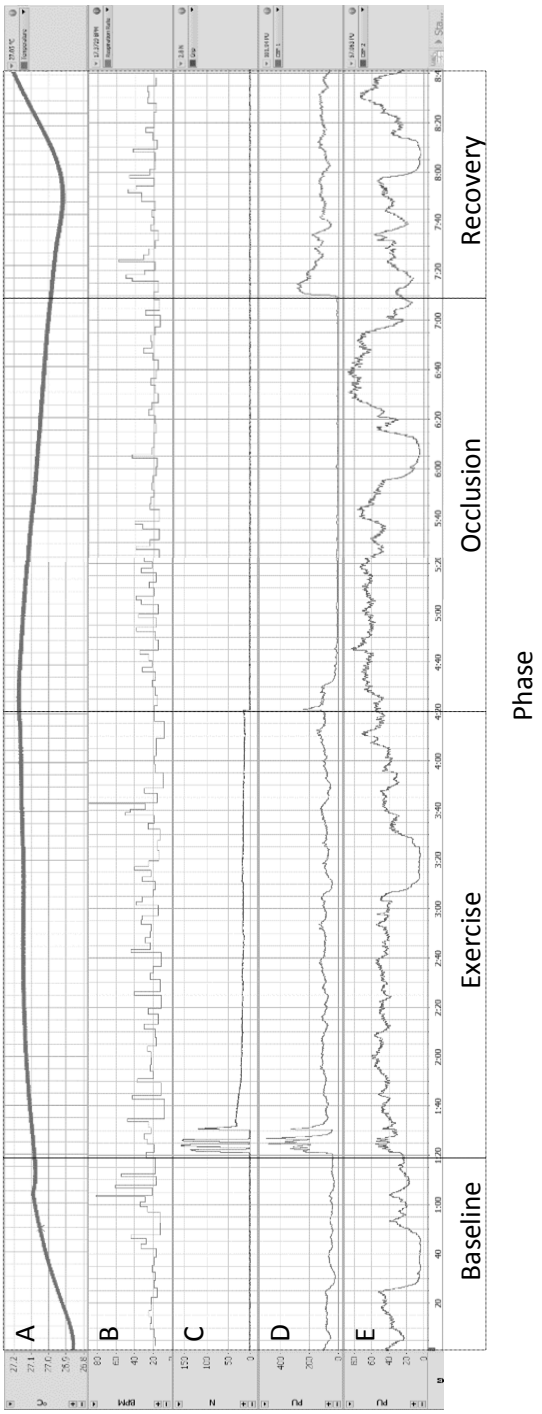

Figure 3.2: Raw Data Traces by Phase. The raw data traces were sectioned by phase and averaged for 30 second intervals for all experimentally measured hemodynamic factors including A. Skin temperature (°C), B. Respiratory rate (BPM), C. Grip force (N), D. Local blood flow (PU), E. Contralateral blood flow (PU).

3.4 DATA ANALYSIS

3.4.1 Change in Vascular Resistance

To examine the relationship between neurostimulation and perfusion under ischemic and non-ischemic tissue conditions, we first determined the importance of dependent study variables, including subject, treatment type, ischemia, and phase, on each other and on the average change in VR ($\Delta\overline{VR}$) using two and three-way ANOVAs for repeated measures. Since the calculation for $\Delta\overline{VR}$ incorporates both flow and pressure measurements, $\Delta\overline{VR}$ is the best approximation of cutaneous perfusion and therefore is the response variable in our statistical analysis. $\Delta\overline{VR}$ was calculated as:

$$\Delta\overline{VR} = \frac{\Delta\overline{MBP}}{\Delta\overline{CBF}}, \left[\frac{mmHg}{PU}\right]$$

where

$$\Delta\overline{MBP} = \Delta\overline{DBP} + \frac{\Delta\overline{SBP} - \Delta\overline{DBP}}{3}, [mmHg]$$

and PU is an arbitrary Perfusion Unit of Doppler velocimetry that approximates cutaneous blood flow.

The $\Delta\overline{VR}$ dataset was left-skewed and required transformation to fit the criteria for normality in ANOVA testing. The data were rectified by adding 10 mmHg/PU to each $\Delta\overline{VR}$value and subsequently transformed using a base 10 logarithm. This transformed the data into a normal distribution for further statistical analysis. Thus, the experimental changes in perfusion were modeled using phase, treatment type, and ischemia as independent variables, subject as a random variable, and $\Delta\overline{VR}$ as the dependent response variable. The shorthand prediction expression is:

$$log_{10}(VR+10) = \overline{\Delta VR} + Phase + Treatment + Phase*Treatment + Ischemia + Phase*Ishcemia + Treatment*Ischemia + Phase*Treatment*Ischemia + Subject$$

This expression is written as a general linear model in the form:

$$y_{ij} = \mu_{ij} + \alpha_1 + \alpha_2 + \alpha_1\alpha_2 + \alpha_3 + \alpha_1\alpha_3 + \alpha_2\alpha_3 + \alpha_1\alpha_2\alpha_3 + \varepsilon_{ij}$$

where

$\mu_{ij} =$ Average Change in Vascular Resistance ($\Delta\overline{VR}$)

$\alpha_1 = \begin{Bmatrix}\alpha_E \\ \alpha_O \\ \alpha_R\end{Bmatrix} =$ Effect of phase (E, O, or R) on $\Delta\overline{VR}$

$\alpha_2 = \begin{Bmatrix}\alpha_T \\ \alpha_I \\ \alpha_P\end{Bmatrix} =$ Effect of treatment type (TENS, IFC, or placebo) on $\Delta\overline{VR}$

$\alpha_3 = \begin{Bmatrix}\alpha_{PECO+} \\ \alpha_{PECO-}\end{Bmatrix}$ Effect of ischemia (PECO+ or PECO-) on $\Delta\overline{VR}$

$\varepsilon_{ij} =$ Residuals, or errors around the predicted trendline

This model allowed us to test the hypothesis that neurostimulation decreases vascular resistance. However, because the effects of treatment phase and ischemic conditions can impact the effects of TENS and IFC on perfusion, we evaluated the interactions between treatment type, treatment phase, and ischemia. We also accounted for between-subject variability by including subject in the model.

Due to the complexity of the model, statistical significance was accepted when $p \leq 0.01$. This allowed for only a 7% probability of falsely obtaining the observed effect in the sample data, assuming our model with 7 fixed input factors was reasonable. No data points were excluded as outliers, and it was reasonable to assume equal variance in the dataset (Levene: $p > 0.05$). The expanded prediction expression may be seen in **Appendix J**.

3.4.2 Change in Pain

Pain data was analyzed for each pain scale in relation to subject, treatment type, ischemia, and phase using two and three-way ANOVAs for repeated measures. The experimental changes in pain were modeled as a function of phase, treatment type, ischemia, and subject. Statistical significance was accepted when $p \leq 0.05$. The shorthand prediction expression is:

$$\Delta Pain = \overline{\Delta Pain} + Phase + Treatment + Phase*Treatment + Ischemia + Phase*Ishcemia + Treatment*Ischemia + Phase*Treatment*Ischemia + Subject$$

This expression is written as a general linear model in the form:

$$y_{ij} = \mu_{ij} + \alpha_1 + \alpha_2 + \alpha_1\alpha_2 + \alpha_3 + \alpha_1\alpha_3 + \alpha_2\alpha_3 + \alpha_1\alpha_2\alpha_3 + \varepsilon_{ij}$$

where

$\mu_{ij} =$ Average Change in Pain ($\Delta Pain$)

$\alpha_1 = \begin{Bmatrix} \alpha_E \\ \alpha_O \\ \alpha_R \end{Bmatrix} =$ Effect of phase (E, O, or R) on $\Delta Pain$

$\alpha_2 = \begin{Bmatrix} \alpha_T \\ \alpha_I \\ \alpha_P \end{Bmatrix} =$ Effect of treatment type (TENS, IFC, or placebo) on $\Delta Pain$

$\alpha_3 = \begin{Bmatrix} \alpha_{PECO+} \\ \alpha_{PECO-} \end{Bmatrix}$ Effect of ischemia (PECO+ or PECO-) on $\Delta Pain$

$\varepsilon_{ij} =$ Residuals, or errors around the predicted trendline

The expanded prediction expression is in **Appendix K**.

This model allowed us to test the hypothesis that neurostimulation decreases pain perception. However, because the effects of treatment phase and ischemic conditions can impact the analgesic effects of TENS and IFC, we evaluated the interactions between

treatment type, treatment phase, and ischemia. We also accounted for between-subject variability by including subject in the model.

3.4.3 Change in Vascular Resistance associated with Change in Pain

The next step was to create a model to examine a possible relationship between changes in pain and changes in cutaneous perfusion associated with neurostimulation under ischemic and non-ischemic tissue conditions. Similar to the first model, inputs included subject, treatment type, ischemia, and phase, with $\Delta\overline{VR}$ as the response variable. However, this model also included pain measurements as a fixed input. The MPQ pain scale was the most successful at detecting changes in pain during exercise and occlusion and was therefore used in this model. However, Δpain during the recovery period is assumed to be zero in this model. If the correlation between Δpain and $\Delta\overline{VR}$ during recovery is of interest, the NRS or Faces pain scale should be used as the response variable. The shorthand prediction expression for the relationship between $\Delta\overline{VR}$ and ΔMPQ pain is:

$$log10(VR+10) = \overline{\Delta VR} + Phase + Ischemia + Treatment + \Delta MPQ + Phase*\Delta MPQ + Ischemia*\Delta MPQ + Treatment*\Delta MPQ + Subject$$

This expression is written as a general linear model in the form:

$$y_o = \beta_o + \alpha_1 + \alpha_2 + \alpha_3 + \beta_1 x_i + \beta_1\alpha_1 + \beta_1\alpha_2 + \beta_1\alpha_3 + \varepsilon_o$$

where

$\beta_o =$ Average Vascular Resistance ($\Delta\overline{VR}$)

$\alpha_1 = \begin{Bmatrix}\alpha_E \\ \alpha_O \\ \alpha_R\end{Bmatrix} =$ Effect of phase on $\Delta\overline{VR}$

$\alpha_2 = \begin{Bmatrix}\alpha_T \\ \alpha_I \\ \alpha_P\end{Bmatrix} =$ Effect of treatment type on $\Delta\overline{VR}$

$\alpha_3 = \begin{Bmatrix} \alpha_{PECO+} \\ \alpha_{PECO-} \end{Bmatrix}$ Effect of ischemia on $\Delta\overline{VR}$

$\beta_1 =$ Effect of ΔMPQ pain on $\Delta\overline{VR}$; slope change

$\varepsilon_o =$ Residuals, or errors around the predicted trendline

The complexity of the model warranted statistical significance at $p \leq 0.01$. No data points were excluded as outliers, and it was reasonable to assume equal variance in the dataset (Levene: $p > 0.05$). The expanded prediction expression is in **Appendix L**.

This model allowed us to test the possible correlation between change in vascular resistance and change in pain. However, because the effects of treatment phase and ischemic conditions have compounding effects on perfusion and pain independent of neurostimulation, we evaluated the interactions between TENS/IFC, treatment phase, and ischemia. We also accounted for between-subject variability by including subject in the model.

3.5 RESULTS

To test the hypothesis that neurostimulation increases perfusion and decreases SNS tone (based on reduction in HR), the absolute changes in local BF, contralateral BF, HR, MBP, VR, and ischemic pain were divided into phase, treatment type, and ischemic condition for analysis, **Appendix M and N**. Change in vascular resistance and change in pain were modeled separately and together in JMP statistical software based on the effects of phase (Exercise, Occlusion, Recovery), treatment type (TENS, IFC, Placebo), and ischemia (PECO+, PECO-), **Appendix O**.

3.5.1 Change in Blood Flow

As expected, blood flow in the treatment arm (termed local blood flow) increased from resting baseline during exercise due to increased metabolic demand, decreased

during occlusion due to manual compression of the arterioles, and increased rapidly during the recovery phase due to reactive hyperemia. However, neither neurostimulation treatment increased blood flow as compared to placebo treatment, though interestingly both TENS and IFC have a general trend towards decreased blood flow in both ischemic and non-ischemic conditions, **Figure 3.3**. During occlusion, TENS and IFC tend to exaggerate ischemia, while during recovery, TENS and IFC tend to dampen reactive hyperemia. Interestingly, both forms of neurostimulation appear to have a latent inhibitory effect on blood flow under non-ischemic conditions after exercise.

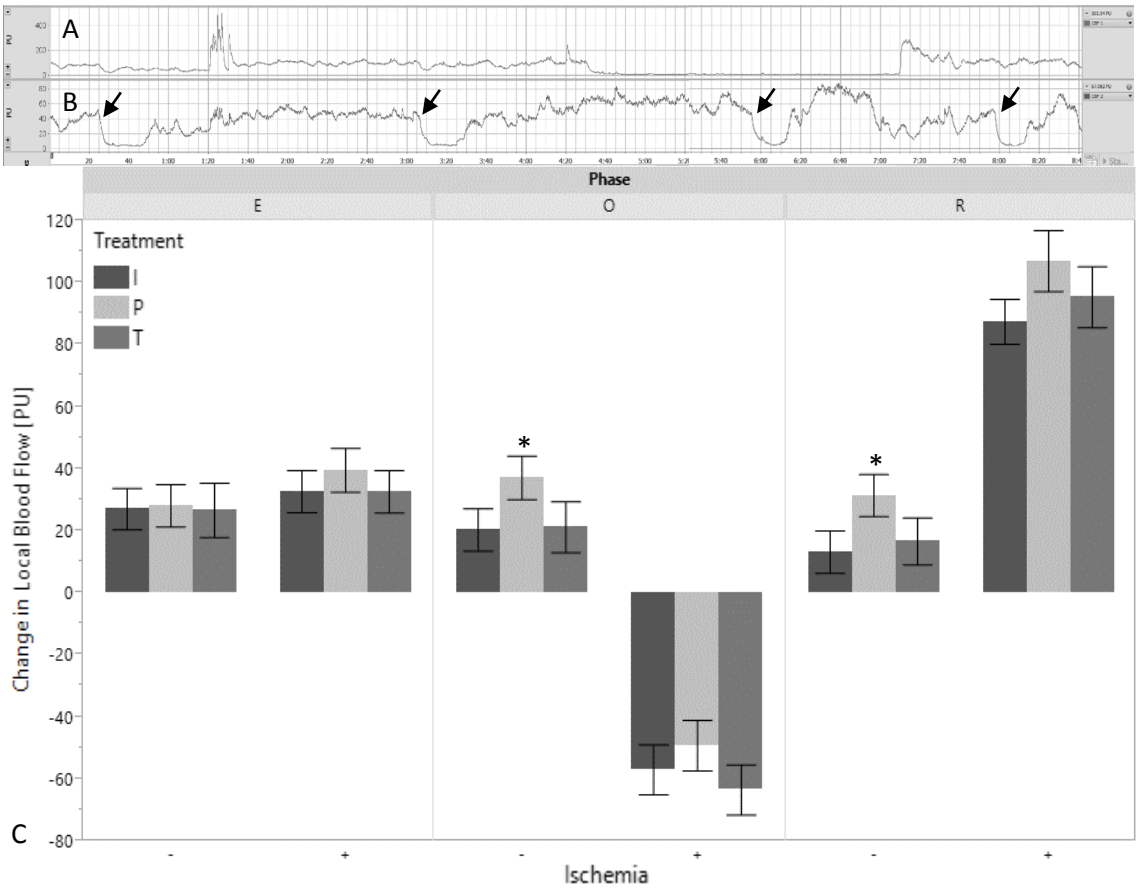

Figure 3.3: Effects of Phase, Treatment, and Ischemia on ΔBlood Flow. A. Local blood flow (PU) data trace showing increased blood flow during exercise, reduced flow

during occlusion, and reactive hyperemia during recovery. B. Contralateral blood flow data trace (PU). Arrows indicate the major drops in perfusion associated with blood pressure measurements. C. Change in local blood flow (PU) by phase, treatment type, and ischemia. Values are shown as mean ± SE. *$p \leq 0.05$ for Δblood flow vs. ischemia & phase.

3.5.2 Change in Vascular Resistance

$\Delta\overline{VR}$ is reflective of changes in both blood flow and pressure and therefore gives a more complete picture of the effects of neurostimulation on perfusion in ischemic tissues. As expected, occlusion increases $\Delta\overline{VR}$, and $\Delta\overline{VR}$ decreases during reactive hyperemia following occlusion, **Figure 3.4**. The increase in $\overline{VR}$ during the exercise phase cannot be attributed to the occlusion, indicating that static exercise itself creates ischemic conditions.

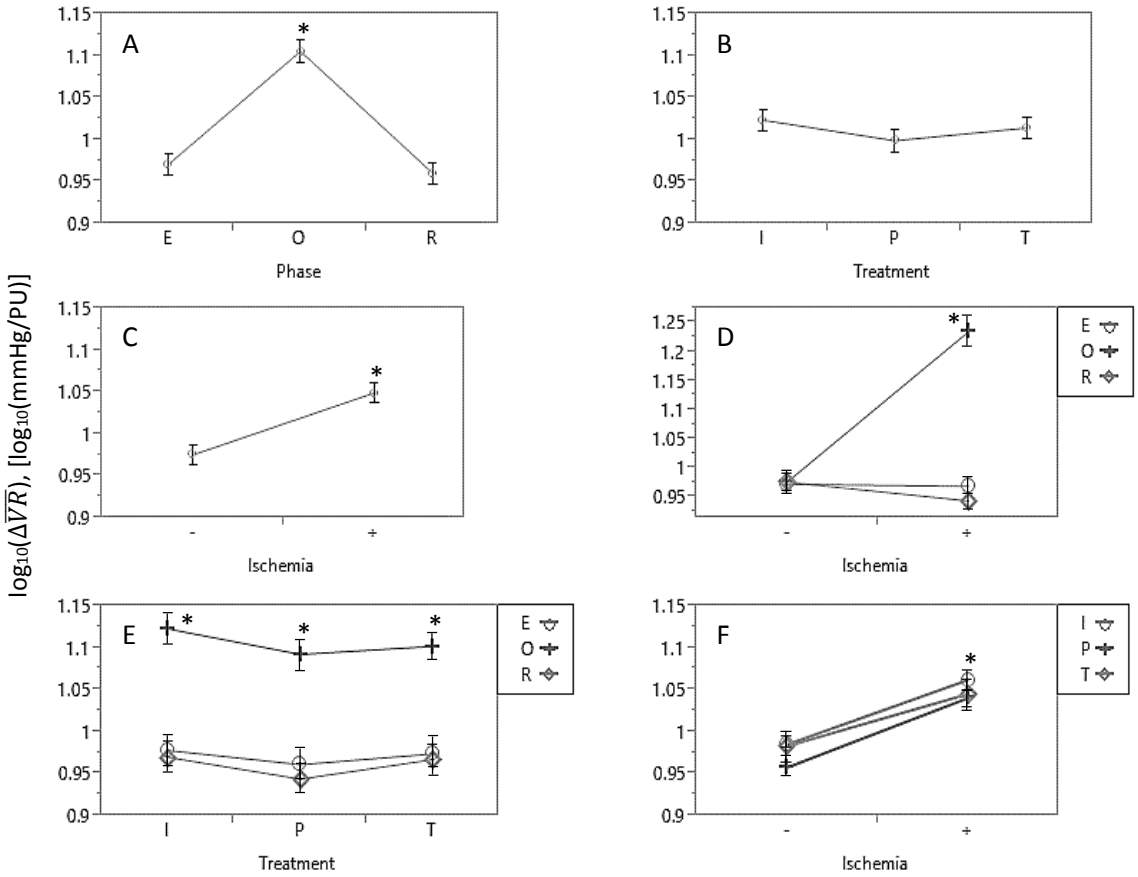

Figure 3.4: Effects of Phase, Treatment, and Ischemia on ΔVascular Resistance. These factors affect the normalized $\Delta\overline{VR}$ both directly and indirectly through interactions. A. Effect of phase on $\Delta\overline{VR}$. B. Effect of treatment type on $\Delta\overline{VR}$. C. Effect of ischemia on $\Delta\overline{VR}$. D. Effect of interaction between phase and ischemia on $\Delta\overline{VR}$. E. Effect of interaction between phase and treatment on $\Delta\overline{VR}$. F. Effect of interaction between treatment and ischemia on $\Delta\overline{VR}$. Values are shown as $\log_{10}$(means) ± SE. *p≤0.05 for $\Delta\overline{VR}$ vs. phase, treatment, & ischemia.

Interestingly, IFC increases $\Delta\overline{VR}$ as compared to placebo under acute ischemic conditions while there is no effect of TENS on $\Delta\overline{VR}$ during occlusion, **Figure 3.5**. Furthermore, the latent effect observed in the blood flow data is reflected in the vascular

resistance data. Both TENS and IFC increase $\Delta\overline{VR}$ 7-9 minutes after static exercise under non-ischemic conditions, though during reactive hyperemia this effect is lifted as the tissues recover from the acute ischemic event.

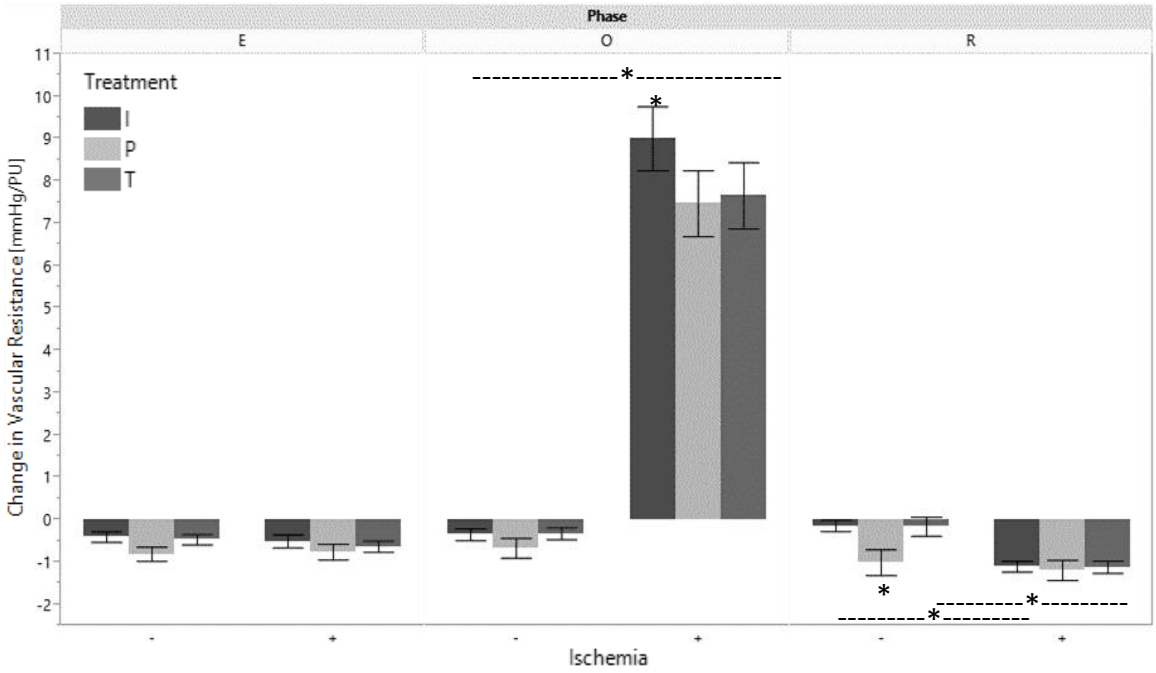

Figure 3.5: Mean Changes in Vascular Resistance for TENS, IFC, and Placebo Treatments during each Phase and Ischemic Condition. IFC increases $\Delta\overline{VR}$ as compared to placebo during ischemia, and both TENS and IFC increase $\Delta\overline{VR}$ under non-ischemic conditions versus ischemic conditions during the recovery phase following acute ischemia. Values are shown as means ± SE. *p≤0.05 for $\Delta\overline{VR}$ vs. phase, treatment, & ischemia.

3.5.3 Change in Pain

As previously mentioned, three separate scales were used to assess pain and paresthesia to discern the effect of neurostimulation on ischemic pain. Pain was assessed during the exercise and occlusion phases using the MPQ scale and every minute using the

NRS and Faces scales. To compare trends from each phase of the treatment, the pain data from the NRS and Faces scales were consolidated into the four main phases of the protocol and normalized to baseline. All three scales captured ischemic pain associated with occlusion, though one of the scales show a difference between TENS, IFC, or placebo treatment types in their effect on ischemic pain, **Figure 3.6, Appendix O Table III.B**. The MPQ scale shows the greatest difference in Δpain during the occlusion phase under ischemic versus non-ischemic conditions, while the NRS scale was the least effective at showing changes in pain. Pain increases during exercise on the MPQ and Faces scales, indicating that exercise induces pain independent of ischemia, **Figure 3.7**.

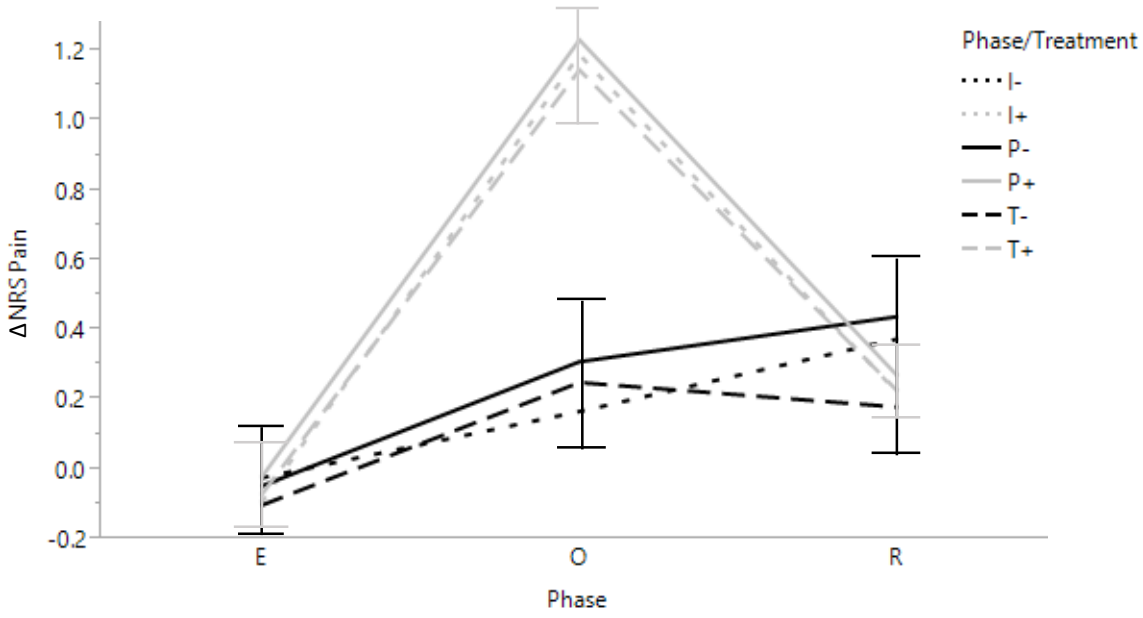

Figure 3.6: Trends in ΔNRS Pain by Treatment Type, Phase, and Ischemia. NRS pain increases during occlusion and remains elevated during recovery for every treatment type. When ischemia is induced, NRS pain increases more during occlusion. Values are shown as means ± SE. *$p \leq 0.001$ for Δpain vs. phase, treatment, & ischemia.

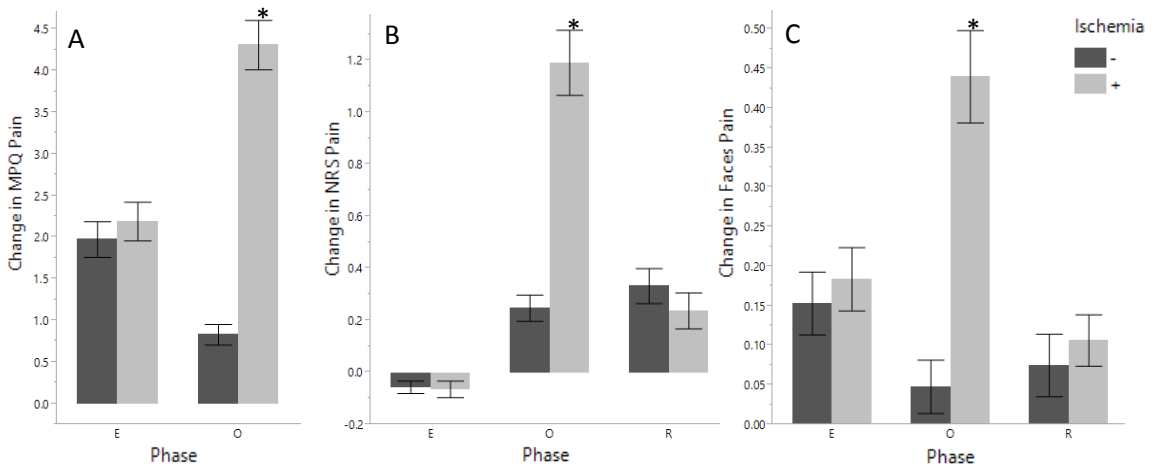

Figure 3.7: Trends in ΔPain by Phase and Ischemia. ΔPain is greatest during the occlusion phase with ischemia as shown by the A. MPQ scale, B. NRS, and C. Faces scale. The other 2-way interactions for Δpain between ischemia and phase have different trends across the different scales. Values are shown as means ± SE. *$p \leq 0.001$ for Δpain vs. phase & ischemia.

While the NRS and Faces scale show no differences in Δpain within the exercise and recovery phases ($p \geq 0.05$), there are different trends in directionality between the two scales. According to the NRS, pain trends toward a decrease during the exercise phase, increases during the occlusion phase, and trends toward an increase in the recovery phase. In contrast, the Faces scale trends toward an increase in pain for all three phases. The NRS model is only able to explain 33% of the variability in change in ischemic pain ($R^2 = 0.33$), indicating that it has little predictive power. The Faces scale is able to explain 61% of the variability ($R^2 = 0.61$), and for this reason only the MPQ and Faces scales are used for further analysis.

3.5.4 Change in Vascular Resistance associated with Change in Pain

To test the hypothesis the analgesic effect is dependent upon decreases in vascular resistance, the relationship between $\Delta\overline{VR}$ and Δpain is analyzed for each experimental phase, treatment type, and ischemic condition, **Appendix O, Table 3.C**.

As expected, ischemic conditions during the occlusion phase invoke a strong positive relationship between $\Delta\overline{VR}$ and Δpain, **Figure 3.8A**. However, during the exercise phase, there is a slightly negative relationship such that $\Delta\overline{VR}$ decreases as Δpain increases. This again suggests an exercise-mediated pain pathway is present and operates independently of ischemic pain mechanisms. Interestingly, $\Delta\overline{VR}$ trends to increase more rapidly in relation to pain with IFC treatment as compared to TENS and placebo, though there are no significant differences in the relationship between $\Delta\overline{VR}$ and Δpain between the three treatment types, **Figure 3.8B**.

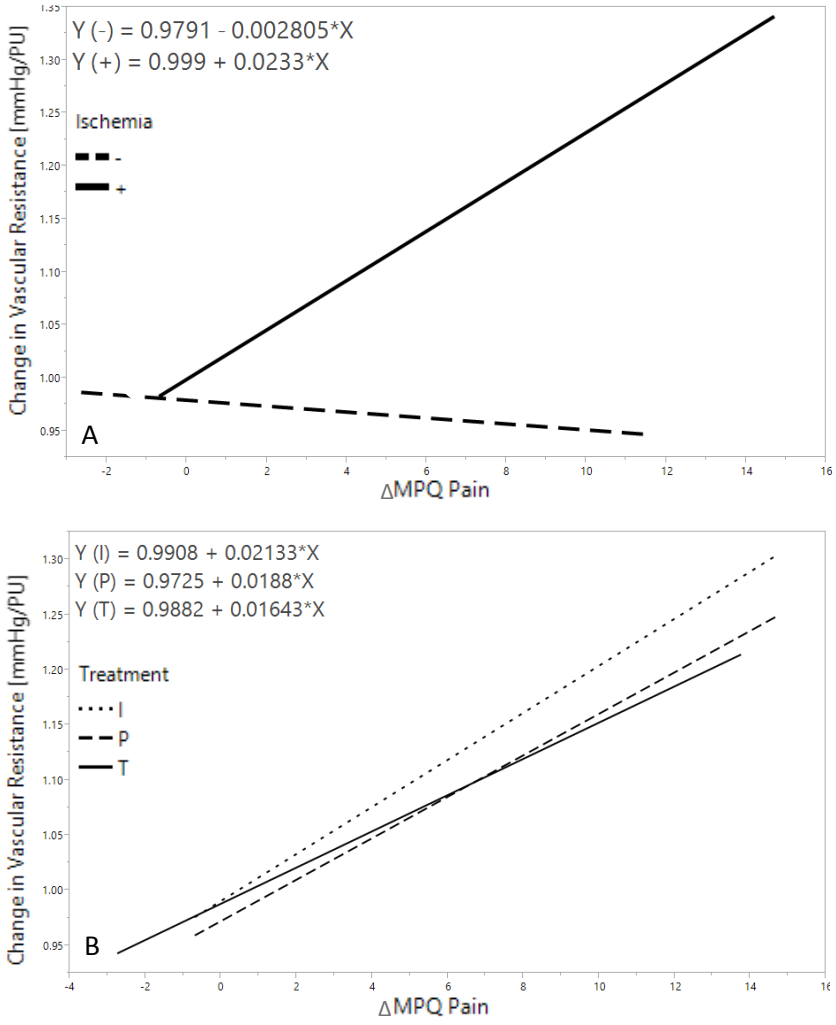

Figure 3.8: Effects of Ischemia, Phase, and Treatment Type on the Relationship between ΔMPQ Pain and $\Delta\overline{VR}$. Ischemia and pain influence the relationship between Δpain and $\Delta\overline{VR}$. A. ΔMPQ Pain and Ischemia. B. ΔMPQ Pain and Treatment.

3.6 DISCUSSION

The main goal of this study was to investigate trends in increased blood flow and decreased vascular resistance and pain associated with neurostimulation under ischemic conditions, possibly due to a decrease in sympathetic tone and increase in C fiber inhibition mediated by Aβ fiber activation. We could not detect any significant

hyperemic or analgesic effects of either TENS or IFC as predicted, though interestingly neurostimulation tended to increase vascular resistance during acute ischemia as well as under non-ischemic conditions after static exercise.

In a similar study in healthy young subjects similar to our study population, TENS increased blood flow and lowered vascular resistance in the calf [32]. The discrepancy between the results of the two studies is most likely not due to statistical power; our study had 45 replicates while the comparator study had 12. Rather, the different results may be due to differences in blood flow measurement techniques, with calf blood flow measured using venous occlusion plethysmography (VOP) as compared to cutaneous palmar blood flow measured using laser Doppler flowmetry (LDF) in our study. Plethysmography is a volume-based measurement, while laser Doppler signals are recorded in arbitrary Perfusion Units (PU) based on a motility standard that does not take tissue volume into account [75]. Thus, VOP may capture whole limb blood flow while LDF is limited to cutaneous perfusion.

Indeed, the relationship between LDF and VOP blood flow measurements during exercise is nonlinear [76]. LDF and VOP measurements are similar during early cutaneous vasodilation, but in later phases LDF values level off while VOP perfusion measurements increase. Furthermore, differences tissue composition between subjects can affect blood refractivity, introducing inter-subject type II error in LDF measurements [77], and therefore between-subject variability must be controlled for by including subject in the data analysis. Although measurements taken from both techniques reflect changes in perfusion, the magnitudes of changes in blood flow during active vasodilation

would not correlate. Thus, it is possible that neurostimulation had a greater hyperemic effect than measured.

We hypothesized that neurostimulation inhibits sympathetic vasoconstriction by inhibiting NE release at peripheral sympathetic nerve terminals. Therefore, neurostimulation ought to affect whole-limb blood flow, not just cutaneous blood flow. To more accurately assess whole-limb hemodynamic responses, future researchers should consider using an alternative system such as VOP to measure tissue perfusion rather than LDF. However, since VOP is too invasive to be feasible for use at Cal Poly, ultrasonic blood flow monitoring may serve as an effective alternative. Ultrasound techniques are used to detect early stages of atherosclerosis in peripheral arteries by measuring real-time blood velocities [78]. Similarly to LDF, ultrasound blood flow profiles will decrease during occlusion and can be used to screen for ischemic conditions. However, ultrasound blood flow measurements are more accurate than those taken with LDF systems [79]. Hand-held ultrasound blood flow measurement systems are affordable within the Cal Poly MEDITEC budget and are feasible for student use [80].

Since our blood flow measurement system may have been limited to cutaneous blood flow, neurostimulation may have a more profound effect on vascular tone throughout the forearm tissue than captured by our measurements. However, trends in vascular resistance and pain were observed with neurostimulation in response to ischemia, **Table 3.2.**

Table 3.2: Experimental Trends in Hemodynamic Factors and Pain during Recovery.

Expected Outcome	Placebo		TENS		IFC	
	PECO-	PECO+	PECO-	PECO+	PECO-	PECO+
Heart Rate	↓	-	↓	-	-	↓
Skin Temperature	↑	↑	↑	↑	↑	↑
Mean Blood Pressure	-	↓	↓	↓	↓	↓
Local Blood Flow	↑	↑↑	↑	↑↑	↑	↑↑
Contralateral Blood Flow	↑	↑	↑	↑	↑	↑
Vascular Resistance	↓	↓	-	↓	-	↓
Ischemic Pain	↑	↑	-	↑	-	↑

3.6.1 Change in Blood Flow

As expected, local tissues experienced a rapid increase in blood flow following occlusion due to the local release of vasodilator metabolites during ischemia. This phenomenon, known as reactive hyperemia, was observed for all three treatment types during the recovery phase. In addition, blood flow increased during exercise as expected, **Figure 3.3**. Compression of intramuscular arterioles during exercise results in the release of endothelial-derived vasodilator metabolites that competitively inhibit the effects of NE and thus inhibit sympathetic vasoconstriction in active tissues [81]. Forearm blood flow is higher at 30% than at 10% maximum voluntary contraction in the forearm following a 2 minute isometric contraction [82], indicating that SNS activity can be prevented by low-intensity exercise. These results followed the same general trends across all groups, **Appendix N**, with very little between-subject variability, **Appendix P**.

We hypothesized that an additional vasodilatory mechanism independent of reactive hyperemia and functional sympatholysis would be present in vessels undergoing

neurostimulation. Transcutaneous neurostimulation may activate peripheral α-2 adrenergic receptors [73], inhibiting norepinephrine release and decreasing local sympathetic tone. However, neurostimulation did not have its expected effect. Both TENS and IFC had a general trend towards decreased blood flow in both ischemic and non-ischemic conditions such that ischemia was more severe during occlusion while reactive hyperemia was dampened.

It is possible that we did not see an increase in blood flow associated with neurostimulation if there was no electrical α-2 adrenergic activation during exercise or if metabolic vasodilation during exercise amplified the hyperemic response and overwhelmed any additional increase in blood flow caused by electrical α-2 adrenergic activation. In order to distinguish between these two possibilities, we must include a non-exercise group in future studies and compare changes in blood flow with and without neurostimulation between the exercise and non-exercise groups.

Although a statistically significant difference eluded our study, the application of TENS and IFC to the ganglions can increase peripheral vasodilatory capacity and reduce blood pressure at the end of exercise in young healthy subjects [39, 32, 52], though there are conflicting reports on their efficacy. The mechanisms behind these observed hemodynamic trends is still unclear, though the blood flow measurement methods of VOP and ultrasound, respectively, may have introduced inconsistencies when comparing studies.

As stated previously, it is possible that the changes in cutaneous blood flow observed using LDF were not reflective of changes in whole-limb perfusion associated with neurostimulation. It is also possible that the increase in local blood flow regardless

of treatment type may be linked to metabolic vasodilator substances released during the isometric handgrip exercise. To isolate the effects of exercise from occlusion and neurostimulation on hyperemia, future studies ought to have both an exercise group and a non-exercise group in addition to blocking by treatment type (TENS, IFC, and placebo). If blood flow is reduced in non-exercise groups as compared to exercise groups, then exercise-induced vasodilation would indeed be a compounding effect.

However, this explanation for the negative blood flow results cannot account for the latency of the inhibitory effect of neurostimulation on exercise hyperemia when the occlusion was not applied. Previous evidence suggests that pre-treatment with TENS has no immediate effect on local blood flow but improves exercise tolerance at later time points [48]. Although the mechanism is unclear, it is possible that latent effects of both TENS and IFC may be associated with latencies in cellular activation. Transcutaneous stimuli have to travel through layers of skeletal muscle and connective tissue before they reach sympathetic neuron cell bodies in the ganglion, and signal impedance may be a time-dependent function of tissue conductance [83]. Therefore, it is possible that the stimuli did not sufficiently activate SNS neurons until later time points. Future studies may consider including a 5 minute pre-conditioning phase before beginning the trial in order to allow for the stimuli to overcome surface impedance and penetrate the ganglion.

3.6.2 Change in Vascular Resistance

The trends observed in the blood flow responses are reflected in the vascular resistance responses. Occlusion increases $\Delta\overline{VR}$ as expected, though interestingly IFC increases $\Delta\overline{VR}$ during recovery while TENS tends to increase $\Delta\overline{VR}$ as well, **Table 3.5**. Although these results are opposite to our original hypothesis that neurostimulation

increases blood flow and decreases $\Delta\overline{VR}$, it does suggest that the hemodynamic effects of IFC may be more exaggerated than those of TENS as we predicted. Furthermore, both TENS and IFC increase $\Delta\overline{VR}$ under non-ischemic conditions after static exercise, though during recovery this effect is diminished as reactive hyperemia takes over following the acute ischemic event. However, the effect of occlusion on $\Delta\overline{VR}$ is so much greater in magnitude than the effect of TENS or IFC that changes in $\Delta\overline{VR}$ associated with either form of neurostimulation could be considered clinically insignificant.

One possible explanation for why we did not see a decrease in $\Delta\overline{VR}$ associated with neurostimulation during acute ischemia as predicted is that peripheral α-2 adrenergic activation via electrical stimulation is affected by hypoxic conditions. As previously mentioned, electrical stimulation activates α-2 adrenoceptors, disrupting N-type calcium current in sympathetic nerve terminals and inhibiting the release of norepinephrine, a known vasoconstrictor. However, without sufficient blood flow, normal functioning of α-2A receptors may be disrupted. For example, forebrain ischemia decreases α -2A receptor binding in the rat hippocampus [84]. A similar event may be occurring in α-2A receptors at the ganglion or at the synapse with smooth muscle cells following peripheral occlusion in healthy young humans, though the mechanism is not clear.

Although we postulated that electrical stimuli have an inhibitory effect on sympathetic signal transmission in Aβ fibers, a second possible explanation for the observed inhibition of vasodilation associated with neurostimulation is that TENS and IFC actually activated peripheral SNS neurons. To our knowledge there are no comparative studies with published data indicating increased sympathetic vascular tone associated with ganglial stimulation; however, TENS treatment has been used to activate

the sympathetics in healthy humans [85], and since the somas of efferent SNS neurons are located in the same ganglia as the somas of efferent Aβ fibers, it is possible that transcutaneous stimulation applied at the ganglia activated efferent vascular SNS fibers as opposed to efferent Aβ fibers and ultimately increased peripheral sympathetic vasoconstriction.

3.6.3 Change in Pain

Pain is present throughout the experimental protocol regardless of phase, ischemic conditions, or the type of neurostimulation applied, **Figure 3.6**. The MPQ scale quantified the greatest magnitude of Δpain during the exercise and occlusion periods, and the Faces scale corroborates a positive Δpain during exercise and occlusion as well as during the recovery period, **Figure 3.7**. These results were the same across TENS, IFC, and placebo treatments both with and without PECO and therefore do not support our hypothesis that transcutaneous neurostimulation decreases ischemic pain.

There is a general consensus that neurostimulation, including TENS and IFC, attenuates both chronic and acute ischemic pain [32, 62, 64, 66, 37]. However, the onset and duration of analgesia may vary considerably between patients [37], and the same protocol may have different degrees of antinociception in acute experimental pain compared with chronic clinical pain [38]. There is also conflicting evidence indicating that neurostimulation does not have a significant analgesic effect on this type of pain. IFC and TENS treatments have shown no differences in analgesia as compared to placebo during ischemic-induced pain tests utilizing the submaximal tourniquet technique (similar to our study) and the cold pressor technique. Furthermore, 50 Hz and 100 Hz TENS treatments have shown no differences in analgesia as compared to IFC treatments

at the same frequency [49, 68, 65, 86]. Therefore the results from our study may be a true negative, as opposed to a false negative, and similarly substantiate the observed non-significance of transcutaneous neurostimulation on acute experimental pain.

One possible explanation for the inability of subjects to detect a cessation in pain is that that cytokines accumulated during the static handgrip exercise and caused pain. While our study was not the first to involve exercise, more than half of our subjects gripped above 30% maximal volumetric contraction and did not adequately decrease their grip strength when instructed. Therefore, it is possible that our subjects experienced greater microdamage to their skeletal muscle tissues and had higher concentrations of algesic cytokines, including arachidonic acid (AA). AA is metabolized into prostaglandin, which inhibits potassium efflux from nociceptors via a G protein, protein kinase A cascade. The sensitized peripheral nociceptors transmit afferent signals to the CNS which are interpreted as pain [87]. Although participants only applied 30% of their maximum handgrip force, the exercise intensity and duration was sufficient to introduce microdamage to the tissues [81, 82]. Analgesic cytokines from the exercise phase may have remained un-metabolized in the tissues long enough to induce pain during the occlusion and recovery phases as well. Therefore, dynamic exercise may be preferable to static exercise for the purpose of our study in order to decrease the buildup of metabolic byproducts.

It is also possible that summation of afferent exercise and ischemic pain signals caused an overall increase in pain. Gate Control Theory postulates that TENS attenuates nociception by stimulating Aβ fibers parallel to C fibers responsible for transmitting pain signals to the brain [41]. However, the Central Summation Theory proposes that

summation of pain may overwhelm the integration center in the thalamus, resulting in increased pain [88]. Therefore, the summation of ischemic and exercise-induced pain may cause an overall increased pain sensation regardless of Aβ fiber activation via neurostimulation. Similar to isolating exercised-induced hyperemic effects, incorporating a non-exercise group in future studies would help isolate the effect of exercise from occlusion and neurostimulation on pain. If pain is reduced in non-exercise groups as compared to exercise groups, then exercise-induced pain would indeed be a compounding effect.

3.6.4 Change in Vascular Resistance associated with Change in Pain

We hypothesized that electrical activation of Aβ fibers would reduce $\overline{VR}$ and pain via parallel mechanisms. As expected, painful ischemic conditions during the occlusion phase invoke a strong positive relationship between $\Delta\overline{VR}$ and Δpain, **Figure 3.8A**. Graded increases in ischemic pain are associated with graded elevations in forearm vascular resistance [89]. This coordination suggests that changes in pain and vascular tone may be mechanistically linked, possibly due to a stress response. When the brain detects a painful stimulus, the hypothalamus signals the adrenal glands to release vasoconstrictive signaling molecules such as adrenaline and NE as part of the "flight or fight" response. We see evidence of this phenomenon in how HR tended to decrease over the course of each experimental trial, **Appendix M, Figure III.C**. Therefore we would expect that vascular resistance would increase in response to an increase in pain.

Interestingly, while there are no significant differences in the relationship between $\Delta\overline{VR}$ and Δpain between the three treatment types, $\Delta\overline{VR}$ trends to increase more rapidly in relation to pain with IFC treatment as compared to TENS and placebo, **Figure 3.8B**. This

indicates that subjects receiving IFC treatment experience increased vascular resistance in response to a painful stimulus. To our knowledge, there is no evidence to date that TENS and IFC have a different effect on the relationship between pain and $\overline{VR}$, though the differences in the trends observed in our study warrant further investigation with a larger sample size. We predicted that our clinical study only needed 45 subjects per treatment group to see a 10% decrease in VR and pain associated with neurostimulation, assuming that the ΔVR and Δpain values observed for the placebo, PECO- group in pilot study IV were valid control measurements. However, it is possible that the true between-subject and within-subject variability associated with our study design are higher than were sampled from pilot study IV, as the student volunteers during the pilot study were homogenous in their age, ethnicity, and physical fitness levels. Therefore, increasing our sample size to 100 subjects would double the predictive power of this study and may increase our ability to observe changes in VR and pain associated with neurostimulation.

CHAPTER 4: SUMMARY

4.1 SYNOPSIS

Peripheral arterial occlusive disease (PAOD) is a pervasive disease characterized by impaired metabolic vasodilation in the peripheries. While intermittent claudication symptoms develop in symptomatic patients, limb ischemia develops in all cases as the disease progresses. The current gold standard of treatment is a combined drug and surgical intervention involving statins, antihypertensive drugs, angioplasty, and stenting. While this approach addresses the impaired blood flow and pain symptoms associated with PAOD, there are often adverse side effects and restenosis.

Neurostimulation may provide a much-needed innovative treatment option for PAOD, as it has a known analgesic effect on both acute and chronic pain and may also increase blood flow. Electrical activation of afferent Aβ fibers, either in the periphery or near the ganglia, inhibits both pain-signal transmission from afferent C fibers and norepinephrine release at sympathetic nerve terminals. The Gate Control Theory explains how Aβ fibers activate an inhibitory interneuron in the dorsal horn of the spinal cord that synapses with ascending spinothalamic (ST) neurons, effectively dampening the pain signal to the brain. Simultaneously, suppression of the ST neurons may result in decreased metaboreflex control as systemic sympathetic vasoconstriction is reduced and mean blood pressure decreases. Aβ fiber activation is also thought to activate α-2 adrenoceptors on primary afferent neurons along the same dermatome, resulting in suppressed sympathetic tone and an increase in local blood flow. Ultimately, electrical stimulation may decrease local vascular resistance (VR), **Figure 1.7**.

The focus of our study was to optimize methods and perform a small-scale clinical study for investigation of hemodynamic and pain responses to neurostimulation during an ischemic event in otherwise healthy subjects. We hypothesized that transcutaneous electrical neurostimulation (TENS) and interferential current (IFC) treatments applied at the C7 and T4 ganglia would result in decreased pain and local vascular resistance in the palms and that IFC may have a greater analgesic and hyperemic effect than TENS due to differences in stimulus current waveforms.

Unfortunately, our findings did not directly support either hypothesis. We found no significant analgesic or hyperemic effects during or following acute ischemia; rather, we saw trends indicating that TENS and IFC increase pain and VR under both ischemic and non-ischemic conditions. Interestingly, IFC increased VR under acute ischemic conditions while TENS had a lesser effect. We also observed a greater increase in VR correlated with an increase in pain with IFC as compared to TENS, indicating that the out-of-phase sinusoidal waveform characteristic of IFC may more readily overcome skin impedance and have a greater effect on sympathetic tone as predicted.

4.2 FUTURE WORK

Considering the outcome of this study, we would propose several changes to the study design that may help to establish clearer conclusions from the data. Future researchers should consider replacing the laser Doppler flowmetry blood flow measurement system with an ultrasound system as it is both feasible for use by students and more accurate at measuring whole-limb perfusion [79]. It would also be advantageous to recruit study participants from a more diverse age range (18-25 years versus 50+ years) and lifestyle (athletic versus sedentary) by targeting recruitment to

professors and student athletes in addition to the general student population, as a healthy young sample population is not reflective of the general PAOD patient population [3].

Many participants were anxious about using neurostimulation for the first time, as evidenced by a general trend towards decreased heart rate over the course of the session, **Appendix M, Figure III.C**. Incorporating an introductory trial run with each participant a day or more prior to the study sessions may allow for less stress-induced sympathetic response during the data collection. Furthermore, including a non-exercise group will be necessary to isolate the effects of exercise from occlusion and neurostimulation on hyperemic and analgesic trends.

It may also be interesting to further investigate the effects of paresthesia on trends in hyperemia and analgesia. Paresthesia introduced significant variability to the pain data as it was often perceived by the participants as an unusual or irritating sensation during testing, **Appendix P**. If the stimulus intensity was set to a subparesthesia level, sympathetic vasoconstriction due to the stress response and non-painful stimuli incorporated into the self-reported pain scores may diminish, possibly resulting in less noise in the blood flow and pain data and more pronounced hyperemia and analgesia associated with neurostimulation. Furthermore, amplifying the baseline pain signal may also improve the signal-to-noise ratio. While the Cal Poly Human Subjects Committee approval only extends to 180 mmHg of pressure on the forearm for the submaximal tourniquet technique, it is possible that moving the site of occlusion to the upper arm may be more painful for most people than an equivalent pressure on the forearm. Interestingly, the amount of pain induced by the submaximal tourniquet technique in our study was less than that captured in comparator studies using a similar numeric rating scale; therefore,

an alternative ischemic pain method should be considered. The cold pressor test may also be used to test the analgesic and hyperemic effects of neurostimulation and may provide a greater painful stimulus that the tourniquet technique [65, 68].

Finally, incorporating a measurable control for skin surface electrode impedance may be important for standardizing treatment across subjects. While future studies ought to consider including a 5 minute pre-conditioning phase in order to control for the latent effects of transcutaneous stimulation and allow time for the stimuli to overcome surface impedance, slight differences in electrode configuration and tissue conductance between patients can alter the electric field distribution and thereby the depth and selectivity of neural activation [83]. Although we attempted to control the shape and depth of the electric field by standardizing electrode placement, a more accurate approach may be to use a multimeter to quantitatively assess surface impedance. While most electromyography (EMG) units have a built-in impedance sensor to direct electrode placement, commercially available TENS units do not [90]. Incorporating a similar user feedback mechanism into future TENS unit designs would be desirable.

Although our study was not able to find significant evidence that IFC has a greater analgesic or hyperemic effects than TENS, nor that transcutaneous neurostimulation in general had such effects, further characterization of the different stimulus waveforms may provide great insight into extracellular electrical control of vascular tone.

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APPENDICES

Appendix A: Informed Consent Form

INFORMED CONSENT TO PARTICIPATE IN A RESEARCH PROJECT:
"The Effects of Transcutaneous Electrical Neurostimulation on Analgesia and Peripheral Perfusion"

A research project on peripheral blood flow and ischemic pain is being conducted by Leah Schafer and Kaylee Keck in the Department of Biomedical Engineering at Cal Poly, San Luis Obispo. The purpose of the study is to measure changes in blood flow, heart rate, and blood pressure due to the application of electrical neurostimulation.

You are being asked to take part in this study by first filling out a short medical history questionnaire. Questions marked with an asterisk (*) are required, but any others you do not wish to answer may be omitted. These questions are directly related to your safety. During each treatment session, you will be hooked up to a neurostimulation device that will be attached to your upper back with electrodes, a blood flow measurement system using skin probes on the upper arms, a respiration belt wrapped around your midsection, and a blood pressure cuff applied to each arm. Appropriate clothing should be worn to ensure proper placement of the electrodes on your upper back. Prior to the treatment session, you will be asked to not consume caffeine for up to 12 hours before the session in an effort to minimize caffeine's effects on blood flow. Once the session begins, you will be asked to squeeze a handgrip force measurement device for a short period of time. You will experience electrical stimulation from the attached electrodes, which you may feel as a warm, tingling sensation on your back. Your participation will involve 6 sessions, with a 10-minute break (5 breaks) in between each session, for a total of 2 hours on one day. In some of these sessions, the neurostimulation device will be hooked up to you, but no current will be applied, as in you will not feel any sensation on your back. This will be randomized. Please be aware that you are not required to participate in this research and you may discontinue your participation at any time without penalty.

The possible risks associated with participation in this study include pain due to temporarily induced ischemia i.e. insufficient blood flow to the tissue, skin irritation from the application of skin probes and electrodes, and possible discomfort and/or stress from gripping the hand force measurement device. If your personal pain tolerance threshold is reached at any point, you may discontinue your participation immediately. If you should experience residual pain or tingling after the duration of the experiment or an allergic reaction at the site of the probes or electrodes, please be aware that you may contact Cal Poly Health and Counseling Services, located in building 27, at (805) 756-1211 for assistance.

Your confidentiality will be protected by recording your medical history, age, gender, height and weight on a document with a corresponding code. This document will be kept as a hard copy only and separate from the corresponding list of codes. Your information will only be accessible to the researchers in this study. If the results of the study are published, any identifying information will be omitted. The incentive associated with this study is a benefit in the form of a $25 Visa gift card to those that choose to

participate and agree to the caffeine restrictions. In the case that you choose to prematurely discontinue your participation due to possible discomfort and/or stress, monetary compensation will still be provided. You may only volunteer once for this study. Depending on the outcome of the study, this could become an additional treatment method for individuals with ischemic pain.

If you have questions regarding this study or would like to be informed of the results when the study is completed, please feel free to contact Leah Schafer at (530) 354-5061 or Dr. Trevor Cardinal at (805) 756-6244. If you have concerns regarding the manner in which the study is conducted, you may contact Dr. Steve Davis, Chair of the Cal Poly Human Subjects Committee, at (805) 756-2754, sdavis@calpoly.edu, or Dr. Dean Wendt, Dean of Research, at (805) 756-1508, dwendt@calpoly.edu.

If you agree to voluntarily participate in this research project as described, please indicate your agreement by signing below. Please keep one copy of this form for your reference, and thank you for your participation in this research.

_____

Signature of Volunteer Date

_____

Signature of Researcher Date

Appendix B: Medical History Questionnaire

MEDICAL HISTORY

*= *Required*

General Information

Participant:

*Name:_____

*Email: _____ Contact phone number: _____

*Dominant Hand: □ Right □ Left

Age: _____

Height: _____

Weight: _____

Sex:
□ Male □ Female

Women only answer the following:
*Are you currently pregnant? □ Yes □ No
Are you currently breast-feeding? □ Yes □ No

Men and women answer the following:
*Have you consumed caffeine in the last 12 hours? □ Yes □ No
*Have you exercised to at least 50% of your maximum heart rate (moderate exercise) in the last:
48 hours? □ Yes □ No
12 hours? □ Yes □ No

List any prescription medications you are currently taking:

_____

Do you have any implantable electrical devices (pacemaker, implantable cardioverter defibrillator, etc.)?

□ Yes □ No If yes, please list: _____

In the past two months, have you experienced any major injury or significant trauma to your arms or upper back? □ Yes □ No If yes, please describe: _____

[over]

Past Medical History

Have you experienced any of the following:

Yes No

□ □ *Dermatitis/eczema (inflammation of the skin)
□ □ *Chronic pain or tingling sensations in your limbs
□ □ *Syncope (fainting)
□ □ *Epilepsy or seizures
□ □ Heart attack
□ □ High blood pressure (hypertension)
□ □ Rheumatic Fever
□ □ Heart murmur (abnormal heart sound)
□ □ Arrhythmia (irregular heartbeat)
□ □ Diseases of the arteries (peripheral artery disease, coronary artery disease)
□ □ Varicose veins (twisted, enlarged veins)
□ □ Diabetes or abnormal blood sugar
□ □ Phlebitis (inflammation of the veins)
□ □ Stroke
□ □ Anemia (low red blood cell count)

Smoking

Have you ever smoked tobacco? □ Yes □ No

If yes, how long did you smoke/how long have you been smoking?_____

How frequently did/do you smoke? _____

Drinking

On average, do you drink more than 1 alcoholic beverage per day (women)/ 2 alcoholic beverages per day (men)? □ Yes □ No

Appendix C: W9 Tax Form

Form **W-9**
(Rev. December 2014)
Department of the Treasury
Internal Revenue Service

Request for Taxpayer Identification Number and Certification

Give Form to the requester. Do not send to the IRS.

Print or type
See **Specific Instructions** on page 2.

1 Name (as shown on your income tax return). Name is required on this line; do not leave this line blank.

2 Business name/disregarded entity name, if different from above

3 Check appropriate box for federal tax classification; check only **one** of the following seven boxes:

☐ Individual/sole proprietor or single-member LLC ☐ C Corporation ☐ S Corporation ☐ Partnership ☐ Trust/estate

☐ Limited liability company. Enter the tax classification (C=C corporation, S=S corporation, P=partnership) ▶ _____

Note. For a single-member LLC that is disregarded, do not check LLC; check the appropriate box in the line above for the tax classification of the single-member owner.

☐ Other (see instructions) ▶

4 Exemptions (codes apply only to certain entities, not individuals; see instructions on page 3):

Exempt payee code (if any) _____

Exemption from FATCA reporting code (if any) _____

(Applies to accounts maintained outside the U.S.)

5 Address (number, street, and apt. or suite no.)

Requester's name and address (optional)

6 City, state, and ZIP code

7 List account number(s) here (optional)

Part I Taxpayer Identification Number (TIN)

Enter your TIN in the appropriate box. The TIN provided must match the name given on line 1 to avoid backup withholding. For individuals, this is generally your social security number (SSN). However, for a resident alien, sole proprietor, or disregarded entity, see the Part I instructions on page 3. For other entities, it is your employer identification number (EIN). If you do not have a number, see *How to get a TIN* on page 3.

Note. If the account is in more than one name, see the instructions for line 1 and the chart on page 4 for guidelines on whose number to enter.

Social security number

___ – ___ – ____

or

Employer identification number

__ – _____

Part II Certification

Under penalties of perjury, I certify that:

1. The number shown on this form is my correct taxpayer identification number (or I am waiting for a number to be issued to me); and
2. I am not subject to backup withholding because: (a) I am exempt from backup withholding, or (b) I have not been notified by the Internal Revenue Service (IRS) that I am subject to backup withholding as a result of a failure to report all interest or dividends, or (c) the IRS has notified me that I am no longer subject to backup withholding; and
3. I am a U.S. citizen or other U.S. person (defined below); and
4. The FATCA code(s) entered on this form (if any) indicating that I am exempt from FATCA reporting is correct.

Certification instructions. You must cross out item 2 above if you have been notified by the IRS that you are currently subject to backup withholding because you have failed to report all interest and dividends on your tax return. For real estate transactions, item 2 does not apply. For mortgage interest paid, acquisition or abandonment of secured property, cancellation of debt, contributions to an individual retirement arrangement (IRA), and generally, payments other than interest and dividends, you are not required to sign the certification, but you must provide your correct TIN. See the instructions on page 3.

Sign Here **Signature of U.S. person** ▶ **Date** ▶

General Instructions

Section references are to the Internal Revenue Code unless otherwise noted.

Future developments. Information about developments affecting Form W-9 (such as legislation enacted after we release it) is at *www.irs.gov/fw9*.

Purpose of Form

An individual or entity (Form W-9 requester) who is required to file an information return with the IRS must obtain your correct taxpayer identification number (TIN) which may be your social security number (SSN), individual taxpayer identification number (ITIN), adoption taxpayer identification number (ATIN), or employer identification number (EIN), to report on an information return the amount paid to you, or other amount reportable on an information return. Examples of information returns include, but are not limited to, the following:

- Form 1099-INT (interest earned or paid)
- Form 1099-DIV (dividends, including those from stocks or mutual funds)
- Form 1099-MISC (various types of income, prizes, awards, or gross proceeds)
- Form 1099-B (stock or mutual fund sales and certain other transactions by brokers)
- Form 1099-S (proceeds from real estate transactions)
- Form 1099-K (merchant card and third party network transactions)
- Form 1098 (home mortgage interest), 1098-E (student loan interest), 1098-T (tuition)
- Form 1099-C (canceled debt)
- Form 1099-A (acquisition or abandonment of secured property)

Use Form W-9 only if you are a U.S. person (including a resident alien), to provide your correct TIN.

If you do not return Form W-9 to the requester with a TIN, you might be subject to backup withholding. See *What is backup withholding?* on page 2.

By signing the filled-out form, you:

1. Certify that the TIN you are giving is correct (or you are waiting for a number to be issued),
2. Certify that you are not subject to backup withholding, or
3. Claim exemption from backup withholding if you are a U.S. exempt payee. If applicable, you are also certifying that as a U.S. person, your allocable share of any partnership income from a U.S. trade or business is not subject to the withholding tax on foreign partners' share of effectively connected income, and
4. Certify that FATCA code(s) entered on this form (if any) indicating that you are exempt from the FATCA reporting, is correct. See *What is FATCA reporting?* on page 2 for further information.

Cat. No. 10231X Form **W-9** (Rev. 12-2014)

Note. If you are a U.S. person and a requester gives you a form other than Form W-9 to request your TIN, you must use the requester's form if it is substantially similar to this Form W-9.

Definition of a U.S. person. For federal tax purposes, you are considered a U.S. person if you are:

- An individual who is a U.S. citizen or U.S. resident alien;
- A partnership, corporation, company, or association created or organized in the United States or under the laws of the United States;
- An estate (other than a foreign estate); or
- A domestic trust (as defined in Regulations section 301.7701-7).

Special rules for partnerships. Partnerships that conduct a trade or business in the United States are generally required to pay a withholding tax under section 1446 on any foreign partners' share of effectively connected taxable income from such business. Further, in certain cases where a Form W-9 has not been received, the rules under section 1446 require a partnership to presume that a partner is a foreign person, and pay the section 1446 withholding tax. Therefore, if you are a U.S. person that is a partner in a partnership conducting a trade or business in the United States, provide Form W-9 to the partnership to establish your U.S. status and avoid section 1446 withholding on your share of partnership income.

In the cases below, the following person must give Form W-9 to the partnership for purposes of establishing its U.S. status and avoiding withholding on its allocable share of net income from the partnership conducting a trade or business in the United States:

- In the case of a disregarded entity with a U.S. owner, the U.S. owner of the disregarded entity and not the entity;
- In the case of a grantor trust with a U.S. grantor or other U.S. owner, generally, the U.S. grantor or other U.S. owner of the grantor trust and not the trust; and
- In the case of a U.S. trust (other than a grantor trust), the U.S. trust (other than a grantor trust) and not the beneficiaries of the trust.

Foreign person. If you are a foreign person or the U.S. branch of a foreign bank that has elected to be treated as a U.S. person, do not use Form W-9. Instead, use the appropriate Form W-8 or Form 8233 (see Publication 515, Withholding of Tax on Nonresident Aliens and Foreign Entities).

Nonresident alien who becomes a resident alien. Generally, only a nonresident alien individual may use the terms of a tax treaty to reduce or eliminate U.S. tax on certain types of income. However, most tax treaties contain a provision known as a "saving clause." Exceptions specified in the saving clause may permit an exemption from tax to continue for certain types of income even after the payee has otherwise become a U.S. resident alien for tax purposes.

If you are a U.S. resident alien who is relying on an exception contained in the saving clause of a tax treaty to claim an exemption from U.S. tax on certain types of income, you must attach a statement to Form W-9 that specifies the following five items:

1. The treaty country. Generally, this must be the same treaty under which you claimed exemption from tax as a nonresident alien.
2. The treaty article addressing the income.
3. The article number (or location) in the tax treaty that contains the saving clause and its exceptions.
4. The type and amount of income that qualifies for the exemption from tax.
5. Sufficient facts to justify the exemption from tax under the terms of the treaty article.

Example. Article 20 of the U.S.-China income tax treaty allows an exemption from tax for scholarship income received by a Chinese student temporarily present in the United States. Under U.S. law, this student will become a resident alien for tax purposes if his or her stay in the United States exceeds 5 calendar years. However, paragraph 2 of the first Protocol to the U.S.-China treaty (dated April 30, 1984) allows the provisions of Article 20 to continue to apply even after the Chinese student becomes a resident alien of the United States. A Chinese student who qualifies for this exception (under paragraph 2 of the first protocol) and is relying on this exception to claim an exemption from tax on his or her scholarship or fellowship income would attach to Form W-9 a statement that includes the information described above to support that exemption.

If you are a nonresident alien or a foreign entity, give the requester the appropriate completed Form W-8 or Form 8233.

Backup Withholding

What is backup withholding? Persons making certain payments to you must under certain conditions withhold and pay to the IRS 28% of such payments. This is called "backup withholding." Payments that may be subject to backup withholding include interest, tax-exempt interest, dividends, broker and barter exchange transactions, rents, royalties, nonemployee pay, payments made in settlement of payment card and third party network transactions, and certain payments from fishing boat operators. Real estate transactions are not subject to backup withholding.

You will not be subject to backup withholding on payments you receive if you give the requester your correct TIN, make the proper certifications, and report all your taxable interest and dividends on your tax return.

Payments you receive will be subject to backup withholding if:

1. You do not furnish your TIN to the requester,
2. You do not certify your TIN when required (see the Part II instructions on page 3 for details),
3. The IRS tells the requester that you furnished an incorrect TIN,
4. The IRS tells you that you are subject to backup withholding because you did not report all your interest and dividends on your tax return (for reportable interest and dividends only), or
5. You do not certify to the requester that you are not subject to backup withholding under 4 above (for reportable interest and dividend accounts opened after 1983 only).

Certain payees and payments are exempt from backup withholding. See *Exempt payee code* on page 3 and the separate Instructions for the Requester of Form W-9 for more information.

Also see *Special rules for partnerships* above.

What is FATCA reporting?

The Foreign Account Tax Compliance Act (FATCA) requires a participating foreign financial institution to report all United States account holders that are specified United States persons. Certain payees are exempt from FATCA reporting. See *Exemption from FATCA reporting code* on page 3 and the Instructions for the Requester of Form W-9 for more information.

Updating Your Information

You must provide updated information to any person to whom you claimed to be an exempt payee if you are no longer an exempt payee and anticipate receiving reportable payments in the future from this person. For example, you may need to provide updated information if you are a C corporation that elects to be an S corporation, or if you no longer are tax exempt. In addition, you must furnish a new Form W-9 if the name or TIN changes for the account; for example, if the grantor of a grantor trust dies.

Penalties

Failure to furnish TIN. If you fail to furnish your correct TIN to a requester, you are subject to a penalty of $50 for each such failure unless your failure is due to reasonable cause and not to willful neglect.

Civil penalty for false information with respect to withholding. If you make a false statement with no reasonable basis that results in no backup withholding, you are subject to a $500 penalty.

Criminal penalty for falsifying information. Willfully falsifying certifications or affirmations may subject you to criminal penalties including fines and/or imprisonment.

Misuse of TINs. If the requester discloses or uses TINs in violation of federal law, the requester may be subject to civil and criminal penalties.

Specific Instructions

Line 1

You must enter one of the following on this line; **do not** leave this line blank. The name should match the name on your tax return.

If this Form W-9 is for a joint account, list first, and then circle, the name of the person or entity whose number you entered in Part I of Form W-9.

a. **Individual.** Generally, enter the name shown on your tax return. If you have changed your last name without informing the Social Security Administration (SSA) of the name change, enter your first name, the last name as shown on your social security card, and your new last name.

Note. ITIN applicant: Enter your individual name as it was entered on your Form W-7 application, line 1a. This should also be the same as the name you entered on the Form 1040/1040A/1040EZ you filed with your application.

b. **Sole proprietor or single-member LLC.** Enter your individual name as shown on your 1040/1040A/1040EZ on line 1. You may enter your business, trade, or "doing business as" (DBA) name on line 2.

c. **Partnership, LLC that is not a single-member LLC, C Corporation, or S Corporation.** Enter the entity's name as shown on the entity's tax return on line 1 and any business, trade, or DBA name on line 2.

d. **Other entities.** Enter your name as shown on required U.S. federal tax documents on line 1. This name should match the name shown on the charter or other legal document creating the entity. You may enter any business, trade, or DBA name on line 2.

e. **Disregarded entity.** For U.S. federal tax purposes, an entity that is disregarded as an entity separate from its owner is treated as a "disregarded entity." See Regulations section 301.7701-2(c)(2)(iii). Enter the owner's name on line 1. The name of the entity entered on line 1 should never be a disregarded entity. The name on line 1 should be the name shown on the income tax return on which the income should be reported. For example, if a foreign LLC that is treated as a disregarded entity for U.S. federal tax purposes has a single owner that is a U.S. person, the U.S. owner's name is required to be provided on line 1. If the direct owner of the entity is also a disregarded entity, enter the first owner that is not disregarded for federal tax purposes. Enter the disregarded entity's name on line 2, "Business name/disregarded entity name." If the owner of the disregarded entity is a foreign person, the owner must complete an appropriate Form W-8 instead of a Form W-9. This is the case even if the foreign person has a U.S. TIN.

Line 2

If you have a business name, trade name, DBA name, or disregarded entity name, you may enter it on line 2.

Line 3

Check the appropriate box in line 3 for the U.S. federal tax classification of the person whose name is entered on line 1. Check only one box in line 3.

Limited Liability Company (LLC). If the name on line 1 is an LLC treated as a partnership for U.S. federal tax purposes, check the "Limited Liability Company" box and enter "P" in the space provided. If the LLC has filed Form 8832 or 2553 to be taxed as a corporation, check the "Limited Liability Company" box and in the space provided enter "C" for C corporation or "S" for S corporation. If it is a single-member LLC that is a disregarded entity, do not check the "Limited Liability Company" box; instead check the first box in line 3 "Individual/sole proprietor or single-member LLC."

Line 4, Exemptions

If you are exempt from backup withholding and/or FATCA reporting, enter in the appropriate space in line 4 any code(s) that may apply to you.

Exempt payee code.

- Generally, individuals (including sole proprietors) are not exempt from backup withholding.
- Except as provided below, corporations are exempt from backup withholding for certain payments, including interest and dividends.
- Corporations are not exempt from backup withholding for payments made in settlement of payment card or third party network transactions.
- Corporations are not exempt from backup withholding with respect to attorneys' fees or gross proceeds paid to attorneys, and corporations that provide medical or health care services are not exempt with respect to payments reportable on Form 1099-MISC.

The following codes identify payees that are exempt from backup withholding. Enter the appropriate code in the space in line 4.

1—An organization exempt from tax under section 501(a), any IRA, or a custodial account under section 403(b)(7) if the account satisfies the requirements of section 401(f)(2)

2—The United States or any of its agencies or instrumentalities

3—A state, the District of Columbia, a U.S. commonwealth or possession, or any of their political subdivisions or instrumentalities

4—A foreign government or any of its political subdivisions, agencies, or instrumentalities

5—A corporation

6—A dealer in securities or commodities required to register in the United States, the District of Columbia, or a U.S. commonwealth or possession

7—A futures commission merchant registered with the Commodity Futures Trading Commission

8—A real estate investment trust

9—An entity registered at all times during the tax year under the Investment Company Act of 1940

10—A common trust fund operated by a bank under section 584(a)

11—A financial institution

12—A middleman known in the investment community as a nominee or custodian

13—A trust exempt from tax under section 664 or described in section 4947

The following chart shows types of payments that may be exempt from backup withholding. The chart applies to the exempt payees listed above, 1 through 13.

IF the payment is for . . .	THEN the payment is exempt for . . .
Interest and dividend payments	All exempt payees except for 7
Broker transactions	Exempt payees 1 through 4 and 6 through 11 and all C corporations. S corporations must not enter an exempt payee code because they are exempt only for sales of noncovered securities acquired prior to 2012.
Barter exchange transactions and patronage dividends	Exempt payees 1 through 4
Payments over $600 required to be reported and direct sales over $5,000[1]	Generally, exempt payees 1 through 5[2]
Payments made in settlement of payment card or third party network transactions	Exempt payees 1 through 4

[1] See Form 1099-MISC, Miscellaneous Income, and its instructions.

[2] However, the following payments made to a corporation and reportable on Form 1099-MISC are not exempt from backup withholding: medical and health care payments, attorneys' fees, gross proceeds paid to an attorney reportable under section 6045(f), and payments for services paid by a federal executive agency.

Exemption from FATCA reporting code. The following codes identify payees that are exempt from reporting under FATCA. These codes apply to persons submitting this form for accounts maintained outside of the United States by certain foreign financial institutions. Therefore, if you are only submitting this form for an account you hold in the United States, you may leave this field blank. Consult with the person requesting this form if you are uncertain if the financial institution is subject to these requirements. A requester may indicate that a code is not required by providing you with a Form W-9 with "Not Applicable" (or any similar indication) written or printed on the line for a FATCA exemption code.

A—An organization exempt from tax under section 501(a) or any individual retirement plan as defined in section 7701(a)(37)

B—The United States or any of its agencies or instrumentalities

C—A state, the District of Columbia, a U.S. commonwealth or possession, or any of their political subdivisions or instrumentalities

D—A corporation the stock of which is regularly traded on one or more established securities markets, as described in Regulations section 1.1472-1(c)(1)(i)

E—A corporation that is a member of the same expanded affiliated group as a corporation described in Regulations section 1.1472-1(c)(1)(i)

F—A dealer in securities, commodities, or derivative financial instruments (including notional principal contracts, futures, forwards, and options) that is registered as such under the laws of the United States or any state

G—A real estate investment trust

H—A regulated investment company as defined in section 851 or an entity registered at all times during the tax year under the Investment Company Act of 1940

I—A common trust fund as defined in section 584(a)

J—A bank as defined in section 581

K—A broker

L—A trust exempt from tax under section 664 or described in section 4947(a)(1)

M—A tax exempt trust under a section 403(b) plan or section 457(g) plan

Note. You may wish to consult with the financial institution requesting this form to determine whether the FATCA code and/or exempt payee code should be completed.

Line 5

Enter your address (number, street, and apartment or suite number). This is where the requester of this Form W-9 will mail your information returns.

Line 6

Enter your city, state, and ZIP code.

Part I. Taxpayer Identification Number (TIN)

Enter your TIN in the appropriate box. If you are a resident alien and you do not have and are not eligible to get an SSN, your TIN is your IRS individual taxpayer identification number (ITIN). Enter it in the social security number box. If you do not have an ITIN, see *How to get a TIN* below.

If you are a sole proprietor and you have an EIN, you may enter either your SSN or EIN. However, the IRS prefers that you use your SSN.

If you are a single-member LLC that is disregarded as an entity separate from its owner (see *Limited Liability Company (LLC)* on this page), enter the owner's SSN (or EIN, if the owner has one). Do not enter the disregarded entity's EIN. If the LLC is classified as a corporation or partnership, enter the entity's EIN.

Note. See the chart on page 4 for further clarification of name and TIN combinations.

How to get a TIN. If you do not have a TIN, apply for one immediately. To apply for an SSN, get Form SS-5, Application for a Social Security Card, from your local SSA office or get this form online at *www.ssa.gov*. You may also get this form by calling 1-800-772-1213. Use Form W-7, Application for IRS Individual Taxpayer Identification Number, to apply for an ITIN, or Form SS-4, Application for Employer Identification Number, to apply for an EIN. You can apply for an EIN online by accessing the IRS website at *www.irs.gov/businesses* and clicking on Employer Identification Number (EIN) under Starting a Business. You can get Forms W-7 and SS-4 from the IRS by visiting IRS.gov or by calling 1-800-TAX-FORM (1-800-829-3676).

If you are asked to complete Form W-9 but do not have a TIN, apply for a TIN and write "Applied For" in the space for the TIN, sign and date the form, and give it to the requester. For interest and dividend payments, and certain payments made with respect to readily tradable instruments, generally you will have 60 days to get a TIN and give it to the requester before you are subject to backup withholding on payments. The 60-day rule does not apply to other types of payments. You will be subject to backup withholding on all such payments until you provide your TIN to the requester.

Note. Entering "Applied For" means that you have already applied for a TIN or that you intend to apply for one soon.

Caution: *A disregarded U.S. entity that has a foreign owner must use the appropriate Form W-8.*

Part II. Certification

To establish to the withholding agent that you are a U.S. person, or resident alien, sign Form W-9. You may be requested to sign by the withholding agent even if items 1, 4, or 5 below indicate otherwise.

For a joint account, only the person whose TIN is shown in Part I should sign (when required). In the case of a disregarded entity, the person identified on line 1 must sign. Exempt payees, see *Exempt payee code* earlier.

Signature requirements. Complete the certification as indicated in items 1 through 5 below.

1. Interest, dividend, and barter exchange accounts opened before 1984 and broker accounts considered active during 1983. You must give your correct TIN, but you do not have to sign the certification.

2. Interest, dividend, broker, and barter exchange accounts opened after 1983 and broker accounts considered inactive during 1983. You must sign the certification or backup withholding will apply. If you are subject to backup withholding and you are merely providing your correct TIN to the requester, you must cross out item 2 in the certification before signing the form.

3. Real estate transactions. You must sign the certification. You may cross out item 2 of the certification.

4. Other payments. You must give your correct TIN, but you do not have to sign the certification unless you have been notified that you have previously given an incorrect TIN. "Other payments" include payments made in the course of the requester's trade or business for rents, royalties, goods (other than bills for merchandise), medical and health care services (including payments to corporations), payments to a nonemployee for services, payments made in settlement of payment card and third party network transactions, payments to certain fishing boat crew members and fishermen, and gross proceeds paid to attorneys (including payments to corporations).

5. Mortgage interest paid by you, acquisition or abandonment of secured property, cancellation of debt, qualified tuition program payments (under section 529), IRA, Coverdell ESA, Archer MSA or HSA contributions or distributions, and pension distributions. You must give your correct TIN, but you do not have to sign the certification.

What Name and Number To Give the Requester

For this type of account:	Give name and SSN of:
1. Individual	The individual
2. Two or more individuals (joint account)	The actual owner of the account or, if combined funds, the first individual on the account[1]
3. Custodian account of a minor (Uniform Gift to Minors Act)	The minor[2]
4. a. The usual revocable savings trust (grantor is also trustee)	The grantor-trustee[1]
b. So-called trust account that is not a legal or valid trust under state law	The actual owner[1]
5. Sole proprietorship or disregarded entity owned by an individual	The owner[3]
6. Grantor trust filing under Optional Form 1099 Filing Method 1 (see Regulations section 1.671-4(b)(2)(i)(A))	The grantor*
For this type of account:	**Give name and EIN of:**
7. Disregarded entity not owned by an individual	The owner
8. A valid trust, estate, or pension trust	Legal entity[4]
9. Corporation or LLC electing corporate status on Form 8832 or Form 2553	The corporation
10. Association, club, religious, charitable, educational, or other tax-exempt organization	The organization
11. Partnership or multi-member LLC	The partnership
12. A broker or registered nominee	The broker or nominee
13. Account with the Department of Agriculture in the name of a public entity (such as a state or local government, school district, or prison) that receives agricultural program payments	The public entity
14. Grantor trust filing under the Form 1041 Filing Method or the Optional Form 1099 Filing Method 2 (see Regulations section 1.671-4(b)(2)(i)(B))	The trust

[1] List first and circle the name of the person whose number you furnish. If only one person on a joint account has an SSN, that person's number must be furnished.

[2] Circle the minor's name and furnish the minor's SSN.

[3] You must show your individual name and you may also enter your business or DBA name on the "Business name/disregarded entity" name line. You may use either your SSN or EIN (if you have one), but the IRS encourages you to use your SSN.

[4] List first and circle the name of the trust, estate, or pension trust. (Do not furnish the TIN of the personal representative or trustee unless the legal entity itself is not designated in the account title.) Also see *Special rules for partnerships* on page 2.

*__Note.__ Grantor also must provide a Form W-9 to trustee of trust.

Note. If no name is circled when more than one name is listed, the number will be considered to be that of the first name listed.

Secure Your Tax Records from Identity Theft

Identity theft occurs when someone uses your personal information such as your name, SSN, or other identifying information, without your permission, to commit fraud or other crimes. An identity thief may use your SSN to get a job or may file a tax return using your SSN to receive a refund.

To reduce your risk:

- Protect your SSN,
- Ensure your employer is protecting your SSN, and
- Be careful when choosing a tax preparer.

If your tax records are affected by identity theft and you receive a notice from the IRS, respond right away to the name and phone number printed on the IRS notice or letter.

If your tax records are not currently affected by identity theft but you think you are at risk due to a lost or stolen purse or wallet, questionable credit card activity or credit report, contact the IRS Identity Theft Hotline at 1-800-908-4490 or submit Form 14039.

For more information, see Publication 4535, Identity Theft Prevention and Victim Assistance.

Victims of identity theft who are experiencing economic harm or a system problem, or are seeking help in resolving tax problems that have not been resolved through normal channels, may be eligible for Taxpayer Advocate Service (TAS) assistance. You can reach TAS by calling the TAS toll-free case intake line at 1-877-777-4778 or TTY/TDD 1-800-829-4059.

Protect yourself from suspicious emails or phishing schemes. Phishing is the creation and use of email and websites designed to mimic legitimate business emails and websites. The most common act is sending an email to a user falsely claiming to be an established legitimate enterprise in an attempt to scam the user into surrendering private information that will be used for identity theft.

The IRS does not initiate contacts with taxpayers via emails. Also, the IRS does not request personal detailed information through email or ask taxpayers for the PIN numbers, passwords, or similar secret access information for their credit card, bank, or other financial accounts.

If you receive an unsolicited email claiming to be from the IRS, forward this message to *phishing@irs.gov*. You may also report misuse of the IRS name, logo, or other IRS property to the Treasury Inspector General for Tax Administration (TIGTA) at 1-800-366-4484. You can forward suspicious emails to the Federal Trade Commission at: *spam@uce.gov* or contact them at *www.ftc.gov/idtheft* or 1-877-IDTHEFT (1-877-438-4338).

Visit IRS.gov to learn more about identity theft and how to reduce your risk.

Privacy Act Notice

Section 6109 of the Internal Revenue Code requires you to provide your correct TIN to persons (including federal agencies) who are required to file information returns with the IRS to report interest, dividends, or certain other income paid to you; mortgage interest you paid; the acquisition or abandonment of secured property; the cancellation of debt; or contributions you made to an IRA, Archer MSA, or HSA. The person collecting this form uses the information on the form to file information returns with the IRS, reporting the above information. Routine uses of this information include giving it to the Department of Justice for civil and criminal litigation and to cities, states, the District of Columbia, and U.S. commonwealths and possessions for use in administering their laws. The information also may be disclosed to other countries under a treaty, to federal and state agencies to enforce civil and criminal laws, or to federal law enforcement and intelligence agencies to combat terrorism. You must provide your TIN whether or not you are required to file a tax return. Under section 3406, payers must generally withhold a percentage of taxable interest, dividend, and certain other payments to a payee who does not give a TIN to the payer. Certain penalties may also apply for providing false or fraudulent information.

Appendix D: Cal Poly Human Subjects Committee Approval Form

All Cal Poly faculty, staff, and student research with human subjects, as well as other research involving human subjects that is conducted at Cal Poly, must be reviewed by the **Cal Poly Human Subjects Committee** for the protection of human subjects, the researchers, and the University. Human subjects research is defined as any systematic investigation of living human subjects that is designed to develop or contribute to generalizable knowledge. While the ethical guidelines for research are applicable to classroom activities, demonstrations, and assignments, the Human Subjects Committee does not review classroom activities unless data will be collected and used in a systematic investigation.

Researchers should complete all items on this approval form and submit it, along with a research protocol (containing the information detailed in Guidelines for Human Subjects Research Protocol), to the Office of Research and Economic Development (Debbie Hart, Bldg. 38, Room 154). Please feel free to attach an additional page if your responses to any of the items require more space. Your answers to the items on this form, as well as the research protocol, should be typed. The Committee will make every effort to respond to your submission within two to four weeks. Committee approval should be received prior to contacting prospective subjects and collecting data. Please read carefully Cal Poly's Policy for the Use of Human Subjects in Research prior to completing this application.

If you require assistance in completing this form, contact the Office of Research and Economic Development at (805) 756-1508.

1. Date: 4/12/15

2. Title of Research Project:

The Effects of Transcutaneous Electrical Neurostimulation on Analgesia and Peripheral Perfusion

3. Type of Research:

- [x] Senior project
- [x] Master's thesis
- [ ] Faculty research
- [ ] Other: *please explain*

4. Name(s) of Researcher(s)

Principal Investigator: Leah Schafer
Department or other affiliation: Biomedical Engineering
Phone: 5303545061 Email: lischafe@calpoly.edu
Position: [ ] Faculty [x] Student
[ ] Other: *please explain*

Additional Researcher: Kaylee Keck
Department or other affiliation: Biomedical Engineering
Phone: 5599205233 Email: kkeck@calpoly.edu
Position: [ ] Faculty [x] Student

☐ Other: *please explain*

Additional Researcher:

Department or other affiliation:

Phone: Email:

Position: ☐ Faculty ☐ Student

☐ Other: *please explain*

Any additional researchers involved in the project should be listed with the descriptive information requested above on a separate sheet.

5. Faculty Advisor (if applicable)

Name: Trevor Cardinal, Ph.D. Email: tcardina@calpoly.edu

Department or other affiliation: Biomedical Engineering Phone: 8057566244

Other thesis committee members if the research is a thesis:

Name: Stuart Rosenberg Email: srosenberg@sjm.com

Department or other affiliation: St. Jude Medical Phone: 8184933629

Name: Melanie Goodman, Ph.D. Email: mgoodman2@sjm.co

Department or other affiliation: St. Jude Medical Phone: 9725264683

Name: Kristen O'Halloran Cardinal, Ph.D. Email: kohallor@calpoly.edu

Department or other affiliation: Biomedical Engineering Phone: 8057562675

6. Is there an *external* funding source for the project:

☒ Yes, and the source is: St. Jude Medical MEDITEC

☐ No

7. Is this a modification of a project previously reviewed by Cal Poly's Human Subjects Committee?

☒ Yes, and the approximate date of the last review was: 2/11/15

☐ No

8. Estimated duration of the project:

Starting date: 5/1/15 Completion date: 7/1/15

9. Describe *any* risks (physical, psychological, social, or economic) that may be involved.
*See **Specific Ethical Criterion #1** in Policy for the Use of Human Subjects in Research for a description of the types of risks.*

The participant will experience ischemic pain i.e. pain resulting from insufficient blood flow to a tissue as a result of acute forearm occlusion by a sphygmomanometer at 180 mmHg for 3 minutes. The participant may experience slight skin irritation with the application of skin probes and electrodes. The participant may experience some discomfort during static handgrip exercise for 3 minutes.

10. Indicate what measures will be taken to minimize risks. *See **Specific Ethical Criterion #1** in Policy for the Use of Human Subjects in Research for a discussion of strategies for minimizing risks.*

The temporarily induced ischemic pain will be assessed every minute using Numeric Rating and Faces pain scales similarly to related published research methodologies. Ischemia will not be held for over 3 minutes at 180 mmHg in accordance with clinical instructions for use. If pain intensity reaches a subject's personal pain tolerance threshold, the trial will be immediately terminated. For skin irritation k a topical antihistamine (Benadryl) may be applied to the area. Handgrip exercise will not exceed 3 minutes.

11. Explain how subjects' *confidentiality* will be protected. *See **Specific Ethical Criterion #5** in Policy for the Use of Human Subjects in Research for a discussion of strategies for minimizing risks.*

Participant information will be recorded in terms of their medical history, age, gender, height, and weight. This information will be recorded in a hard copy of the medical questionnaire document with a corresponding code for each patient. These documents will be kept in Dr. Trevor Cardinal's locked office or in a locked drawer in the testing lab. All data recordings will be stored electronically on the P.I.'s personal computer and accessible only to Leah Schafer, Kaylee Keck, and Dr. Cardinal.

12. Describe any *incentives* for participation that will be used. *See **Specific Ethical Criterion #2** in Policy for the Use of Human Subjects in Research for a discussion of the use of incentives in research.*

A $25 Visa gift card will be offered to participants that agree to the caffeine restrictions. In the case that a participant chooses to prematurely discontinue their participation due to possible discomfort and/or stress, monetary compensation will still be provided.

13. Will *deception* of subjects be involved in the research procedures?

[x] Yes* [ ] No

If so, explain the deception and how it will be handled. See **Specific Ethical Criterion #3 in Policy for the Use of Human Subjects in Research for a discussion of the use of deception in research:*

The study is designed to be single-blinded such that the participant does not know whether they are in a placebo (control) trial or treatment trial. The control trial will involve attaching neurostimulation electrodes just as in the treatment trial; however, no electrical stimulation will occur during control. This is necessary to determine if the neurostimulation itself is actually causing the changes witnessed.

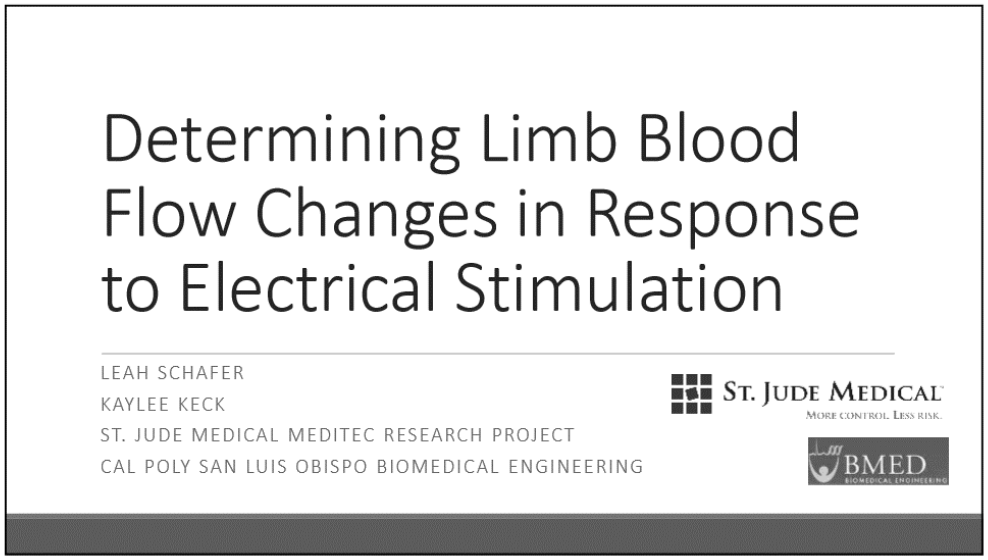
14. Type of review requested:

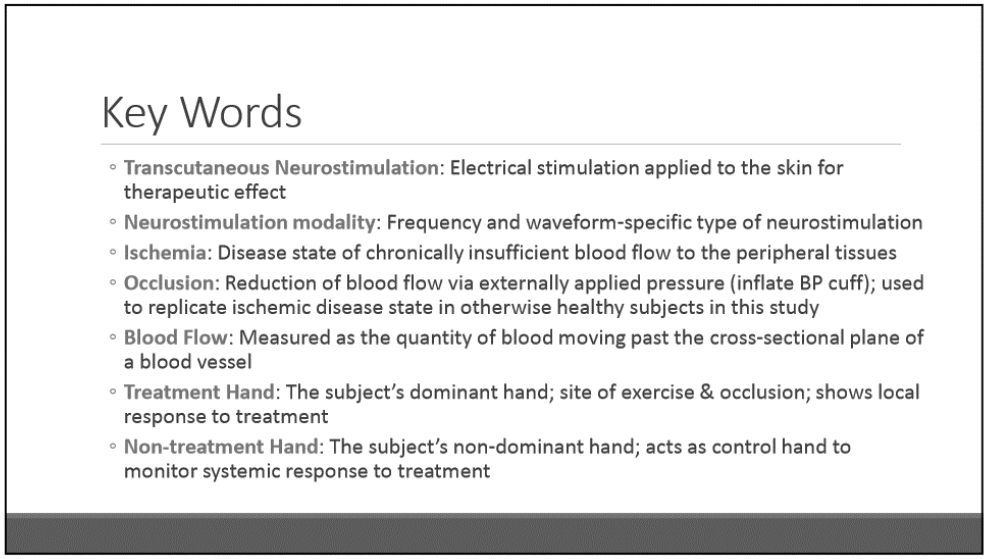
[ ] Exempt from further review* [x] Expedited review [ ] Full review

See ***Types of Review*** in Policy for the Use of Human Subjects in Research for a discussion of the criteria for exempt, expedited, and full reviews.

***The research protocol submitted for a project presumed to be exempt may be abbreviated but should contain sufficient information to support the conclusion that the project meets the criteria for exemption.*

Appendix E: Pilot Study Experimental Design Summary Presentation





Project Goal

Understand the role neurostimulation plays in a subject's pain and blood flow under a variety of experimental conditions.

More specifically:

- Determine if the modality of neurostimulation, under both ischemic and non-ischemic conditions, in the occlusion phase and the recovery phase of the trial, affects a subject's pain and blood flow, as compared to the baseline phase of the trial, in either the treatment hand or the non-treatment hand.
- Determine if ischemic and non-ischemic conditions, under the different neurostimulation modalities, in the occlusion phase and the recovery phase of the trial, affects a subject's pain and blood flow, as compared to the baseline phase of the trial, in either the treatment hand or the non-treatment hand.

Experimental Design

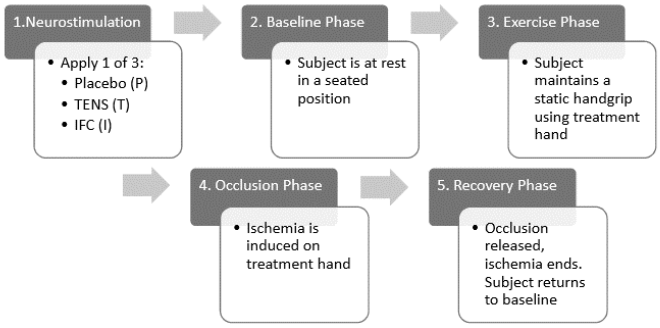
Randomized Block Design

Responses:

- Pain: categorical data
- Blood Flow: continuous data

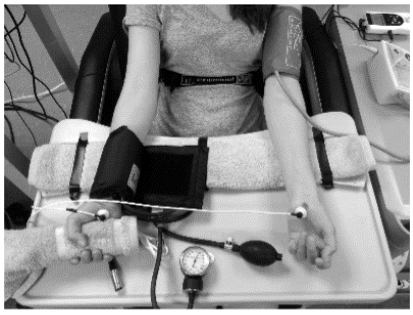
Factors:

- Subject (n=10)
- Neurostimulation modality (P, T, I)
- Ischemia (+, -)
- Hand (treatment, non-treatment)
- Time
  - Baseline (BL) Phase
  - Exercise (E) Phase
    - 3 time points: E1, E2, E3
  - Occlusion (O) Phase
    - 3 time points : O1, O2, O3
  - Recovery (R) Phase



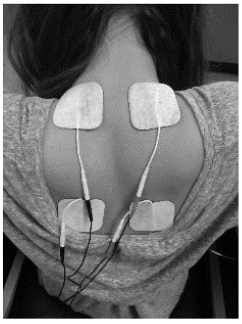
Blood Flow Data Acquisition

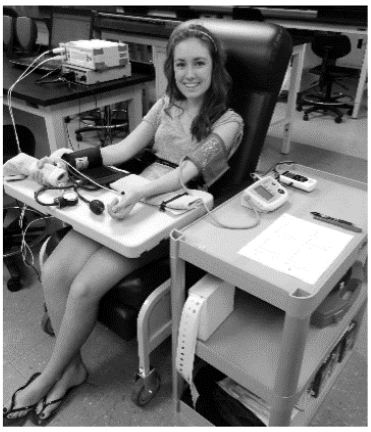
Blood flow is monitored at the palms of each hand using a Laser Doppler Perfusion System



Experimental Setup

- Neurostimulation is applied by placing electrodes on subject's back
- Data is collected on both treatment arm & non-treatment arm





Pain Data Acquisition

Pain is monitored using short-form McGill Pain Questionnaire (SF-MPQ)

- Descriptive word association for pain
- Possible responses and relative weights:
  - None → 1
  - Mild → 2
  - Moderate → 3
  - Severe → 4
- Score is sum of all responses according to relative weights
- Acquired during Exercise & Occlusion Phases

How would you describe your pain based on the following words? Check one of the columns for each descriptor:

Descriptor	None	Mild	Moderate	Severe
Throbbing				
Shooting	—	—	—	—
Stabbing				
Sharp	—	—	—	—
Cramping				
Gnawing	—	—	—	—
Hot-burning				
Aching	—	—	—	—
Heavy	—	—	—	—
Tender	—	—	—	—
Splitting				
Tiring-exhausting				
Sickening	—	—	—	—
Fearful	—	—	—	—
Punishing-cruel				

Appendix F: Protocol

I. Setup
   1. Turn on the laptop.
   2. Connect the power supply to PowerLab.
   3. Connect the USB cable from PowerLab to the laptop.
   4. Connect the respiration belt to Input 1 on the front panel of PowerLab.
   5. Connect the Hand Dynamometer to Input 2 on the front panel of PowerLab.
   6. Connect the power supply to the Laser Doppler Flow (LDF) system and turn it on.
   7. Connect the skin probes to Channels 1 and 2 on the LDF system.
   8. Connect the BNC cables from the LDF system to Inputs 3 and 4 on the front panel of PowerLab.
   9. Turn on the PowerLab system.
   10. Open LabChart on the laptop and open the customized settings file.
       i. The raw breath signal in millivolts (mV), the respiratory rate in breaths per minute (BPM), the handgrip force in Newtons (N), CBF 1 in perfusion units (PU), and CBF 2 in PU should all be displayed in LabChart at this point.

II. Application
   1. Seat the participant in a chair with both arms supinated and gently resting on the tray. Ensure that they are comfortable and properly positioned before continuing.
   2. Apply the electrodes to the C7 and T4 vertebrae locations, approximately 3 cm to the left and right of the vertebral column (Figure 1).†
   3. Wrap the respiration belt around the participant's chest, just below the xiphoid process.
   4. Attach the skin probe connected to Channel 1 of the LDF system to the left arm, 2 cm below the crease of the wrist.
   5. Attach the skin probe connected to Channel 2 of the LDF system to the right arm, 2 cm below the crease of the wrist.
   6. Wrap the cuff connected to the manual sphygmomanometer around the participant's left forearm, 2 cm below the crease of the elbow.
   7. Wrap the cuff connected to the blood pressure monitor around the participant's right arm.
   8. Instruct the participant to loosely grip the Hand Dynamometer in their dominant hand.
   9. Instruct the participant to squeeze the Hand Dynamometer as hard as possible for a second or two, and then relax their grip.*
   10. Determine Maximum Voluntary Contraction (MVC) by recording the average of three handgrip trials and calculate 25% of MVC.

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* Adapted from BMED 460 – "Muscle Stimulation Fatigue Student Protocol" by Trevor Cardinal
† Adapted from "Effect of transcutaneous electrical nerve stimulation on muscle metaboreflex in healthy young and older subjects." by Vieira, et. al.

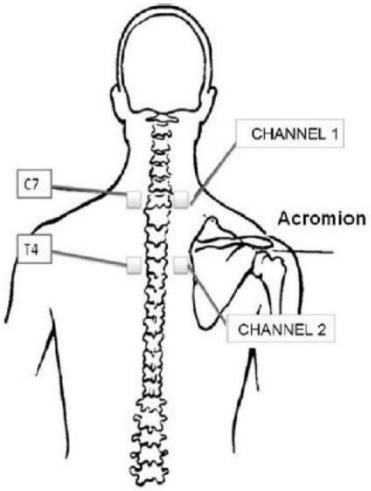

Figure 1: TENS Electrode Placement at the C7 and T4 Regions*

III. Treatment

1. Begin treatment according to assigned group code.†
2. Every minute, assess the intensity of the participant's pain via the NPRS. In addition, halfway through each interval of the treatment i.e. "baseline", "exercise", "occlusion", and "recovery", record the participant's blood pressure and heart rate from the monitor.
3. Set the stimulation frequency to 100 Hz, pulse duration to 200 μs, and slowly adjust the intensity to just above sensory threshold (no pain or muscle contraction) by asking the participant when he/she begins to feel a strong, but comfortable tingling sensation.
4. Begin 1 min metronome and instruct participant to verbalize his or her pain level every min.
5. Begin 1.5 min metronome and collect BP & HR data every 1.5 min.
6. Begin recording baseline blood flow for 3 minutes at resting heart rate.
7. Place the hand dynamometer in the participant's left hand. Instruct the participant to perform a static handgrip exercise for 3 minutes at 25% MVC.
8. Five seconds before exercise completion, inflate the sphygmomanometer cuff to 180 mmHg.[!]
9. Maintain cuff inflation at 180 mmHg for 3 minutes, while still recording blood flow.
10. Deflate the cuff immediately and record for 3 minutes.
11. Stop recording.
12. Insert comments for "baseline", "exercise", "occlusion", and "recovery" at the end of each interval.
13. Detach all equipment from the participant and wait at least 10 minutes before beginning the next treatment.

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* Adapted from "Effect of transcutaneous electrical nerve stimulation on muscle metaboreflex in healthy young and older subjects" Vieira, et. al.

† Group codes: Placebo/PECO- (P-), Placebo/PECO+ (P+), TENS/PECO- (T-), TENS/PECO+ (T+), IFC/PECO- (I-), IFC/PECO+(I+)

[!] Only if PECO+ group

Appendix G: Sample of Data Master

Chronology	Subject	Dom Hand	Age	BMI	Sex	Caffeine	8 hr Exerci	2 hr Exerci	Phase	Trx	Ischemia	Intensity	Breath Mean	spiration R Mean	Grip Mean	CBF 1 Mean	CBF 1 Diff	CBF 2 Mean	Skin Temp	Systolic	Diastolic	Pulse	MAP	Vascular Resistance	VR Diff
												mA	mV	BPM	N	PU	PU	PU	°C	mmMg	mmMg	BPM	mmMg	mmMg/PU	
1	1	R	22	25.8245	M	0	1	0	B	P	-	0	2.062	#NUM!	11.2245	65.881		26.7749	28.6683	122	66	58	84.6667	1.2851454	
2	1	R	22	25.8245	M	0	1	0	E	P	-	0	1.5975	19.6483	62.9786	69.4718	3.5908	23.6553	29.8251	114	70	57	84.6667	1.2187199	-0.06643
3	1	R	22	25.8245	M	0	1	0	O	P	-	0	1.4186	17.9707	10.7873	88.5421	22.6611	30.746	29.5961	115	71	55	85.6667	0.9675247	-0.31762
4	1	R	22	25.8245	M	0	1	0	R	P	-	0	1.256	16.5782	10.2885	82.3855	16.5045	26.1193	29.4125	116	69	60	84.6667	1.0276889	-0.25746
5	1	R	22	25.8245	M	0	1	0	B	I	+	7	1.7228	#NUM!	10.0211	52.4971		26.3459	30.0161	118	75	64	89.3333	1.7016813	
6	1	R	22	25.8245	M	0	1	0	E	I	+	7	0.9703	18.6306	38.315	109.723	57.2258	26.1473	32.2703	119	71	69	87	0.7929065	-0.90877
7	1	R	22	25.8245	M	0	1	0	O	I	+	7	0.6992	16.921	12.6756	7.3079	-45.1892	27.4168	31.9496	113	74	66	87	11.904925	10.2032
8	1	R	22	25.8245	M	0	1	0	R	I	+	7	0.6218	#NUM!	11.3661	191.145	138.648	32.3849	31.6109	116	69	60	84.6667	0.4429447	-1.25874
9	1	R	22	25.8245	M	0	1	0	B	T	+	9	1.5986	#NUM!	10.2929	89.8651		49.4443	28.1859	84	65	58	71.3333	0.7937824	
10	1	R	22	25.8245	M	0	1	0	E	T	+	9	0.3762	18.9246	31.7593	84.4036	-5.4615	19.4081	30.9916	120	74	68	89.3333	1.0584067	0.26462
11	1	R	22	25.8245	M	0	1	0	O	T	+	9	0.0026	18.8158	11.1058	6.672	-83.1931	19.9779	31.3239	120	70	65	86.6667	12.989608	12.1958
12	1	R	22	25.8245	M	0	1	0	R	T	+	9	-0.1788	#NUM!	10.6625	164.064	74.1992	17.9594	30.7785	116	74	58	88	0.5363751	-0.25741
13	1	R	22	25.8245	M	0	1	0	B	I	-	10	-0.121	16.5727	9.8825	39.743		17.9419	28.6947	104	75	56	84.6667	2.1303542	
14	1	R	22	25.8245	M	0	1	0	E	I	-	10	-0.2672	18.6923	35.8969	47.2371	7.4941	20.4908	30.2231	102	77	63	85.3333	1.8064897	-0.32386
15	1	R	22	25.8245	M	0	1	0	O	I	-	10	-0.3657	16.8318	9.8283	71.6247	31.8817	19.621	30.265	97	75	67	82.3333	1.1495103	-0.98084
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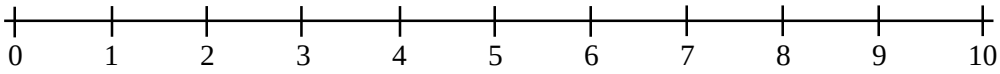
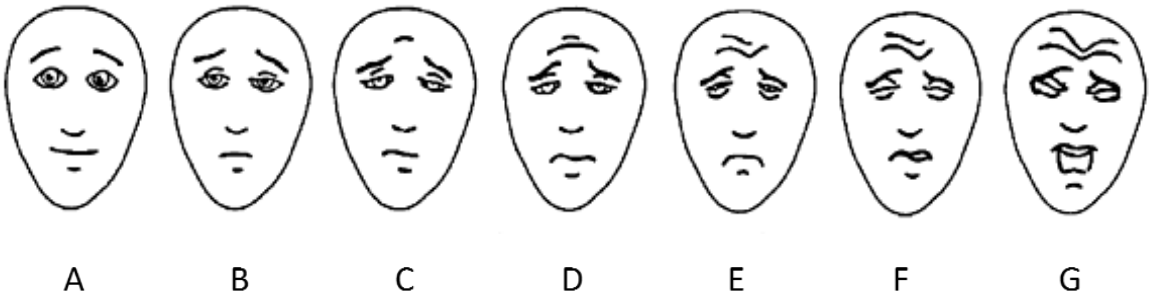
17	1	R	22	25.8245	M	0	1	0	B	P	+	0	-0.1952	13.2763	10.0738	41.5564		14.9052	28.6683	116	75	57	88.6667	2.1336465	
18	1	R	22	25.8245	M	0	1	0	E	P	+	0	-0.3161	17.1422	37.0612	60.4243	18.8679	16.4421	29.8251	120	73	63	88.6667	1.4674008	-0.66625
19	1	R	22	25.8245	M	0	1	0	O	P	+	0	-0.5416	15.6897	9.8027	10.7616	-30.7948	16.6875	29.5961	113	74	57	87	8.0842997	5.95065
20	1	R	22	25.8245	M	0	1	0	R	P	+	0	-0.6824	13.4095	9.2171	108.49	66.9335	15.9338	29.4125	112	75	58	87.3333	0.8049904	-1.32866
21	1	R	22	25.8245	M	0	1	0	B	T	-	9	-0.2572	14.6246	10.6489	28.2923		11.9757	28.0128	107	77	58	87	3.0750416	
22	1	R	22	25.8245	M	0	1	0	E	T	-	9	-0.6122	17.3807	36.1723	46.9891	18.6968	12.1786	28.9639	115	79	58	91	1.9366193	-1.13842
23	1	R	22	25.8245	M	0	1	0	O	T	-	9	-0.6988	18.7997	9.8086	35.7725	7.4802	11.9722	28.9608	109	70	56	83	2.320218	-0.75482
24	1	R	22	25.8245	M	0	1	0	R	T	-	9	-0.7577	#NUM!	9.4562	26.1478	-2.1445	10.1687	29.0057	112	72	61	85.3333	3.2634995	0.18846
1	2	R	23	21.4104	M	1	0	0	B	T	+	5	-2.8333	#NUM!	8.5748	118.665		26.2731	28.1859	113	67	58	82.3333	0.6938323	
2	2	R	23	21.4104	M	1	0	0	E	T	+	5	-3.1437	35.5095	46.7793	279.33	160.665	29.144	30.9916	121	71	55	87.6667	0.3138465	-0.37999
3	2	R	23	21.4104	M	1	0	0	O	T	+	5	-3.3906	37.388	8.0984	3.6298	-115.035	33.1506	31.3239	120	75	55	90	24.794755	24.1009
4	2	R	23	21.4104	M	1	0	0	R	T	+	5	-3.6732	#NUM!	7.9807	262.215	143.55	13.1129	30.7785	112	72	61	85.3333	0.3254332	-0.3684
5	2	R	23	21.4104	M	1	0	0	B	I	+	7	-4.1948	#NUM!	8.0289	139.366		73.4679	31.0283	109	64	51	79	0.5668515	
6	2	R	23	21.4104	M	1	0	0	E	I	+	7	-4.5885	33.087	33.4652	144.057	4.6908	64.9773	31.5362	122	80	56	94	0.652519	0.08567
7	2	R	23	21.4104	M	1	0	0	O	I	+	7	-4.5583	31.6867	10.6825	5.3706	-133.996	41.6142	31.4106	117	73	54	87.6667	16.32344	15.7566
8	2	R	23	21.4104	M	1	0	0	R	I	+	7	-4.8688	25.0401	8.4878	251.55	112.184	52.8235	31.6825	112	64	51	80	0.3180282	-0.24882
9	2	R	23	21.4104	M	1	0	0	B	P	-	0	-4.9485	25.6523	8.4893	112.027		46.1772	30.963	110	69	57	82.6667	0.7379193	
10	2	R	23	21.4104	M	1	0	0	E	P	-	0	-5.1441	17.1887	38.3512	114.298	2.271	71.2637	32.5373	119	81	54	93.6667	0.8194974	0.08158
11	2	R	23	21.4104	M	1	0	0	O	P	-	0	-5.5157	26.5189	8.102	213.587	101.56	68.1124	32.9203	113	72	52	85.6667	0.4010856	-0.33683
12	2	R	23	21.4104	M	1	0	0	R	P	-	0	-5.7223	36.9865	7.9958	193.492	81.4655	62.9518	32.8292	109	66	54	80.3333	0.4151761	-0.32274
13	2	R	23	21.4104	M	1	0	0	B	I	-	7	-5.177	17.0211	8.3998	83.9243		19.5508	29.8996	113	71	49	85	1.0128175	
14	2	R	23	21.4104	M	1	0	0	E	I	-	7	-5.6802	22.3643	30.8316	189.379	105.455	13.1529	32.6173	115	79	59	91	0.4805169	-0.5323
15	2	R	23	21.4104	M	1	0	0	O	I	-	7	-5.8896	12.8785	7.8821	126.855	42.9305	13.0054	32.7423	104	65	53	78	0.6148762	-0.39794
16	2	R	23	21.4104	M	1	0	0	R	I	-	7	-5.9211	#NUM!	7.9885	155.827	71.9029	21.0794	33.6618	109	66	50	80.3333	0.5155283	-0.49729
17	2	R	23	21.4104	M	1	0	0	B	P	+	0	-5.8156	#NUM!	8.3342	66.69		29.3971	30.4642	119	73	50	88.3333	1.3245364	
18	2	R	23	21.4104	M	1	0	0	E	P	+	0	-6.6979	23.6194	35.1872	163.98	97.2904	24.4592	31.1588	118	78	52	91.3333	0.5569771	-0.76756
19	2	R	23	21.4104	M	1	0	0	O	P	+	0	-6.5771	19.759	9.5479	7.5056	-59.1844	54.9067	32.5624	115	71	53	85.6667	11.4137	10.0892
20	2	R	23	21.4104	M	1	0	0	R	P	+	0	-6.9526	#NUM!	8.6565	252.226	185.536	19.9041	32.8692	115	75	53	88.3333	0.3502157	-0.97432

Appendix H: Numeric Pain Scale (NRS) and Faces Pain Scale





Appendix I: Modified Short-Form McGill Pain and Paresthesia Questionnaire

mSF-MP Questionnaire

Participant:_____ Date:_____ Treatment:_____

How would you describe your pain based on the following words? Check one of the columns for each descriptor:

Descriptor	None	Mild	Moderate	Severe
Throbbing	_____	_____	_____	_____
Shooting	_____	_____	_____	_____
Stabbing	_____	_____	_____	_____
Sharp	_____	_____	_____	_____
Cramping	_____	_____	_____	_____
Gnawing	_____	_____	_____	_____
Hot-burning	_____	_____	_____	_____
Aching	_____	_____	_____	_____
Heavy	_____	_____	_____	_____
Tender	_____	_____	_____	_____
Splitting	_____	_____	_____	_____
Tiring-exhausting	_____	_____	_____	_____
Sickening	_____	_____	_____	_____
Fearful	_____	_____	_____	_____
Punishing-cruel	_____	_____	_____	_____
Tingling	_____	_____	_____	_____
Tickling	_____	_____	_____	_____
Pricking	_____	_____	_____	_____
Itching	_____	_____	_____	_____

Appendix J: Prediction Expression for Changes in Vascular Resistance

Prediction Expression:

$log_{10}(VR+10) = Average\ change\ in\ VR + Phase + Treatment + Phase*Treatment + Ischemia + Phase*Ishcemia + Treatment*Ischemia + Phase*Treatment*Ischemia + Subject$

Expanded Prediction Expression:

$log_{10}(VR+10) =$

1.01221563634817

+ Match(Phase) { "E" ⇒ -0.0413459913717, "O" ⇒ 0.09339575224448, "R" ⇒ -0.0520497608728, else ⇒ . }

+ Match(Treatment) { "I" ⇒ 0.01120887418798, "P" ⇒ -0.0132045540026, "T" ⇒ 0.00199567981465, else ⇒ . }

+ Match(Phase) { "E" ⇒ Match(Treatment) { "I" ⇒ -0.0042072461413, "P" ⇒ 0.00335051791028, "T" ⇒ 0.00085672823099, else ⇒ . }, "O" ⇒ Match(Treatment) { "I" ⇒ 0.00585615143786, "P" ⇒ -0.0001389616697, "T" ⇒ -0.0057171897681, else ⇒ . }, "R" ⇒ Match(Treatment) { "I" ⇒ -0.0016489052966, "P" ⇒ -0.0032115562406, "T" ⇒ 0.00486046153714, else ⇒ . }, else ⇒ . }

+ Match(Ischemia) { "-" ⇒ -0.036976767736, "+" ⇒ 0.03697676773596, else ⇒ . }

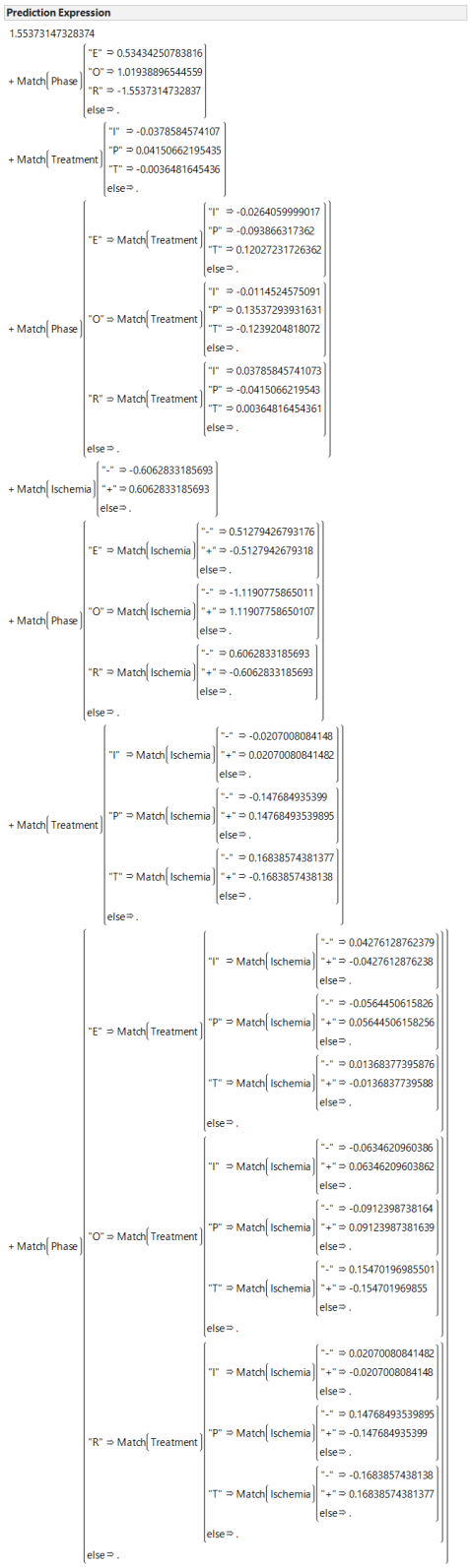
+ Match(Phase) { "E" ⇒ Match(Ischemia) { "-" ⇒ 0.03877783027436, "+" ⇒ -0.0387778302744, else ⇒ . }, "O" ⇒ Match(Ischemia) { "-" ⇒ -0.0919068042252, "+" ⇒ 0.09190680422523, else ⇒ . }, "R" ⇒ Match(Ischemia) { "-" ⇒ 0.05312897395087, "+" ⇒ -0.0531289739509, else ⇒ . }, else ⇒ . }

+ Match(Treatment) { "I" ⇒ Match(Ischemia) { "-" ⇒ -0.001543066227, "+" ⇒ 0.00154306622698, else ⇒ . }, "P" ⇒ Match(Ischemia) { "-" ⇒ -0.0044742333567, "+" ⇒ 0.00447423335674, else ⇒ . }, "T" ⇒ Match(Ischemia) { "-" ⇒ 0.00601729958372, "+" ⇒ -0.0060172995837, else ⇒ . }, else ⇒ . }

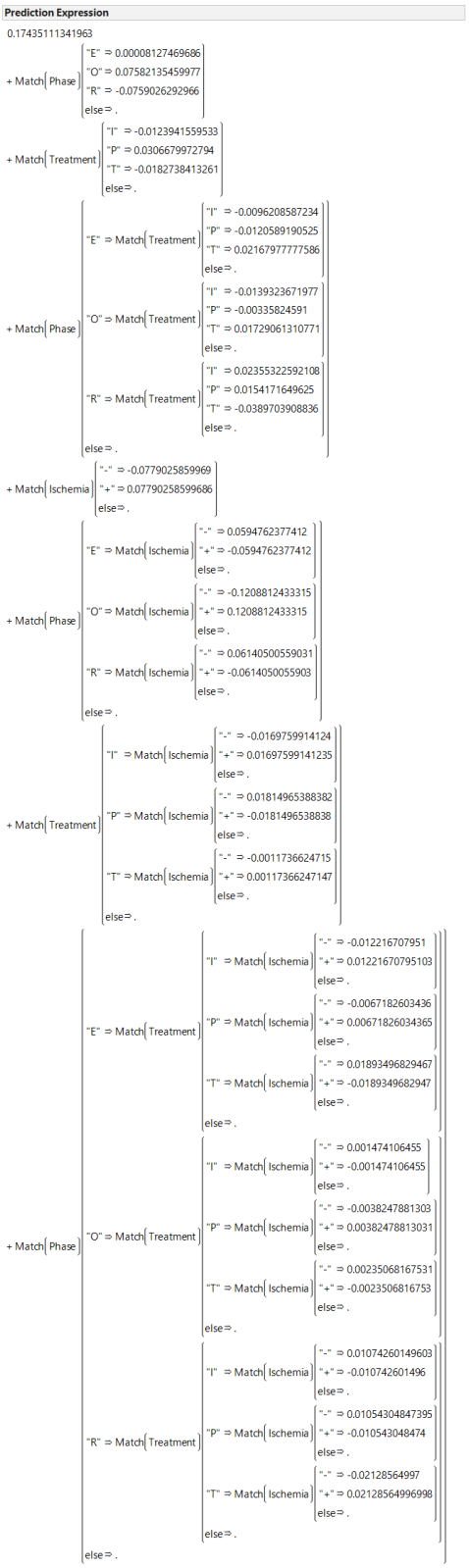
+ Match(Phase) { "E" ⇒ Match(Treatment) { "I" ⇒ Match(Ischemia) { "-" ⇒ 0.00222626446709, "+" ⇒ -0.0022262644671, else ⇒ . }, "P" ⇒ Match(Ischemia) { "-" ⇒ 0.00149396383228, "+" ⇒ -0.0014939638323, else ⇒ . }, "T" ⇒ Match(Ischemia) { "-" ⇒ -0.0037202282994, "+" ⇒ 0.00372022829937, else ⇒ . }, else ⇒ . }, "O" ⇒ Match(Treatment) { "I" ⇒ Match(Ischemia) { "-" ⇒ -0.0100527457338, "+" ⇒ 0.01005274573379, else ⇒ . }, "P" ⇒ Match(Ischemia) { "-" ⇒ 0.00534653420769, "+" ⇒ -0.0053465342077, else ⇒ . }, "T" ⇒ Match(Ischemia) { "-" ⇒ 0.0047062115261, "+" ⇒ -0.0047062115261, else ⇒ . }, else ⇒ . }, "R" ⇒ Match(Treatment) { "I" ⇒ Match(Ischemia) { "-" ⇒ 0.0078264812667, "+" ⇒ -0.0078264812667, else ⇒ . }, "P" ⇒ Match(Ischemia) { "-" ⇒ -0.00684049804, "+" ⇒ 0.00684049803996, else ⇒ . }, "T" ⇒ Match(Ischemia) { "-" ⇒ -0.0009859832267, "+" ⇒ 0.00098598322674, else ⇒ . }, else ⇒ . }, else ⇒ . }

Appendix K: Prediction Expression for ΔMPQ and ΔFaces Pain

ΔMPQ Pain:



ΔFaces Pain:



Appendix L: Prediction Expression for Changes in Vascular Resistance and Pain

Prediction Expression:

$log10(VR+10) = \overline{\Delta VR} + Phase + Ischemia + Treatment + MPQ + Phase*MPQ + Ischemia*MPQ + Treatment*MPQ + Subject$

Expanded Prediction Expression:

log10(VR+10) =

0.9953115896553

+ Match(Phase) { "E" ⇒ -0.015277510385, "O" ⇒ 0.09805180417847, "R" ⇒ -0.0827742937935, else ⇒ . }

+ Match(Treatment) { "I" ⇒ 0.01034794007025, "P" ⇒ -0.0122419219832, "T" ⇒ 0.00189398191291, else ⇒ . }

+ Match(Ischemia) { "-" ⇒ -0.0259600298943, "+" ⇒ 0.02596002989433, else ⇒ . }

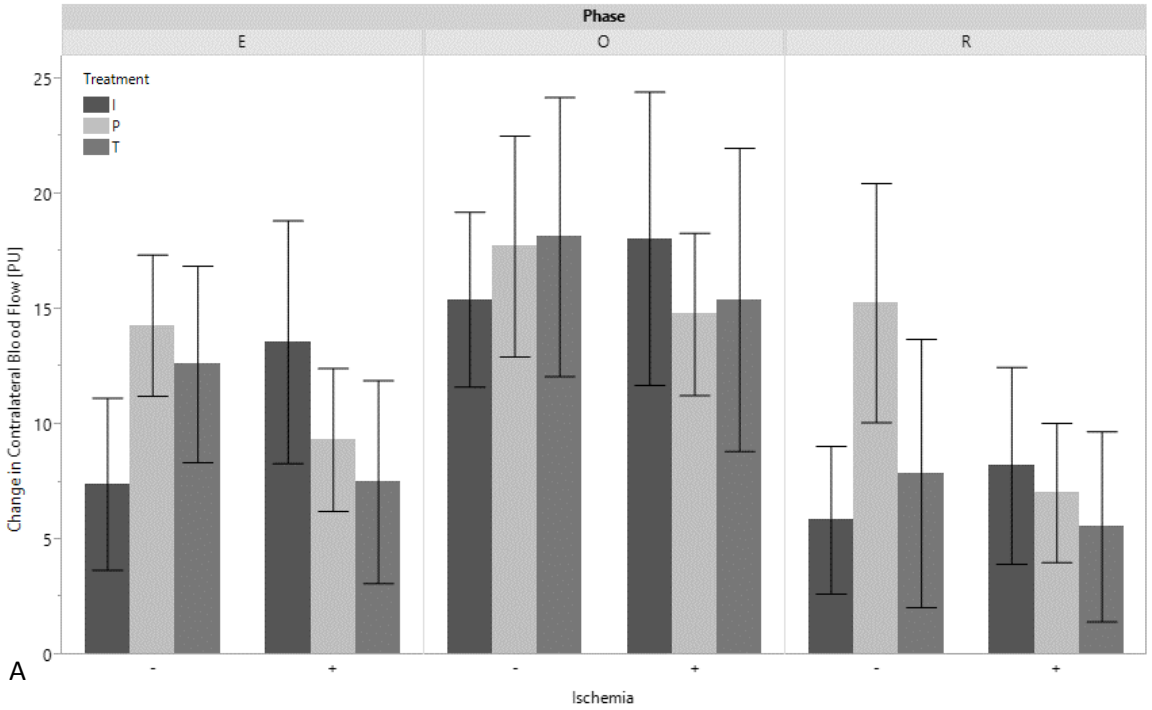
+ -0.0076033602066 * MPQ Pain

+ (MPQ Pain - 1.48987341772152) * Match(Phase) { "E" ⇒ 0.00774400503742, "O" ⇒ 0.02515285469848, "R" ⇒ -0.0328968597359, else ⇒ . }

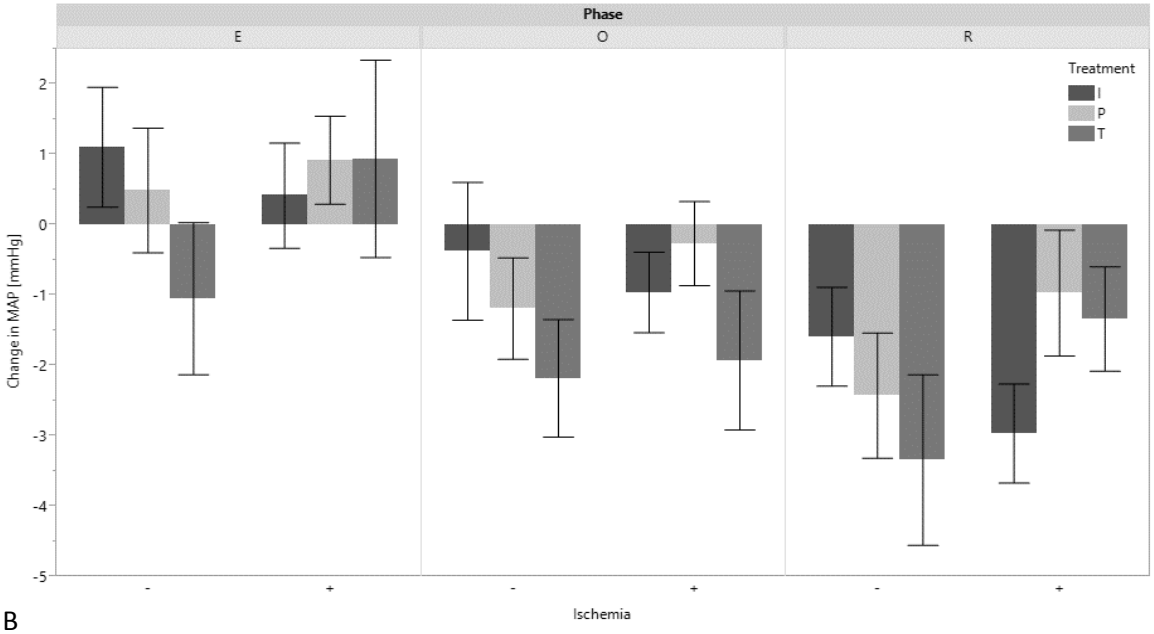
+ (MPQ Pain - 1.48987341772152) * Match(Treatment) { "I" ⇒ 0.00187556552148, "P" ⇒ -0.0011442408181, "T" ⇒ -0.0007313247034, else ⇒ . }

+ (MPQ Pain - 1.48987341772152) * Match(Ischemia) { "-" ⇒ -0.004122751043, "+" ⇒ 0.00412275104302, else ⇒ . }

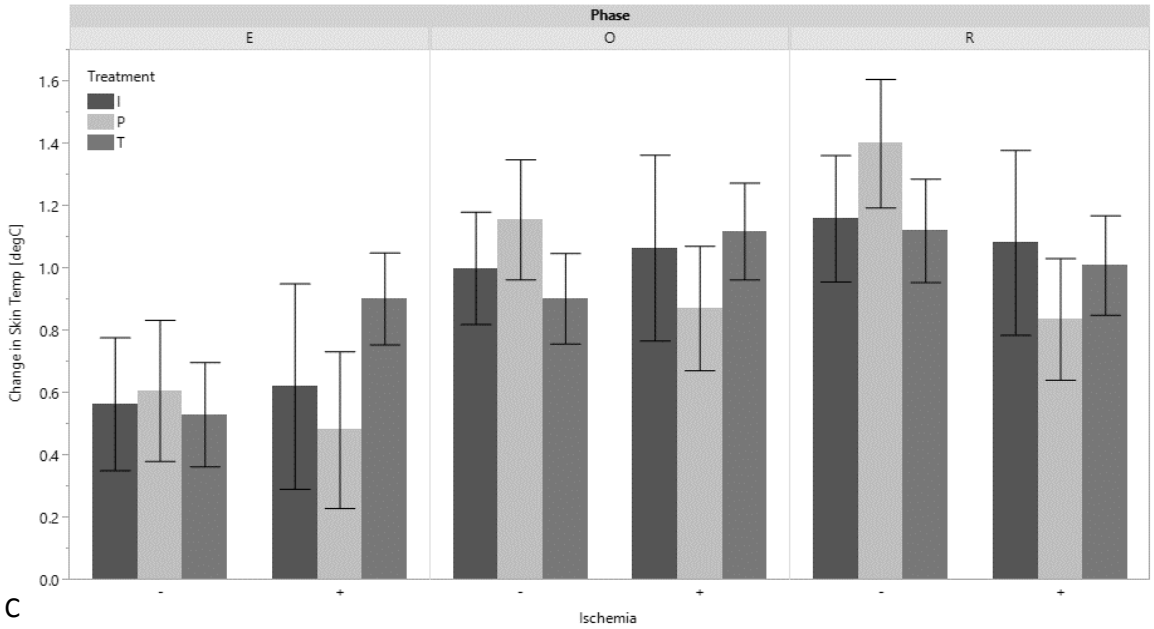
Appendix M: Mean Changes in Hemodynamic Factors and Pain for Treatment Type by Ischemia and Phase



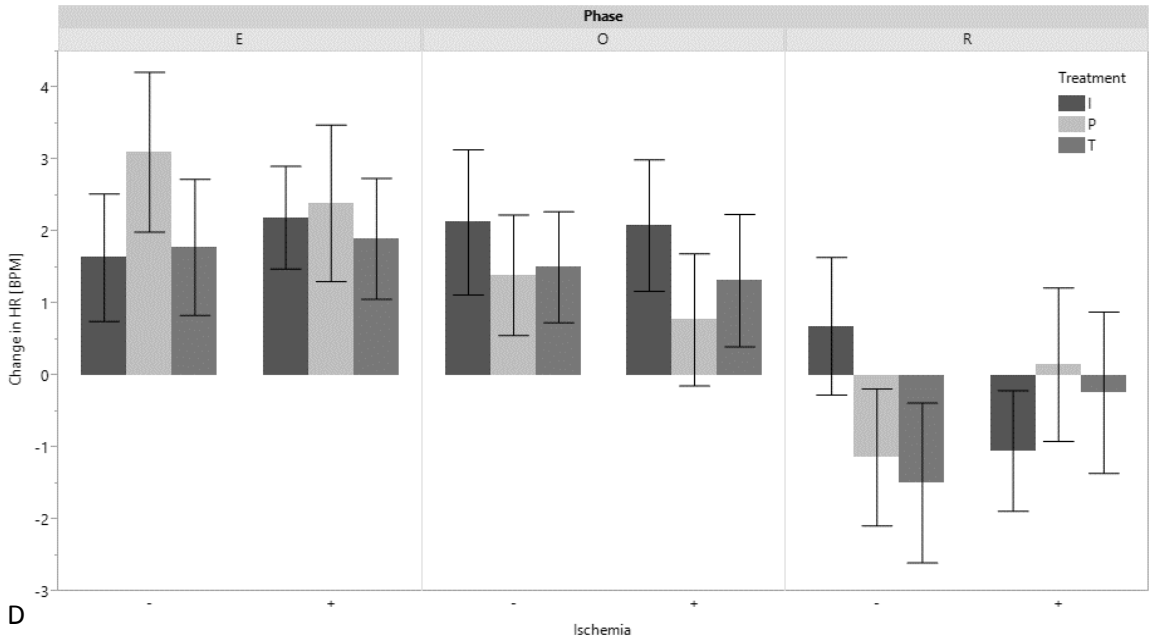
A



B

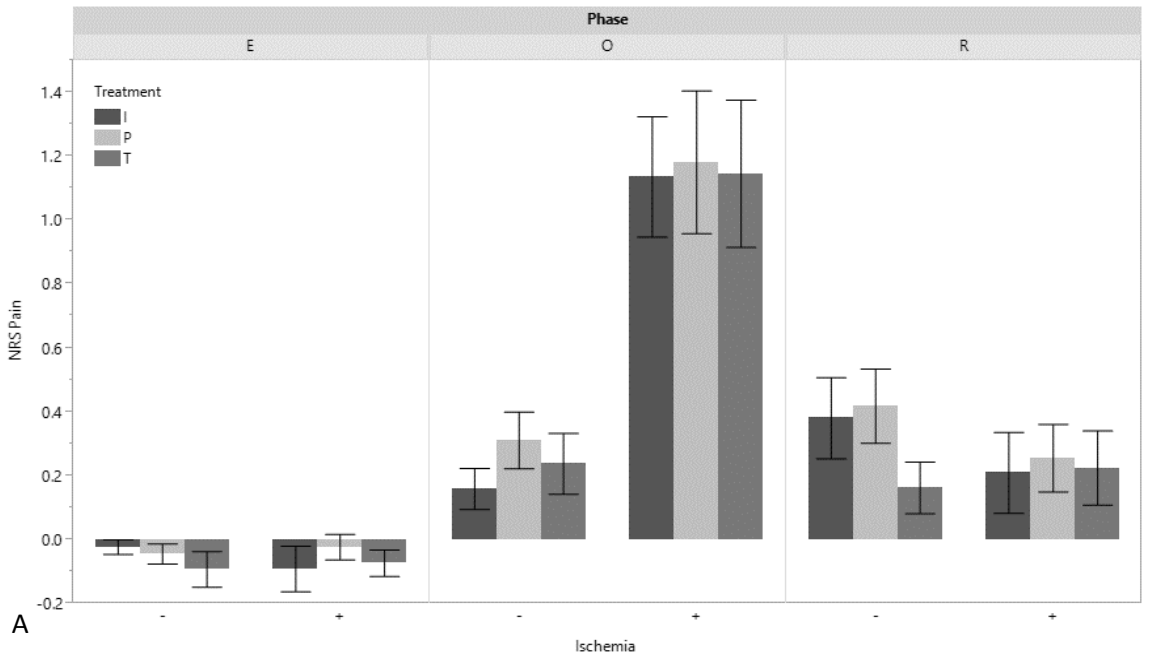


C

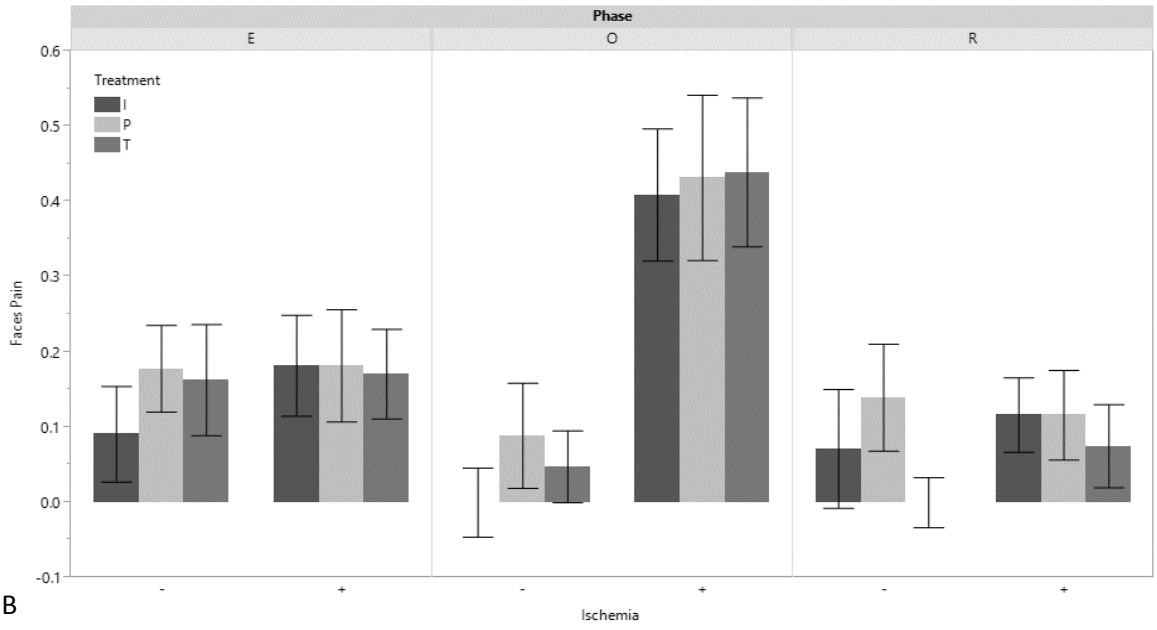


D

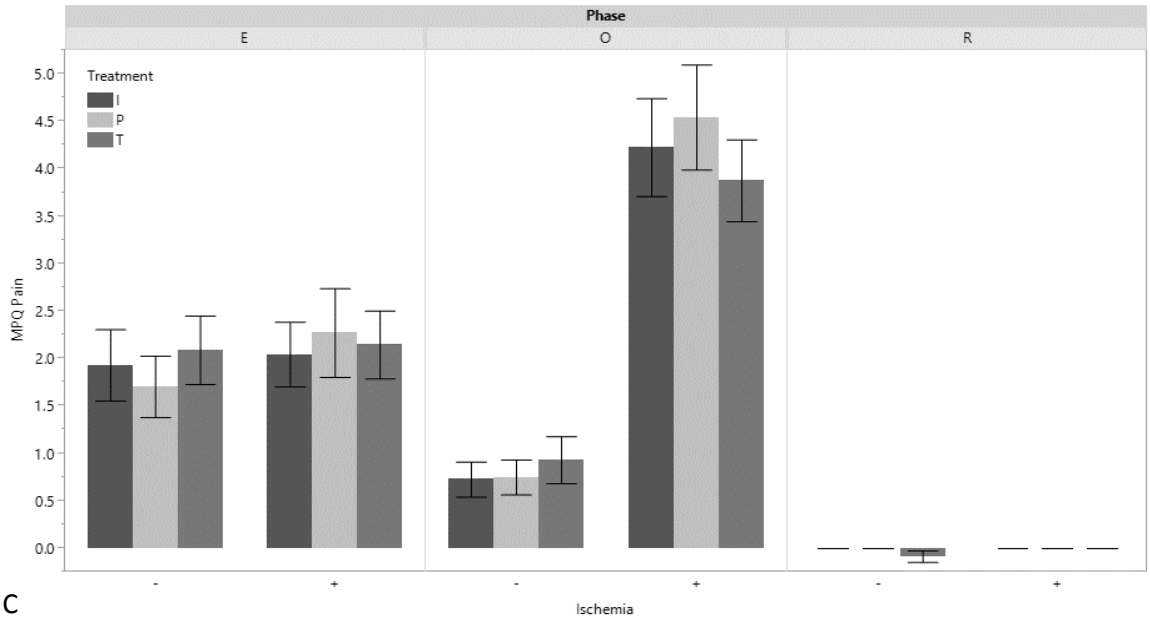
Figure 3.9: Mean Changes in Non-Significant Hemodynamic Factors for Treatment Types by Ischemia and Phase. A. Change in contralateral blood flow. B. Change in local blood flow. C. Change in mean arterial pressure (MAP). D. Change in Skin Temperature. E. Change in heart rate. Values are shown as means ± SE.


Phase
E
O
R
Treatment
I
P
T
NRS Pain
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-
+
Ischemia

A


Phase
E
O
R
Treatment
I
P
T
Faces Pain
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-
+
Ischemia

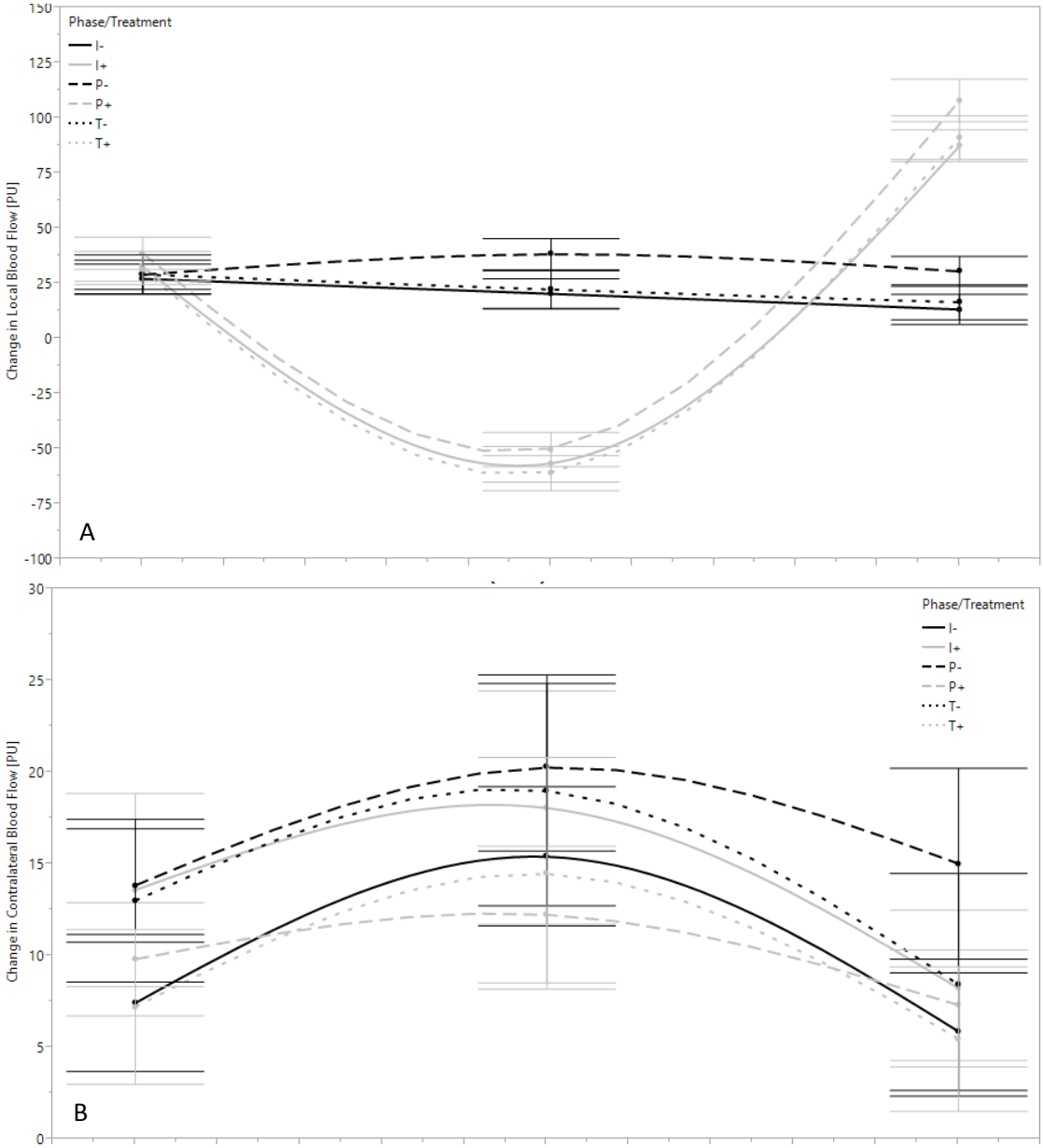
B

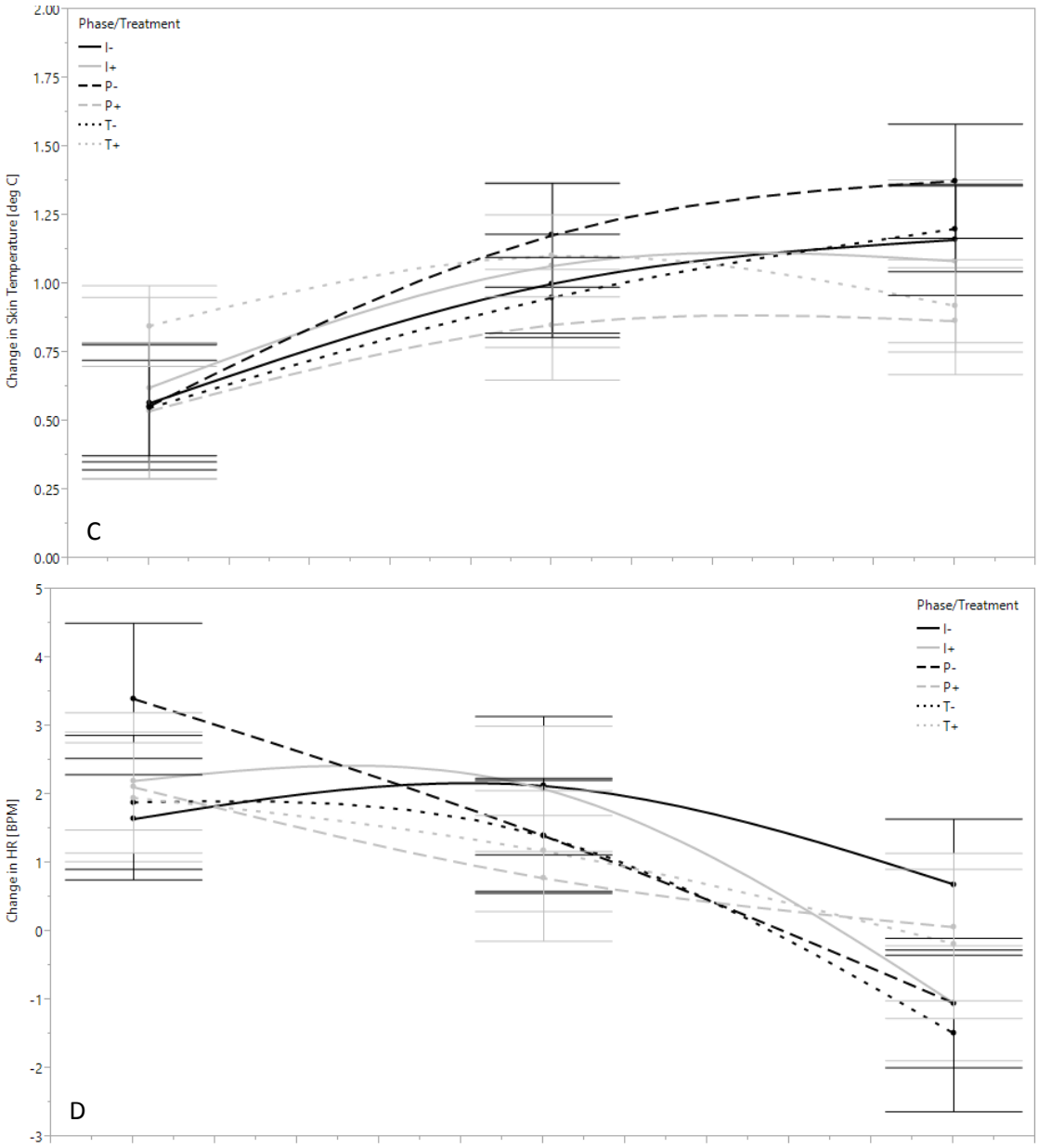


C

Figure 3.10: Mean Changes in Pain for Treatment Type by Ischemia and Phase. A. Change in Faces pain. B. Change in NRS Pain. C. Change in MPQ Pain. Values are shown as means ± SE.

Appendix N: Hemodynamic and Pain Trends during Exercise, Occlusion, and Recovery




Phase/Treatment
I-
I+
P-
P+
T-
T+
Change in Skin Temperature [deg C]
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
C
Change in HR [BPM]
5
4
3
2
1
0
-1
-2
-3
D

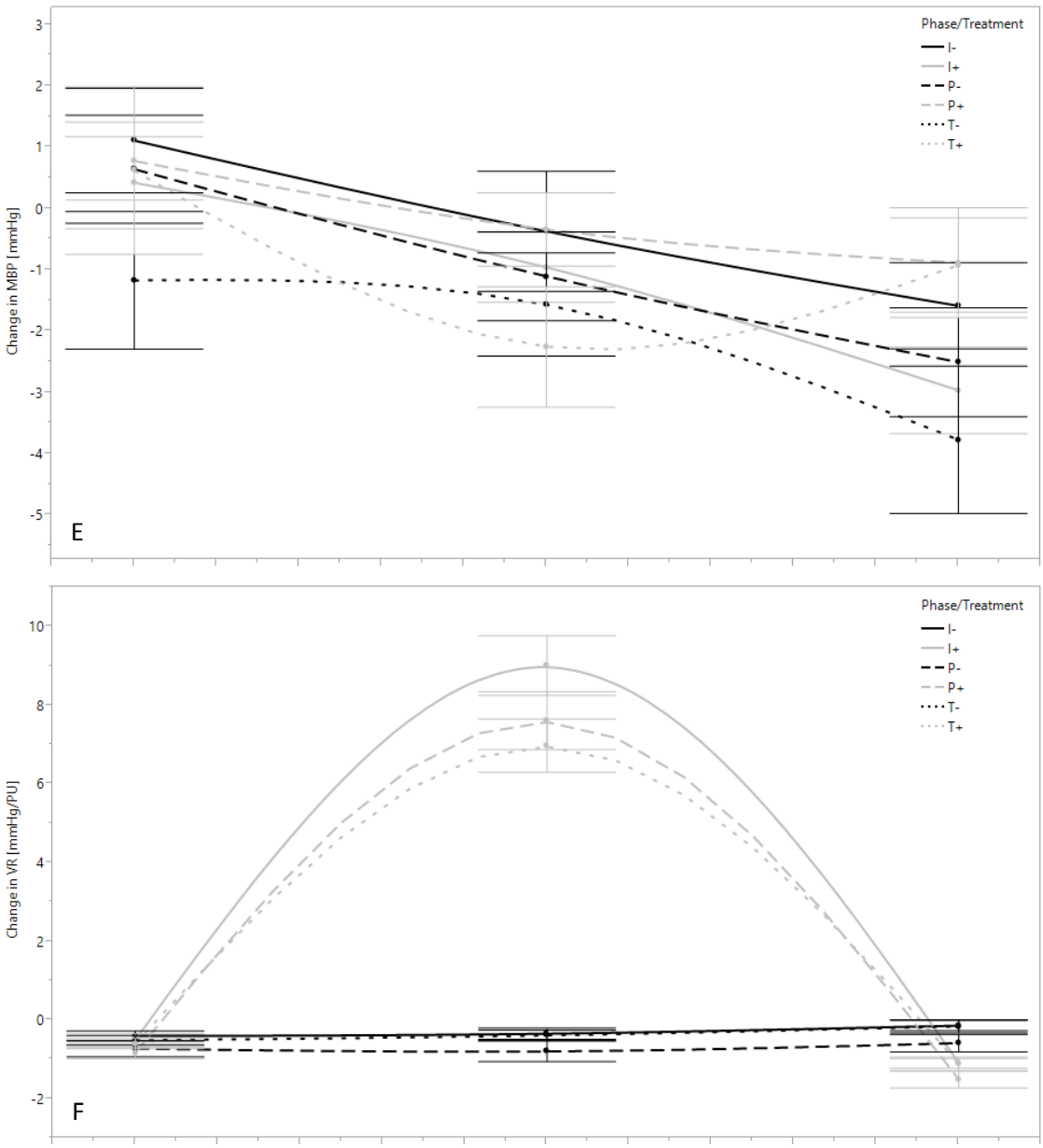
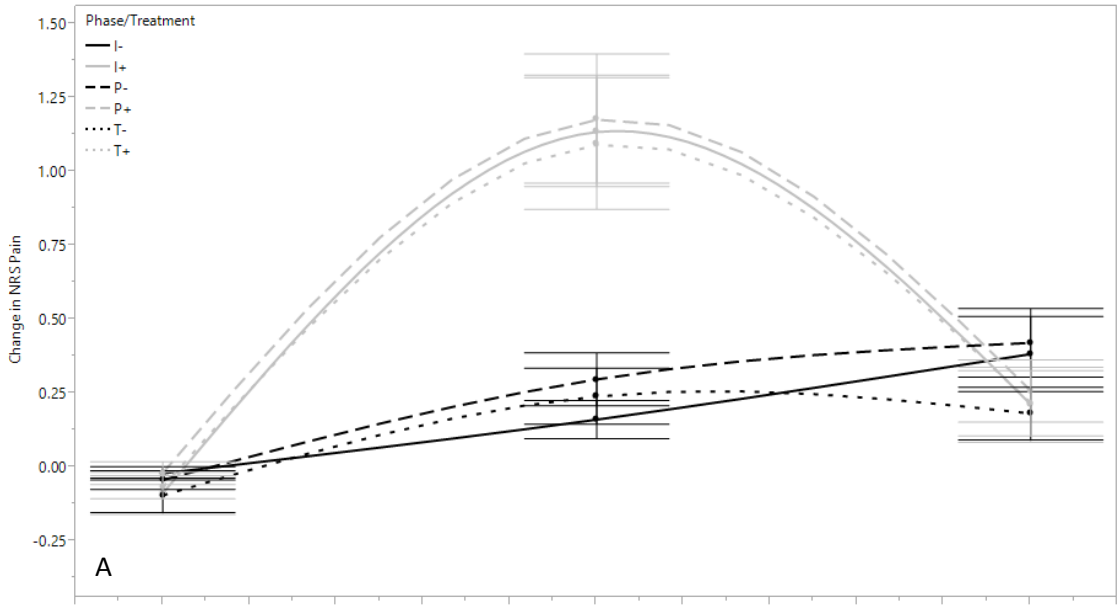
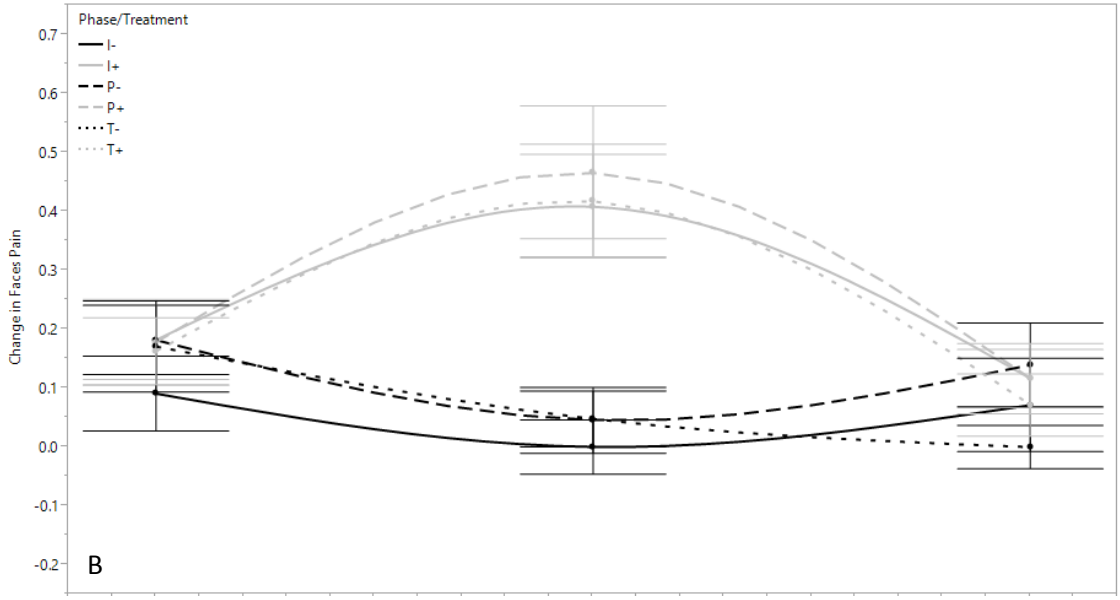


Figure 3.11: Trends in Hemodynamic Factors during Exercise, Occlusion, and Recovery Phases. A. Local blood flow. B. Contralateral blood flow. C. Skin temperature. D. Heart rate (HR). E. Mean blood pressure (MBP). F. Vascular resistance (VR). Absolute changes in hemodynamic factors during the static handgrip exercise, after exercise with (PECO+, gray lines) and without (PECO-, black lines) circulatory occlusion, and during recovery in healthy young individuals with IFC (continuous lines), TENS (dotted lines), and placebo (dashed lines). Values are shown as means ± SE.


Phase/Treatment
I-
I+
P-
P+
T-
T+
Change in NRS Pain
1.50
1.25
1.00
0.75
0.50
0.25
0.00
-0.25
A


Phase/Treatment
I-
I+
P-
P+
T-
T+
Change in Faces Pain
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
B

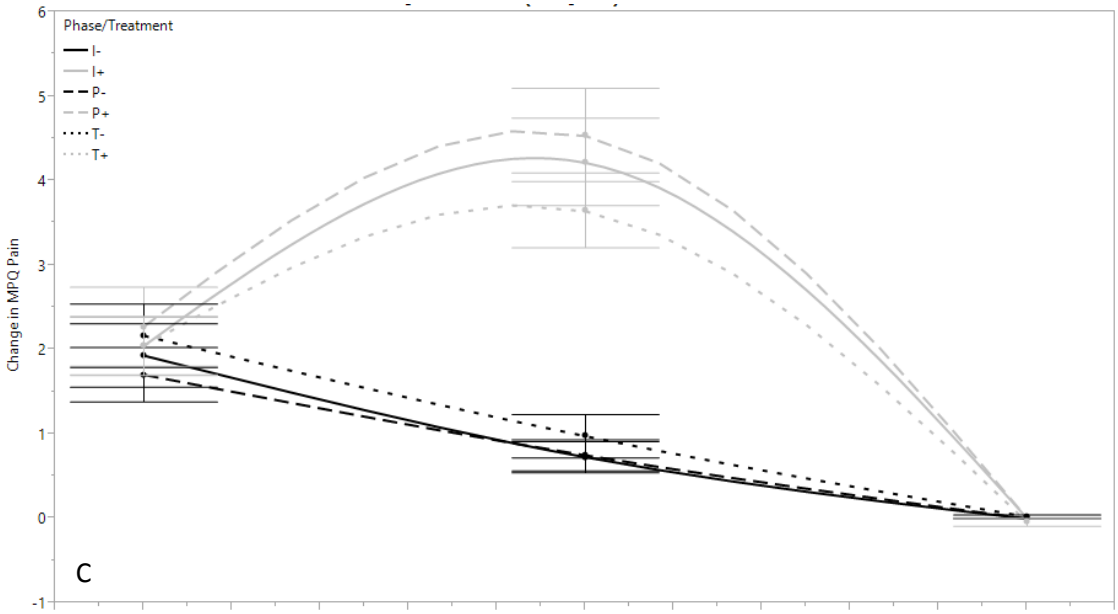


Figure 3.12: Change in Pain during Exercise, Occlusion, and Recovery Phases. A. NRS pain. B. Faces pain. C. MPQ pain. Absolute changes in pain during the static handgrip exercise, after exercise with (PECO+, gray lines) and without (PECO-, black lines) circulatory occlusion, and during recovery in healthy young individuals with IFC (continuous lines), TENS (dotted lines), and placebo (dashed lines). Values are shown as means ± SE.

Appendix O: Effects of Phase, Treatment Type, and Ischemia on $\Delta\overline{VR}$ and Pain

Different levels of each dependent variable, i.e. the O, E, and R levels of the phase variable, have a different effect on $\Delta\overline{VR}$ and Δpain. These effects are denoted by the fixed effect coefficient α.

Table 3.3: Effects of Phase, Treatment Type, and Ischemia on $\Delta\overline{VR}$. The average $\Delta\overline{VR}$ predicted by the dataset is offset by a value α for different phases, treatment types, and ischemic conditions. Using Tukey-Kramer's for post hoc comparisons, levels that do not share a letter have different effects on $\Delta\overline{VR}$ ($p \leq 0.01$).

Factor	p Value	Level	Tukey			Fixed Effect Coeff	Effect on $\Delta\overline{VR}$, [μmHg/PU]
Phase	<0.0001	O	A			α_O	+93.4
		E		B		α_E	-41.3
		R		B		α_R	-52.0
Treatment Type	0.0005	I	A			α_I	+11.2
		T	A	B		α_T	+2.00
		P		B		α_P	-13.2
Ischemia	<0.0001	PECO+	A			α_{PECO+}	+37.0
		PECO-		B		α_{PECO-}	-37.0
Phase*Ischemia	<0.0001	O,+	A			$\alpha_O\alpha_{PECO+}$	+91.9
		O,-		B		$\alpha_O\alpha_{PECO-}$	-91.1
		R,-		B		$\alpha_R\alpha_{PECO-}$	+53.1
		E,-		B	C	$\alpha_E\alpha_{PECO-}$	+38.7
		E,+		B	C	$\alpha_E\alpha_{PECO+}$	-38.7
		R,+			C	$\alpha_R\alpha_{PECO+}$	-53.1

Table 3.4: Effects of Phase and Ischemia on Change in Pain. The average pain predicted by the dataset is offset by a value α for different phases and ischemic conditions, while treatment type has no significant effect. Using Tukey-Kramer's for post hoc comparisons, levels that do not share a letter have different effects on change in pain ($p \leq 0.01$).

Factor	p value	Level	Tukey	Effect Coeff	Effect, α [ΔMPQ Pain]	Effect, α [ΔFaces Pain]
Phase	<.0001	O	A	α_O	+1.02	+75.8
		E	B	α_E	+0.534	+0.0813
		R	C	α_R	-	-75.9
Ischemia	<.0001	+	A	α_+	+0.606	+77.9
		-	B	α_-	-0.606	-77.9
Ischemia*	<.0001	O,+	A	$\alpha_O\alpha_+$	+1.12	+121
Phase		E,+	B	$\alpha_E\alpha_+$	+0.513	+59.5
		E,-	B	$\alpha_E\alpha_-$	-0.513	-59.5
		R,-	B C	$\alpha_R\alpha_-$	-	+61.4
		R,+	B C	$\alpha_R\alpha_+$	-	-61.4
		O,-	C	$\alpha_O\alpha_-$	-1.12	-121

Table 3.5: Effects of Pain on $\Delta\overline{VR}$. The relationship between $\Delta\overline{VR}$ and MPQ pain is dependent on the interactions between phase and treatment type effects, α, and a slope coefficient, β. For every 1 μmHg/PU increase in $\Delta\overline{VR}$, different phases or ischemic conditions have a different effect on the slope, β, describing the relationship between $\Delta\overline{VR}$ and ΔMPQ pain. Using Tukey-Kramer's for post hoc comparisons, levels that do not share a letter have different effects on $\Delta\overline{VR}$ ($p \leq 0.05$).

Factor	p value	Level	Tukey	Fixed Effect	Slope Effect, β [μmHg/PU·MPQ]
ΔMPQ Pain*Ischemia	0.0121	PECO+	A	$\beta_1\alpha_{PECO+}$	-2.81
		PECO-	B	$\beta_1\alpha_{PECO-}$	+23.3
ΔMPQ Pain*Phase	<.0001	O	A	$\beta_1\alpha_O$	+23.6
		E	B	$\beta_1\alpha_E$	-1.42

Appendix P: Variability in Pain associated with Paresthesia

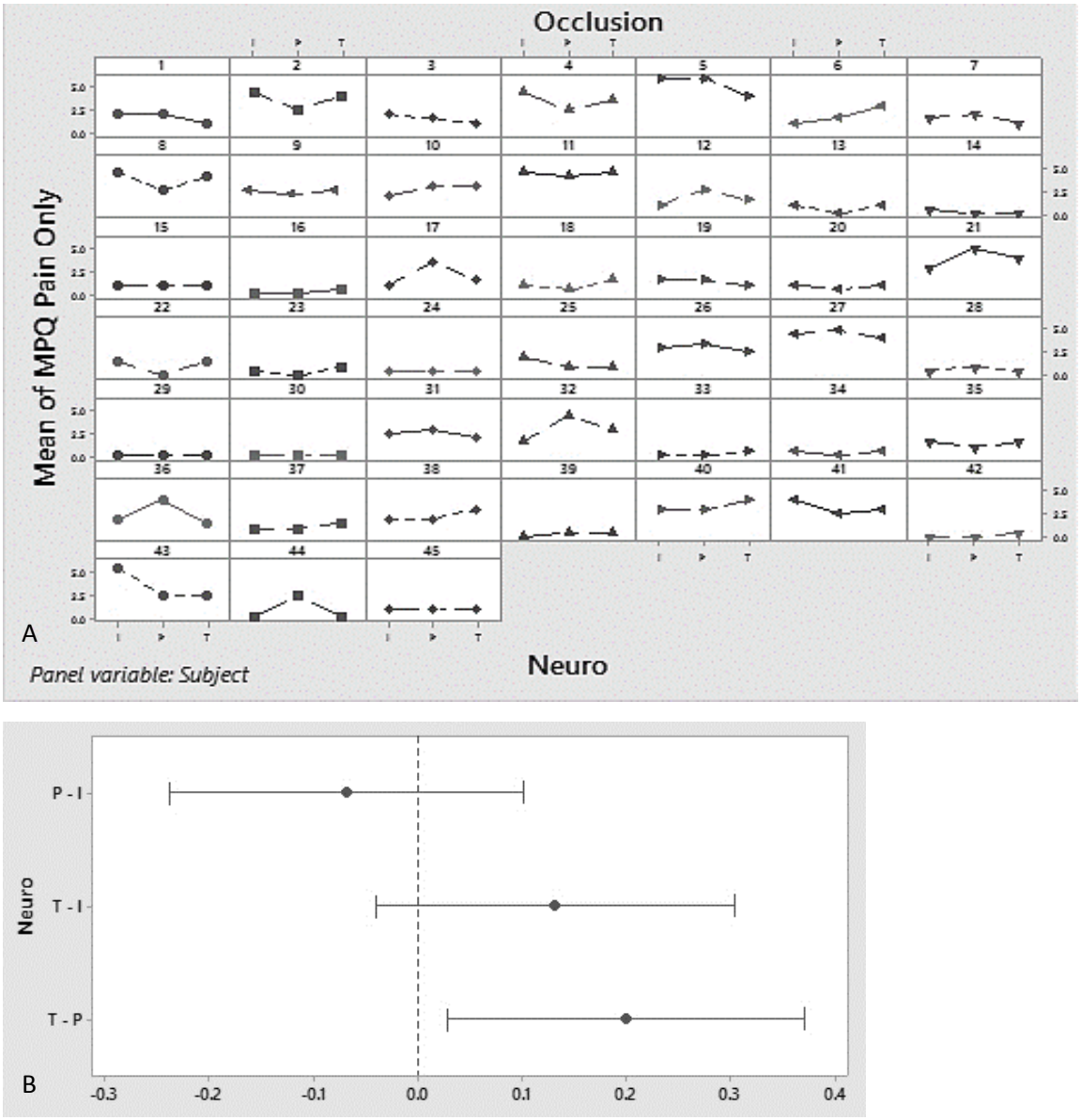


Figure 3.13: Paresthesia and Pain Variability between Subjects. A. Significant between-subject variability occurred in self-reported changes in MPQ pain when paresthesia descriptors were included in the questionnaire. B. Tukey post-hoc comparisons show that participants felt significant paresthesia with TENS treatment as compared to placebo (T-P). Values shown as means ± SE.