Michigan Technological University

Digital Commons @ Michigan Tech

Dissertations, Master's Theses and Master's Reports

2020

Sympathetic Neural Responses to Acute Thermal Sensations

Hannah August Cunningham

Michigan Technologial University

Recommended Citation

SYMPATHETIC NEURAL RESPONSES TO ACUTE THERMAL SENSATIONS

By

Hannah A. Cunningham

A THESIS

Submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

In Kinesiology

MICHIGAN TECHNOLOGICAL UNIVERSITY

2020

This thesis has been approved in partial fulfillment of the requirements for the Degree of MASTER OF SCIENCE in Kinesiology.

Department of Kinesiology and Integrative Physiology

Thesis Co-Advisor: *Jason R. Carter*

Thesis Co-Advisor: *John J. Durocher*

Committee Member: *Yeonwoo Rho*

Committee Member: *Scott Lempka*

Department Chair: *Megan C. Frost*

Table of Contents

List of figures

List of tables

Acknowledgements

List of abbreviations

CNS	Central Nervous System
PNS	Peripheral Nervous System
ANS	Autonomic Nervous System
SSNA	Skin Sympathetic Nerve Activity
MSNA	Muscle Sympathetic Nerve Activity
HRV	Heart Rate Variability
CPT	Cold Pressor Test
ESS	Epworth Sleepiness Scale
STAI	State-Trait Anxiety Inventory
ISI	Insomnia Severity Index
CES-Depression	Center for Epidemiologic Studies Depression Scale
ANOVA	Analysis of Variance
IQR	Interquartile Range
SAP	Systolic Arterial Pressure
DAP	Diastolic Arterial Pressure
HR	Heart Rate
BP	Blood Pressure

Abstract

The influence of thermal stimuli on the sympathetic nervous system is variable and largely depends on the change in temperature and timing of the stimuli. Core temperature changes yield increased muscle sympathetic nerve activity (MSNA) while changes in skin temperature yield variable MSNA responses. The MSNA responses to acute heating or cooling sensations remains unclear. Twenty-three participants (11 women, 12 men; age 24±1 years, BMI 26±1 kg/m^2) underwent a thermal protocol that included four trials each of cool sensation threshold, warm sensation threshold, and heat pain (12 total trials). Continuous blood pressure (finger plethysmography), heart rate (electrocardiography), and MSNA (via microneurography) were recorded throughout all trials. Data was assessed with a Shapiro-Wilk test and log transformations were utilized for non-normal distributed data. T-tests were used to compare physiological data for cool and warm sensation thresholds, and repeated measures ANOVA to compare multiple heat pain data points. MSNA was significantly attenuated during the immediate recovery of cool threshold and warm threshold. MSNA was inhibited during the sensation of heat pain and systolic arterial pressure was reduced during the recovery from heat pain. There were no significant differences between men and women for any variables and responses. These results indicate that acute thermal sensations result in the inhibition of MSNA.

1   Introduction and Broad Impact

Acute and chronic pain are known to impact a large proportion of the United States public. Recent evidence from the 2016 National Health Interview Survey reported 20.4% (i.e., 50.0 million) of U.S. adults had chronic pain, while 8.0% of U.S. adults (i.e., 19.6 million) had high-impact chronic pain (i.e., pain that interferes with day-to-day activities)[1]. An individual with chronic pain is at higher risk for several comorbidities, including heart disease[2], obesity[3], and depression.[4] Economic burdens in 2008 was estimated at a minimum of $560 billion dollars, more than the cost of cancer ($243 billion), diabetes ($188 billion) and heart disease ($309 billion).[5] It is imperative to conduct rigorous, high quality research on pain processing to alleviate this economic and clinical burden. The design of treatments for chronic pain can benefit by understanding how individuals process pain and what variables influence this response.

1.1   Basic Structure of the Nervous System

The human nervous system is composed of a vast network of neurons receiving and sending signals throughout every organ system of the body. It is separated into two distinct regions: the central nervous system (CNS), consisting of the brain and spinal cord, and the peripheral nervous system (PNS), including afferent and efferent motor and sensory neurons. The PNS can be further subdivided into the somatic nervous system and the autonomic nervous system (ANS). The somatic nervous system is responsible for the perception and propagation of voluntary motor responses, also including reflexes. For example, during a painful situation, such as burning your hand on a hot object, you will, by reflex, snatch your hand away from the offending object. This is a somatic nervous system response.[6]

The other branch of the PNS, the autonomic nervous system, is responsible for the regulation of involuntary responses related to the cardiovascular system, respiratory system, gastrointestinal system, and other key systems for maintaining homeostasis. In the case of the burning hand example, just after pulling your hand away, you might notice an increase in heart rate and/or breathing rate. These responses are due to the ANS. The two branches of this system, the sympathetic and parasympathetic branches, act in an opposing manner to one another. The sympathetic induces a rapid "fight or flight" or arousal response, resulting in pupil dilation, increased respiratory rate and bronchodilation, increased heart rate and contractility, glucose release for energy, and inhibition of the digestive and urinary tracts. The parasympathetic nervous system leads the "rest and digest" response, causing pupil constriction, decreased respiratory rate and heart rate, and stimulation of the digestive and urinary tracts.[6]

The sympathetic nervous system is of particular interest to researchers and clinicians due to it being implicated in a number of chronic diseases and conditions, including chronic pain. Over sensitization of the sympathetic branch of the PNS is thought to be a contributing mechanism of chronic pain. In a healthy individual, the sympathetic nervous

system is activated in response to pain in order to inhibit the pain experience.[7,8] Disturbances to this pathway can result in central sensitization, a condition where the nervous system is in a persistent state of hyperactivity.

The SNS response to stimuli can be characterized through the measurement of skin sympathetic nerve activity (SSNA) or muscle sympathetic nerve activity (MSNA). The primary role of SSNA is to regulate body temperature through sweat release and blood flow in the skin. In contrast, MSNA plays a significant role in beat-to-beat blood pressure regulation and the innervation of vascular smooth muscle leading to the regulation of peripheral vascular resistance. MSNA will be discussed more in Section 1.4.

1.2 Anatomy of Pain

1.2.1 Primary Somatosensory Neurons, Nociceptors and Transmitting Fibers

Current theories of pain postulate that specialized receptors within the peripheral nervous system respond to specific stimuli and transmit sensory information to the central nervous system. The somatosensory system is comprised of specialized receptors, many of which terminate with free nerve endings in the dermis of the skin. There are three types of afferent nerve fibers: Aβ fibers, Aδ fibers, and C fibers. Aβ fibers and Aδ fibers have large diameter, myelinated axons, conducting information at 5–30 m/s.[9] Aβ respond to non-noxious, mechanical stimuli. In contrast, C fibers have small diameter, unmyelinated axons, conducting sensory information at 0.4–1.4m/s.[9] Aδ fibers and C fibers are sensitive to noxious thermal, mechanical, and chemical stimuli.

Nociceptors are specialized primary sensory neurons that respond to stimuli with the potential to cause tissue damage. Nociceptive information is transmitted by Aδ fibers and C fibers, with the majority of nociceptors being C fibers. Due to their conducting speed, Aδ fibers are responsible for the fast-pain response.[10] Both fibers respond to a range of nociceptive inputs, including thermal, mechanical, and chemical stimuli. Nociceptors that respond to a combination of these inputs are called polymodal.[11] Nociceptors transmit information in all-or-nothing action potentials when triggered by an adequate stimulus. However, this signal transmission does not always lead to the perception of pain. Central influences, from the brain and spinal cord, can have a significant role attenuating the signal transmitted by pain-specific fibers.[12,13]

1.2.2 Central Nervous System Response to Pain

Somatosensory fibers synapse in the dorsal root ganglion and excite second-order neurons within the spinal cord. At this level, spinal reflexes, such as the withdrawal reflex, are activated. This quick response system prevents further harm to the tissue without processing in the brainstem. Nociceptive input is conveyed by the spinal cord to the brainstem along two distinct tracts: spinothalamic and spinoreticulothalamic. The

spinoreticulothalamic pathway transmits nociceptive information from the dorsal horns to the reticular formation of the brainstem and further relays information to the thalamus.[10,14] The reticular formation responds to nociceptive inputs by regulating the autonomic response to the stimuli. This includes responses associated with breathing and heart rate, creating the classic fight or flight response. The spinothalamic tract and the orofacial equivalent, trigeminothalamic tract, convey information to the medial and lateral thalamus.[10,14] This pathway results in cognitive, perceptive and emotional responses to pain, processed and transmitted to higher brain centers by the thalamus. The medial thalamus projects to the limbic system and hypothalamus and the lateral thalamus to neocortical somatosensory areas.[10,14]

In addition to creating the autonomic, perceptive, and emotional responses to nociceptive stimuli, the central nervous system is also responsible for modulating nociceptive input.[8] Much like a "gate", there exists descending pathways from the brain to the spinal cord to inhibit pain or facilitate the pain response. The ability of the human body to generate these descending pathways, inhibit or excitatory, may be a key factor in individual variations in pain and contribute to diseased states (e.g., chronic pain, fibromyalgia).[8,15-17]

1.3 Methods to Measure Autonomic Nervous System Activation

1.3.1 Norepinephrine Urinary and Plasma Measurements

Norepinephrine is the main neurotransmitter used by the sympathetic nervous system and has therefore been used to evaluate sympathetic nerve activity with measurements of urinary and plasma norepinephrine.[18] Twenty-four hour urinary excretion of norepinephrine allows researchers to infer sympathetic activity, however this method is too slow to measure acute responses, does not isolate the location of norepinephrine release (e.g., heart or kidneys), and largely depends on kidney function[18].

Plasma norepinephrine levels also provides a limited window-of-insight into sympathetic activity. Conditions that change sympathetic tone (e.g. head up tilt, sleep) produce parallel changes in plasma norepinephrine.[18,19] However, plasma levels of norepinephrine depend on the neurotransmitter's secretion, clearance, and reuptake, and therefore are not a definitive of indicator of norepinephrine activity in the body. This method has low sensitivity and high intra-individual variability when compared to better measures of sympathetic nervous system activity (i.e., microneurography).[20] In addition, plasma measurements are not sensitive to rapid, acute increases in sympathetic activity. Hypertension is known to elevate sympathetic activity (evaluated using microneurography), though hypertensive individuals have the same plasma norepinephrine values as healthy individuals.[21] The pitfalls of measuring norepinephrine in urine and plasma led researchers to develop the methods to measure norepinephrine release more directly.

1.3.2 Plasma Norepinephrine Kinetics

The plasma norepinephrine spillover technique expands on basic measurement of the presence of norepinephrine in plasma by utilizing radiotracer methodology to quantify rate of release of norepinephrine from sympathetic nerve endings into the plasma.[22] The methodology includes infusing a known concentration of titrated, radiotracer norepinephrine into the bloodstream until the individual has reached steady state conditions. Using kinetic derived equations, researchers can quantify the norepinephrine spillover rate and clearance rate for the entire body. This technique can also be used to identify organ specific sources of whole-body norepinephrine. Elser et al.[23,24] reported the lungs contribute to the greatest amount to whole body norepinephrine spillover (~30-40%) while the adrenal (2%) and the heart (3%) contribute the least. Since its creation in the 1980s, this technique remains to be the gold standard for measuring sympathetic activity. However, it is incredibly expensive, invasive, and requires the presence of a licensed medical professional.

1.3.3 Heart Rate Variability

Heart rate variability (HRV) is a convenient and popular method to estimate parasympathetic and sympathetic innervation of the heart because it only requires an electrocardiogram. The sympathetic and parasympathetic nervous system innervate the heart to increase and decrease heart rate, respectively. Innervation is constantly fluctuating and researchers can get an approximate snapshot by examining the frequency analysis of the R-R interval, where there are two distinct peaks, one between 0.04–0.15 Hz and another between 0.15–0.4 Hz.[25] The lower frequency range, LF, is modified by the sympathetic and parasympathetic nervous system.[26] The high-frequency range, HF, is modified by the parasympathetic nervous system.[26] HRV is not a direct measure of sympathetic activity and must be used with great caution when interpreting results. Studies have shown its lack of reproducibility.[27,28] Many investigators misinterpret the LF spectrum as only sympathetic innervation and not correctly as a combination of sympathetic and parasympathetic. While this technique is cost effective and produces a reliable interpretation of parasympathetic innervation, it is not a robust method of measuring sympathetic activity.

1.3.4 Microneurography

Microneurography is a highly specialized technique that allows for direct and continuous recordings of post-ganglionic sympathetic nerve activity to the vasculature.[29,30] It is minimally invasive compared to norepinephrine spillover and more cost effective. The most common recordings site is the peroneal nerve, accessed at the popliteal or fibular site. Other locations include the radial, median, and ulnar nerves of the arm and some facial cranial nerves.[30]

The methodology of the technique includes inserting a microelectrode directly into the nerve to measure multi-fiber or single fiber recordings. The uninsulated tip of the active

electrode is inserted directly into the nerve, while a reference electrode is placed nearby, serving as a ground. The electrodes are connected to a pre-amplifier which is connected to an additional amplifier. The signal is amplified and filtered, to display the signal as a mean voltage neurogram. Researchers can visually and acoustically identify "bursts" of sympathetic activity as they travel down the nerve. This signal can be quantified as bursts over time, the number of bursts normalized to heart rate, and by amplitude and/or area of the bursts (i.e., total MSNA). This technique can be used to measure MSNA or SSNA.

The sympathetic response measured at the peroneal nerve is highly correlated with total sympathetic outflow to different limbs and with norepinephrine spillover at the heart and kidneys.[31,32] Therefore signals measured at the peroneal site are often used as estimates to signals received at the heart and kidney, major sites of blood pressure regulation, and at other limbs. The results from this technique are highly reproducible at baseline and during stressors.[33]

1.4 Muscle Sympathetic Nerve Activity

Muscle sympathetic nerve activity (MSNA) is involved in the beat-to-beat control of blood pressure, and operates primarily through the baroreflex. Briefly, specialized pressure sensors in the carotid bodies of the neck and aortic arch detect transient, beat-to-beat changes in blood pressure and respond by modulating heart rate, contractility, and total peripheral resistance. The primary goal is to maintain hemodynamic homeostasis. By sensing fluctuations in blood pressure, the carotid bodies modulate their firing patterns to activate changes in sympathetic outflow. When blood pressure is high, the carotid bodies are activated and inhibit sympathetic activity, effectively lowering blood pressure through vasodilation via MSNA.[6]

1.4.1 MSNA Response to Non-Noxious Heating and Cooling

Historically, the first studies examining the human body response to thermal changes were done with whole body heating or cooling. As it became clear that there are autonomic and cardiovascular responses to whole body heat and cold stress, researchers began to focus on localized heat and cold stress.

1.4.1.1 Whole Body Heating

Whole body heating can be accomplished by raising ambient room temperatures or having participants wear suits that circulate hot/warm water. Experimental designs vary; some experimenters look for a rise in core temperature and others a rise in skin temperature. Several studies have measured MSNA in conjunction with whole body heating, with most studies reporting that whole body heating raises body temperature and augments heart rate and sympathetic activity.[34-43] However, the influence of whole body heating on blood pressure is equivocal, with studies reporting no change in blood pressure[34,36,41-43], a decrease[35,40,44], or an increase in systolic pressure.[37] Whole body

heating triggers robust vasodilation, resulting in a drop in total vascular resistance and thus blood pressure. This serves as the trigger for the sympathetic nervous system to increase outflow thereby increasing MSNA via the baroreflex to compensate for the drop in blood pressure. By raising core body temperature by 0.6°C and skin temperature by 3.5°C, Cui et al.,[34] saw MSNA burst frequency double. Similar results were seen by Gagnon et al.,[35] in which participants wore a tube-lined suit with warm water till their core temperature increased by 1.2°C and MSNA burst frequency tripled. In conclusion, whole body heating that raises core body temperature induces a significant autonomic response resulting in marked and robust increases of MSNA.

1.4.1.2 Whole Body Cooling

Opposite to whole body heating, whole body cooling lowers the body temperature below normal using ambient cooling or cold-water suits. While the literature is consistent that whole body heating augments MSNA, the response to whole body cooling remains equivocal. Some studies report an overall increase in sympathetic activity using ambient cooling[45] or no change using water perfusion suits.[46,47] Studies by Jian Cui and Craig Crandall show modest increases in MAP with no observable changes in MSNA or heart rate.[46] Greaney et al.,[47] reported similar results with increases in blood pressure and no change in MSNA and heart rate during whole body cooling. All groups utilized water perfusion suits to cool skin temperature to ~30°C, held constant throughout the experiment. Similar increases in MAP have been observed by other research groups.[45,48]

The method and magnitude of cooling might explain the conflicting results of MSNA responsiveness during these studies. Some studies cool the body to a constant hypothermic temperature,[46,47] while others measure hemodynamics and sympathetic activity as the body temperature is dropping.[37,48] The range of results suggest that sympathetic responses depends on the magnitude of cooling, as different populations of thermal and/or pain receptors are stimulated with different cooling paradigms.

1.4.1.3 Localized Heating

With the consensus that whole body heating augments MSNA, with variable effect on blood pressure, the next logical step is to examine localized heating. This method involves the application of heat to a portion of the skin (e.g. lower leg or back). This is a common therapy for physical therapists and athletic trainers with clinical benefits.[49,50] Many studies have examined local heating in conjunction with exercise. For example, during hand grip exercise, the application of heat to the working forearm increases the MSNA response compared to a neutral stimulus.[51-53]

One study, examining the influence of local heating without the influence of exercise, observed an opposite effect.[54] Applying mild heat to the lower leg with ipsilateral measurements of MSNA at the peroneal nerve, Takahashi et al.,[54] reported a decrease in burst frequency and total MSNA at the onset of local heat (see Figure 1).
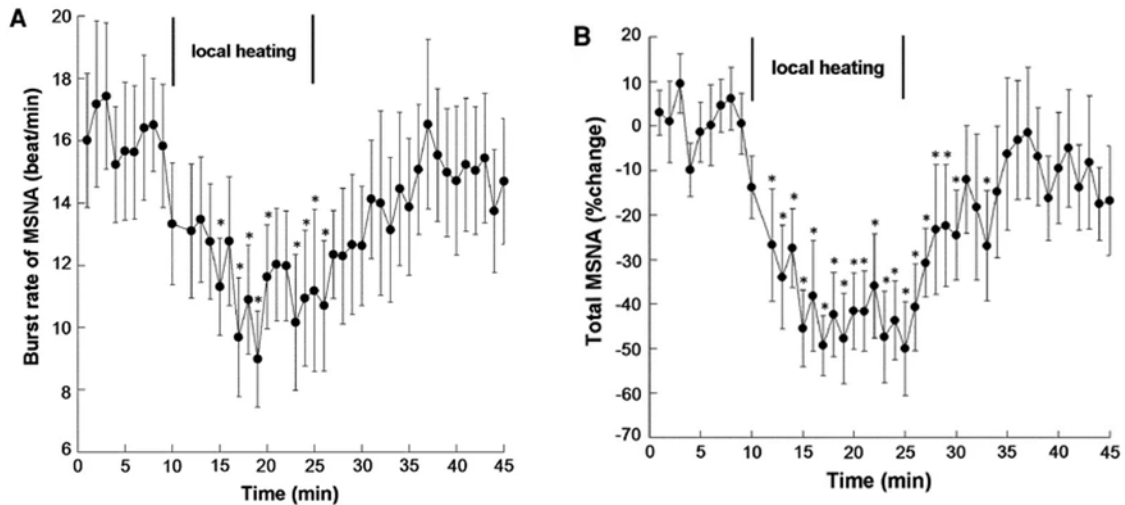
Figure 1. Muscle sympathetic nerve response during localized heating of the leg. Reprinted by permission from Springer Nature: Springer nature. European Journal of Applied Physiology. Takahashi, N. et al. Local heat application to the leg reduces muscle sympathetic nerve activity in human. 111, 2203-2211. (2011).[54] See Appendix G for full attribution and copyright licensing information.

This attenuation continued throughout the stimulus, with MSNA returning to baseline values during recovery. The percentage change in total MSNA correlated significantly with the change in skin temperature (i.e., as skin temperature increased, MSNA decreased). Importantly, the researchers saw no change in core body temperature, and therefore speculated increasing skin temperature modulated MSNA. Heart rate and blood pressure were consistent throughout the local heating and recovery. They concluded the threshold to initiate a change in MSNA is different than that needed to initiate a change in heart rate or systemic blood pressure.

1.4.1.4 Localized Cooling

The cold pressor test (CPT) is a common laboratory technique used to induce localized cooling. Typically done through the submersion of the hand into a bucket of cold water, it causes reproducible increases in heart rate, blood pressure and sympathetic activity.[55] By using progressively colder water temperatures, Kregel et al.,[56] were able to specifically target nociceptors in the skin. Using water temperatures of 28°C and 21°C, they were able to activate non-noxious, low threshold (35°C-20°C), cold sensitive fibers (Aδ and C fibers). By cooling the water temperature to 14°C, 7°C, and 0°C, they activated noxious, high threshold (<20°C), nociceptive fibers (primarily C fibers). During warmer water temperatures (28°C -14°C), they observed a brief inhibition of MSNA during the initial 30 seconds of water immersion (see Figure 2). Because there were no significant differences in blood pressure and body temperature during this interval, they concluded this inhibition was mediated by the stimulation of Aδ and C sensory afferents.

7
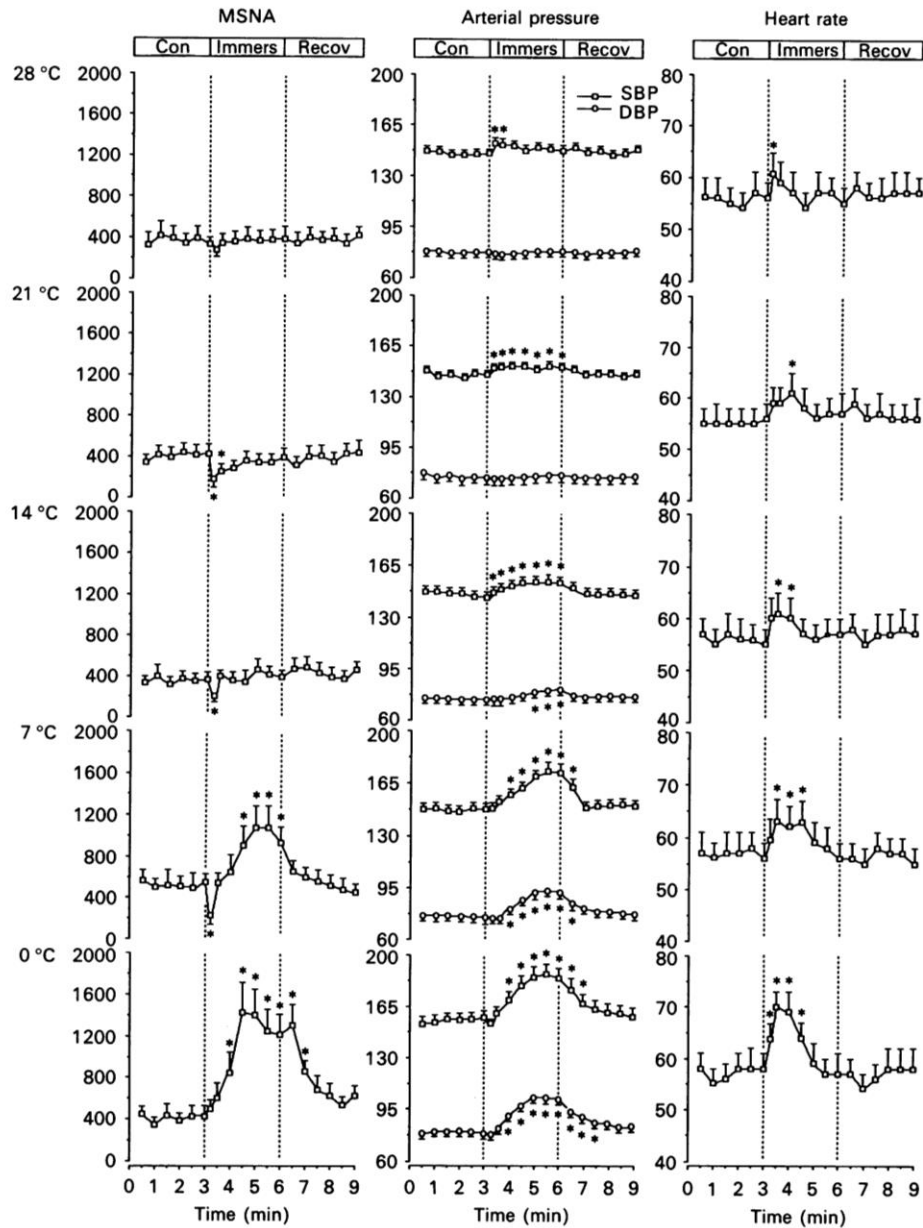
Figure 2. Muscle sympathetic nerve response during cold pressor test at varying temperatures. Reprinted by permission from John Wiley and Sons: John Wiley and Sons. Journal of Physiology. Kregel, K. C., Seals, D. R. & Callister, R. Sympathetic nervous system activity during skin cooling in humans: relationship to stimulus intensity and pain sensation. 454, 359-371, (1992).[56] See Appendix G for full attribution and copyright licensing information.

Conversely, Ishida et al., [57] reported a rise in MSNA during localized cooling of the leg with ipsilateral measurement of MSNA (see Figure 3). Similar to Kregel et al.[56], the authors concluded the rise in MSNA was due to local mechanisms and activation rather than activation through the vasomotor control center in the brain. The authors also

8

reported an attenuation from baseline of MSNA during recovery from the cooling
stimulus.
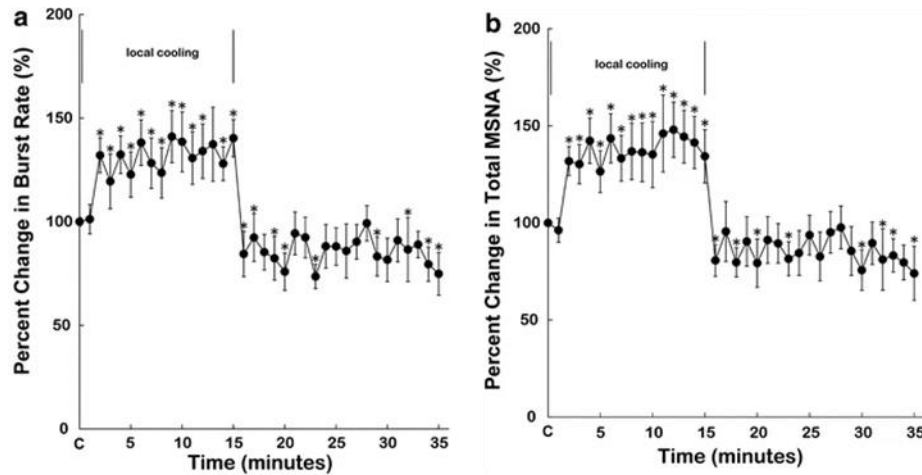
Figure 3. Muscle sympathetic nerve response during localized cooling of the leg. Reprinted by permission
from Springer Nature: Springer Nature. European Journal of Applied Physiology. Ishida, K. et al.
Suppression of activation of muscle sympathetic nerve during non-noxious local cooling after the end of
local cooling in normal adults. 116, 851-858, (2016).[57] See Appendix G for full attribution and copyright
licensing information. European journal of applied physiology

1.4.1 MSNA response to Pain

Early evidence using the cold pressor test as a pain stimulus suggests augmented
sympathetic activity and blood pressure occur in parallel with increasing pain. Kregel
observed that immersion of the hand in non-noxious 14 °C water did not increase MSNA,
whereas submersion in noxious 7 °C water induced increased MSNA.[56] This early work,
shows pain perception and cardiovascular and autonomic regulation centers are activated
in parallel, potentially one triggering the other. This is further evidenced by the many
pain disorders with elevated sympathetic activity.[58-60]

The few studies that induce pain concurrent with measurements of sympathetic activity
confirm this relationship between pain and increased hemodynamic and autonomic
variables. Other studies have shown the association between increased pain with
corresponding increases in MSNA, blood pressure, and heart rate during the cold pressor
test.[61,62] Other pain modalities, including pressure to the nail-bed,[63] instillation of soap
solution into one eye,[63] mechanical pressure on the skin,[64] and saline infusion[65,66]
demonstrate consistent increases in MSNA and blood pressure. With regards to cutaneous
thermal pain, prolonged cold and heat pain stimulation (~5 min) elicited rapid increases
in MSNA burst frequency during the initial minute of stimulus onset that plateaued for
the remainder of the experiment.[67] Though the stimulus was painful, the researchers
observed no change in heart rate for either condition, and an increase in diastolic blood
pressure during only the heat pain condition.

A common model for long lasting pain is through the infusion of saline. By controlling infusion depth, researchers can effectively mimic deep or superficial pain. When saline is infused at different depths of the leg, Burton et al. [66] observed increased MSNA burst frequency. The increased MSNA was comparable between depths, suggesting location of pain might not have a differential effect on MSNA response. Though individuals consistently display an increase in burst frequency, further work from the same lab suggest divergent responses in total MSNA (i.e., burst amplitude).[68] Some individuals had parallel increases in MSNA, blood pressure and heart rate, while others did not. The responses are relatively consistent between experimental sessions with ~73% of individuals having a similar response during a second experimental session.[69] Further studies suggest the divergent responses to pain via infusion of saline results from differential activation of a neural pathways, further highlighting the role of the brain in processing and generating physiological responses.[70]

It is imperative to understand the physiological responses driven by the experience of pain, not only to design creative, cost effective treatments for chronic pain, but to also understand how these healthy responses, such as those seen in the sympathetic nervous system, are altered during diseased states. It is reasonable to predict these healthy adaptations are replaced by maladaptive variations.

1.4.2 Influence of Sex

As discussed in the introduction, chronic pain is a serious condition affecting about 1 in 5 Americans.[1] Women are at higher risk to develop chronic pain[71], with ~6 million more women reporting chronic pain then men in the U.S.[1] Several pain conditions only occur in women, such as endometriosis, vulvodynia and menstrual pain. Conditions found in both sexes have a higher prevalence in women than men.[71,72]

The observation of sex differences in experimental procedures complements the epidemiological data. Women and men experience pain differently, and women have the tendency to report more pain to the same stimuli. Experimental protocols have a range of modalities, including electrical[73,74], pressure[75-77], thermal[78-82], and chemical stimuli.[83,84] Several comprehensive reviews have been written on the subject.[71,85] Explanations for this reproducible difference between men and women are an amalgamation of biological and psychosocial differences. Biological differences include varying influence of sex hormones, cortical processing of noxious stimuli or difference in the opioidergic system. Psychosocial influences include coping mechanisms and cultural expectations for gender roles.

Sex hormones and their receptors have a significant, complex role in pain, and incidentally, sympathetic activity. Estrogen and progesterone exert a pro-nociceptive and anti-nociceptive influence, while testosterone is more anti-nociceptive.[86] The exact mechanisms of action are not completely understood. However, the influence of sex hormones is apparent. Pain in women has been shown to change throughout the ovarian cycle.[87,88].

The ovarian cycle has a robust influence on the sympathetic nervous system, largely due to estrogen and progesterone.[89-91] Initial studies reported no difference between phases[91-94] or sympathoexcitation during the mid-luteal phase.[95,96] These inconsistencies are likely explained by variable surges in sex hormones from participant to participant. A multi-study, retrospective analysis (n=30) by Carter and colleagues[89] concluded the mid-luteal phase of the ovarian cycle is associated with heightened sympathetic activity at rest, with the response dependent on surges of estrogen and progesterone. This and other work suggest estradiol exerts a sympathoinhibitory effect,[97,98] and progesterone a sympathoexcitatory effect.[99,100] Moreover, sympathetic activity is comparable at rest between the early follicular phase and the placebo phase of oral contraceptive.[90]

Given the influence the ovarian cycle has on pain and sympathetic activity and the similar levels of sympathoexcitation during the early follicular phase in naturally, eumenorrheic women, we controlled for menstrual phase was controlled in the present study, with all female subjects tested 2-5 days after the start of menses.

1.5 Premise

While a significant portion of the literature examines the responses to heating and cooling during longer protocols (>5 minutes), the response to acute thermal sensations (<60 seconds) remains unclear. The purpose of this study is to determine hemodynamic and sympathetic response to acute thermal sensations. Given the known differences in the perception of pain between men and women, this study attempted to equally recruit men and women in order to investigate sex differences. Our **primary hypothesis** was that the sensation of heat pain would augment MSNA, blood pressure and heart rate, and that cool and warm threshold sensation would have no influence on these parameters. Our **secondary hypothesis** was that women would have an augmented pain response compared to men.

11

2 Methodology

2.1 Participants

Thirty-one participants (17 men and 14 women) were recruited to participate in this study. All participants were nonsmokers, had no history of cardiovascular disease, diabetes or any pain conditions, and were not prescribed any cardiovascular or antihypertensive medications. All female participants were eumenorrheic (~26-30 day cycle length). One participant used an intrauterine device. Women were tested during their early follicular phase (2-5 days after initiating menstruation). For eight participants (4 women and 4 men), a quality nerve recording site was not obtained, thus data from those participants were not included in the final analysis. Warm threshold data was not collected in one male participant due to equipment malfunction. Thus, our final sample size was 23 participants for cool threshold and heat pain (11 women and 12 men), and 22 participants for warm sensation (11 women and 11 men).

Participant eligibility was evaluated through study orientation where they were informed of the purpose of the study and potential risks. Informed consent was obtained during this visit. This study was reviewed by Michigan Technological University's Institutional Review Board.

2.2 Experimental Design

Following informed consent, eligible participants arrived at the laboratory after no exercise, alcohol, or caffeine in the prior 12 hours and food a minimum of 3 hours prior. The following surveys were conducted to collect information for potential sub-analyses and/or future research related to the laboratories focus on sleep, anxiety and sympathetic activity: Epworth Sleepiness Scale (ESS), a State-Trait Anxiety Inventory (STAI), the Center for Epidemiologic Studies Depression Scale (CES-Depression), and the Insomnia Severity Index (ISI).

Participants were then equipped for an autonomic function test. Briefly, participants were situated comfortably in the supine position on a padded laboratory tilt table and instrumented with a 3-lead electrocardiogram, finger plethysmography, and microneurography to measure beat-to-beat blood pressure, pneumobelt to monitor respiratory rate, and post-ganglionic MSNA, respectively. Once quality MSNA recordings were confirmed, participants were provided a 10 min non-recorded rest to ensure hemodynamic and autonomic recordings returned to baseline levels following the microneurography procedure. Three consecutive automated sphygmomanometer blood pressure recordings were taken to calibrate the finger plethysmography unit for continuous blood pressure recordings. Following the non-recorded rest and blood pressure calibrations, a 5-minute resting supine baseline was recorded prior to the thermal test battery.

Cool sensation, heat sensation and heat pain thresholds were assessed using a precise, computer-controlled thermode (Q-sense, somatosensory analyzer, Medoc, Israeli) for generating and recording responses to thermal stimuli. The thermode was securely attached to the palm, via a Velcro strap, and remained there for the entirety of the experiment.

Cool sensation trials were conducted first. Cool sensation threshold is the temperature the participant first perceives as "cool". From a baseline temperature of 32°C, the thermode was cooled at a rate of 0.5°C/sec. The participant was instructed to click a controller with their free hand as soon as they felt the cool sensation. Four trials were conducted with an inter-stimulus interval of ~100 seconds. A standardized script was used to conduct each trial, developed from suggested standards set by the German Research Network on Neuropathic Pain.[101] Subjects were prompted with the following language prior to the first cool sensation trial:

> *"First we will test your ability to perceive cold sensations. Please press*
> *the stop button immediately once you perceive a change in temperature*
> *to cool or cooler for the first time."* [101]

Following completion of each trial, the thermode returned to 32°C until the next trial.

After four trials of cool sensation, four trials of warm sensation were conducted. Warm sensation threshold is the temperature the participant first perceives as "warm". From a baseline temperature of 32°C, the thermode was warmed at a rate of 0.5°C/sec. Subjects were prompted with the following language prior to the first warm sensation trial:

> *"Now we will test your ability to perceive warm sensations. Please*
> *press the stop button immediately once you perceive a change in*
> *temperature to warm/warmer for the first time."* [101]

Following completion of each trial, the thermode returned to 32°C until the next trial. The inter-stimulus interval was ~100 seconds.

Heat pain threshold is the temperature the participant identifies the heat as painful. Similar protocols were followed for heat pain threshold as for cool and warm sensation thresholds. The inter-stimulus interval was ~110 seconds due to the time it took for the thermode to cool down after each trial. The following language was used to describe the heat pain trials:

> *"Now we will test as to when you perceive the warming of the*
> *thermode as painful. Your skin will be slowly warmed. At some point in*
> *time you will feel a second sensation on top of the "warm" or "hot"*
> *sensation. The impression of "warmth" or "heat" will change its*
> *quality towards an additional impression of a "burning", "stinging",*
> *or "aching" sensation. Please press the stop button immediately once*
> *you perceive such a change. Please do not wait to press the stop button*

until the sensation has become unbearably painful. We want the first moment you detect the warmth as painful." [101]

Throughout the protocol, participants were unable to view the program screen and were only aware when each trial would begin.

2.3 Measurements

2.3.1 MSNA, Heart Rate and Blood Pressure

Data were imported and analyzed with WinCPRS (Absolute Aliens; Turku, Finland). R-waves were detected and marked in the time series. Bursts of MSNA were automatically detected on the basis of amplitude using a signal-to-noise ratio of 3:1, with a 0.5-s search window centered on a 1.3-s expected burst peak latency from the previous R-wave. Potential bursts were initially marked and detected by a team of graduate students (H. Cunningham, I. Greenlund, and J. Bigalke), with final confirmation and editing by the laboratory's senior investigator (J. Carter). MSNA is expressed as burst frequency (bursts/min), burst incidence (bursts/100 heartbeats), and total MSNA (i.e., the sum of the normalized burst amplitude/min)

Blood pressure, heart rate and MSNA were averaged for two intervals for cool and warm threshold sensations: 1) during a 30 second baseline prior to stimulus application, 2) during a 30 second recovery (post threshold). For the heat pain trial, we measured blood pressure, heart rate and MSNA during: 1) a 30 second baseline prior to stimulus onset, 2) stimulus application, and 3) a 30 second recovery (post threshold). Thirty second baseline and recovery periods were chosen due to device constraints, only 100 seconds were allowed to be programmed between each trial, and to prevent baseline and recovery overlap. Data during the application of cool and warm threshold were not used due to the briefness of the interval (<5 seconds). This interval is not sufficient to gather physiological data for the stated objective and hypotheses. In contrast, heat pain intervals were long enough to gather sufficient physiological data.

2.4 Statistical Analyses

All data were analyzed statistically using commercial software (SPSS 25.0 IBM; Armonk, NY). Independent samples t-tests were used to compare demographic data between men and women. Repeated measures analysis of variance (ANOVA) was used to assess consistency of hemodynamic and MSNA data for each trial of sensory stimuli (e.g., determine consistency within the four trials of cold sensation) (see Appendix B). Following confirmation of no time effect, the four trials were averaged for further analysis. Assumption of normality was tested for each outcome of interest using a Shapiro Wilk test (Appendix C). When assumptions of normality were violated, variables (i.e., burst incidence and total MSNA for cool and warm threshold, and burst frequency, burst incidence, and total MSNA for heat pain) were transformed using a log

14

transformation. Heat pain variables (burst frequency, burst incidence, and total MSNA) were transformed using a log transformation with the addition of a small constant (i.e. 1) to accommodate zero values. For heat pain, a repeated measures ANOVA with time as the within factor and sex (men vs. women) as the between factor was used to compare hemodynamic and MSNA measurements (Appendix D, E and F). Post-hoc analysis using least square differences were performed when significant time or time $\times$ sex interactions were detected (Appendix F). For thermal threshold analyses (i.e., warm and cool threshold), paired t-tests were used to compare responses between baseline and recovery (Appendix D and E). Data are presented as means $\pm$ SE.

3 Results

3.1 Participant Characteristics

Table 3.1 compares participant characteristics between men and women during the experimental session. Both groups were age- and BMI-matched, and had similar seated blood pressures. Age and BMI is similar between groups. Women scored higher on the STAI-state and ISI questionnaires, while men had a higher score on the ESS.

Table 3.1. Baseline characteristic of participants during first visit.

Variable		Men	Women	Sex, P-Value
Age, yr		23 ± 1	26 ± 2	0.115
BMI, kg/m^2		26 ± 1	25 ± 1	0.925
SAP, mmHg		109 ± 2	107 ± 3	0.098
DAP, mmHg		64 ± 2	65 ± 2	0.466
HR, beats/min		71 ± 4	73 ± 4	0.974
STAI-state, a.u.				
	Raw	25 ± 1	31 ± 3	*0.037
	Standard	39 ± 1	46 ± 2	0.056
	Percentile	15 ± 3	38 ± 8	*0.002
STAI-trait, a.u.				
	Raw	29 ± 1	34 ± 2	0.145
	Standard	43 ± 1	48 ± 2	0.145
	Percentile	32 ± 5	45 ± 8	0.195
Depression, a.u.		5 ± 1	9 ± 1	0.306
Insomnia, a.u.		4 ± 1	7 ± 2	*0.013
Epworth Sleepiness, a.u.		6 ± 1	5 ± 1	*0.011

Values are means ± SE; n = 12 men and 11 women. BMI, body mass index; SAP, systolic arterial blood pressure; DAP, diastolic arterial pressure; HR, heart rate; STAI, state-trait anxiety inventory; a.u., arbitrary units. Independent t-tests were used to compare variables between men and women.

3.2 Cool and Warm Threshold

Table 3.2 presents cool and warm sensation threshold temperatures, in addition to the time to threshold (i.e., seconds for subjects to detect a perceived change in temperature).

Table 3.2. Mean temperature and time to stimulus for cool and warm sensation.

Variable	Mean	Range	Sex, p-value
Cool Sensation			
Temperature (°C)	30.9 ± 0.1	29.27 – 31.58	0.386
Time (s)	2.3 ± 0.3	0.84 – 5.47	
Warm Sensation			
Temperature (°C)	33.1 ± 0.2	32.55 – 36.16	0.107
Time(s)	2.1 ± 0.3	1.11 – 8.32	

Mean values are means ± SE. Independent t-tests were used to compare variables between men and women.

3.2.1 Hemodynamic Responses

Figure 4 shows the systolic arterial pressure (SAP), diastolic arterial pressure (DAP), and heart rate (HR) response to cool sensation during the 30 second baseline and post sensation. SAP significantly dropped from baseline following cool sensation (baseline, 109.7 ± 1.9 mmHg; post sensation, 108.6 ± 1.9 mmHg, p=0.005). This was also observed for DAP (baseline, 62.2 ± 1.8 mmHg; post sensation, 61.2 ± 1.9 mmHg, p<0.001). There was a significant elevation in HR post sensation (baseline, 62.6 ± 1.8 bpm; post sensation 63.3 ± 1.8 bpm, p=0.040). Finally, men and women did not vary in response to cool sensation (time × sex, p>0.05).
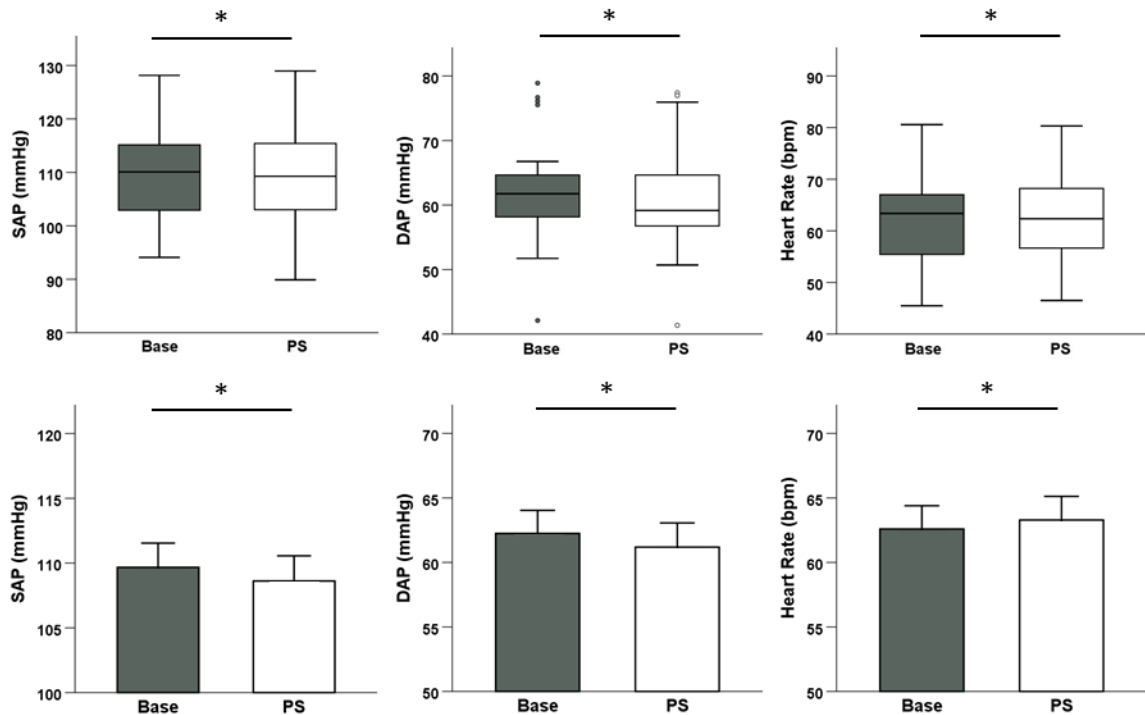
Figure 4. Blood pressure and heart rate responses to cool sensation represented as boxplots and bar graphs (mean ± SE). *Top panel*: The line in the boxplots represents the median and the box represents the interquartile range (IQR; the difference between 25th and 75th percentile). The whiskers extend from the

upper and lower edge of the box to the highest and lowest values which are no greater than 1.5 times the IQ range. *Bottom panel*: Systolic and diastolic arterial pressures were significantly attenuated during the 30 second post sensation (p=0.005, and p<0.001, respectively). Heart rate was significantly elevated during post sensation (p=0.040). SAP, systolic arterial pressure; DAP, diastolic arterial pressure; PS, post sensation. *p < 0.05

There were no significant changes in SAP, DAP, or HR following warm sensation (Figure 5). There were no significant sex differences for warm sensation (time $\times$ sex, p>0.05).
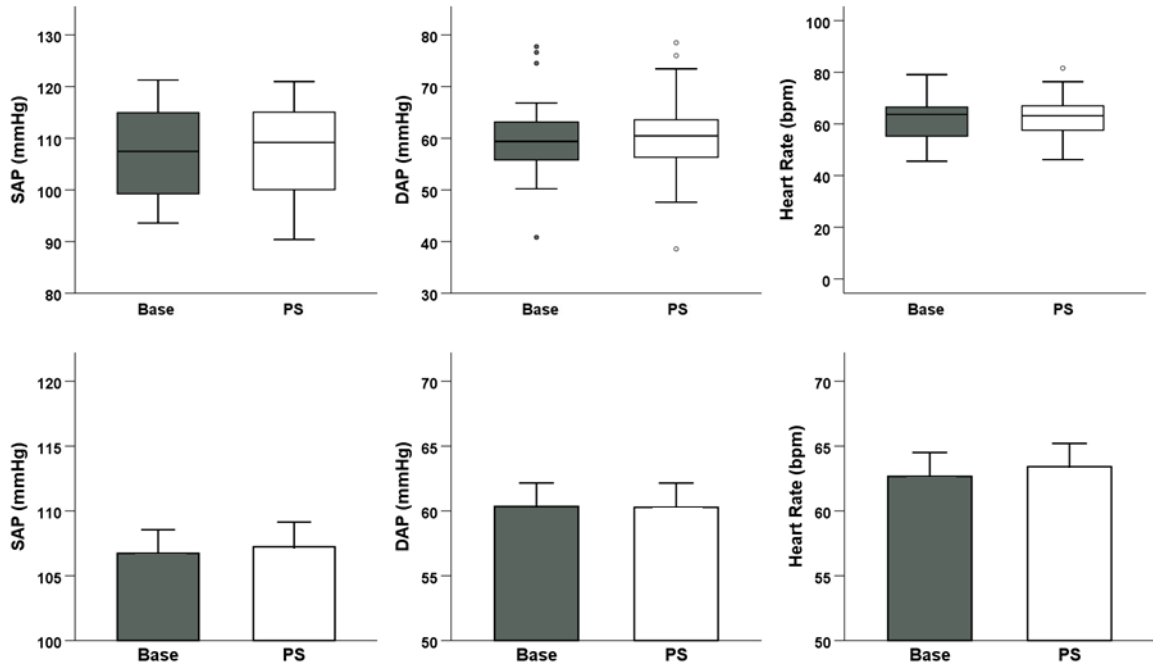
Figure 5. Blood pressure and heart rate responses to warm sensation represented as boxplots and bar graphs (mean ± SE). *Top panel*: The line in the boxplots represents the median and the box represents the interquartile range (IQR; the difference between 25th and 75th percentile). The whiskers extend from the upper and lower edge of the box to the highest and lowest values which are no greater than 1.5 times the IQ range. *Bottom panel*: Systolic arterial pressure, diastolic arterial pressure, and heart rate were not significantly altered during 30 seconds post-threshold. SAP, systolic arterial pressure; DAP, diastolic arterial pressure; PS, post sensation. *p < 0.05

3.2.2 MSNA Responses

Figure 6 compares the change in MSNA response to cool sensation during the 30 second baseline and post sensation. There was a significant reduction in MSNA burst frequency following cool sensation (baseline, 15.9 ± 1.8 bursts/min; post sensation, 13.6 ± 1.6 burst/min, p=0.001). This was also observed when MSNA was represented as burst incidence (baseline, 26.5 ± 3.3 bursts/100hb versus post sensation, 22.5 ± 2.8 bursts/100hb, p=0.001). Total MSNA was significantly reduced following cool sensation (baseline, 58.5 ± 7.2 a.u., post threshold, 50.4 ± 6.3 a.u., p=0.000). There were no significant sex differences for cool sensation (time $\times$ sex, p>0.05).
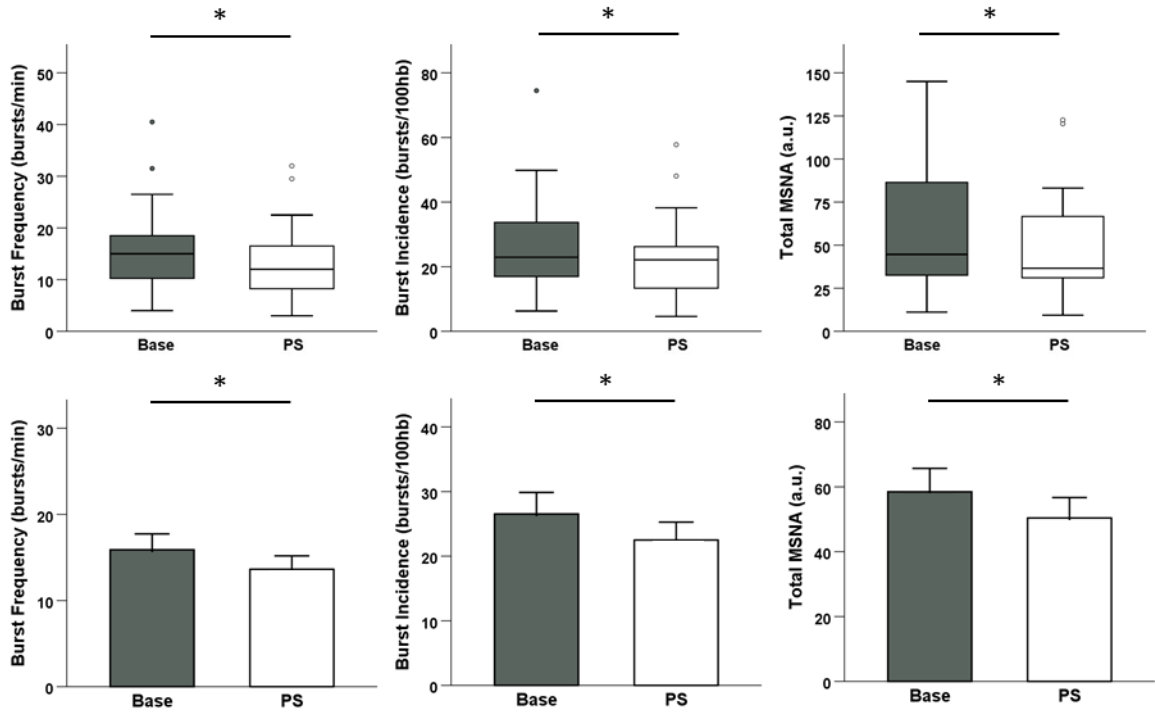
Figure 6. MSNA responses to cool sensation represented as boxplots and bar graphs (mean ± SE). *Top panel*: The line in the boxplots represents the median and the box represents the interquartile range (IQR; the difference between 25th and 75th percentile). The whiskers extend from the upper and lower edge of the box to the highest and lowest values which are no greater than 1.5 times the IQ range. *Bottom panel*: Burst frequency was attenuated during recovery for cool threshold (p=0.001). This was also observed when MSNA was represented as burst incidence (p<0.001) and total MSNA (p<0.001). MSNA, muscle sympathetic nerve activity. *p < 0.05
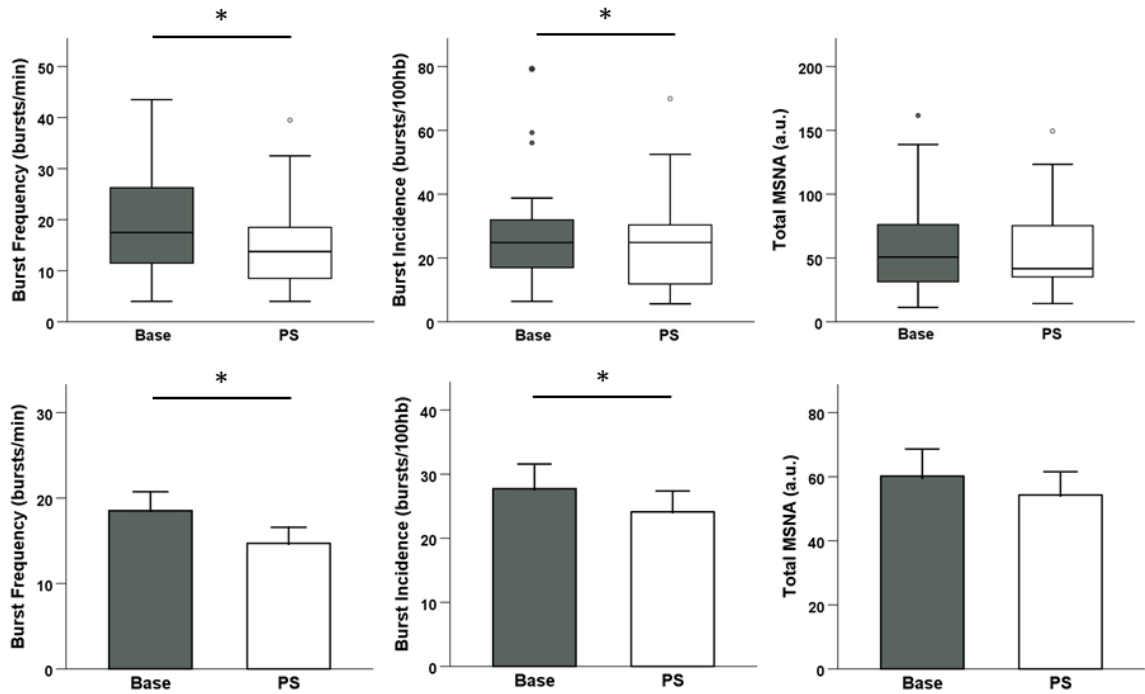
Figure 7 compares the change in MSNA response to warm sensation during the 30 second baseline and post sensation. There was a significant reduction in MSNA burst frequency following warm sensation (baseline, 18.5 ± 2.2 burst/min; post sensation, 14.7 ± 1.9 bursts/min, p=0.003). This was also observed when MSNA was represented as burst incidence (baseline 27.7 ± 3.8 bursts/100hb versus post sensation, 24.1 ± 3.3 bursts/100hb, p=0.010). Total MSNA was trending for warm sensation (baseline, 60.2 ± 8.4 a.u., post sensation, 54.3 ±7.3 a.u., p=0.054). There were no significant sex differences for warm sensation (time × sex, p>0.05).
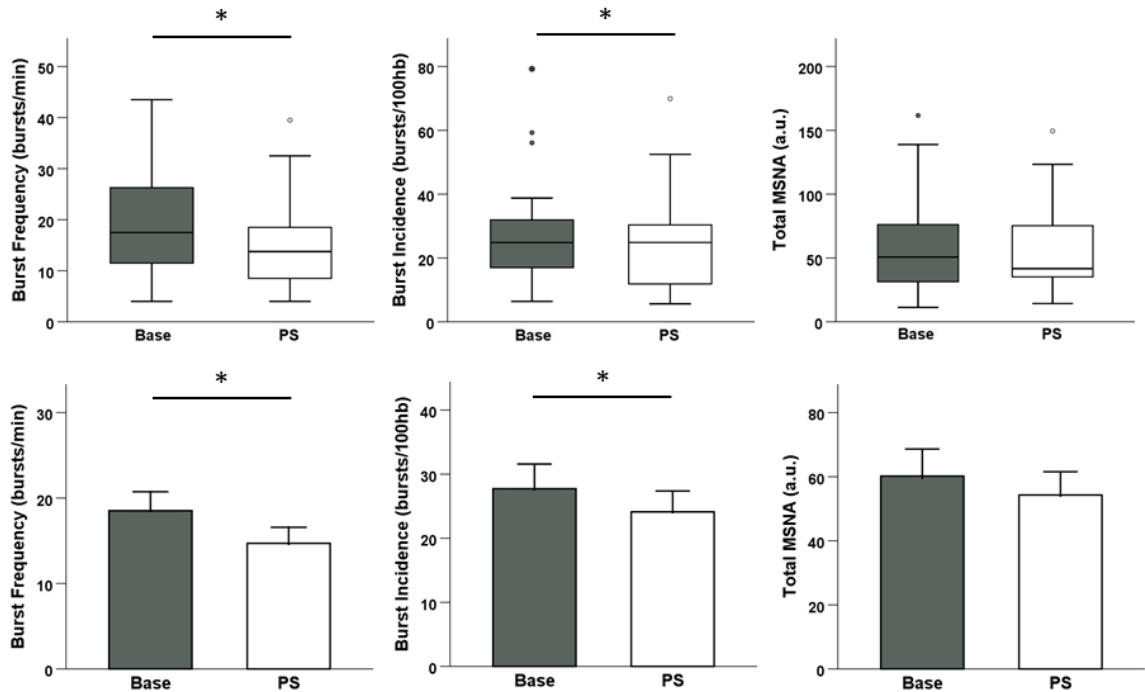
Figure 7. MSNA responses to warm sensation represented as boxplots and bar graphs (mean ± SE). *Top panel*: The line in the boxplots represents the median and the box represents the interquartile range (IQR; the difference between 25th and 75th percentile). The whiskers extend from the upper and lower edge of the box to the highest and lowest values which are no greater than 1.5 times the IQ range. *Bottom panel*: Burst frequency was attenuated during recovery for warm sensation (p=0.003). This was also observed when MSNA was represented as burst incidence (p=0.010). Total MSNA was trending for warm sensation (p=0.054). MSNA, muscle sympathetic nerve activity. *p < 0.05

3.3 Heat Pain

Table 3.3 reports the average and range of heat pain thresholds, as well as the time to heat pain threshold.

Table 3.3. Average temperature and time to stimulus for heat pain.

Variable	Average	Range	Sex, p-value
Heat Pain			
Temperature (°C)	41.6 ± 0.7	35.73 – 48.22	0.358
Time (s)	19.1 ± 1.5	7.27 – 32.44	

Average values are means ± SE. Independent t-tests were used to compare variables between men and women.

3.3.1 Hemodynamic Responses

Figure 8 demonstrates the SAP, DAP, and HR responses to heat pain during the 30 second baseline, the sensation, and 30 second recovery. There was a significant time

effect for SAP (p=0.008) and HR (p<0.001), and a trending time effect for DAP (p=0.065). SAP was significantly augmented during the sensation compared to recovery (sensation, 110.1 ± 2.3 mmHg; recovery, 107.9 ± 2.3 mmHg; p=0.006). HR was augmented during recovery (64.3 ± 1.8 bpm) compared to baseline (62.7 ± 1.8, p=0.003) and sensation (61.4 ± 1.8 bpm, p = 0.000). There were no significant sex differences for BP or HR (time $\times$ sex, p>0.05)
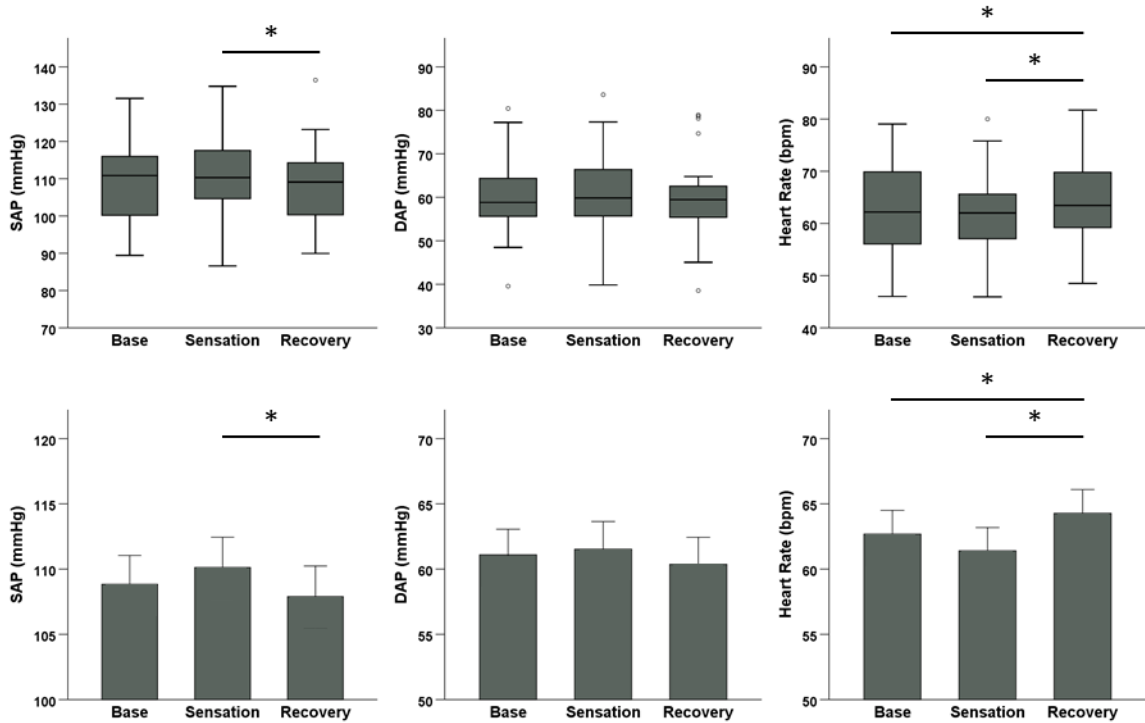
Figure 8. Blood pressure and heart rate responses to heat pain represented as boxplots and bar graphs (mean $\pm$ SE). *Top panel*: The line in the boxplots represents the median and the box represents the interquartile range (IQR; the difference between 25th and 75th percentile). The whiskers extend from the upper and lower edge of the box to the highest and lowest values which are no greater than 1.5 times the IQ range. *Bottom panel*: There was a significant time effect for systolic arterial pressure (p=0.008), and heart rate (p<0.001). Systolic arterial pressure was significantly attenuated during the recovery compared to the sensation (p=0.006). Heart rate was significantly augmented during recovery compared to baseline (p=0.003) and sensation (p<0.001). SAP, systolic arterial pressure; DAP, diastolic arterial pressure. *p < 0.05

3.3.2 MSNA Responses

Figure 9 illustrates the MSNA responses to heat pain during the 30 second baseline, the sensation, and 30 second recovery. There was a significant time effect for burst frequency (p=0.017), burst incidence (p=0.031), and total MSNA (p=0.022). MSNA burst frequency was attenuated during the sensation (13.4 ± 1.9 bursts/min) compared to baseline (16.9 ± 2.1 bursts/min; p=0.004) and recovery (16.6 ± 1.9 bursts/min; p=0.033). When MSNA was represented as burst incidence, the response was significantly attenuated during sensation (22.2 ± 3.3 bursts/100hb) compared to baseline (28.1 ± 3.7

bursts/100hb; p=0.010) and trending compared to recovery (27.1 ± 3.3 burst/100hb; p=0.063). Total MSNA was attenuated during the sensation (48.0 ± 7.4 a.u.) compared to baseline (63.6 ± 8.7 a.u., p=0.005), and recovery (59.7 ± 7.2 a.u.; p=0.049). There were no sex differences for these responses (time × sex, p>0.05).
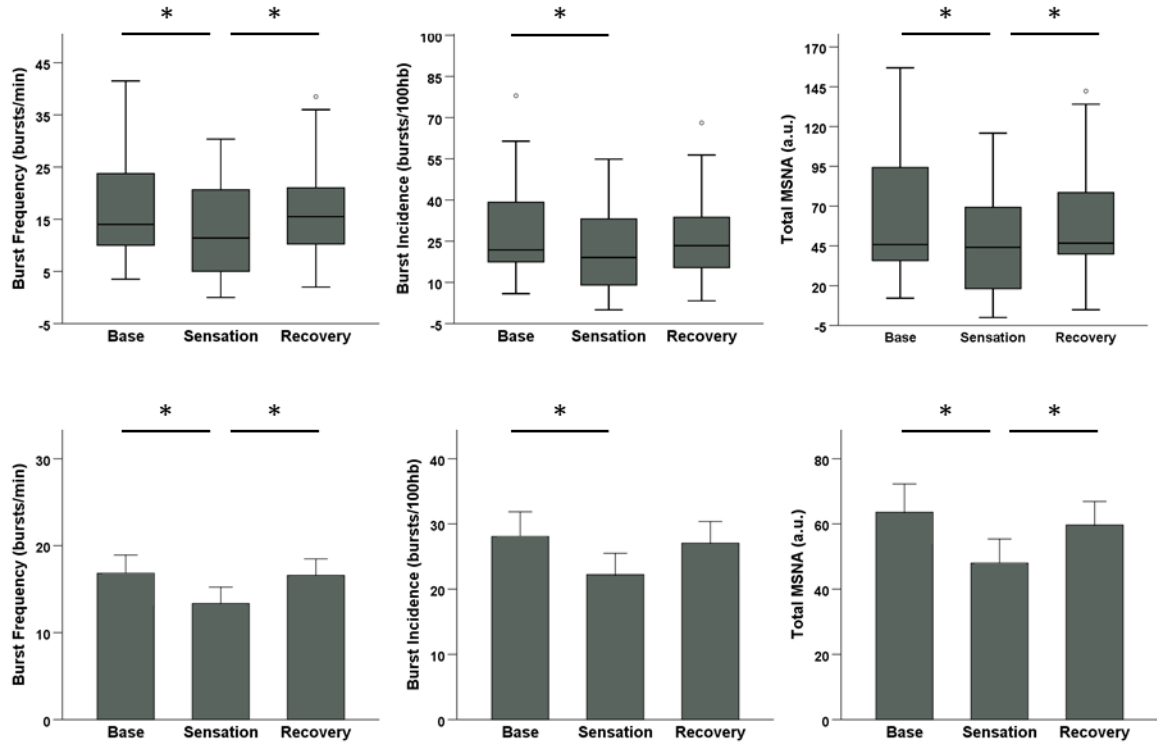
Figure 9. MSNA responses to heat pain represented as boxplots and bar graphs (mean ± SE). *Top panel*: The line in the boxplots represents the median and the box represents the interquartile range (IQR; the difference between 25th and 75th percentile). The whiskers extend from the upper and lower edge of the box to the highest and lowest values which are no greater than 1.5 times the IQ range. *Bottom panel*: There was a significant time effect for burst frequency (p=0.017), burst incidence (p=0.031), and total MSNA (p=0.022). MSNA burst frequency was attenuated during the sensation compared to baseline (p=0.004) and recovery (p=0.033). MSNA burst incidence was significantly attenuated during sensation compared to baseline (p=0.008) and trending compared to recovery (p=0.063). Total MSNA was attenuated during the sensation compared to baseline (p=0.005) and recovery (p=0.049). MSNA, muscle sympathetic nerve activity. *p < 0.05

Figure 10 depicts a representative neurogram of the MSNA response during the heat pain stimuli in one participant.
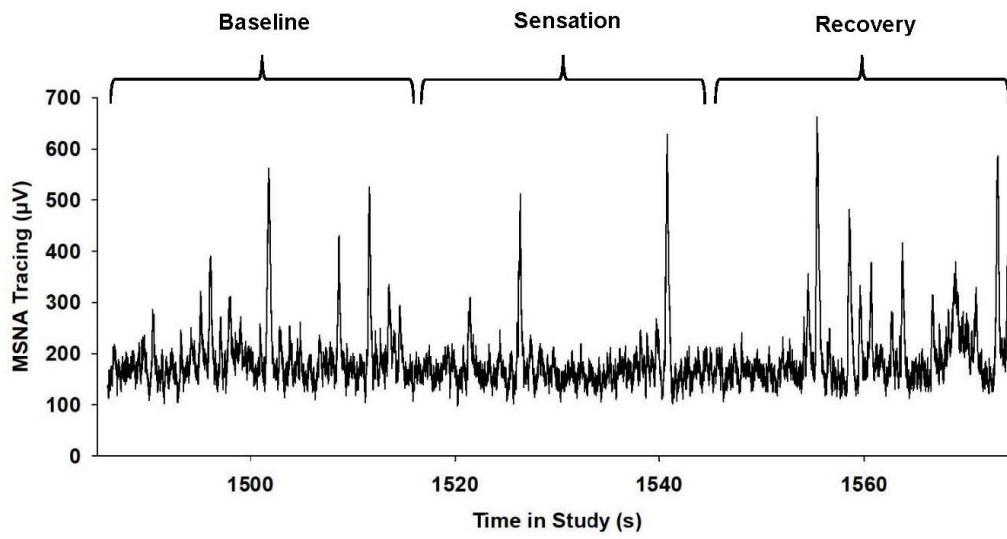
Figure 10. Representative neurogram from participant during heat pain.

4 Discussion

To our knowledge, this is the first study to examine the influence of *acute* thermal threshold sensations (warm and cool sensation) on muscle sympathetic nerve activity, blood pressure and heart rate. The present study was designed to test the effect thermal thresholds on sympathetic activity and hemodynamics. The following are primary findings: 1) blood pressure and MSNA were attenuated during the immediate recovery of cool threshold; 2) MSNA was attenuated during the immediate recovery of warm threshold; 3) SAP was attenuated during the recovery of phase of heat pain; 4) MSNA was inhibited during the sensation phase of heat pain. These results were in contrast to our initial hypothesis, in which we anticipated cool and warm sensation having no influence on physiological variables and heat pain eliciting an augmented response. A unique element of the present study was the ability to look at transient changes in sympathetic activity as a result of acute thermal stimuli. These responses are often lost when experiments apply thermal stimuli for longer periods of time (i.e., >2 minutes).

4.1 Participant Demographics

While age, BMI, and blood pressure were similar between men and women, women scored higher on the STAI-state and ISI questionnaire and men higher on the ESS. However, these differences are minute in nature for two specific reasons: 1) the aforementioned differences do not correlate with any significant differences in classification, and 2) STAI-state, ISI, and ESS scores are all below pathological indices.

4.2 Cool and Warm Threshold Sensation

4.2.1 Inhibition of MSNA during Post-Threshold

The thresholds reached during cool and warm sensation were non-noxious. These brief sensations lasted ~2 seconds and yet resulted in a significant inhibition of MSNA. This reduction in MSNA is analogous to that observed by Kregel et al.,[56] which demonstrated that during the 30 second onset of CPT via non-noxious temperatures, there was a 47 % reduction in MSNA, followed by a rapid return to control levels. In the present study, there was an average drop of $13 \pm 3\%$ in MSNA immediately following cool threshold. While participants in Kregel et al.[56] had their hands submerged throughout the 30 seconds, similar mechanisms could be occurring to explain the inhibition of MSNA.

This study advances the growing body of literature focused on the human response to thermal cooling and heating. Historically, experiments have been conducted using whole body and localized heating (>5 min). To our knowledge, this is the first study to examine the response to acute thermal thresholds. The results indicate variable autonomic responses potentially due to the stimulus application interval and the stimulus temperature.

The magnitude of heating/cooling seems to have a large influence on the hemodynamic and autonomic response. The whole-body cooling/heating paradigm alters core body temperature, whereas localized heating/cooling only alters skin temperature. This difference in magnitude of heating/cooling seems to have a large influence on the MSNA response. Core body changes result in consistent increases of MSNA.[34,35] Changes in only skin temperature exert variable responses in MSNA depending on the modality, with the following observations: 1) no change of MSNA during whole body cooling (skin only)[46,47], 2) decrease of MSNA during localized heating[54], 3) and increased MSNA during localized cooling.[57] In the present study, the acute thermal thresholds applied by 9.0 cm^2 skin thermode is unlikely to produce large changes in skin temperature as observed in during localized heating/cooling paradigms.[54] However, the thermal stimuli were still detectable by the participant.

It is also likely that the length of cooling and heating contributes to the diverse responses in the literatures, and within the present study. In our study, acute (<10 seconds) thermal cooling and heating yielded an inhibition of MSNA during recovery (cool and warm threshold). Other studies apply heat and cool for longer than 10 minutes.[34,35,46,47,54,57] The acute nature of the thermal stimuli used in the present study is an additional factor that might explain variable autonomic responses.

Transient inhibition of MSNA has been reported following an acute sensory stimuli, which complements anecdotal reports of MSNA being inhibited by peripheral stimuli.[102,103] Using non-noxious cutaneous (electrical stimulation on the tip of the middle finger) and visual (a flash of light in the eye) stimuli presented in a random order, Donadio et al.[104] reported an inhibition of MSNA amplitude for one or two MSNA bursts. This was also observed when the cutaneous stimulus was applied repetativly.[105] This inhibitory effect was not observed in all participants, and was only present the first few seconds after the stimulus. Though researchers only observed reductions in MSNA amplitude during the first two seconds, and did not average for 30 second intervals as we did in the present study, this response could contribute to the reduction of MSNA observed in the present study.

Donadio et al.[104] postulate the MSNA inhibition is related to an arousal response. The human stress response is adapted as a quick response system to "fight or flight" and includes physiological responses such as vasoconstriction to the digestive system, pupillary dilation, vasodilation to the muscles, increases in heart rate and blood pressure, and a rush of adrenaline throughout the body. It is possible the inhibition of MSNA observed in the present study is paradoxically related to this fight or flight response, as noted by Donadio and his collegues.[104-106]

4.2.2 Reduction of Blood Pressure during Post-Threshold

Blood pressure was reduced only during the immediate recovery from cool threshold. Previous work demonstrates that DAP is highly coupled to with MSNA, with a strong association between beat-to-beat variations in DAP and MSNA bursts during resting

conditions.[107] This coupling between DAP and MSNA is rather complex, with one leading the other and vice versa.[108] It is possible the inhibition of MSNA in the present study is contributing to the attenuation of blood pressure.

4.3 Heat Pain

4.3.1 Inhibition of MSNA during Sensation Application

The inhibition of MSNA during the application of the heat pain stimulus is at odds with the general relationship between pain and MSNA, in which pain typically increases MSNA.[63-66] However, the application of the heat pain stimulus in the present study is analogous to localized heating until the point of pain, whereby the stimulus is removed the moment pain is experienced. From that lens, our results mirror that of Takahashi et al., [54] who reported a 72% reduction of MSNA during 15 minutes of non-noxious heat application. In the present study, in the average 19 seconds the heat stimulus was applied, MSNA dropped on average $21 \pm 7\%$.

In contrast, Lautenschläger et al.[67] used participants' heat pain threshold as the temperature for the 5 minute heat stimulus. MSNA was significantly increased from this noxious stimulus. The acute nature of the pain stimulus in the present study might explain the deviation from the typical relationship between pain and MSNA. Unlike the study conducted by Lautenschläger et al.,[67] the painful stimulus was removed the moment pain was experienced. This might explain why an inhibition of MSNA was observed during the sensation period of the pain stimulus in our study.

4.4 Sex Differences

Contrary to our hypothesis, we did not observe any sex differences for hemodynamic and MSNA responses to cool and warm sensation and heat pain. Though many studies indicate women report and experience more pain, stimulation method seems to be a key factor in determining sex differences. Using an identical protocol including baseline temperature and rate of 0.7°C/sec increase or decrease, Lautenbacher and Rollman also reported no sex differences in heat pain, warmth and cold thresholds.[109] Inoue et al.[110] used a rate of 0.3°C/sec and observed women were more sensitive to cool and warm stimuli. For the present study, a low sample size could be masking potential sex differences.

4.5 Limitations

One limitation of the present study is the difficulty to equate the results to real world conditions. The human body is consistently detecting and simultaneously processing a number of peripheral stimuli. The many peripheral stimuli we experience in a single moment – including touch and pressure from clothes, ambient room temperature, and

visceral signals within the body – summate to create our perceptual and physical experience. Internal influences from the central and peripheral nervous system amplify and inhibit these signals, allowing for perceptual focus on those most important to the current circumstance. This study was designed to *isolate* specific thermal stimuli in a controlled laboratory setting. As such, it minimizes many interfering factors. Every stimulus was described beforehand and anticipated. It is unknown how multiple stimuli or an un-controlled environment might influence these results.

4.6 Implications

Given the literature and the results of the present study, it is evident the human body has variable responses depending on the nature of the stimuli. In healthy, young subjects, we observed that a variety of *acute* thermal sensations, including cool and warm sensation and heat pain, induced an inhibition of MSNA. This research has broader implications for pain research. With the opioid epidemic, there has been a renewed interest in developing alternative treatment methods for chronic and acute pain. It is crucial to understand how various thermal stimuli influence the human body's cardiovascular and nervous system, and what physiological reactions are evoked as a result. Understanding how the human body responds to various stimuli in a healthy state can help us better understand responses in diseased states, and potentially contribute to the development of preventative and therapeutic treatments options.

4.7 Future Directions

Numerous opportunities exist in the realm of acute peripheral stimuli and their influences on the nervous system. While this study used thermal sensations, other potential avenues might include pressure, electrical, and mechanical stimuli. Moreover, the location of the stimuli might also have an influence. The thenar eminence is specialized with many free nerve endings that allow us to easily navigate touching and moving our world, and a "thick" epidermis. Other parts of the body, such as the arm or the face, might generate different responses to the same peripheral stimuli. Since afferent nerve fibers (e.g., mechanoreceptors, chemoreceptors, and nociceptors) are stimulated by many different types of innocuous and noxious stimuli (i.e., polymodal), it is reasonable to expect different responses between stimuli. Future work should investigate how other physical sensations influence the peripheral nervous system.

4.8 Summary

The design of treatments for chronic pain can benefit by understanding how individuals process pain and what variables influence this response. Through the measurement of MSNA, we can get an indication of the autonomic response to a stimulus. The present study measured the autonomic response to acute thermal sensation, including cool and warm sensation and heat pain. All three stimuli induced a small, yet significant, reductions of MSNA. These findings are in contrast to the literature suggesting whole

body thermal sensations increase MSNA. This is evidence of an internal response that is variable on the modality, time, and intensity of heating/cooling.

5 Reference List

1   Dahlhamer, J. *et al.* Prevalence of Chronic Pain and High-Impact Chronic Pain Among Adults - United States, 2016. *MMWR Morb Mortal Wkly Rep* **67**, 1001-1006, doi:10.15585/mmwr.mm6736a2 (2018).

2   Fayaz, A., Watt, H. C., Langford, R. M. & Donaldson, L. J. The Association Between Chronic Pain and Cardiac Disease: A Cross-sectional Population Study. *The Clinical journal of pain* **32**, 1062-1068, doi:10.1097/ajp.0000000000000359 (2016).

3   Okifuji, A. & Hare, B. D. The association between chronic pain and obesity. *Journal of pain research* **8**, 399-408, doi:10.2147/jpr.S55598 (2015).

4   Gormsen, L., Rosenberg, R., Bach, F. W. & Jensen, T. S. Depression, anxiety, health-related quality of life and pain in patients with chronic fibromyalgia and neuropathic pain. *European journal of pain (London, England)* **14**, 127.e121-128, doi:10.1016/j.ejpain.2009.03.010 (2010).

5   Gaskin, D. J. & Richard, P. The economic costs of pain in the United States. *J Pain* **13**, 715-724, doi:10.1016/j.jpain.2012.03.009 (2012).

6   Hall, J. E. *Guyton and hall textbook of medical physiology*. (ELSEVIER - HEALTH Science, 2020).

7   Tracey, I. & Mantyh, P. W. The cerebral signature for pain perception and its modulation. *Neuron* **55**, 377-391, doi:10.1016/j.neuron.2007.07.012 (2007).

8   Millan, M. J. Descending control of pain. *Progress in neurobiology* **66**, 355-474, doi:10.1016/s0301-0082(02)00009-6 (2002).

9   Djouhri, L. & Lawson, S. N. Abeta-fiber nociceptive primary afferent neurons: a review of incidence and properties in relation to other afferent A-fiber neurons in mammals. *Brain research. Brain research reviews* **46**, 131-145, doi:10.1016/j.brainresrev.2004.07.015 (2004).

10   Lautenbacher, S. F., Roger B. *Pathophysiology of Pain Perception*. (Springer Verlag, 2012).

11   Dubin, A. E. & Patapoutian, A. Nociceptors: the sensors of the pain pathway. *J Clin Invest* **120**, 3760-3772, doi:10.1172/jci42843 (2010).

12   Urban, M. O. & Gebhart, G. F. Central mechanisms in pain. *The Medical clinics of North America* **83**, 585-596, doi:10.1016/s0025-7125(05)70125-5 (1999).

13   Woolf, C. J. & Ma, Q. Nociceptors--noxious stimulus detectors. *Neuron* **55**, 353-364, doi:10.1016/j.neuron.2007.07.016 (2007).

14   Millan, M. J. The induction of pain: an integrative review. *Progress in neurobiology* **57**, 1-164, doi:https://doi.org/10.1016/S0301-0082(98)00048-3 (1999).

15   Julien, N., Goffaux, P., Arsenault, P. & Marchand, S. Widespread pain in fibromyalgia is related to a deficit of endogenous pain inhibition. *Pain* **114**, 295-302, doi:10.1016/j.pain.2004.12.032 (2005).

16   Daenen, L. *et al.* Dysfunctional pain inhibition in patients with chronic whiplash-associated disorders: an experimental study. *Clinical rheumatology* **32**, 23-31, doi:10.1007/s10067-012-2085-2 (2013).

17   Chen, Q. & Heinricher, M. M. Descending Control Mechanisms and Chronic Pain. *Current rheumatology reports* **21**, 13, doi:10.1007/s11926-019-0813-1 (2019).

18   Esler, M. *et al.* Assessment of human sympathetic nervous system activity from measurements of norepinephrine turnover. *Hypertension* **11**, 3-20, doi:10.1161/01.hyp.11.1.3 (1988).

19   Maling, T. J. B., Dollery, C. T. & Hamilton, C. A. Clonidine and Sympathetic Activity during Sleep. *Clinical Science* **57**, 509-514, doi:10.1042/cs0570509 (1979).

20   Grassi, G. *et al.* Comparison between reproducibility and sensitivity of muscle sympathetic nerve traffic and plasma noradrenaline in man. *Clinical science (London, England : 1979)* **92**, 285-289, doi:10.1042/cs0920285 (1997).

21   Goldstein, D. S. Plasma catecholamines and essential hypertension. An analytical review. *Hypertension* **5**, 86-99, doi:10.1161/01.hyp.5.1.86 (1983).

22   Grassi, G. & Esler, M. How to assess sympathetic activity in humans. *Journal of hypertension* **17**, 719-734, doi:10.1097/00004872-199917060-00001 (1999).

23   Esler, M. *et al.* Measurement of total and organ-specific norepinephrine kinetics in humans. *The American journal of physiology* **247**, E21-28, doi:10.1152/ajpendo.1984.247.1.E21 (1984).

24   Esler, M. *et al.* Contribution of individual organs to total noradrenaline release in humans. *Acta physiologica Scandinavica. Supplementum* **527**, 11-16 (1984).

25   Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North

American Society of Pacing and Electrophysiology. *Circulation* **93**, 1043-1065 (1996).

26   Akselrod, S. *et al.* Hemodynamic regulation: investigation by spectral analysis. *The American journal of physiology* **249**, H867-875, doi:10.1152/ajpheart.1985.249.4.H867 (1985).

27   Lord, S. W. *et al.* Low-frequency heart rate variability: reproducibility in cardiac transplant recipients and normal subjects. *Clinical science (London, England : 1979)* **100**, 43-46 (2001).

28   Goldstein, D. S., Bentho, O., Park, M. Y. & Sharabi, Y. Low-frequency power of heart rate variability is not a measure of cardiac sympathetic tone but may be a measure of modulation of cardiac autonomic outflows by baroreflexes. *Experimental physiology* **96**, 1255-1261, doi:10.1113/expphysiol.2010.056259 (2011).

29   Vallbo, A. B. & Hagbarth, K. E. Impulses recorded with micro-electrodes in human muscle nerves during stimulation of mechanoreceptors and voluntary contractions. *Electroencephalography and clinical neurophysiology* **23**, 392 (1967).

30   Carter, J. R. Microneurography and sympathetic nerve activity: a decade-by-decade journey across 50 years. *Journal of neurophysiology* **121**, 1183-1194, doi:10.1152/jn.00570.2018 (2019).

31   Wallin, B. G., Thompson, J. M., Jennings, G. L. & Esler, M. D. Renal noradrenaline spillover correlates with muscle sympathetic activity in humans. *The Journal of physiology* **491 (Pt 3)**, 881-887, doi:10.1113/jphysiol.1996.sp021265 (1996).

32   Wallin, B. G. *et al.* Simultaneous measurements of cardiac noradrenaline spillover and sympathetic outflow to skeletal muscle in humans. *The Journal of physiology* **453**, 45-58, doi:10.1113/jphysiol.1992.sp019217 (1992).

33   Fonkoue, I. T. & Carter, J. R. Sympathetic neural reactivity to mental stress in humans: test-retest reproducibility. *Am J Physiol Regul Integr Comp Physiol* **309**, R1380-1386, doi:10.1152/ajpregu.00344.2015 (2015).

34   Cui, J., Blaha, C. & Sinoway, L. I. Whole body heat stress attenuates the pressure response to muscle metaboreceptor stimulation in humans. *Journal of applied physiology (Bethesda, Md. : 1985)* **121**, 1178-1186, doi:10.1152/japplphysiol.00212.2016 (2016).

35   Gagnon, D., Schlader, Z. J. & Crandall, C. G. Sympathetic activity during passive heat stress in healthy aged humans. *The Journal of physiology* **593**, 2225-2235, doi:10.1113/jp270162 (2015).

36   Cui, J., Wilson, T. E. & Crandall, C. G. Baroreflex modulation of sympathetic nerve activity to muscle in heat-stressed humans. *Am J Physiol Regul Integr Comp Physiol* **282**, R252-258, doi:10.1152/ajpregu.00337.2001 (2002).

37   Yamazaki, F. & Sone, R. Modulation of arterial baroreflex control of heart rate by skin cooling and heating in humans. *Journal of applied physiology (Bethesda, Md. : 1985)* **88**, 393-400, doi:10.1152/jappl.2000.88.2.393 (2000).

38   Crandall, C. G., Etzel, R. A. & Farr, D. B. Cardiopulmonary baroreceptor control of muscle sympathetic nerve activity in heat-stressed humans. *The American journal of physiology* **277**, H2348-2352, doi:10.1152/ajpheart.1999.277.6.h2348 (1999).

39   Crandall, C. G. Heat stress and baroreflex regulation of blood pressure. *Medicine and science in sports and exercise* **40**, 2063-2070, doi:10.1249/MSS.0b013e318180bc98 (2008).

40   Keller, D. M., Cui, J., Davis, S. L., Low, D. A. & Crandall, C. G. Heat stress enhances arterial baroreflex control of muscle sympathetic nerve activity via increased sensitivity of burst gating, not burst area, in humans. *The Journal of physiology* **573**, 445-451, doi:10.1113/jphysiol.2006.108662 (2006).

41   Low, D. A., Keller, D. M., Wingo, J. E., Brothers, R. M. & Crandall, C. G. Sympathetic nerve activity and whole body heat stress in humans. *Journal of applied physiology (Bethesda, Md. : 1985)* **111**, 1329-1334, doi:10.1152/japplphysiol.00498.2011 (2011).

42   Cui, J. *et al.* Muscle sympathetic responses during orthostasis in heat-stressed individuals. *Clinical autonomic research : official journal of the Clinical Autonomic Research Society* **21**, 381-387, doi:10.1007/s10286-011-0126-6 (2011).

43   Cui, J. *et al.* Heat stress attenuates the increase in arterial blood pressure during the cold pressor test. *Journal of applied physiology (Bethesda, Md. : 1985)* **109**, 1354-1359, doi:10.1152/japplphysiol.00292.2010 (2010).

44   Lucas, R. A., Sarma, S., Schlader, Z. J., Pearson, J. & Crandall, C. G. Age-related changes to cardiac systolic and diastolic function during whole-body passive hyperthermia. *Experimental physiology* **100**, 422-434, doi:10.1113/expphysiol.2014.083014 (2015).

45   Fagius, J. & Kay, R. Low ambient temperature increases baroreflex-governed sympathetic outflow to muscle vessels in humans. *Acta physiologica Scandinavica* **142**, 201-209, doi:10.1111/j.1748-1716.1991.tb09148.x (1991).

46   Cui, J., Durand, S. & Crandall, C. G. Baroreflex control of muscle sympathetic nerve activity during skin surface cooling. *Journal of applied physiology (Bethesda, Md. : 1985)* **103**, 1284-1289, doi:10.1152/japplphysiol.00115.2007 (2007).

47   Greaney, J. L., Stanhewicz, A. E., Kenney, W. L. & Alexander, L. M. Muscle sympathetic nerve activity during cold stress and isometric exercise in healthy older adults. *Journal of applied physiology (Bethesda, Md. : 1985)* **117**, 648-657, doi:10.1152/japplphysiol.00516.2014 (2014).

48   Wilson, T. E. *et al.* Skin-surface cooling elicits peripheral and visceral vasoconstriction in humans. *Journal of applied physiology (Bethesda, Md. : 1985)* **103**, 1257-1262, doi:10.1152/japplphysiol.00401.2007 (2007).

49   Petrofsky, J., Laymon, M. & Lee, H. Local heating of trigger points reduces neck and plantar fascia pain. *Journal of back and musculoskeletal rehabilitation* **33**, 21-28, doi:10.3233/bmr-181222 (2020).

50   Aciksoz, S., Akyuz, A. & Tunay, S. The effect of self-administered superficial local hot and cold application methods on pain, functional status and quality of life in primary knee osteoarthritis patients. *Journal of clinical nursing* **26**, 5179-5190, doi:10.1111/jocn.14070 (2017).

51   Ackerley, R. *et al.* Cutaneous warmth, but not touch, increases muscle sympathetic nerve activity during a muscle fatigue hand-grip task. *Exp Brain Res* **238**, 1035-1042, doi:10.1007/s00221-020-05779-x (2020).

52   Kuipers, N. T., Sauder, C. L., Kearney, M. L. & Ray, C. A. Interactive effect of aging and local muscle heating on renal vasoconstriction during isometric handgrip. *American journal of physiology. Renal physiology* **297**, F327-332, doi:10.1152/ajprenal.00165.2009 (2009).

53   Ray, C. A. & Gracey, K. H. Augmentation of exercise-induced muscle sympathetic nerve activity during muscle heating. *Journal of Applied Physiology* **82**, 1719-1733, doi:10.1152/jappl.1997.82.6.1719 (1997).

54   Takahashi, N. *et al.* Local heat application to the leg reduces muscle sympathetic nerve activity in human. *European journal of applied physiology* **111**, 2203-2211, doi:10.1007/s00421-011-1852-0 (2011).

55   Fagius, J., Karhuvaara, S. & Sundlöf, G. The cold pressor test: effects on sympathetic nerve activity in human muscle and skin nerve fascicles. *Acta*

physiologica Scandinavica **137**, 325-334, doi:10.1111/j.1748-1716.1989.tb08760.x (1989).

56   Kregel, K. C., Seals, D. R. & Callister, R. Sympathetic nervous system activity during skin cooling in humans: relationship to stimulus intensity and pain sensation. *The Journal of physiology* **454**, 359-371, doi:10.1113/jphysiol.1992.sp019268 (1992).

57   Ishida, K. *et al.* Suppression of activation of muscle sympathetic nerve during non-noxious local cooling after the end of local cooling in normal adults. *European journal of applied physiology* **116**, 851-858, doi:10.1007/s00421-016-3343-9 (2016).

58   Adlan, A. M., Paton, J. F., Lip, G. Y., Kitas, G. D. & Fisher, J. P. Increased sympathetic nerve activity and reduced cardiac baroreflex sensitivity in rheumatoid arthritis. *The Journal of physiology* **595**, 967-981, doi:10.1113/jp272944 (2017).

59   Jewson, J. L. *et al.* Pain duration is associated with increased muscle sympathetic nerve activity in patients with Achilles tendinopathy. *Scandinavian journal of medicine & science in sports* **27**, 1942-1949, doi:10.1111/sms.12820 (2017).

60   Zamunér, A. R. *et al.* Relationship between sympathetic activity and pain intensity in fibromyalgia. *Clinical and experimental rheumatology* **33**, S53-57 (2015).

61   Victor, R. G., Leimbach, W. N., Jr., Seals, D. R., Wallin, B. G. & Mark, A. L. Effects of the cold pressor test on muscle sympathetic nerve activity in humans. *Hypertension* **9**, 429-436, doi:10.1161/01.hyp.9.5.429 (1987).

62   Mizushima, T. *et al.* Muscle sympathetic nerve activity during cold pressor test in patients with cerebrovascular accidents. *Stroke* **29**, 607-612, doi:10.1161/01.str.29.3.607 (1998).

63   Nordin, M. & Fagius, J. Effect of noxious stimulation on sympathetic vasoconstrictor outflow to human muscles. *The Journal of physiology* **489** (**Pt 3**), 885-894, doi:10.1113/jphysiol.1995.sp021101 (1995).

64   Schobel, H. P. *et al.* Hemodynamic and sympathetic nerve responses to painful stimuli in normotensive and borderline hypertensive subjects. *Pain* **66**, 117-124, doi:10.1016/0304-3959(96)03079-5 (1996).

65   Burton, A. R., Fazalbhoy, A. & Macefield, V. G. Sympathetic Responses to Noxious Stimulation of Muscle and Skin. *Front Neurol* **7**, 109, doi:10.3389/fneur.2016.00109 (2016).

66   Burton, A. R., Birznieks, I., Bolton, P. S., Henderson, L. A. & Macefield, V. G. Effects of deep and superficial experimentally induced acute pain on muscle sympathetic nerve activity in human subjects. *The Journal of physiology* **587**, 183-193, doi:10.1113/jphysiol.2008.162230 (2009).

67   Lautenschlager, G. *et al.* The impact of baroreflex function on endogenous pain control: a microneurography study. *The European journal of neuroscience* **42**, 2996-3003, doi:10.1111/ejn.13096 (2015).

68   Fazalbhoy, A., Birznieks, I. & Macefield, V. G. Individual differences in the cardiovascular responses to tonic muscle pain: parallel increases or decreases in muscle sympathetic nerve activity, blood pressure and heart rate. *Experimental physiology* **97**, 1084-1092, doi:10.1113/expphysiol.2012.066191 (2012).

69   Fazalbhoy, A., Birznieks, I. & Macefield, V. G. Consistent interindividual increases or decreases in muscle sympathetic nerve activity during experimental muscle pain. *Experimental Brain Research* **232**, 1309-1315, doi:10.1007/s00221-014-3847-7 (2014).

70   Kobuch, S., Fazalbhoy, A., Brown, R., Henderson, L. A. & Macefield, V. G. Central circuitry responsible for the divergent sympathetic responses to tonic muscle pain in humans. *Human Brain Mapping* **38**, 869-881, doi:10.1002/hbm.23424 (2017).

71   Fillingim, R. B., King, C. D., Ribeiro-Dasilva, M. C., Rahim-Williams, B. & Riley, J. L., 3rd. Sex, gender, and pain: a review of recent clinical and experimental findings. *J Pain* **10**, 447-485, doi:10.1016/j.jpain.2008.12.001 (2009).

72   Mogil, J. S. Laboratory environmental factors and pain behavior: the relevance of unknown unknowns to reproducibility and translation. *Lab Anim (NY)* **46**, 136-141, doi:10.1038/laban.1223 (2017).

73   al'Absi, M., France, C., Harju, A., France, J. & Wittmers, L. Adrenocortical and nociceptive responses to opioid blockade in hypertension-prone men and women. *Psychosom Med* **68**, 292-298, doi:10.1097/01.psy.0000203240.64965.bd (2006).

74   Nyklícek, I., Vingerhoets, A. J. & Van Heck, G. L. Hypertension and pain sensitivity: effects of gender and cardiovascular reactivity. *Biological psychology* **50**, 127-142, doi:10.1016/s0301-0511(99)00006-x (1999).

75   Chesterton, L. S., Barlas, P., Foster, N. E., Baxter, G. D. & Wright, C. C. Gender differences in pressure pain threshold in healthy humans. *Pain* **101**, 259-266, doi:10.1016/s0304-3959(02)00330-5 (2003).

76   Fillingim, R. B. *et al.* Sex-related psychological predictors of baseline pain perception and analgesic responses to pentazocine. *Biological psychology* **69**, 97-112, doi:10.1016/j.biopsycho.2004.11.008 (2005).

77   Lautenbacher, S., Nielsen, J., Andersen, T. & Arendt-Nielsen, L. Spatial summation of heat pain in males and females. *Somatosensory & motor research* **18**, 101-105, doi:10.1080/135578501012006192-1 (2001).

78   Myers, C. D., Robinson, M. E., Riley, J. L., 3rd & Sheffield, D. Sex, gender, and blood pressure: contributions to experimental pain report. *Psychosom Med* **63**, 545-550, doi:10.1097/00006842-200107000-00004 (2001).

79   Lowery, D., Fillingim, R. B. & Wright, R. A. Sex differences and incentive effects on perceptual and cardiovascular responses to cold pressor pain. *Psychosom Med* **65**, 284-291, doi:10.1097/01.psy.0000033127.11561.78 (2003).

80   Edwards, R. R., Haythornthwaite, J. A., Sullivan, M. J. & Fillingim, R. B. Catastrophizing as a mediator of sex differences in pain: differential effects for daily pain versus laboratory-induced pain. *Pain* **111**, 335-341, doi:10.1016/j.pain.2004.07.012 (2004).

81   Bragdon, E. E. *et al.* Group differences in pain modulation: pain-free women compared to pain-free men and to women with TMD. *Pain* **96**, 227-237, doi:10.1016/s0304-3959(01)00451-1 (2002).

82   Girdler, S. S. *et al.* Cigarette smoking, stress-induced analgesia and pain perception in men and women. *Pain* **114**, 372-385, doi:10.1016/j.pain.2004.12.035 (2005).

83   Aufiero, M., Stankewicz, H., Quazi, S., Jacoby, J. & Stoltzfus, J. Pain Perception in Latino vs. Caucasian and Male vs. Female Patients: Is There Really a Difference? *The western journal of emergency medicine* **18**, 737-742, doi:10.5811/westjem.2017.1.32723 (2017).

84   Otto, A., Emery, K. & Cote, J. N. Sex differences in perceptual responses to experimental pain before and after an experimental fatiguing arm task. *Biology of sex differences* **10**, 39, doi:10.1186/s13293-019-0253-7 (2019).

85   Bartley, E. J. & Fillingim, R. B. Sex differences in pain: a brief review of clinical and experimental findings. *British journal of anaesthesia* **111**, 52-58, doi:10.1093/bja/aet127 (2013).

86   Craft, R. M. Modulation of pain by estrogens. *Pain* **132 Suppl 1**, S3-12, doi:10.1016/j.pain.2007.09.028 (2007).

87   Goolkasian, P. Cyclic changes in pain perception: an ROC analysis. *Perception & psychophysics* **27**, 499-504, doi:10.3758/bf03198677 (1980).

88   Hellström, B. & Lundberg, U. Pain perception to the cold pressor test during the menstrual cycle in relation to estrogen levels and a comparison with men. *Integrative physiological and behavioral science : the official journal of the Pavlovian Society* **35**, 132-141, doi:10.1007/bf02688772 (2000).

89   Carter, J. R., Fu, Q., Minson, C. T. & Joyner, M. J. Ovarian cycle and sympathoexcitation in premenopausal women. *Hypertension* **61**, 395-399, doi:10.1161/hypertensionaha.112.202598 (2013).

90   Harvey, R. E. *et al.* Oral Contraceptive Use, Muscle Sympathetic Nerve Activity, and Systemic Hemodynamics in Young Women. *Hypertension* **66**, 590-597, doi:10.1161/hypertensionaha.115.05179 (2015).

91   Carter, J. R., Lawrence, J. E. & Klein, J. C. Menstrual cycle alters sympathetic neural responses to orthostatic stress in young, eumenorrheic women. *American journal of physiology. Endocrinology and metabolism* **297**, E85-91, doi:10.1152/ajpendo.00019.2009 (2009).

92   Fu, Q. *et al.* Menstrual cycle effects on sympathetic neural responses to upright tilt. *The Journal of physiology* **587**, 2019-2031, doi:10.1113/jphysiol.2008.168468 (2009).

93   Jarvis, S. S. *et al.* Sympathetic activation during early pregnancy in humans. *The Journal of physiology* **590**, 3535-3543, doi:10.1113/jphysiol.2012.228262 (2012).

94   Lawrence, J. E., Ray, C. A. & Carter, J. R. Vestibulosympathetic reflex during the early follicular and midluteal phases of the menstrual cycle. *American journal of physiology. Endocrinology and metabolism* **294**, E1046-1050, doi:10.1152/ajpendo.00056.2008 (2008).

95   Middlekauff, H. R., Park, J. & Gornbein, J. A. Lack of effect of ovarian cycle and oral contraceptives on baroreceptor and nonbaroreceptor control of sympathetic nerve activity in healthy women. *Am J Physiol Heart Circ Physiol* **302**, H2560-2566, doi:10.1152/ajpheart.00579.2011 (2012).

96   Minson, C. T., Halliwill, J. R., Young, T. M. & Joyner, M. J. Influence of the menstrual cycle on sympathetic activity, baroreflex sensitivity, and vascular transduction in young women. *Circulation* **101**, 862-868, doi:10.1161/01.cir.101.8.862 (2000).

97   He, X. R., Wang, W., Crofton, J. T. & Share, L. Effects of 17beta-estradiol on sympathetic activity and pressor response to phenylephrine in ovariectomized

rats. *The American journal of physiology* **275**, R1202-1208, doi:10.1152/ajpregu.1998.275.4.R1202 (1998).

98   Vongpatanasin, W., Tuncel, M., Mansour, Y., Arbique, D. & Victor, R. G. Transdermal estrogen replacement therapy decreases sympathetic activity in postmenopausal women. *Circulation* **103**, 2903-2908, doi:10.1161/01.cir.103.24.2903 (2001).

99   Ettinger, S. M. *et al.* Effects of the ovarian cycle on sympathetic neural outflow during static exercise. *Journal of applied physiology (Bethesda, Md. : 1985)* **85**, 2075-2081, doi:10.1152/jappl.1998.85.6.2075 (1998).

100   Minson, C. T., Halliwill, J. R., Young, T. M. & Joyner, M. J. Sympathetic activity and baroreflex sensitivity in young women taking oral contraceptives. *Circulation* **102**, 1473-1476, doi:10.1161/01.cir.102.13.1473 (2000).

101   Rolke, R. A., K., Magerl, W.; Treede, R.-D.; Pfau, D.; Klein, T.; Blunk, J. A.; Geber, C.; Krumova, E.; Limbeck, C.; Maier, C.; Westermann, A.; Schuh - Hofer, S.; Tiede, W.   (ed University Medicine Mannheim) (2010).

102   Delius, W., Hagbarth, K. E., Hongell, A. & Wallin, B. G. General characteristics of sympathetic activity in human muscle nerves. *Acta physiologica Scandinavica* **84**, 65-81, doi:10.1111/j.1748-1716.1972.tb05158.x (1972).

103   Stjernberg, L., Blumberg, H. & Wallin, B. G. Sympathetic activity in man after spinal cord injury. Outflow to muscle below the lesion. *Brain : a journal of neurology* **109 (Pt 4)**, 695-715, doi:10.1093/brain/109.4.695 (1986).

104   Donadio, V., Kallio, M., Karlsson, T., Nordin, M. & Wallin, B. G. Inhibition of human muscle sympathetic activity by sensory stimulation. *The Journal of physiology* **544**, 285-292, doi:10.1113/jphysiol.2002.019596 (2002).

105   Donadio, V., Karlsson, T., Elam, M. & Wallin, B. G. Interindividual differences in sympathetic and effector responses to arousal in humans. *The Journal of physiology* **544**, 293-302, doi:10.1113/jphysiol.2002.020099 (2002).

106   Wallin, B. G. *et al.* Arousal increases baroreflex inhibition of muscle sympathetic activity. *Acta physiologica Scandinavica* **177**, 291-298, doi:10.1046/j.1365-201X.2003.01071.x (2003).

107   Sundlöf, G. & Wallin, B. G. Human muscle nerve sympathetic activity at rest. Relationship to blood pressure and age. *The Journal of physiology* **274**, 621-637, doi:10.1113/jphysiol.1978.sp012170 (1978).

108   Carter, J. R. & Cooke, W. H. Sympathetic nerve activity and blood pressure: who leads, who follows and why sex matters. *The Journal of physiology* **594**, 4705-4706, doi:10.1113/jp272569 (2016).

109   Lautenbacher, S. & Rollman, G. B. Sex differences in responsiveness to painful and non-painful stimuli are dependent upon the stimulation method. *Pain* **53**, 255-264, doi:10.1016/0304-3959(93)90221-a (1993).

110   Inoue, Y. *et al.* Sex differences in age-related changes on peripheral warm and cold innocuous thermal sensitivity. *Physiology & behavior* **164**, 86-92, doi:10.1016/j.physbeh.2016.05.045 (2016).

A Raw Data

Table A.1. Raw data for subject characteristics

Subject	Sex	Age	Height (cm)	Weight (kg)	BMI (kg/m2)	SAP (mmHg)	DAP (mmHg)	HR (bpm)
1	f	20	167.64	77.73	27.66	104	65	74
2	f	38	165.10	87.09	31.95	103	60	64
3	m	25	180.34	103.87	31.94	118	60	65
4	m	20	167.64	86.36	30.73	110	71	71
5	f	24	167.64	60.33	21.47	119	62	72
6	m	21	193.04	106.36	28.54	117	67	66
7	f	27	160.02	62.73	24.50	109	63	81
8	m	23	185.42	89.09	25.91	106	54	50
9	m	22	182.88	77.27	23.10	124	75	72
10	m	27	162.56	88.00	33.30	109	67	65
11	m	20	190.50	94.35	26.00	108	59	68
12	m	19	185.42	78.93	22.96	114	67	84
13	f	19	162.56	71.21	26.95	96	66	69
14	f	19	165.10	58.51	21.47	106	66	98
15	m	30	168.00	69.40	24.59	101	60	72
16	f	28	161.50	61.24	23.48	103	61	59
17	f	20	165.10	60.33	22.13	120	66	83
18	m	18	176.53	62.27	19.98	107	72	110
19	f	20	160.02	58.06	22.67	124	80	53
20	f	31	167.64	86.82	30.89	91	59	71
21	m	21	172.72	69.55	23.31	98	58	71
22	m	33	172.72	75.00	25.14	113	61	63
23	f	35	152.40	45.91	19.77	101	72	74

BMI, body mass index

SAP, systolic arterial pressure

DAP, diastolic arterial pressure

HR, heart rate

Table A.2. Raw data for subject characteristics.

Subject	State-Trait Anxiety Inventory (state)	State-Trait Anxiety Inventory (trait)	Center for Epidemiologic Studies Depression Scale	Insomnia Severity Index	Epworth Sleepiness Scale
1	26	31	9	8	6
2	27	30	4	5	5
3	21	25	2	1	2
4	20	23	1	2	2
5	39	47	18	16	4
6	29	34	7	1	6
7	32	33	10	1	5
8	22	25	6	3	2
9	26	29	4	2	13
10	29	33	10	4	1
11	26	26	6	5	7
12	25	30	4	4	5
13	22	26	3	5	6
14	20	22	3	2	4
15	26	36	9	5	7
16	33	34	9	0	6
17	29	32	10	6	6
18	20	25	2	2	5
19	37	40	11	13	7
20	51	47	12	4	7
21	26	34	2	3	9
22	24	29	6	11	17
23	27	30	14	19	4

Table A.3. Raw data for blood pressure and heart rate response during cool sensation.

Subject	SAP (mmHg) Base	SAP (mmHg) REC	DAP (mmHg) Base	DAP (mmHg) REC	HR (bpm) Base	HR (bpm) REC
1	103.95	104.15	65.48	65.83	75.88	77.70
2	99.13	97.65	59.38	57.83	61.78	61.60
3	114.78	116.48	76.10	77.40	61.88	61.50
4	102.18	102.75	62.83	63.08	57.83	58.83
5	122.10	121.13	51.73	50.70	50.38	50.98
6	115.53	115.35	61.75	59.03	54.80	55.18
7	104.43	101.28	54.05	52.10	62.23	61.48
8	95.98	95.05	42.10	41.35	50.80	51.88
9	128.15	128.98	78.90	76.98	66.30	64.55
10	112.80	111.45	58.05	56.48	65.15	67.30
11	113.48	113.65	75.53	75.95	55.73	57.08
12	122.05	117.50	60.15	57.58	69.85	73.20
13	102.90	103.63	63.85	64.20	63.83	64.25
14	109.30	106.58	54.68	52.48	73.78	76.95
15	102.93	103.28	61.43	61.83	71.40	69.15
16	113.58	113.40	60.08	59.05	55.15	55.83
17	113.50	111.68	66.75	65.10	66.85	67.40
18	110.10	109.28	58.28	57.03	80.58	80.33
19	118.15	115.75	76.68	75.70	45.48	46.50
20	94.10	89.90	61.83	59.15	63.38	66.35
21	109.75	108.05	55.85	54.70	64.38	62.35
22	115.95	115.48	62.35	61.75	55.03	56.23
23	97.58	95.75	63.85	62.03	67.18	69.03

SAP, systolic arterial pressure

DAP, diastolic arterial pressure

HR, heart rate

REC, recovery

44

Table A.4. Raw data for blood pressure and heart rate response during warm sensation.

Subject	SAP (mmHg) Base	SAP (mmHg) REC	DAP (mmHg) Base	DAP (mmHg) REC	HR (bpm) Base	HR (bpm) REC
1	101.98	103.03	63.98	64.98	74.85	76.35
2	98.98	97.93	58.05	56.63	64.70	60.70
3	115.10	115.88	77.75	78.50	63.43	63.35
4	100.43	100.73	61.18	61.13	63.73	59.82
5	121.28	120.98	53.73	53.28	52.35	52.83
6	107.55	115.05	57.30	60.48	55.70	57.58
7	99.25	96.33	50.23	47.63	63.68	63.55
8	93.85	92.08	40.83	38.58	49.58	53.40
9	.	.	.	.	.	.
10	108.95	109.70	56.13	56.33	65.23	67.03
11	115.23	113.50	74.53	73.45	55.23	60.08
12	114.95	117.30	55.33	55.90	74.53	72.73
13	99.90	100.05	61.13	60.45	62.93	59.83
14	107.38	108.60	53.55	54.55	70.43	74.00
15	98.75	101.60	60.25	61.80	70.70	71.38
16	111.05	110.70	56.88	57.08	52.18	55.48
17	114.63	113.75	66.83	66.05	66.53	66.48
18	101.83	108.68	55.80	57.38	79.08	81.60
19	116.00	115.05	76.63	76.00	45.58	46.18
20	93.58	90.40	63.15	61.95	65.05	66.48
21	112.58	111.85	58.53	57.93	63.53	62.98
22	118.40	117.93	62.85	62.50	55.25	57.00
23	96.40	98.08	63.03	63.58	64.45	66.20

SAP, systolic arterial pressure

DAP, diastolic arterial pressure

HR, heart rate

REC, recovery

Table A.5. Raw data for blood pressure and heart rate response during heat pain.

Subject	SAP (mmHg)			DAP (mmHg)			HR (bpm)		
	BASE	Sensation	REC	BASE	Sensation	REC	BASE	Sensation	REC
1	103.98	106.03	100.10	65.80	67.75	62.60	73.13	80.00	76.73
2	98.13	96.98	98.63	58.48	56.23	57.60	61.03	57.20	60.80
3	114.00	113.95	116.80	76.40	76.02	78.95	64.15	62.30	62.30
4	101.08	103.23	100.53	61.18	63.10	60.38	59.50	60.58	61.45
5	124.78	122.95	123.18	54.33	52.68	53.10	54.20	50.75	52.68
6	110.88	118.08	111.65	58.45	59.85	57.93	53.75	48.30	54.40
7	98.15	95.75	93.28	48.48	46.70	45.05	62.18	62.20	63.45
8	95.43	96.73	94.53	39.58	39.85	38.55	50.70	50.70	52.33
9	131.53	134.78	136.45	74.88	76.30	78.13	63.98	60.70	67.70
10	107.30	109.40	109.08	55.53	57.35	57.25	71.08	63.03	69.20
11	117.18	117.08	113.35	77.23	77.33	74.68	59.08	62.85	61.50
12	119.00	118.20	115.55	55.35	54.45	54.45	76.30	68.50	72.95
13	99.33	107.25	102.75	59.73	65.08	62.53	57.93	56.93	61.05
14	112.45	112.25	113.10	57.83	58.33	59.48	73.33	70.40	79.60
15	101.03	110.28	101.78	61.53	67.73	61.50	71.28	71.65	70.38
16	110.85	109.50	109.10	57.95	55.83	56.35	52.45	52.10	56.08
17	114.78	111.78	113.15	66.35	63.58	64.78	68.68	67.00	70.98
18	107.45	110.88	103.43	55.53	55.58	53.33	79.05	75.80	81.73
19	121.35	126.75	119.13	80.43	83.60	78.63	46.00	45.93	48.50
20	89.43	86.58	89.95	58.83	58.38	60.30	61.80	61.15	64.88
21	111.50	110.28	110.63	55.70	54.35	54.05	63.90	62.00	65.75
22	117.70	118.70	115.18	62.80	64.20	61.60	53.38	58.00	57.60
23	96.00	95.43	90.18	62.90	60.85	57.30	64.85	64.20	66.40

SAP, systolic arterial pressure

DAP, diastolic arterial pressure

HR, heart rate

REC, recovery

Hb, heart beat

Table A.6. Raw data for muscle sympathetic nerve activity during cool sensation.

Subject	BURST FREQUENCY (burst/100hb) Base	REC	BURST INCIDENCE (burst/min) Base	REC	TOTAL MSNA (a.u.) Base	REC
1	7.00	6.00	9.33	7.78	28.38	22.80
2	12.50	9.00	20.88	14.83	38.60	29.25
3	19.00	16.00	30.88	26.25	85.25	78.30
4	25.50	18.00	44.50	31.48	90.33	66.68
5	5.50	6.00	11.00	11.78	24.43	22.85
6	22.50	21.00	41.55	38.23	89.68	83.15
7	26.50	22.50	42.93	36.38	93.75	66.20
8	12.00	13.50	24.13	26.15	33.63	34.78
9	15.10	16.50	22.93	25.93	44.25	47.68
10	18.00	15.00	27.53	22.23	72.30	67.55
11	9.50	8.00	17.18	14.05	31.48	26.85
12	9.00	8.00	13.33	11.33	39.13	33.48
13	4.00	3.00	6.28	4.60	11.10	9.38
14	15.00	10.50	20.85	13.75	61.85	44.83
15	17.00	16.50	23.88	24.10	62.55	66.65
16	17.50	12.00	32.35	22.13	87.55	60.43
17	11.00	8.50	16.73	12.93	40.83	34.85
18	14.50	11.00	18.03	13.75	44.65	32.90
19	15.50	12.00	35.08	26.00	47.83	36.63
20	5.50	4.00	9.20	6.20	22.53	15.48
21	31.50	29.50	49.83	48.08	123.60	120.50
22	40.50	32.00	74.50	57.78	145.03	122.68
23	11.50	15.00	17.35	21.73	26.00	35.15

REC, recovery

MSNA, muscle sympathetic nerve activity

Hb, heart beat

Table A.7. Raw data for muscle sympathetic nerve activity during warm sensation.

Subject	BURST FREQUENCY (burst/100hb)		BURST INCIDENCE (burst/min)		TOTAL MSNA (a.u.)	
	Base	REC	Base	REC	Base	REC
1	26.63	8.50	10.35	11.20	28.53	36.28
2	26.28	15.00	21.28	25.00	37.15	40.15
3	17.50	18.00	27.75	28.10	74.78	76.78
4	30.05	19.00	31.23	32.20	75.75	75.30
5	8.50	6.50	16.98	11.88	31.40	26.13
6	33.00	18.50	59.28	32.43	119.73	74.15
7	17.00	20.00	26.93	31.63	53.75	62.25
8	11.50	13.00	23.43	24.73	31.68	37.48
9	.	.	.	.	.	.
10	20.50	20.00	31.93	30.08	82.88	76.78
11	6.50	4.50	11.88	7.43	22.63	17.15
12	6.00	4.00	8.28	5.63	23.43	20.50
13	4.00	5.00	6.40	8.75	11.33	14.30
14	13.50	11.50	19.53	15.93	57.35	52.60
15	18.50	13.50	26.25	19.20	76.13	56.40
16	18.00	16.00	35.48	30.35	89.45	79.53
17	13.50	10.00	20.45	15.48	49.93	40.83
18	15.00	14.00	18.90	17.28	45.78	42.55
19	17.50	12.00	38.78	26.40	51.55	35.18
20	5.00	4.00	8.13	6.53	19.15	16.38
21	35.00	32.50	56.08	52.48	138.88	123.35
22	43.50	39.50	79.28	69.93	161.60	149.45
23	20.50	18.50	31.58	28.03	41.40	40.73

REC, recovery

MSNA, muscle sympathetic nerve activity

Hb, heart beat

Table A.8. Raw data for muscle sympathetic nerve activity during heat pain.

Subject	BURST FREQUENCY (burst/100hb)			BURST INCIDENCE (burst/min)			TOTAL MSNA (a.u.)		
	BASE	Sensation	REC	BASE	Sensation	REC	BASE	Sensation	REC
1	5.00	0.00	10.00	6.93	0.00	13.05	19.60	0.00	40.03
2	13.00	10.60	14.00	21.58	19.08	23.08	41.13	25.30	39.8
3	26.00	27.83	23.00	39.83	45.38	36.78	108.88	115.83	94.73
4	23.50	17.85	19.50	39.63	29.70	32.05	95.25	73.63	76.05
5	9.50	4.48	10.50	17.70	8.78	20.10	38.03	17.58	44.48
6	28.00	27.05	28.00	52.80	54.88	52.35	114.08	100.85	99.50
7	24.00	16.78	21.00	38.73	26.88	33.68	73.80	51.05	64.53
8	10.50	5.68	16.00	20.90	11.40	31.15	27.05	15.08	42.43
9	14.00	21.5	14.50	22.05	35.20	21.83	43.28	64.90	42.10
10	24.50	22.85	21.03	34.80	36.25	31.20	110.48	92.23	88.65
11	10.00	2.80	15.50	17.38	4.48	25.58	37.90	8.98	55.00
12	10.00	4.25	8.00	13.10	6.30	11.65	45.85	24.00	46.75
13	4.50	5.18	2.00	8.20	9.28	3.28	12.18	13.75	4.85
14	12.50	11.95	9.50	17.53	16.88	12.23	47.28	39.03	29.18
15	14.00	11.23	16.00	19.85	16.13	22.45	68.18	50.68	68.58
16	21.00	11.40	18.00	41.83	22.08	33.73	93.33	54.73	81.03
17	14.50	13.13	9.00	21.88	19.45	12.93	55.03	45.38	37.48
18	10.00	10.15	14.50	12.70	13.33	17.68	33.68	27.95	42.28
19	10.00	3.40	11.00	21.83	7.33	23.38	29.53	10.25	32.95
20	3.50	4.83	3.50	5.90	8.08	5.55	16.75	18.70	13.53
21	37.50	23.93	36.00	61.43	37.15	56.38	148.78	108.90	142.28
22	41.50	30.35	38.50	77.98	52.53	68.10	156.90	100.98	134.05
23	20.00	19.8	22.50	31.20	31.00	34.05	45.50	44.08	52.23

REC, recovery

MSNA, muscle sympathetic nerve activity

Hb, heart beat

B Repeated Measures to Average Four Trials for Each Threshold

B.1 Systolic Pressure - Cool Threshold Recovery

Mauchly's Test of Sphericity[a]

Measure: MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b] Greenhouse-Geisser	Huynh-Feldt	Lower-bound
time	.819	4.150	5	.528	.876	1.000	.333

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	87.949	3	29.316	1.979	.126
	Greenhouse-Geisser	87.949	2.629	33.459	1.979	.134
	Huynh-Feldt	87.949	3.000	29.316	1.979	.126
	Lower-bound	87.949	1.000	87.949	1.979	.173
Error(time)	Sphericity Assumed	977.816	66	14.815		
	Greenhouse-Geisser	977.816	57.829	16.909		
	Huynh-Feldt	977.816	66.000	14.815		
	Lower-bound	977.816	22.000	44.446		

B.2 Diastolic Pressure - Cool Sensation Recovery

Mauchly's Test of Sphericity[a]

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b] Greenhouse-Geisser	Huynh-Feldt	Lower-bound
time	.571	11.608	5	.041	.758	.850	.333

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	39.823	3	13.274	2.294	.086
	Greenhouse-Geisser	39.823	2.274	17.515	2.294	.105
	Huynh-Feldt	39.823	2.550	15.619	2.294	.097
	Lower-bound	39.823	1.000	39.823	2.294	.144
Error(time)	Sphericity Assumed	381.862	66	5.786		
	Greenhouse-Geisser	381.862	50.020	7.634		
	Huynh-Feldt	381.862	56.091	6.808		
	Lower-bound	381.862	22.000	17.357		

B.3 Heart Rate - Cool Sensation Recovery

Mauchly's Test of Sphericity[a]

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
						Epsilon[b]	
time	.888	2.452	5	.784	.929	1.000	.333

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	40.923	3	13.641	1.329	.273
	Greenhouse-Geisser	40.923	2.787	14.685	1.329	.274
	Huynh-Feldt	40.923	3.000	13.641	1.329	.273
	Lower-bound	40.923	1.000	40.923	1.329	.261
Error(time)	Sphericity Assumed	677.650	66	10.267		
	Greenhouse-Geisser	677.650	61.310	11.053		
	Huynh-Feldt	677.650	66.000	10.267		
	Lower-bound	677.650	22.000	30.802		

B.4 Burst Frequency - Cool Sensation Recovery

Mauchly's Test of Sphericity[a]

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b] Greenhouse-Geisser	Huynh-Feldt	Lower-bound
time	.895	2.288	5	.808	.931	1.000	.333

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	168.562	3	56.187	1.990	.124
	Greenhouse-Geisser	168.562	2.794	60.331	1.990	.129
	Huynh-Feldt	168.562	3.000	56.187	1.990	.124
	Lower-bound	168.562	1.000	168.562	1.990	.172
Error(time)	Sphericity Assumed	1863.958	66	28.242		
	Greenhouse-Geisser	1863.958	61.467	30.324		
	Huynh-Feldt	1863.958	66.000	28.242		
	Lower-bound	1863.958	22.000	84.725		

B.5 Burst Incidence - Cool Sensation Recovery

Mauchly's Test of Sphericity[a]

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b] Greenhouse-Geisser	Huynh-Feldt	Lower-bound
time	.929	1.522	5	.911	.956	1.000	.333

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	636.692	3	212.231	2.212	.095
	Greenhouse-Geisser	636.692	2.867	222.074	2.212	.098
	Huynh-Feldt	636.692	3.000	212.231	2.212	.095
	Lower-bound	636.692	1.000	636.692	2.212	.151
Error(time)	Sphericity Assumed	6331.185	66	95.927		
	Greenhouse-Geisser	6331.185	63.075	100.376		
	Huynh-Feldt	6331.185	66.000	95.927		
	Lower-bound	6331.185	22.000	287.781		

B.6 Total MSNA - Cool Sensation Recovery

Mauchly's Test of Sphericity[a]

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b] Greenhouse-Geisser	Huynh-Feldt	Lower-bound
time	.763	5.598	5	.348	.863	.989	.333

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	3663.159	3	1221.053	2.486	.068
	Greenhouse-Geisser	3663.159	2.590	1414.522	2.486	.078
	Huynh-Feldt	3663.159	2.966	1235.229	2.486	.069
	Lower-bound	3663.159	1.000	3663.159	2.486	.129
Error(time)	Sphericity Assumed	32422.158	66	491.245		
	Greenhouse-Geisser	32422.158	56.973	569.080		
	Huynh-Feldt	32422.158	65.243	496.948		
	Lower-bound	32422.158	22.000	1473.734		

B.7   Systolic Pressure - Warm Sensation Recovery

Mauchly's Test of Sphericity[a]

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b] Greenhouse-Geisser	Huynh-Feldt	Lower-bound
time	.646	8.617	5	.126	.760	.858	.333

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	32.859	3	10.953	.799	.499
	Greenhouse-Geisser	32.859	2.280	14.410	.799	.470
	Huynh-Feldt	32.859	2.573	12.770	.799	.483
	Lower-bound	32.859	1.000	32.859	.799	.381
Error(time)	Sphericity Assumed	863.086	63	13.700		
	Greenhouse-Geisser	863.086	47.886	18.024		
	Huynh-Feldt	863.086	54.034	15.973		
	Lower-bound	863.086	21.000	41.099		

B.8　Diastolic Pressure - Warm Sensation Recovery

Mauchly's Test of Sphericity[a]

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
time	.818	3.963	5	.555	.888	1.000	.333

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	26.048	3	8.683	1.139	.340
	Greenhouse-Geisser	26.048	2.665	9.775	1.139	.338
	Huynh-Feldt	26.048	3.000	8.683	1.139	.340
	Lower-bound	26.048	1.000	26.048	1.139	.298
Error(time)	Sphericity Assumed	480.322	63	7.624		
	Greenhouse-Geisser	480.322	55.961	8.583		
	Huynh-Feldt	480.322	63.000	7.624		
	Lower-bound	480.322	21.000	22.872		

B.9 Heart Rate - Warm Sensation Recovery

Mauchly's Test of Sphericity[a]

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
					Epsilon[b]		
time	.371	19.562	5	.002	.678	.751	.333

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	202.060	3	67.353	3.844	.014
	Greenhouse-Geisser	202.060	2.034	99.355	3.844	.029
	Huynh-Feldt	202.060	2.254	89.662	3.844	.024
	Lower-bound	202.060	1.000	202.060	3.844	.063
Error(time)	Sphericity Assumed	1103.957	63	17.523		
	Greenhouse-Geisser	1103.957	42.708	25.849		
	Huynh-Feldt	1103.957	47.325	23.327		
	Lower-bound	1103.957	21.000	52.569		

B.10 Burst Frequency - Warm Sensation Recovery

Mauchly's Test of Sphericity[a]

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b] Greenhouse-Geisser	Huynh-Feldt	Lower-bound
time	.159	36.311	5	.000	.473	.497	.333

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	659.470	3	219.823	1.602	.198
	Greenhouse-Geisser	659.470	1.419	464.668	1.602	.220
	Huynh-Feldt	659.470	1.492	441.875	1.602	.219
	Lower-bound	659.470	1.000	659.470	1.602	.220
Error(time)	Sphericity Assumed	8646.735	63	137.250		
	Greenhouse-Geisser	8646.735	29.804	290.122		
	Huynh-Feldt	8646.735	31.341	275.891		
	Lower-bound	8646.735	21.000	411.749		

B.11   Burst Incidence - Warm Sensation Recovery

Mauchly's Test of Sphericity[a]

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
						Epsilon[b]	
time	.826	3.760	5	.585	.893	1.000	.333

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	673.146	3	224.382	1.855	.146
	Greenhouse-Geisser	673.146	2.678	251.348	1.855	.154
	Huynh-Feldt	673.146	3.000	224.382	1.855	.146
	Lower-bound	673.146	1.000	673.146	1.855	.188
Error(time)	Sphericity Assumed	7622.199	63	120.987		
	Greenhouse-Geisser	7622.199	56.241	135.528		
	Huynh-Feldt	7622.199	63.000	120.987		
	Lower-bound	7622.199	21.000	362.962		

B.12 Total MSNA - Warm Sensation Recovery

Mauchly's Test of Sphericity^a

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon^b Greenhouse-Geisser	Huynh-Feldt	Lower-bound
time	.724	6.380	5	.272	.814	.930	.333

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	5517.292	3	1839.097	3.534	.020
	Greenhouse-Geisser	5517.292	2.443	2258.239	3.534	.028
	Huynh-Feldt	5517.292	2.789	1978.421	3.534	.023
	Lower-bound	5517.292	1.000	5517.292	3.534	.074
Error(time)	Sphericity Assumed	32782.118	63	520.351		
	Greenhouse-Geisser	32782.118	51.307	638.942		
	Huynh-Feldt	32782.118	58.563	559.771		
	Lower-bound	32782.118	21.000	1561.053		

B.13 Systolic Pressure - Heat Pain Sensation

Mauchly's Test of Sphericity[a]

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b] Greenhouse-Geisser	Huynh-Feldt	Lower-bound
time	.762	5.355	5	.375	.861	.992	.333

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	21.386	3	7.129	.455	.715
	Greenhouse-Geisser	21.386	2.582	8.281	.455	.686
	Huynh-Feldt	21.386	2.976	7.186	.455	.713
	Lower-bound	21.386	1.000	21.386	.455	.507
Error(time)	Sphericity Assumed	987.212	63	15.670		
	Greenhouse-Geisser	987.212	54.232	18.204		
	Huynh-Feldt	987.212	62.500	15.795		
	Lower-bound	987.212	21.000	47.010		

B.14   Diastolic Pressure - Heat Pain Sensation

Mauchly's Test of Sphericity[a]

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b] Greenhouse-Geisser	Huynh-Feldt	Lower-bound
time	.722	6.419	5	.268	.858	.988	.333

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	4.439	3	1.480	.155	.926
	Greenhouse-Geisser	4.439	2.574	1.725	.155	.902
	Huynh-Feldt	4.439	2.964	1.497	.155	.924
	Lower-bound	4.439	1.000	4.439	.155	.697
Error(time)	Sphericity Assumed	600.069	63	9.525		
	Greenhouse-Geisser	600.069	54.049	11.102		
	Huynh-Feldt	600.069	62.252	9.639		
	Lower-bound	600.069	21.000	28.575		

B.15   Heart Rate - Heat Pain Sensation

Mauchly's Test of Sphericity[a]

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b] Greenhouse-Geisser	Huynh-Feldt	Lower-bound
time	.466	15.074	5	.010	.704	.785	.333

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	42.201	3	14.067	1.050	.377
	Greenhouse-Geisser	42.201	2.112	19.981	1.050	.362
	Huynh-Feldt	42.201	2.354	17.927	1.050	.367
	Lower-bound	42.201	1.000	42.201	1.050	.317
Error(time)	Sphericity Assumed	843.941	63	13.396		
	Greenhouse-Geisser	843.941	44.353	19.028		
	Huynh-Feldt	843.941	49.437	17.071		
	Lower-bound	843.941	21.000	40.188		

B.16 Burst Frequency - Heat Pain Sensation

Mauchly's Test of Sphericity[a]

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
time	.720	6.488	5	.262	.822	.940	.333

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	105.386	3	35.129	.495	.687
	Greenhouse-Geisser	105.386	2.467	42.713	.495	.652
	Huynh-Feldt	105.386	2.821	37.358	.495	.676
	Lower-bound	105.386	1.000	105.386	.495	.490
Error(time)	Sphericity Assumed	4473.442	63	71.007		
	Greenhouse-Geisser	4473.442	51.813	86.339		
	Huynh-Feldt	4473.442	59.240	75.514		
	Lower-bound	4473.442	21.000	213.021		

B.17   Burst Incidence - Heat Pain Sensation

Mauchly's Test of Sphericity[a]

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b] Greenhouse-Geisser	Huynh-Feldt	Lower-bound
time	.701	7.017	5	.220	.798	.907	.333

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	249.664	3	83.221	.375	.771
	Greenhouse-Geisser	249.664	2.393	104.314	.375	.726
	Huynh-Feldt	249.664	2.722	91.707	.375	.752
	Lower-bound	249.664	1.000	249.664	.375	.547
Error(time)	Sphericity Assumed	13987.981	63	222.031		
	Greenhouse-Geisser	13987.981	50.261	278.306		
	Huynh-Feldt	13987.981	57.170	244.672		
	Lower-bound	13987.981	21.000	666.094		

B.18   Total MSNA - Heat Pain Sensation

Mauchly's Test of Sphericity[a]

| Within Subjects Effect | Mauchly's W | Approx. Chi-Square | df | Sig. | Epsilon[b] | | |
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
time	.895	2.192	5	.822	.927	1.000	.333

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	31841944.959	3	10613981.653	.444	.723
	Greenhouse-Geisser	31841944.959	2.781	11450081.015	.444	.708
	Huynh-Feldt	31841944.959	3.000	10613981.653	.444	.723
	Lower-bound	31841944.959	1.000	31841944.959	.444	.513
Error(time)	Sphericity Assumed	1506687339.416	63	23915672.054		
	Greenhouse-Geisser	1506687339.416	58.400	25799590.719		
	Huynh-Feldt	1506687339.416	63.000	23915672.054		
	Lower-bound	1506687339.416	21.000	71747016.163		

B.19   Systolic Pressure - Heat Pain Recovery

Mauchly's Test of Sphericity[a]

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b] Greenhouse-Geisser	Huynh-Feldt	Lower-bound
time	.816	4.017	5	.547	.873	1.000	.333

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	13.728	3	4.576	.304	.823
	Greenhouse-Geisser	13.728	2.619	5.242	.304	.796
	Huynh-Feldt	13.728	3.000	4.576	.304	.823
	Lower-bound	13.728	1.000	13.728	.304	.587
Error(time)	Sphericity Assumed	949.057	63	15.064		
	Greenhouse-Geisser	949.057	54.997	17.257		
	Huynh-Feldt	949.057	63.000	15.064		
	Lower-bound	949.057	21.000	45.193		

B.20   Diastolic Pressure - Heat Pain Recovery

Mauchly's Test of Sphericity[a]

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
						Epsilon[b]	
time	.808	4.194	5	.522	.871	1.000	.333

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	12.434	3	4.145	.493	.688
	Greenhouse-Geisser	12.434	2.612	4.760	.493	.663
	Huynh-Feldt	12.434	3.000	4.145	.493	.688
	Lower-bound	12.434	1.000	12.434	.493	.490
Error(time)	Sphericity Assumed	529.144	63	8.399		
	Greenhouse-Geisser	529.144	54.853	9.647		
	Huynh-Feldt	529.144	63.000	8.399		
	Lower-bound	529.144	21.000	25.197		

B.21　Heart Rate - Heat Pain Recovery

Mauchly's Test of Sphericity[a]

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b] Greenhouse-Geisser	Huynh-Feldt	Lower-bound
time	.671	7.864	5	.164	.797	.906	.333

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	21.116	3	7.039	.450	.718
	Greenhouse-Geisser	21.116	2.390	8.835	.450	.675
	Huynh-Feldt	21.116	2.718	7.769	.450	.700
	Lower-bound	21.116	1.000	21.116	.450	.510
Error(time)	Sphericity Assumed	985.972	63	15.650		
	Greenhouse-Geisser	985.972	50.190	19.645		
	Huynh-Feldt	985.972	57.075	17.275		
	Lower-bound	985.972	21.000	46.951		

B.22   Burst Frequency - Heat Pain Recovery

Mauchly's Test of Sphericity[a]

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
						Epsilon[b]	
time	.638	8.859	5	.115	.765	.864	.333

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	175.623	3	58.541	1.376	.258
	Greenhouse-Geisser	175.623	2.294	76.546	1.376	.263
	Huynh-Feldt	175.623	2.591	67.769	1.376	.261
	Lower-bound	175.623	1.000	175.623	1.376	.254
Error(time)	Sphericity Assumed	2680.484	63	42.547		
	Greenhouse-Geisser	2680.484	48.181	55.633		
	Huynh-Feldt	2680.484	54.421	49.254		
	Lower-bound	2680.484	21.000	127.642		

B.23   Burst Incidence - Heat Pain Recovery

Mauchly's Test of Sphericity[a]

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
time	.605	9.915	5	.078	.741	.832	.333

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	456.205	3	152.068	1.184	.323
	Greenhouse-Geisser	456.205	2.223	205.261	1.184	.318
	Huynh-Feldt	456.205	2.497	182.666	1.184	.321
	Lower-bound	456.205	1.000	456.205	1.184	.289
Error(time)	Sphericity Assumed	8090.550	63	128.421		
	Greenhouse-Geisser	8090.550	46.674	173.342		
	Huynh-Feldt	8090.550	52.447	154.261		
	Lower-bound	8090.550	21.000	385.264		

B.24   Total MSNA - Heat Pain Recovery

Mauchly's Test of Sphericity[a]

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
time	.885	2.399	5	.792	.926	1.000	.333

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	54293658.337	3	18097886.112	1.166	.330
	Greenhouse-Geisser	54293658.337	2.777	19553119.862	1.166	.329
	Huynh-Feldt	54293658.337	3.000	18097886.112	1.166	.330
	Lower-bound	54293658.337	1.000	54293658.337	1.166	.292
Error(time)	Sphericity Assumed	977935743.328	63	15522789.577		
	Greenhouse-Geisser	977935743.328	58.311	16770962.271		
	Huynh-Feldt	977935743.328	63.000	15522789.577		
	Lower-bound	977935743.328	21.000	46568368.730		

C   Tests of Normality

Variable Name	Data
cs_sap	Baseline systolic pressure for cool sensation
cs_dap	Baseline diastolic pressure for cool sensation
cs_hr	Baseline heart rate for cool sensation
cs_fre	Baseline MSNA burst frequency for cool sensation
cs_inc	Baseline MSNA burst incidence for cool sensation
cs_amp	Baseline total MSNA for cool sensation
ws_sap	Baseline systolic pressure for warm sensation
ws_dap	Baseline diastolic pressure for warn sensation
ws_hr	Baseline heart rate for warm sensation
ws_freq	Baseline MSNA burst frequency for warm sensation
ws_inc	Baseline MSNA burst incidence for warm sensation
ws_amp	Baseline total MSNA for warm sensation
hp_sap	Baseline systolic pressure for heat pain
hp_dap	Baseline diastolic pressure for heat pain
hp_hr	Baseline heart rate for heat pain
hp_fre	Baseline MSNA burst frequency for heat pain
hp_inc	Baseline MSNA burst incidence for heat pain
hp_amp	Baseline total MSNA for heat pain

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
cs_sap	.115	23	.200[*]	.974	23	.777
cs_dap	.165	23	.105	.940	23	.177
cs_hr	.115	23	.200[*]	.987	23	.985
cs_fre	.145	23	.200[*]	.923	23	.077
cs_inc	.168	23	.090	.900	23	.025
cs_amp	.186	23	.038	.915	23	.053

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
ws_sap	.166	22	.118	.933	22	.141
ws_dap	.153	22	.199	.942	22	.215
ws_hr	.194	22	.031	.961	22	.501
ws_freq	.152	22	.200[*]	.949	22	.300
ws_inc	.181	22	.059	.878	22	.011
ws_amp	.165	22	.123	.894	22	.023

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
hp_sap	.116	23	.200[*]	.982	23	.934
hp_dap	.163	23	.115	.930	23	.111
hp_hr	.098	23	.200[*]	.979	23	.886
hp_fre	.200	23	.018	.912	23	.044
hp_inc	.239	23	.001	.895	23	.020
hp_amp	.218	23	.006	.896	23	.021

D   Tests on Normally Distributed Data

Variable Name	Data
cs_sap_base	Systolic pressure during cool sensation baseline period
cs_sap_rec	Systolic pressure during cool sensation recovery period
cs_dap_base	Diastolic pressure during cool sensation baseline period
cs_dap_rec	Diastolic pressure during cool sensation recovery period
cs_hr_base	Heart rate during cool sensation baseline period
cs_hr_rec	Heart rate during cool sensation recovery period
cs_freq_rec	MSNA burst frequency during cool sensation baseline period
cs_fre_rec	MSNA burst frequency during cool sensation recovery period
ws_s_base	Systolic pressure during warm sensation baseline period
ws_s_rec	Systolic pressure during warm sensation recovery period
ws_d_base	Diastolic pressure during warm sensation baseline period
ws_d_rec	Diastolic pressure during warm sensation recovery period
ws_h_base	Heart rate during warm sensation baseline period
ws_h_rec	Heart rate during warm sensation recovery period
ws_f_base	MSNA burst frequency during warm sensation baseline period
ws_f_rec	MSNA burst frequency during warm sensation recovery period

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	cs_sap_base - cs_sap_rec	1.05217	1.60846	.33539	.35662	1.74772	3.13	22	.005
Pair 2	cs_dap_base - cs_dap_rec	1.05870	1.12643	.23488	.57159	1.54580	4.50	22	.000
Pair 3	cs_map_base - cs_map_rec	-.69674	1.52694	.31839	-1.35704	-.03644	-2.18	22	.040

Pair 4	cs_freq_base - cs_freq_rec	2.26522	2.76908	.57739	1.06778	3.46266	3.92	22	.001
Pair 5	ws_s_base - ws_s_rec	-.50682	2.64011	.56287	-1.67738	.66374	-.90	21	.378
Pair 6	ws_d_base - ws_d_rec	.06818	1.32234	.28192	-.51811	.65447	.24	21	.811
Pair 7	ws_h_base - ws_h_rec	-.74205	2.37722	.50682	-1.79604	.31195	-1.46	21	.158
Pair 8	ws_f_base - ws_f_rec	3.81591	5.32804	1.13594	1.45359	6.17823	3.359	21	.003

D.1 Repeated Measures Test on Systolic Pressure during Heat Pain

Mauchly's Test of Sphericity[a]

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b] Greenhouse-Geisser	Epsilon[b] Huynh-Feldt	Epsilon[b] Lower-bound
time	.913	1.909	2	.385	.920	1.000	.500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	57.730	2	28.865	5.420	.008
	Greenhouse-Geisser	57.730	1.840	31.373	5.420	.010
	Huynh-Feldt	57.730	2.000	28.865	5.420	.008
	Lower-bound	57.730	1.000	57.730	5.420	.029
Error(time)	Sphericity Assumed	234.320	44	5.325		
	Greenhouse-Geisser	234.320	40.483	5.788		
	Huynh-Feldt	234.320	44.000	5.325		
	Lower-bound	234.320	22.000	10.651		

Pairwise Comparisons

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b] Lower Bound	95% Confidence Interval for Difference[b] Upper Bound
1	2	-1.283	.719	.088	-2.775	.208
	3	.949	.572	.112	-.238	2.136
2	1	1.283	.719	.088	-.208	2.775
	3	2.232*	.738	.006	.702	3.762
3	1	-.949	.572	.112	-2.136	.238
	2	-2.232*	.738	.006	-3.762	-.702

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

D.2   Repeated Measures Test on Diastolic Pressure during Heat Pain

Mauchly's Test of Sphericity[a]

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b] Greenhouse-Geisser	Epsilon[b] Huynh-Feldt	Epsilon[b] Lower-bound
time	.977	.478	2	.787	.978	1.000	.500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	15.748	2	7.874	2.911	.065
	Greenhouse-Geisser	15.748	1.956	8.051	2.911	.066
	Huynh-Feldt	15.748	2.000	7.874	2.911	.065
	Lower-bound	15.748	1.000	15.748	2.911	.102
Error(time)	Sphericity Assumed	119.020	44	2.705		
	Greenhouse-Geisser	119.020	43.031	2.766		
	Huynh-Feldt	119.020	44.000	2.705		
	Lower-bound	119.020	22.000	5.410		

Pairwise Comparisons

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b] Lower Bound	Upper Bound
1	2	-.429	.488	.389	-1.441	.583
	3	.728	.451	.121	-.207	1.663
2	1	.429	.488	.389	-.583	1.441
	3	1.157*	.514	.035	.091	2.223
3	1	-.728	.451	.121	-1.663	.207
	2	-1.157*	.514	.035	-2.223	-.091

D.3   Repeated Measures Test on Heart Rate during Heat Pain

Mauchly's Test of Sphericity[a]

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b] Greenhouse-Geisser	Huynh-Feldt	Lower-bound
time	.798	4.736	2	.094	.832	.892	.500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	95.603	2	47.802	10.756	.000
	Greenhouse-Geisser	95.603	1.664	57.452	10.756	.000
	Huynh-Feldt	95.603	1.784	53.598	10.756	.000
	Lower-bound	95.603	1.000	95.603	10.756	.003
Error(time)	Sphericity Assumed	195.542	44	4.444		
	Greenhouse-Geisser	195.542	36.609	5.341		
	Huynh-Feldt	195.542	39.241	4.983		
	Lower-bound	195.542	22.000	8.888		

Pairwise Comparisons

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b] Lower Bound	95% Confidence Interval for Difference[b] Upper Bound
1	2	1.279	.731	.094	-.237	2.795
	3	-1.598[*]	.483	.003	-2.601	-.596
2	1	-1.279	.731	.094	-2.795	.237
	3	-2.877[*]	.625	.000	-4.175	-1.580
3	1	1.598[*]	.483	.003	.596	2.601
	2	2.877[*]	.625	.000	1.580	4.175

82

E   Statistics on Transformed Data

Variable Name	Data
cs_inc_base	Transformed MSNA burst incidence during cool sensation baseline period
cs_inc_recovery	Transformed MSNA burst incidence during cool sensation recovery period
cs_amp_base	Transformed total MSNA during cool sensation baseline period
cs_amp_recovery	Transformed total MSNA during cool sensation recovery period
ws_inc_base	Transformed MSNA burst incidence during warm sensation baseline period
ws_inc_recovery	Transformed MSNA burst incidence during warm sensation recovery period
ws_amp_base	Transformed total MSNA during warm sensation baseline period
ws_amp_recovery	Transformed total MSNA during warm sensation recovery period

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	cs_inc_base	1.3506	23	.26326	.05489
	cs_inc_recovery	1.2756	23	.27557	.05746
Pair 2	cs_amp_base	1.6912	23	.27144	.05660
	cs_amp_recovery	1.6261	23	.27276	.05687
Pair 3	ws_inc_base	1.3581	22	.28551	.06087
	ws_inc_recovery	1.2985	22	.28724	.06124
Pair 4	ws_amp_base	1.6907	22	.29219	.06229
	ws_amp_recovery	1.6552	22	.27474	.05857

Paired Samples Test

| | | Paired Differences | | | | | | | |
| | | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference | | t | df | Sig. (2-tailed) |
					Lower	Upper			
Pair 1	cs_inc_base - cs_inc_recovery	.07501	.07743	.01615	.04152	.10849	4.646	22	.000
Pair 2	cs_amp_base - cs_amp_recovery	.06505	.07314	.01525	.03342	.09668	4.265	22	.000
Pair 3	ws_inc_base - ws_inc_recovery	.05963	.09893	.02109	.01577	.10349	2.827	21	.010
Pair 4	ws_amp_base - ws_amp_recovery	.03540	.08122	.01732	-.00061	.07141	2.045	21	.054

E.1 Repeated Measures Test on Transformed Burst Frequency during Heat Pain

Mauchly's Test of Sphericity[a]

| Within Subjects Effect | Mauchly's W | Approx. Chi-Square | df | Sig. | Epsilon[b] | | |
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
time	.256	28.614	2	.000	.573	.584	.500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	.297	2	.148	6.186	.004
	Greenhouse-Geisser	.297	1.147	.259	6.186	.017
	Huynh-Feldt	.297	1.169	.254	6.186	.016
	Lower-bound	.297	1.000	.297	6.186	.021
Error(time)	Sphericity Assumed	1.056	44	.024		
	Greenhouse-Geisser	1.056	25.229	.042		
	Huynh-Feldt	1.056	25.717	.041		
	Lower-bound	1.056	22.000	.048		

Pairwise Comparisons

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	.140*	.044	.004	.048	.231
	3	.001	.025	.962	-.050	.052
2	1	-.140*	.044	.004	-.231	-.048
	3	-.139*	.061	.033	-.265	-.012
3	1	-.001	.025	.962	-.052	.050
	2	.139*	.061	.033	.012	.265

E.2 Repeated Measures Test on Transformed Burst Incidence during Heat Pain

Mauchly's Test of Sphericity[a]

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
						Epsilon[b]	
time	.244	29.627	2	.000	.569	.580	.500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	.298	2	.149	4.983	.011
	Greenhouse-Geisser	.298	1.139	.262	4.983	.031
	Huynh-Feldt	.298	1.160	.257	4.983	.030
	Lower-bound	.298	1.000	.298	4.983	.036
Error(time)	Sphericity Assumed	1.315	44	.030		
	Greenhouse-Geisser	1.315	25.056	.052		
	Huynh-Feldt	1.315	25.516	.052		
	Lower-bound	1.315	22.000	.060		

Pairwise Comparisons

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	.145*	.050	.008	.041	.249
	3	.012	.026	.647	-.042	.067
2	1	-.145*	.050	.008	-.249	-.041
	3	-.133	.068	.063	-.274	.008
3	1	-.012	.026	.647	-.067	.042
	2	.133	.068	.063	-.008	.274

E.3 Repeated Measures Test on Total MSNA during Heat Pain

Mauchly's Test of Sphericity[a]

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
time	.144	40.673	2	.000	.539	.545	.500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	.517	2	.259	5.858	.006
	Greenhouse-Geisser	.517	1.078	.480	5.858	.022
	Huynh-Feldt	.517	1.089	.475	5.858	.021
	Lower-bound	.517	1.000	.517	5.858	.024
Error(time)	Sphericity Assumed	1.943	44	.044		
	Greenhouse-Geisser	1.943	23.709	.082		
	Huynh-Feldt	1.943	23.960	.081		
	Lower-bound	1.943	22.000	.088		

Pairwise Comparisons

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b] Lower Bound	Upper Bound
1	2	.192*	.062	.005	.065	.320
	3	.018	.028	.520	-.040	.077
2	1	-.192*	.062	.005	-.320	-.065
	3	-.174*	.083	.049	-.347	-.001
3	1	-.018	.028	.520	-.077	.040
	2	.174*	.083	.049	.001	.347

F   Statistics on Sex Differences

Variable Name	Data
cool_sap	Systolic pressure during cool sensation recovery
cool_dap	Diastolic pressure during cool sensation recovery
cool_hr	Heart rate during cool sensation recovery
cool_freq	MSNA frequency during cool sensation recovery
warm_sap	Systolic pressure during warm sensation recovery
warm_dap	Diastolic pressure during warm sensation recovery
warm_hr	Heart rate during warm sensation recovery
warm_freq	MSNA frequency during warm sensation recovery

| | | Levene's Test for Equality of Variances | | | | | | |
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
cool_sap	Equal variances assumed	.013	.909	1.695	21	.105	1.09280	.64471
	Equal variances not assumed			1.700	20.987	.104	1.09280	.64278
cool_dap	Equal variances assumed	1.085	.310	.933	21	.361	.44083	.47230
	Equal variances not assumed			.945	20.369	.356	.44083	.46665
cool_hr	Equal variances assumed	1.306	.266	-1.253	21	.224	-.78917	.62983
	Equal variances not assumed			-1.272	19.888	.218	-.78917	.62063

cool_freq	Equal variances assumed	.105	.749	-.283	21	.780	-.33409	1.18083
	Equal variances not assumed			-.285	20.868	.778	-.33409	1.17174
warm_sap	Equal variances assumed	4.198	.054	1.898	20	.072	2.01636	1.06239
	Equal variances not assumed			1.898	14.609	.078	2.01636	1.06239
warm_dap	Equal variances assumed	.612	.443	1.403	20	.176	.77455	.55190
	Equal variances not assumed			1.403	18.491	.177	.77455	.55190
warm_hr	Equal variances assumed	.161	.692	.495	20	.626	.51091	1.03286
	Equal variances not assumed			.495	19.907	.626	.51091	1.03286
warm_freq	Equal variances assumed	.149	.703	.112	20	.912	.26000	2.32769
	Equal variances not assumed			.112	19.315	.912	.26000	2.32769

F.1 Repeated Measures Test on Transformed Burst Incidence during Cool Sensation

Mauchly's Test of Sphericity^a

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon^b Greenhouse-Geisser	Huynh-Feldt	Lower-bound
time	1.000	.000	0	.	1.000	1.000	1.000

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	.066	1	.066	22.821	.000
	Greenhouse-Geisser	.066	1.000	.066	22.821	.000
	Huynh-Feldt	.066	1.000	.066	22.821	.000
	Lower-bound	.066	1.000	.066	22.821	.000
time * sex_cool	Sphericity Assumed	.005	1	.005	1.748	.200
	Greenhouse-Geisser	.005	1.000	.005	1.748	.200
	Huynh-Feldt	.005	1.000	.005	1.748	.200
	Lower-bound	.005	1.000	.005	1.748	.200
Error(time)	Sphericity Assumed	.061	21	.003		
	Greenhouse-Geisser	.061	21.000	.003		
	Huynh-Feldt	.061	21.000	.003		
	Lower-bound	.061	21.000	.003		

F.2 Repeated Measures Test on Transformed Total MSNA during Cool Sensation

Mauchly's Test of Sphericity^a

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon^b Greenhouse-Geisser	Huynh-Feldt	Lower-bound
time	1.000	.000	0	.	1.000	1.000	1.000

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	.050	1	.050	20.060	.000
	Greenhouse-Geisser	.050	1.000	.050	20.060	.000
	Huynh-Feldt	.050	1.000	.050	20.060	.000
	Lower-bound	.050	1.000	.050	20.060	.000
time * sex_cool	Sphericity Assumed	.006	1	.006	2.560	.125
	Greenhouse-Geisser	.006	1.000	.006	2.560	.125
	Huynh-Feldt	.006	1.000	.006	2.560	.125
	Lower-bound	.006	1.000	.006	2.560	.125
Error(time)	Sphericity Assumed	.052	21	.002		
	Greenhouse-Geisser	.052	21.000	.002		
	Huynh-Feldt	.052	21.000	.002		
	Lower-bound	.052	21.000	.002		

F.3 Repeated Measures Test on Transformed Burst Incidence during Warm Sensation

Mauchly's Test of Sphericity[a]

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b] Greenhouse-Geisser	Huynh-Feldt	Lower-bound
time	1.000	.000	0	.	1.000	1.000	1.000

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	.039	1	.039	7.952	.011
	Greenhouse-Geisser	.039	1.000	.039	7.952	.011
	Huynh-Feldt	.039	1.000	.039	7.952	.011
	Lower-bound	.039	1.000	.039	7.952	.011
time * sex_cool	Sphericity Assumed	.004	1	.004	.890	.357
	Greenhouse-Geisser	.004	1.000	.004	.890	.357
	Huynh-Feldt	.004	1.000	.004	.890	.357
	Lower-bound	.004	1.000	.004	.890	.357
Error(time)	Sphericity Assumed	.098	20	.005		
	Greenhouse-Geisser	.098	20.000	.005		
	Huynh-Feldt	.098	20.000	.005		
	Lower-bound	.098	20.000	.005		

F.4 Repeated Measures Test on Transformed Total MSNA during Warm Sensation

Mauchly's Test of Sphericity[a]

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b] Greenhouse-Geisser	Huynh-Feldt	Lower-bound
time	1.000	.000	0	.	1.000	1.000	1.000

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	.014	1	.014	4.192	.054
	Greenhouse-Geisser	.014	1.000	.014	4.192	.054
	Huynh-Feldt	.014	1.000	.014	4.192	.054
	Lower-bound	.014	1.000	.014	4.192	.054
time * sex_cool	Sphericity Assumed	.003	1	.003	1.057	.316
	Greenhouse-Geisser	.003	1.000	.003	1.057	.316
	Huynh-Feldt	.003	1.000	.003	1.057	.316
	Lower-bound	.003	1.000	.003	1.057	.316
Error(time)	Sphericity Assumed	.066	20	.003		
	Greenhouse-Geisser	.066	20.000	.003		
	Huynh-Feldt	.066	20.000	.003		
	Lower-bound	.066	20.000	.003		

Variable Name	Data
cs_inc_base	Transformed MSNA incidence during cool sensation baseline
cs_inc_recovery	Transformed MSNA incidence during cool sensation recovery
cs_amp_base	Transformed Total MSNA during cool sensation baseline
cs_amp_recovery	Transformed Total MSNA during cool sensation recovery
ws_inc_base	Transformed MSNA incidence during warm sensation baseline
ws_inc_recovery	Transformed MSNA incidence during warm sensation recovery
ws_amp_base	Transformed Total MSNA during warm sensation baseline
ws_amp_recovery	Transformed Total MSNA during warm sensation recovery

F.5   Independent Samples T-Test for Transformed Burst Incidence and Total MSNA during Cool Sensation

Independent Samples Test

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | 95% Confidence Interval of the Difference | |
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
cs_inc_base	Equal variances assumed	.512	.482	2.231	21	.037	.22565	.10113	.01534	.43595
	Equal variances not assumed			2.210	19.282	.039	.22565	.10210	.01217	.43912
cs_inc_recovery	Equal variances assumed	.661	.425	2.618	21	.016	.26767	.10222	.05509	.48026
	Equal variances not assumed			2.590	18.983	.018	.26767	.10334	.05138	.48397
cs_amp_base	Equal variances assumed	.253	.620	2.297	21	.032	.23812	.10368	.02250	.45373
	Equal variances not assumed			2.274	19.237	.035	.23812	.10469	.01917	.45706
cs_amp_recovery	Equal variances assumed	.002	.966	2.896	21	.009	.28532	.09851	.08046	.49018
	Equal variances not assumed			2.881	20.159	.009	.28532	.09902	.07887	.49177

F.6 Independent Samples T-Test for Transformed Burst Incidence and Total MSNA during Warm Sensation

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means					95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
ws_inc_base	Equal variances assumed	.029	.866	1.509	20	.147	.17841	.11820	-.06815	.42497
	Equal variances not assumed			1.509	19.709	.147	.17841	.11820	-.06839	.42520
ws_inc_recovery	Equal variances assumed	.343	.565	1.139	20	.268	.13850	.12162	-.11520	.39220
	Equal variances not assumed			1.139	18.273	.270	.13850	.12162	-.11675	.39374
ws_amp_base	Equal variances assumed	.800	.382	1.979	20	.062	.23105	.11674	-.01247	.47458
	Equal variances not assumed			1.979	19.358	.062	.23105	.11674	-.01299	.47510

		.98 3	.33 3	1.74 9	20	.096	.19549	.11180	- .0377 2	.4287 0
ws_amp_recovery	Equal variances assumed									
	Equal variances not assumed			1.74 9	18.71 8	.097	.19549	.11180	- .0387 5	.4297 3

F.7 Repeated Measures Test on Systolic Pressure during Heat Pain

Mauchly's Test of Sphericity[a]

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b] Greenhouse-Geisser	Epsilon[b] Huynh-Feldt	Epsilon[b] Lower-bound
time	.912	1.832	2	.400	.920	1.000	.500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	56.332	2	28.166	5.339	.009
	Greenhouse-Geisser	56.332	1.839	30.632	5.339	.011
	Huynh-Feldt	56.332	2.000	28.166	5.339	.009
	Lower-bound	56.332	1.000	56.332	5.339	.031
time * sex	Sphericity Assumed	12.757	2	6.378	1.209	.309
	Greenhouse-Geisser	12.757	1.839	6.937	1.209	.307
	Huynh-Feldt	12.757	2.000	6.378	1.209	.309
	Lower-bound	12.757	1.000	12.757	1.209	.284
Error(time)	Sphericity Assumed	221.563	42	5.275		
	Greenhouse-Geisser	221.563	38.619	5.737		
	Huynh-Feldt	221.563	42.000	5.275		
	Lower-bound	221.563	21.000	10.551		

F.8 Repeated Measures Test on Diastolic Pressure during Heat Pain

Mauchly's Test of Sphericity[a]

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b] Greenhouse-Geisser	Epsilon[b] Huynh-Feldt	Epsilon[b] Lower-bound
time	.968	.658	2	.720	.969	1.000	.500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	15.686	2	7.843	2.877	.067
	Greenhouse-Geisser	15.686	1.937	8.097	2.877	.069
	Huynh-Feldt	15.686	2.000	7.843	2.877	.067
	Lower-bound	15.686	1.000	15.686	2.877	.105
time * sex	Sphericity Assumed	4.513	2	2.256	.828	.444
	Greenhouse-Geisser	4.513	1.937	2.329	.828	.441
	Huynh-Feldt	4.513	2.000	2.256	.828	.444
	Lower-bound	4.513	1.000	4.513	.828	.373
Error(time)	Sphericity Assumed	114.508	42	2.726		
	Greenhouse-Geisser	114.508	40.683	2.815		
	Huynh-Feldt	114.508	42.000	2.726		
	Lower-bound	114.508	21.000	5.453		

F.9   Repeated Measures Test on Heart Rate during Heat Pain

Mauchly's Test of Sphericity[a]

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
time	.771	5.200	2	.074	.814	.914	.500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	95.980	2	47.990	10.647	.000
	Greenhouse-Geisser	95.980	1.627	58.977	10.647	.001
	Huynh-Feldt	95.980	1.829	52.480	10.647	.000
	Lower-bound	95.980	1.000	95.980	10.647	.004

time * sex	Sphericity Assumed	6.233	2	3.117	.691	.506
	Greenhouse-Geisser	6.233	1.627	3.830	.691	.479
	Huynh-Feldt	6.233	1.829	3.408	.691	.495
	Lower-bound	6.233	1.000	6.233	.691	.415
Error(time)	Sphericity Assumed	189.309	42	4.507		
	Greenhouse-Geisser	189.309	34.176	5.539		
	Huynh-Feldt	189.309	38.407	4.929		
	Lower-bound	189.309	21.000	9.015		

F.10 Repeated Measures Test on Burst Frequency during Heat Pain

Mauchly's Test of Sphericity[a]

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
time	.382	18.274	2	.000	.618	.671	.500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	.191	2	.096	4.849	.013
	Greenhouse-Geisser	.191	1.236	.155	4.849	.030
	Huynh-Feldt	.191	1.343	.142	4.849	.027
	Lower-bound	.191	1.000	.191	4.849	.040
time * sex_cool	Sphericity Assumed	.031	2	.015	.786	.462
	Greenhouse-Geisser	.031	1.236	.025	.786	.410
	Huynh-Feldt	.031	1.343	.023	.786	.419
	Lower-bound	.031	1.000	.031	.786	.386
Error(time)	Sphericity Assumed	.788	40	.020		
	Greenhouse-Geisser	.788	24.725	.032		
	Huynh-Feldt	.788	26.858	.029		
	Lower-bound	.788	20.000	.039		

F.11 Repeated Measures Test on Burst Incidence during Heat Pain

Mauchly's Test of Sphericity[a]

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
time	.368	18.984	2	.000	.613	.665	.500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	.163	2	.082	3.870	.029
	Greenhouse-Geisser	.163	1.226	.133	3.870	.053
	Huynh-Feldt	.163	1.330	.123	3.870	.049
	Lower-bound	.163	1.000	.163	3.870	.063
time * sex_cool	Sphericity Assumed	.038	2	.019	.899	.415
	Greenhouse-Geisser	.038	1.226	.031	.899	.373
	Huynh-Feldt	.038	1.330	.029	.899	.380
	Lower-bound	.038	1.000	.038	.899	.354
Error(time)	Sphericity Assumed	.845	40	.021		
	Greenhouse-Geisser	.845	24.513	.034		
	Huynh-Feldt	.845	26.594	.032		
	Lower-bound	.845	20.000	.042		

F.12 Repeated Measures Test on Total MSNA during Heat Pain

Mauchly's Test of Sphericity[a]

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Epsilon[b] Huynh-Feldt	Lower-bound
time	.265	25.224	2	.000	.576	.620	.500

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	.250	2	.125	6.194	.005
	Greenhouse-Geisser	.250	1.153	.217	6.194	.017
	Huynh-Feldt	.250	1.240	.202	6.194	.015
	Lower-bound	.250	1.000	.250	6.194	.022
time * sex_cool	Sphericity Assumed	.027	2	.013	.663	.521
	Greenhouse-Geisser	.027	1.153	.023	.663	.445
	Huynh-Feldt	.027	1.240	.022	.663	.455
	Lower-bound	.027	1.000	.027	.663	.425
Error(time)	Sphericity Assumed	.808	40	.020		
	Greenhouse-Geisser	.808	23.056	.035		
	Huynh-Feldt	.808	24.791	.033		
	Lower-bound	.808	20.000	.040		

G Request for Permission to Reproduce Previously Published Material

G.1 Permission for Figure 1.

License Details

This Agreement between Michigan Technological University -- Hannah Cunningham ("You") and Springer Nature ("Springer Nature") consists of your license details and the terms and conditions provided by Springer Nature and Copyright Clearance Center.

Print Copy

License Number	4858260331626
License date	Jun 29, 2020
Licensed Content Publisher	Springer Nature
Licensed Content Publication	European Journal of Applied Physiology
Licensed Content Title	Local heat application to the leg reduces muscle sympathetic nerve activity in human
Licensed Content Author	Noriyo Takahashi et al
Licensed Content Date	Feb 12, 2011
Type of Use	Thesis/Dissertation
Requestor type	academic/university or research institute
Format	print and electronic
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	1
Will you be translating?	no
Circulation/distribution	1 - 29
Author of this Springer Nature content	no
Title	Graduate Student
Institution name	Michigan Technological University
Expected presentation date	Jul 2020
Portions	Figure 5
Requestor Location	Michigan Technological University 1400 Townsend Drive
	HOUGHTON, MI 49931 United States Attn: Michigan Technological University
Total	**0.00 USD**

G.2 Permission for Figure 2.

G.3　　Permission for Figure 3.

License Details

This Agreement between Michigan Technological University -- Hannah Cunningham ("You") and Springer Nature ("Springer Nature") consists of your license details and the terms and conditions provided by Springer Nature and Copyright Clearance Center.

Print　Copy

License Number	4858260426924
License date	Jun 29, 2020
Licensed Content Publisher	Springer Nature
Licensed Content Publication	European Journal of Applied Physiology
Licensed Content Title	Suppression of activation of muscle sympathetic nerve during non-noxious local cooling after the end of local cooling in normal adults
Licensed Content Author	Kazuya Ishida et al
Licensed Content Date	Feb 19, 2016
Type of Use	Thesis/Dissertation
Requestor type	academic/university or research institute
Format	print and electronic
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	1
Will you be translating?	no
Circulation/distribution	1 - 29
Author of this Springer Nature content	no
Title	Graduate Student
Institution name	Michigan Technological University
Expected presentation date	Jul 2020
Portions	Figure 4
Requestor Location	Michigan Technological University 1400 Townsend Drive
	HOUGHTON, MI 49931 United States Attn: Michigan Technological University
Total	0.00 USD