The Effects of Transcutaneous Electrical Nerve Stimulation (TENS) on Heart Rate Variability (HRV) in Response to Mechanical Cutaneous Nociception

By

Garrett Morrison

A Thesis
Submitted to
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In Partial Fulfillment of the Requirements for the Degree

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Abstract

The study of heart rate variability (HRV) has been proposed as an accurate measure of autonomic activity. To date, detailed data illustrating HRV responses to nociception does not exist. The use of HRV to study the effects of nociception on the autonomic nervous system (ANS) may further our understanding of this complex relationship and have the potential to serve as an objective indicator of pain. Further, the interaction between transcutaneous electrical nerve stimulation (TENS), an electrophysiological modality used for pain control, and the ANS is not well understood. It is well known that the ANS is involved in painful states usually through increases of sympathetic activity. Whether TENS affects the SNS, or produces analgesia through its effects on the SNS is unclear. Research investigating the effects of TENS on the ANS in response to nociception is also very limited. The primary objective of this study was to use time domain and frequency domain measures of HRV and skin conductance measures to evaluate the ANS in response to mechanical cutaneous nociception. The effects of TENS on the ANS responding to a sympathetic perturbation produced by a nociceptive stimulus was also studied. Athletes and non-athletes have been known to have different autonomic behaviors and characteristics, therefore differences in ANS behavior in response to noxious stimuli and TENS intervention was studied between athlete and non-athlete groups. METHODS: 10 participants (5 athletes, 5 non-athletes) completed three separate trials in random order. The three trials studied the effects of TENS on ANS, the effects of nociception on the ANS, and the effects of TENS following nociception on ANS. The Polar S810i heart rate

monitor was used for HRV acquisition. Skin conductance measures were recorded from the upper extremities as an index of autonomic (sympathetic) activity, and a small battery clip was used to pinch the participant's skin which produced the mechanical cutaneous nociceptive stimuli. RESULTS: A significant decrease in low frequency (LF) power was seen in the HRV frequency domain following mechanical cutaneous nocicpetion ($p<0.05$). No significant changes in time domain HRV were seen in response to the nociceptive stimulus. TENS failed to significantly affect HRV measures, both when applied to the unperturbed ANS and when applied to the perturbed ANS following the nociceptive stimulus. The mechanical cutaneous nociceptive stimulus produced significant increases in SNS activity seen through skin conductance (ipsilateral $p<0.001$, contralateral $p<0.001$). Application of TENS following exposure to nociception resulted in a prolonged duration of increased SNS activity when compared to SNS activity of nociception alone (ipsilateral; $p<0.023$, contralateral; $p<0.004$). A significant increase in the magnitude of SNS activity was seen on the contralateral side ($p<0.026$) when TENS followed nociception versus nociception alone. Higher values of SNS activity were seen ipsilaterally but not statistically significant ($p<0.067$). No significant differences were seen between athlete and non-athlete groups when HRV and skin conductance response to nociception and / or TENS was compared. CONCLUSIONS: The low frequency power component of HRV is affected by the application of a mechanical cutaneous nociceptive stimulus. TENS appears to increase and prolong SNS activity in response to acute mechanical cutaneous nociception. The effects of

TENS on the ANS may differ depending on the presence of autonomic perturbations. Further research is required to examine time domain measures of HRV as indices of autonomic behavior.

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Introduction

The focus of pain research has been growing rapidly over the past few years. New findings continue to add to our understanding of this complex topic and how it affects human beings. Many researchers have suggested that the Autonomic Nervous System (ANS) may play a significant role in the physiology of pain. Several publications present findings suggesting that the ANS is involved with the development of both acute and chronic painful conditions. Although our understanding of the pain and ANS relationship continues to develop, there remains a lack of clinical research in the rehabilitation field in terms of how modern medical treatments affect the ANS and pain. Studies performed in the rehabilitation field mainly utilize measures of skin conductance, skin temperature, photoplethsymography, blood pressure, and heart rate to monitor ANS functioning in response to a variety of treatments. Experiments using these techniques show little consistency in their results, thus, the use of interventions and their specific effects on the ANS remain in question.

The study of heart rate variability (HRV) has been proposed as an accurate measure of autonomic activity. Measures of HRV represent the summation of sympathetic and parasympathetic effects at the sinus node of the heart and it provides information towards dominant autonomic tones within the individual. Autonomic tones can be monitored in a number of physiological conditions such as exercise, psychological tasks, and pain. To date, detailed data illustrating HRV responses to nociception does not exist. The use of HRV to study the effects of

nociception on the ANS would provide new information in the pain research field. Transcutaneous Electrical Nerve Stimulation (TENS) is a treatment modality commonly used to treat many types of pain. Testing the effects of TENS on autonomic perturbations due to nociception would add to the depth of research in the area of the effects of TENS on the ANS. HRV along with typical measures of the ANS will provide a unique assessment battery that will permit precise characterization of ANS activity in response to nociception and TENS. It will also produce reliable and valid findings allowing more definitive conclusions with reference to the ANS and TENS to be made based on the best scientific information available. The ability to monitor ANS activity in response to a nociceptive stimulus and to test the effects of TENS on the nociceptive autonomic modulation offers a powerful experimental approach for studying the effects of TENS on the Sympathetic Nervous System (SNS) and pain (Reeves, Graff-Radford, & Shipman, 2004). This approach integrated with the use of HRV is novel, and will add important findings to the current body of knowledge in this research area.

The purpose of this study is to provide further understanding in the relationship between nociception and the autonomic nervous system in terms of sympathetic and parasympathetic responses to mechanical nociception. The effects of Transcutaneous Electrical Nerve Stimulation (TENS) on the autonomic nervous system responding to nociception will also be studied. HRV and skin conductance will be used to monitor ANS activity.

Literature Review

Sympathetic Nervous System and Pain

The ANS has been recognized as a significant part of the human response to pain. Hess and Brugger (1943) first described the defense reaction which is a general response of the SNS to stress. The defense reaction is an integrated response which consists of autonomic, endocrine and motor components. This general reaction would produce similar responses across various stressors characterized by:

- Increase in cardiac output
- Piloerection
- Sweat secretion
- Decrease blood flow through viscera and skin
- Catecholamine release
- Vasopressin and ACTH release

Any noxious, tissue-damaging stimulus, whether applied to the body surface, deep somatic tissue, or visceral structures, affects the SNS as outlined above (Janig, 1985).

Specific reactions to noxious stimuli have been reported in the presence of the defense reaction. Noxious stimulation of the skin (either thermal or mechanical) elicits well-defined reflexes in sympathetic neurons that supply skeletal muscle and skin, specifically sudomotor neurons are excited and cutaneous vasoconstrictor neurons are inhibited (Janig, 1985). Current research

techniques such as skin conductance measures and photoplethsymography (digital pulse volume) are intended to quantify these specific responses in painful situations and provide an index of autonomic (sympathetic) tone. These specific sympathetic reflexes are relayed through the spinal cord and may be controlled by descending pathways from the brain (Janig, 1985). Little understanding is known about how the descending pathways from supraspinal structures segmentally affect the SNS, although current research continues to add to our comprehension. Specific sympathetic reflexes such as sudomotor responses are controlled by descending pathways. Conventional measures of sympathetic activity may not reflect SNS activity accurately especially in treatment scenarios when multiple descending pathways become activated. HRV may serve as a more sensitive measure of widespread SNS activity.

Sympathetic dysfunction has been implicated in acute and chronic pain conditions. Changes in autonomic function have been documented in peripheral and central sensitization (Wright, 1999). Researchers believe that abnormal functioning of the SNS can possibly create and/or contribute to pathological pain. Possible links may exist between the experience of pain and alterations of sympathetic function and suggest that sympathetic outflow may influence or maintain afferent activity in nociceptive neurons (Campbell, Meyer, Davis, & Raja, 1992; Roberts, 1986). Studies by Thomas, Siahamis, Millicant, and Boyle (1992), and Mani, Cooper, Kidd, Cole, & Cawley (1989) found alterations in SNS function and abnormalities of somatosympathetic reflex responses in patients with

musculoskeletal disorders. Under normal physiological conditions, there appears to be no communication between sympathetic postganglionic neurons and afferent neurons (Janig & Koltzenburg, 1992). However in pathophysiological conditions, increased alpha-adrenergic sensitivity in injured nociceptors has been demonstrated experimentally (Janig, Levine, & Michaelis, 1996; Devor, 1995). Increased alpha-adrenergic sensitivity in nociceptors may lead to a hyperalgesic state in the presence of increased SNS function. This indirect hyperalgesia mediated by sympathetic postganglionic noradrenergic neurons has been referred to as sympathetic-dependant hyperalgesia (Levine, Yetunde, & Heller, 1992). It appears that noradrenaline acts to stimulate prostaglandin release, which in turn induces nociceptor sensitization (Janig et al., 1996). The development of nociceptor sensitization due to SNS dysfunction may lead to complex pain states involving central sensitization such as complex regional pain syndrome.

Research conducted in the area of pain and the ANS concur that the SNS plays a significant role in pathological pain states. Sympathetic dysfunction may influence or maintain peripheral and central sensitization. Clinicians who treat pain need to know the effects of their interventions on the ANS and more specifically the SNS for comprehensive treatment of painful conditions. Typical methods of SNS activity quantification may not reflect accurate SNS response during interventions or other physiological conditions due to influences from descending pathways. HRV may provide a better index to overall ANS response (widespread SNS effects vs. local SNS effects). Ignoring the ANS in a clinical

approach to pain treatment may undermine the clinician's primary purpose of understanding, controlling, and improving their client's pain.

Heart Rate Variability

Within the last decade, the study of heart rate variability (HRV) has become immensely popular among researchers. The use of HRV began as an objective measure in myocardial infarction reasearch used to identify sympathetic activity that could lead to heart beat irregularities. HRV is now recognized as a very informative measure of ANS functioning providing the researcher with insight to Sympathetic Nervous System (SNS) and Parasympathetic Nervous System (PNS) activity. The cardiovascular system is mostly controlled by autonomic regulation through the activity of sympathetic and parasympathetic pathways of the ANS (Aubert, Seps, & Beckers, 2003). Heart rate responds dynamically to physiologic perturbations mediated by these efferent parasympathetic and sympathetic nerve impulses (Appel, Berger, Saul, Smith, & Cohen, 1989). The HRV measure will change accordingly based upon specific sympathetic or parasympathetic effects. The basic time domain HRV measure is taken from a normal electrocardiogram strip. HRV reflects the variability of time between R-R intervals in milliseconds (msec) measured between consecutive R waves.

Kleiger, Stein, and Bigger Jr. (2005) define HRV as the cyclic fluctuation of the R-R intervals. Multiple studies show that SNS perturbations decrease HRV

(less variability between R-R intervals) while PNS perturbations increase HRV (more variability between R-R intervals) (Martinelli et al., 2005; Terkelsen, Molgaard, Hansen, Andersen, & Jensen, 2005; Lucini, Norbiato, Clerici, & Pagani, 2002).

HRV has emerged as the prevalent technique to assess autonomic influences. Support for its utility as an index of autonomic tone come from data which demonstrates that R-R interval variability is abolished following complete SNS and PNS pharmacological blockade (Goldberger, 1999; Ori, Monir, Weiss, Sahyouni, & Singer, 1992). Table 1 displays time domain parameters of HRV that are commonly used in research.

Time Domain Measures of HRV	
SDNN	Standard deviation of all normal to normal R-R (NN) intervals
SDANN	Standard deviation of 5-minute average NN intervals
ASDNN (index)	Mean of the standard deviation of all NN intervals for all 5 –minute segments in 24 hours
rMSSD	Square root of the mean of the squares of successive NN interval differences
NN50	The number of NN intervals differing by > 50 ms from the preceding interval
pNN50	The percentage of intervals > 50 ms different from preceding interval
Night-day difference	Mean night R-R interval minus mean day R-R interval

Table 1: Time domain measures of HRV. SDNN value is the time domain measure referred to throughout the document and used in the experiment.

Time domain measures of HRV should be considered only as the net result of all autonomic influences on the sinus node without regard to individual levels of parasympathetic and sympathetic tone or their modulation (Goldberger, 1999). It is not possible to objectify SNS or PNS responses or presume a specific relationship between sympathetic and parasympathetic tone using time domain measures (Kollai, Jokkel, Bonyhay, Tomcsanyi, & Naszlady, 1994). HRV measures are affected by barorecptor and chemoreceptor activity, muscle

afferents, local tissue metabolism, circulating hormones, circadian variations, and a number of environmental factors (Levy & Martin, 1979). Researchers must exercise caution when attempting to interpret what is truly being measured due to these possible co-existing variables.

The physiologic basis for HRV is seen at the sinus node. SNS and PNS stimuli arrive at the sinus node in the form of direct innervations from autonomic ganglia, and through autocrine, paracrine, and endocrine substances. The sinus node acts as the final summing element of sympathetic and parasympathetic stimuli and their instantaneous relation is reflected in the actual inter-beat interval (R-R interval) (Hejjel, 2001). The two different systems have different latent periods and time courses at the sinus node (Kollai & Koizumi, 1979; Pickering & Davis, 1973). These different latencies and time courses dictate how the SNS and PNS influences individually affect the length of the R-R interval. PNS influence on heart rate is mediated by release of acetylcholine on muscarinic receptors. The parasympathetic influence is brief in nature due to the high content of acetylcholinesterase at the sinus node. SNS influence is much slower and is mediated by the release of adrenaline and noradrenaline onto beta-adrenergic receptors which are far more widespread than parasympathetic innervation (Warner & Cow, 1962). Constant interaction at the sinus node between SNS and PNS in resting conditions where parasympathetic tone is dominant suggests that the R-R interval will experience rapid adjustments and thus high variability (increase HRV) due to the behavior of the muscarinic receptors. Upon a

sympathetic perturbation when the SNS influence is dominant at the sinus node (dominant beta adrenergic receptor activity), the R-R interval experiences slow adjustments and the overall result is a decrease in HRV.

The use of HRV as a measure of ANS activity in pain research remains a relatively unexplored area. We could find no publications to date reporting on the effects of pain on HRV. It is well known that the ANS responds to stressors in a stereotypical manner. Experiments performed by Lucini et al. (2002) illustrate the effects of a psychological stressor on HRV. Their results show a significant decrease in HRV when psychological stress is present. The decrease in HRV with stress is also accompanied by significant increase in salivary cortisol which is a well known marker associated with a SNS response. They conclude that individuals experiencing psychological stress have a clear activation of the hypothalamic-pituitary-adrenal axis and a shift toward a sympathetic dominant tone. A significant decrease in HRV concurrently with a sympathetic dominant tone in response to psychological stress has also been shown by other researchers (Terkelsen et al., 2005; Terkelsen, Andersen, Molgaard, Hansen, and Jensen, 2004).

HRV response to physical exercise as a stressor is another area that has been studied. Heart rate during exercise is regulated by increased sympathetic modulation and withdrawal of parasympathetic activity (Persson, 1996). A decrease in HRV is seen in healthy subjects during and immediately following

exercise (Raczak et al., 2005). Exercise stress causes hormones such as cortisol and catecholamines (norepinephrine) to significantly increase which corresponds with the stereotypical stress response seen across all stressful situations.

Montano et al. (1994) reports sympathetic modulations characterized by a decrease in HRV while subjecting individuals to the orthostatic test. The orthostatic test causes a baroreceptor reflex response that is mediated almost entirely by the SNS. HRV remains high in supine with parasympathetic activity dominant at rest. Orthostatic stress increases in standing, increasing sympathetic activity and therefore decreasing HRV.

Noxious stimuli and the resulting pain increase the activity of the sympathetic nervous system, thus, noxious stressors (sympathetic perturbations) would appear to produce similar responses in terms of HRV, to psychological stress, physical exercise type stress and orthostatic stress because of the stereotypical autonomic response. The above research supports a decrease in HRV in individuals who are experiencing stress from different origins. The decrease in HRV is associated with sympathetic dominant influences by the hormonal/catecholamine mediated stereotypical SNS response. The above premise suggests that a decrease in HRV will be seen in the presence of pain which is known to produce sympathetic modulations.

Review of the HRV literature indicates that time domain measures are a direct reflection of autonomic fluctuations. HRV may be a useful tool to examine these autonomic fluctuations under different physiological circumstances (Akselrod et al., 1981). Scientific grounds exist for the use of HRV in pain research to explore sympathetic and parasympathetic modulations in various pain and treatment situations. This novel use of HRV may lead to significant findings that would contribute to our understanding of pain, its treatment, and the ANS.

Autonomic Regulation: Athletes vs. Non-athletes

The study of HRV has been used by researchers to highlight differences in autonomic regulation in athletic and non-athletic populations. Many of these studies analyze HRV data through power spectral analysis in the frequency domain. Quantitative information on the autonomic control of the heart can be obtained from HRV power spectral analysis related to the separation of different components in various physiological conditions (Camm et al., 1996; Kamath & Fallen, 1993; Saul, 1990). Three components in the HRV power spectrum are commonly described:

- High Frequency (HF >0.15Hz) peak at respiratory frequency that corresponds to respiratory sinus arrhythmia
- Low Frequency (LF 0.04 -0.15Hz) peak centered at about 0.1Hz that is related to arterial pressure control
- Very Low Frequency (VLF <0.04Hz) considered to be an expression of the peripheral vasomotor regulation

A great deal of argument arises among researchers towards the significance of each of these components. It has been proposed that specific sympathetic and parasympathetic modulations can be identified by measuring the power of specific frequency oscillations. Although this type of analysis is common in physiological HRV studies, its validity as an indicator of specific sympathetic and parasympathetic modulations remains controversial. Recent publications continue to add to our understanding of the interpretation of power spectral analysis. Camm et al. (1996) have shown that agreement exists that the HF peak is a reasonable index of parasympathetic activity. Experiments by Perini and Veicsteinas (2003) examining HRV during different physiological conditions using frequency domain analysis provide further support that the HF peak represents parasympathetic activity.

The significance and the interpretation of the LF and the VLF oscillations are equivocal. Current understanding shows the LF spectrum representing a combination of parasympathetic activity and sympathetic effects believed to be from the baroreflex (Grasso, Schena, Gulli, & Cevese, 1997). The VLF spectrum has not received much attention from researchers and is believed to be an indicator of thermoregulation and other slow physiological processes.

Numerous researchers have examined the differences in HRV between athletic and non-athletic populations. Strong evidence demonstrates that

individuals who are physically active show "improved autonomic control". Improved autonomic control is distinguished by greater parasympathetic dominance at rest and during recovery from stress. Improved autonomic control (increased PNS effects) is beneficial due to various health risks associated with prolonged increase of SNS activity (Bernardi, Porta, Spicuzza, & Sleight, 2005). Studies by Aubert, Beckers, and Ramaekers (2001) show significantly higher time domain measures of HRV between athletes and sedentary groups at rest, these differences were also found in the frequency spectrum by significantly larger HF components in aerobically trained athletes. Experiments by Shin, Minamitani, Onishi, Yamazaki, and Lee (1997) used a spectral analysis approach to elucidate autonomic differences between athletes (aerobically trained) and non-athletes. Their results demonstrated significantly greater HF power in athletes compared to non-athletes at rest. They concluded that endurance training results in enhanced parasympathetic activity in athletes. Several other research groups have also concluded, through HRV measurement, that endurance training results in enhanced parasympathetic tone (Macor, Fagard, & Amery, 1996; Puig et al., 1993; Goldsmith, Bigger Jr., Steinman, & Fleiss, 1992). Similarly, Dixon, Kamath, McCartney, and Fallen (1992) present interesting data comparing endurance trained athletes to sedentary controls after exposure to exercise stress. Their results illustrate significantly higher HF power in athletes both at rest and after experiencing physical exercise (during recovery) when compared to sedentary controls.

Increased PNS activity at rest represents improved autonomic control among athletically trained individuals which is shown to have health benefits. Improved autonomic control in athletes also contributes to their ability to recover from exercise stress better than non-athletes. Athletes have the ability to return their autonomic tone to baseline levels after experiencing physical stress significantly faster than sedentary individuals, thus decreasing sympathetic effects and increasing parasympathetic effects. Noxious stimuli and the resulting pain is a known physical stressor causing a stereotypical sympathetic response. Athletically trained individuals may have the ability to recover from a noxious autonomic stressor faster than a sedentary individual as a result of their improved autonomic control. The ability to decrease sympathetic modulations due to noxious stimuli may prevent nociceptor sensitization and sympathetic-mediated hyperalgesia. Treatment modalities that affect the ANS (decrease SNS response) in individuals experiencing pain may be more efficacious in those who are athletically trained due to their ability to promptly attenuate SNS modulations.

The Effects of TENS on the Sympathetic Nervous System

TENS is an electrophysiological modality used for pain control. It is widely used in all types of health care facilities to treat pain arising from many different conditions. The use of TENS remains controversial with many studies presenting conflicting findings raising question to its clinical effectiveness. Neurophysiological mechanisms in which TENS exerts its effects have been illustrated best in animal models. Current paradigms regarding these mechanisms

arise from the gate control theory and endogenous opioid release (Sluka & Walsh, 2003). The interaction between TENS and the ANS is not well understood. It is well known that the ANS is involved in painful states, usually through increases of sympathetic activity. The SNS plays an important role in mediating pain, and whether TENS produces analgesia through its effects on the SNS is unclear (Reeves et al., 2004). Anatomically, relationships exist between sensory afferent pain fibers and sympathetic fibers. The nerves are distributed in similar regions in the viscera and cutaneous tissue and travel similar paths in the CNS. Pain fibers may course through sympathetic ganglia en route to the spinal cord (Stanton-Hicks, 1990). A physiological basis for TENS affecting the SNS may exist, and an increasing amount of research has focused on elucidating the effects of TENS on the SNS.

Most research performed in the area of TENS and the SNS use measures of skin conductance and peripheral blood flow (skin temperature and photoplethysmography). Coles, Donchin, and Porges (1986) state that the above techniques represent the gold standard in measurement of peripheral SNS activity. Skin conductance is a measure of sudomotor activity predominantly mediated by cholinergic stimulation of the SNS (Fowles et al., 1981). Peripheral blood flow in the finger tips is controlled almost entirely by sympathetic α-adrenergic stimulation causing peripheral vasoconstriction (Stallworth, Horne, & Plonk Jr., 1981). Skin temperature is the product of cutaneous blood flow which directly reflects autonomic (sympathetic) function. All of the above non-invasive

measures provide valid measures of ANS behavior for comparison across various research applications.

HRV as a measure of ANS behavior in response to TENS has been reported in a limited number of studies. Buoncore, Mortara, La Rovere, and Casale (1992) published their work illustrating the effects of TENS on HRV. Ten normal subjects were recruited and HRV data was measured before and after a 30 minute TENS treatment on the left forearm along the course of the median nerve. Subjects were not exposed to any autonomic perturbations, although subject positioning was not discussed as a possible orthostatic stressor. Their results showed no significant changes in time domain measures of HRV after TENS treatment. This finding suggests that TENS has no effect on overall ANS tone when individuals are at rest. Reeves et al. (2004) designed an experiment that would allow TENS to be tested on a dominant sympathetic tone in response to a psychophysiological perturbation (anticipation of noxious electrical shock). This study investigated the effects of high-frequency/low-intensity TENS, low-frequency/high-intensity TENS, and sham TENS on the perception of experimental pain and SNS function in healthy volunteers. The results of Reeves et al. (2004) show that the three TENS conditions failed to affect the psychophysiological SNS response and concluded that no support was found for TENS effecting ANS function in the experimental condition. Indergrand and Morgan (1994) reported that high frequency TENS, at intensities just above or below motor threshold, did not affect local blood flow, and thus TENS showed no

effect on the SNS in healthy volunteers. Several other studies show similar findings where TENS failed to exert an inhibitory effect on the SNS (Nolan, Hartsfield, Witters, & Watson, 1993; Johnson, Hajela, Ashton, & Thompson, 1991; Tracy, Currier, & Threlkeld, 1988). Conversely, several researchers have reported that TENS did have an affect on the SNS. Olyaei, Talebian, Hadian, Bagheri, and Momadjed (2004) presented results from an experiment examining the effect of TENS on sympathetic skin response. Skin conductance and skin temperature was recorded pre-TENS, immediately after TENS, 5 minutes post-TENS, and 10 minutes post-TENS ipsilateraly. After 20 minutes of TENS applied to the right wrist, They found a significant decrease in the latency and the amplitude of both skin conductance and skin temperature measures which were indicative of a decrease in SNS activity. Olyaei et al. (2004) concluded that TENS has an inhibitory effect on the SNS which leads to a decrease in overall sympathetic tone in healthy subjects. Electrical stimulation at intensities that could activate Aδ fibers was found to decrease SNS activity by increasing local blood flow and decreasing vascular resistance (Schaible & Grubb, 1993). Transient increases in blood flow with low frequency TENS were observed at the area of stimulation if the intensity exceeded the motor threshold (Sherry, Oehrlein, Hegge, & Morgan, 2001). Laser doppler imaging studies show changes in blood flow in response to TENS in healthy volunteers. Increases in blood flow were observed with either high or low frequency TENS set at intensities reported to be non-painful (Wilkstrom, Svedmen, Svensson, & Tanweer, 1999). Multiple investigators have also shown that TENS decreases sympathetic tone (Cramp,

Gilsenan, Lowe, & Walsh, 2000; Noble, Henderson, Cramp, Walsh, & Lowe, 2000; Currier et al., 1986).

A review of the literature on the effects of TENS on the ANS generates more questions than it does answers. Interpretation of the results among different studies remains extremely complicated. Comparison of the results across different studies is difficult due to variations in methodology, lack of appropriate controls, differences in electrode placement, differences in TENS stimulation parameters, positioning of, and type of physiological recording devices, and differences in treatment, stabilization, and baseline times (Cramp et al., 2000). It is possible that TENS may have little effect on the ANS in healthy subjects, but in situations where the ANS is perturbed, such as in response to noxious stimuli, application of TENS may show effects on the ANS. Research looking at the effects of TENS and its effects on the ANS in the presence of nociception is very limited. This direction of research would provide the most clinically relevant findings in regards to the use of TENS for acute pain control and its effects on noxious ANS perturbations. Critics of TENS affecting the SNS argue that TENS induced changes in SNS activity are localized to the site of stimulation which may be related to segmental innervations. If TENS truly affected the ANS/SNS the effects should be widespread in the individual. The use of HRV allows the measurement of systemic changes in autonomic tone as a more sensitive measure than previously used heart rate and blood pressure. HRV in conjunction with skin

conductance will provide a comprehensive measure to detect both local and widespread effects of TENS on ANS function.

Technique/Methodology Review

The study of HRV is traditionally performed through the use of an electrocardiogram (ECG). Ambulatory ECG units are common because of their ease of application into a variety of experimental paradigms. Recent literature supports the ECG method as the gold standard in collecting HRV data. One of the world's leading heart rate monitor manufacturers, Polar (Finland), has developed a portable heart rate monitor with the ability to record HRV data. The model S810i can store the duration (ms) of up to 30,000 R-R intervals. This heart rate monitor is relatively inexpensive compared to an ECG apparatus, it can be physically attached to individuals with little to no interference upon most activities and, it can be used in many different applications because of its compact size and ease of operation. The Polar S810i is starting to be incorporated into research by a number of different investigators due to the virtue of its versatility. To investigate the validity of HRV data collected from heart rate monitors, the Polar S810i was compared to an ambulatory ECG unit. Kingsley, Lewis, and Marson (2005) compared HRV data collected at rest and during a graded exercise test to volitional failure on both an ambulatory ECG and a Polar S810i heart rate monitor. Data was studied for agreement in time and frequency domain parameters. Their results showed no significant differences between the R-R intervals identified by S810i and ECG for any subject at rest or at any relative

exercise intensity. Agreement of the R-R interval duration between these two units was ±15 ms per R-R interval. They concluded that the Polar S810i and the ambulatory ECG provide consistent measurement of R-R intervals. This study supports the validity of R-R interval measurements on the Polar S810i heart rate monitor (Kingsley et al., 2005).

There are essentially two general methodologies by which HRV analysis can be performed, those being 24 hour ambulatory recordings and short-term (usually 5 to 15 minute) recordings. In physiological HRV research, the five minute recordings are usually employed to allow the investigator to immediately see specific responses to perturbations controlled within the experiment. Arguments surrounding the reliability of HRV in short-term recordings have surfaced due to discrepancies found within the literature. Sandercock, Bromley, and Brodie (2005) systematically reviewed the available literature concerning reliability of HRV data from short-term recordings and found that short-term HRV is a moderately reliable measurement. They express that most studies indicating poor reliability can be explained, at least in part by data collection protocols. The authors then report that there is evidence to suggest that optimal data collection conditions exist for specific phenomena, and potential investigators should bear this in mind during research design. Findings reported by Schroeder et al. (2004) support the use of at least five minute recordings when studying HRV. Their results are in agreement with previous guidelines set out by

the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (1996).

Respiration is intrinsically related to HRV measures. The heart and lungs receive autonomic innervations from common nerves and, respiratory rate is known to affect heart rate and thus HRV. There is currently no consensus as to whether respiratory rate should be controlled or left spontaneous in order to obtain reproducible HRV measures. Kowalewski and Urban (2004) outline that spontaneous respiration showed high reproducibility during HRV measurements made within the same day as well as in the short term and long term. Time domain parameters seem to be less affected by spontaneous respiration as compared to frequency domain measures, therefore, caution must be taken when attempting to quantify frequency oscillations in the power spectrum density if respiration is not controlled.

High reproducibility in HRV measures has been shown in studies using time domain parameters during spontaneous respiration. An experiment design that uses time domain parameters and is collected in five minute intervals for short term situations should produce accurate results and permit a valid and reliable indication of autonomic activity.

Summary

HRV has many diverse applications in its role as a measure of ANS activity. This valuable tool remains relatively new in the field of pain research. Its ability to identify dominant autonomic tones and provide an overall index to an individual's autonomic control will allow researchers to better understand ANS behavior in response to nociception and pain. It is well known that pain is a physical stressor that causes an increase in SNS activity. This increase in SNS activity as a result of pain may in fact influence or contribute to pathological painful conditions. The ability to affect sympathetic activity with a treatment intervention in a clinical setting may allow enhanced success in the treatment of pain. Research findings regarding the effects of TENS on the ANS in response to pain remain equivocal. The use of HRV to study the effects of TENS on the ANS in response to pain may provide a more sensitive dimension of autonomic assessment then the typical measures of autonomic (sympathetic) activity. HRV may also have the ability to reveal potentially unique autonomic responses in athletes compared to non-athletes in response to pain and TENS.

Purpose, Objectives and Hypothesis

Purpose

The purpose of this study was to provide further understanding in the relationship between nociception and the autonomic nervous system in terms of sympathetic and parasympathetic responses to mechanical nociception. HRV was used as a tool to measure the ANS response to nociceptive stimuli. The effects of Transcutaneous Electrical Nerve Stimulation (TENS) on the autonomic nervous system responding to nociception was also studied.

Objectives

1) To characterize autonomic cardiovascular modulations in response to a mechanical cutaneous nociceptive stimulus through time and frequency domain measures of heart rate variability (HRV).
2) To monitor local sympathetic responses to both mechanical cutaneous nociception through measures of skin conductance.
3) To examine the effects of TENS on autonomic cardiovascular modulations and skin conductance in response to mechanical cutaneous nociception in both athletic and non-athletic populations.

Hypothesis

I. A brief acute mechanical noxious stimulation will result in a recruitment of sympathetic activity (dominant sympathetic tone) which would be reflected

in increased skin conductance and decreased HRV, and potentially a change in the power spectrum seen within the frequency domain.

II. Athletes, that have a presumably increased parasympathetic bias, will have HRV changes to noxious stimulation that differ from the non-athelete who have presumably no such bias.

III. TENS will have no effect on sympathetic activity (skin or HRV) alone but will decrease the heightened sympathetic activity in response to the noxious stimulus, assuming TENS acts via decreasing the sympathetic response that could augment the pain.

Rationale of Study

The study of HRV as a quantitative indicator for pain is a unique approach that represents a new direction in research towards the understanding of pain and the autonomic nervous system. To date, we could find no documented studies of HRV in pain research. The above experiment provides valuable information on autonomic changes to nociception but also probes the effectiveness of a common modality used for pain control (TENS) and report on its efficacy towards autonomic aspects of pain. The results obtained may eventually assist clinicians in using the best approach to treating pain in terms of modality selection and configuration.

Limitations and Assumptions

The use of HRV as an index of autonomic tone is well supported in the literature. This study utilizes HRV to identify dominant autonomic tones in response to nociception and a treatment intervention directed towards pain. Time domain parameters of HRV (standard deviation) identify a dominant sympathetic or parasympathetic tone, however it is unclear whether a sympathetic dominant tone is a result of an increase in SNS activity or due to a decrease in PNS activity. Moreover, a reciprocal relationship between the two divisions can not be assumed.

Large hormonal fluctuations in females due to the menstrual cycle may affect HRV. This variable is not easily controlled and thus females were excluded from this study. Extrapolation of results towards this population must be made with caution. HRV is subject to variability from circadian rhythms and diurnal variations in hormones. All attempts have been made to insure all experimentation occured around a similar time of day in attempts to better control for these issues. Unfortunately the possibility of unexplained variation within the HRV data will always exist as a result of these factors.

Within this study, resting heart rate and a subjective report was used for stratification into athletic and non-athletic groups. Training bradycardia is a phenomenon seen within highly trained athletes that produces a resting heart below 60 beats per minute. It is considered rare for normal non-trained individuals to have resting heart rates below 60 beats per minute. Some subjects

may masquerade as athletes due to an innately low resting heart rate and be mistakenly classified into the athletic cluster. Although these individuals may report regular physical activity, they will not demonstrate the training adaptations and autonomic control seen in athletes with a true training bradycardia. This scenario may be confounding to the results of the experiment. Detailed metabolic assessment may be required to objectively classify athletes and non-athletes, which would allow for greater control within the experimental design.

It is well known that the training bradycardia is a result of endurance training. Strict resistance training regimes typically do not produce a training bradycardia in individuals. Individuals reporting regular participation in resistance training type activities with resting heart rates above 60 beats per minute were excluded. An accurate classification within the study's inclusion criteria is not possible, therefore this group may seriously confound the results. Individuals who report regular resistance training type activities with resting heart rates below 60 beats per minute were included in the athletic group. It was assumed that their training regime provided endurance type adaptations due to the presence of the training bradycardia.

The mechanical cutaneous nociceptive stimulus used in this study produces pain in most people. It is hoped that this model of pain will correlate with the pain in some conditions seen clinically. This of course is an assumption and in reality it is unknown whether pain seen clinically behaves physiologically

similar to the experimental pain applied in this study. Conclusions made based on experimental pain are helpful in furthering our understanding in this complex area. However, statements concerned with the behavior of clinical pain based on experimental pain are uncertain. There may also be different autonomic modulations with different modalities of nociceptive pain.

Notwithstanding the above mentioned limitations, this study provides novel findings in regards to ANS behavior in response to nociception and the effects of TENS. The findings are clinically relevant in terms of improving our understanding of the physiological effects of TENS and its use to treat pain.

Description of Paper

The Methods, Results, Discussion, and Conclusions parts of the thesis are presented in two separate manuscripts. The first manuscript entitled *Heart Rate Variability (HRV) in Response to Mechanical Cutaneous Nociception*, and the second manuscript entitled *The Effects of Transcutaneous Electrical Nerve Stimulation (TENS) on the Autonomic Nervous System Following Exposure to Acute Mechanical Cutaneous Nociception.* Manuscript 1 focuses on the experiment's first objective, that being to characterize autonomic cardiovascular modulations in response to a mechanical cutaneous nociceptive stimulus through time and frequency domain measures of heart rate variability (HRV), and compare differences between athlete and non-athlete groups within the above criteria. Manuscript 2 focuses on the remaining objectives, to monitor local sympathetic responses to both mechanical cutaneous nociception through measures of skin conductance, and examine the effects of TENS on the ANS in response to mechanical cutaneous nociception. A general discussion regarding both manuscripts follows the manuscripts.

Manuscript 1

Heart Rate Variability (HRV) in Response to Mechanical Cutaneous Nociception

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Abstract

The study of heart rate variability (HRV) has been proposed as an accurate measure of autonomic activity. To date, detailed data illustrating HRV responses to nociception does not exist. The use of HRV to study the effects of nociception on the autonomic nervous system (ANS) may further our understanding of this complex relationship and have the potential to serve as an objective indicator of pain. The primary objective of this study was to use time domain and frequency domain measures of HRV to evaluate the ANS responding to mechanical cutaneous nociception and, transcutaneous electrical nerve stimulation (TENS). Athletes and non-athletes have been shown to have different autonomic behaviors and characteristics, therefore differences in ANS behavior in response to nociceptive perturbations and TENS intervention was also studied between athlete and non-athlete groups. METHODS: 10 participants (5 athletes, 5 non-athletes) completed three separate trials in random order. The three trials studied the effects of TENS on ANS, the effects of nociception on the ANS, and the effects of TENS following nociception on ANS. The Polar S810i heart rate monitor was used for HRV acquisition, and a small battery clip was used to pinch the skin which produced a mechanical cutaneous nociceptive stimuli. RESULTS: A significant decrease in low frequency (LF) power was seen following mechanical cutaneous nocicpetion ($p<0.05$). No significant changes in time domain HRV were seen in response to the nociceptive stimulus. TENS failed to significantly affect HRV measures both when applied to the unperturbed ANS and when applied to the perturbed ANS following the nociceptive stimulus. No significant

differences were seen between athlete and non-athlete groups when HRV response to nociception and / or TENS was compared. CONCLUSIONS: The low frequency power component of HRV is affected by the application of a mechanical cutaneous nociceptive stimulus. Further research is required to examine time domain measures of HRV as indices of autonomic behavior.

Introduction

The study of heart rate variability (HRV) has been proposed as an accurate measure of autonomic activity. Measures of HRV represent the summation of sympathetic and parasympathetic effects at the sinus node of the heart and provide information towards dominant autonomic tones within the individual. Autonomic tones can be monitored in a number of physiological conditions such as exercise, psychological tasks, and pain. To date, detailed data illustrating HRV responses to nociception does not exist. The use of HRV to study the effects of nociception on the autonomic nervous system (ANS) would provide new information in the pain research field. Research conducted in the area of pain and the ANS concur that the sympathetic nervous system (SNS) plays a significant role in pathological pain states where sympathetic dysfunction can influence or maintain peripheral and central sensitization (Wright, 1999; Devor, 1995; Janig, & Koltzenburg, 1992). Clinicians who treat pain need to know the effects of their interventions on the ANS and more specifically the SNS for comprehensive treatment of pain. The study of HRV as a measure of ANS function may have the potential to serve as an objective indicator of pain.

The cardiovascular system is mostly controlled by autonomic regulation through the activity of sympathetic and parasympathetic pathways of the ANS (Aubert, Seps, & Beckers, 2003; Hainsworth, 1998). Heart rate responds dynamically to physiologic perturbations mediated by these efferent parasympathetic and sympathetic nerve impulses (Pumprla, Howorka, & Groves,

2002; Appel, Berger, Saul, Smith, & Cohen, 1989). The variability in time measured between consecutive R waves in milliseconds (msec) yields HRV between R-R intervals. The HRV measure will change accordingly based upon specific sympathetic or parasympathetic effects. Support for its utility as an index of autonomic tone comes from data which demonstrate that R-R interval variability is abolished following complete SNS and parasympathetic nervous system (PNS) pharmacological blockade (Goldberger, 1999; Ori, Monir, Weiss, Sahyouni, & Singer, 1992).

Multiple studies show that perturbations of the SNS decrease HRV (less variability between R-R intervals) while the PNS dominance results in increased HRV (Martinelli, Chacon-Mikahil, Martins, Lima-Filho, Golfetti, Paschoal, & Gallo-Junior, 2005; Terkelsen, Molgaard, Hansen, Andersen, & Jensen, 2005; Lucini, Norbiato, Clerici, & Pagani, 2002). Experiments performed by Lucini et al. (2002) illustrate the effects of a psychological stressor on HRV. They studied a well-established model of mild real life stress by measuring HRV, salivary cortisol, and cytokine profiles in university students both pre and post-university examination. Their results show a significant decrease in HRV when psychological stress is present. This decrease in HRV is also accompanied by significant increases in salivary cortisol which is a well known marker associated with a SNS response (Lucini et al., 2002). A significant decrease in HRV concurrent with a sympathetic dominant tone in response to psychological stress

has also been shown by other researchers (Terkelsen et al., 2005; Terkelsen, Andersen, Molgaard, Hansen, and Jensen, 2004).

HRV in response to physical exercise, as a physical stressor is another area of study that has been receiving more attention. Decreases in HRV are seen in healthy participants during and immediately following exercise (Raczak, Pinna, La Rovere, Maestri, Danilowicz-Szymanowicz, Ratkowski, Figura-Chmielewska, Szwoch, & Ambroch-Dorniak, 2005; Kamath, & Fallen, 1993). Exercise stress causes hormones such as cortisol and catecholamines (norepinephrine) to significantly increase which parallels the stereotypical ANS stress response. Montano, Ruscone, Porta, Lombardi, Pagani, and Malliani, (1994) report sympathetic modulations characterized by a decrease in HRV while subjecting individuals to an orthostatic test. The orthostatic test causes a baroreceptor reflex response that is mediated almost entirely by the SNS. HRV values in the time domain are higher in a supine position resembling a parasympathetic dominance. HRV readily decreases with exposure to orthostatic stress upon standing, thus reflecting a sympathetic dominance. The above research supports a decrease in HRV in response to sympathetic perturbations in individuals who are experiencing psychological, physical exercise and orthostatic stress. Noxious stimuli increases the activity of the sympathetic nervous system (Janig, 1985), thus, noxious stressors (sympathetic perturbations) should produce similar responses in terms of HRV because of the stereotypical autonomic response. This suggests that a decrease in HRV would be seen in response to nociception which

is known to produce sympathetically dominant modulations of the ANS (Terkelsen et al. 2004). The ability to characterize ANS behavior in response to noxious stimuli, and more importantly, evaluate the treatment effects of a modality used for pain control, would provide valuable insight into the relationship between pain, the ANS, and therapeutic effects of clinical modalities. Measures of skin conductance and peripheral blood flow (skin temperature and photoplethysmography) represent the gold standard in measurement of peripheral SNS activity (Coles, Donchin, and Porges, 1986). Skin conductance is a measure of sudomotor activity predominantly mediated by cholinergic stimulation of the SNS (Fowles, Christie, Edelberg, Grings, Lykken, & Venables, 1981). Skin conductance recordings provide valid measures of SNS behavior and will allow for meaningful comparison across research applications including HRV and experimental nociception.

The study of HRV has been used by many researchers to highlight differences in autonomic regulation in athletic and non-athletic populations. Many of these studies analyze HRV data through power spectral analysis in the frequency domain. Quantitative information on the autonomic control of the heart can be obtained from HRV power spectral analysis related to the separation of different components comprised of oscillation frequencies in various physiological conditions (Camm, Malik, Bigger, Brethardt, Cerutti, Cohen, Coumel, Fallen, Kennedy, Kleiger, Lombardi, Malliani, Moss, Rottman, Schmidt, Schwartz, & Singer, 1996; Kamath & Fallen, 1993; Saul, 1990). The HRV power

spectrum can be described using three frequency components. High Frequency (HF) components, >0.15Hz, peak at respiratory frequency that correspond to respiratory sinus arrhythmia. The Low Frequency (LF) components from 0.04 - 0.15Hz, have the peak centered at about 0.1Hz and are related to arterial pressure control. Finally, Very Low Frequency (VLF) components at <0.04Hz are considered to be an expression of peripheral vasomotor regulation.

It has been proposed that specific sympathetic and parasympathetic modulations can be identified by measuring the power of specific frequency oscillations. Camm et al. (1996) states that the HF peak is a reasonable index of parasympathetic activity after reviewing studies showing disappearance of the HF peak upon pharmacological blockade of cardiovascular parasympathetic receptors using atropine. Others using frequency domain analysis in examining HRV during different physiological conditions further support that the HF peak demonstrates parasympathetic activity (Perini and Veicsteinas, 2003). While there is general agreement on the interpretation of the HF components in the analysis of HRV, the significance and interpretation of the LF and the VLF oscillations are equivocal. Current understanding shows the LF spectrum represents a combination of parasympathetic activity and sympathetic effects conveyed via the baroreflex (Grasso, Schena, Gulli, & Cevese, 1997; Koh, Brown, Beightol, Ha, & Eckberg, 1994; Madwed, Albrecht, Mark, & Cohen, 1989). The VLF spectrum has not received much attention from researchers and is believed to be an indicator of thermoregulation and other slow physiological processes (Aubert et al., 2003).

Previous evidence demonstrates that individuals who are physically active display "improved autonomic control" through changes in heart rate control by means of neurocardiac mechanisms (Shin, Minamtani, Onishi, Yamazaki, & Lee, 1997; Dixon, Kamath, McCartney, & Fallen, 1992). Improved autonomic control is distinguished by greater parasympathetic dominance at rest and during recovery from stress. Improved autonomic control (increased PNS dominance) is thought to be beneficial because of the various health risks associated with prolonged increases of SNS activity (Bernardi, Porta, Spicuzza, & Sleight, 2005). Studies show significantly larger HF components in aerobically trained athletes (Aubert, Beckers, and Ramaekers, 2001; Shin, Minamitani, Onishi, Yamazaki, and Lee, 1997). A spectral analysis approach was used to elucidate autonomic differences between athletes (aerobically trained) and non-athletes. Significantly greater HF power was found in athletes compared to non-athletes at rest. Thus, endurance training resulted in enhanced parasympathetic activity in athletes (Shin, Minamitani, Onishi, Yamazaki, and Lee, 1997). Similarly, Dixon, Kamath, McCartney, and Fallen (1992) present interesting data comparing endurance trained athletes to sedentary controls after exposure to exercise stress. Their results illustrate significantly higher HF power in athletes both at rest and after experiencing physical exercise (during recovery) when compared to sedentary controls. Several other research groups have also concluded that endurance training results in enhanced parasympathetic tone as evidenced by higher proportions of the HF component (Macor, Fagard, & Amery, 1996; Puig, Freitas,

Carvalho, Puga, Ramos, Fernandes, Costa, & Freitas, 1993; Goldsmith, Bigger Jr., Steinman, & Fleiss, 1992). Thus, it appears that athletes have the ability to promptly return their autonomic tone to baseline levels after experiencing physical stress significantly faster than sedentary individuals. Athletically trained individuals may also recover from a noxious stressor faster than a sedentary individual as a result of their improved autonomic control. Similarly, the ability to decrease sympathetic modulations due to noxious stimuli may decrease nociceptor sensitization associated with increased SNS activity, and therefore decrease sympathetic-mediated hyperalgesia (sympathetic dysfunction). Treatment modalities that affect the ANS in individuals experiencing pain may have different effects in those who are athletically trained versus sedentary individuals due to differences in autonomic behavior between the two groups.

The objective of the present study was to characterize autonomic cardiovascular modulations in response to a mechanical cutaneous nociceptive stimulus through time and frequency domain measures of heart rate variability (HRV) in both athletic and non-athletic populations. Sympathetic responses to mechanical cutaneous nociception were monitored through measures of skin conductance. Finally, both the time and frequency domain measures of HRV and changes in skin conductance in response to this mechanical cutaneous nociception were used to assess the sympathetic response to transcutaneous electrical nerve stimulation (TENS), a commonly employed modality in the treatment of pain.

Participants

Ten male university students were recruited for the study. Five participants were recruited into the athlete group, and 5 participants were recruited into the non-athlete group. Subjects reporting participation in endurance type activities three times per week or greater with a resting heart rate below 60 beats per minute were placed in the athlete group. Individuals reporting no frequent participation in endurance type activities with resting heart rates above 65 beats per minute were included in the non-athlete group. Individuals reporting regular participation in endurance type activities with resting heart rates above 60 beats per minute were excluded from the study. The use of resting heart rate to distinguish between athletes and non-athletes is a simple method that exploits training bradycardia, a cardiovascular adaptation seen in endurance trained athletes. Participants were between the ages of 19 to 29 years, in good health and free of illness on testing days. Participants were excluded from the study with reports of any painful conditions, tobacco and/or alcohol addiction, drug or medication use, contraindications to TENS, or the presence of psychological distress. They were asked to maintain their normal daily routines during participation in the study. Prior to participation, all participants signed a written informed consent according to a protocol approved by the Faculty of Medicine Human Research Ethics Board of the University of Manitoba (H2005:136). Table 2 summarizes subject demographics.

Methods

HRV was recorded using the Polar S810i heart rate monitor (Polar Electro Oy, Finland). The chest strap was affixed to the subject at the levels of the 5th intercostal space. Data was transmitted from the chest transmitter to the wrist receiver which was positioned beside the subject to allow operation by investigators. All data was initially stored in the wrist receiver. Default settings were maintained for all participants as outlined by the manufacturer with the exception of recording rate which was set to "R-R interval". The watch function on the wrist receiver was used as the experiment timer. Stored data in the wrist receiver was uploaded to a PC through the Polar IR interface where data files were saved in Polar Precision Performance software (Polar Electro Oy, Finland) for offline analysis.

Skin conductance measurements were made using the Flexcomp Infiniti and SC-Flex/Pro skin conductance sensors (Thought Technology, Canada). Skin conductance was recorded in micro-Siemens (μS) through two electrodes placed on the participants 2nd and 5th digits of the hand bilaterally. The Flexcomp Infiniti encoder was linked to a PC where data was stored in the Biograph Infiniti software (Thought Technology, Canada). Skin conductance was sampled at 32 Hz.

A mechanical cutaneous nociceptive stimuli was applied using a small battery clip. The clip was attached to a pinch of skin on the dominant upper extremity within the C_7 dermatome (lateral elbow). Pressure applied to the skin

was monitored for consistency between participants by testing the clip on a pinch dynamometer. The clip used in this experiment produced 2.4 Kg of force on the pinch dynamometer (Therapeutic Instruments, USA). The pressure exerted on the pinch of skin produced significant local nociception.

The Dynex V (Empi, USA) was the TENS unit used in the experiment. Standard 2" x 2" carbon rubber electrodes were attached to the participant's skin using hypoallergenic conductive gel (Empi, USA) and surgical tape (3M, USA). Electrodes were positioned in a 2-channel cross-over configuration (interferential) within the C_7 dermatome centering the electrode configuration to the battery clip position. Positive polarity electrodes were positioned proximally. The Dynex V uses a pulsed current with a balanced, asymmetrical, biphasic waveform. The TENS parameters used can be defined as "conventional". Unit configurations were set as follows: Normal Stimulation Mode, 100 μs pulse duration, 100 Hz frequency/rate. Amplitude was set at "sensory plus" threshold which is defined by increasing intensity until a motor response is visualized, then lowering the intensity to the point where the motor response is no longer present. This method allows standardization between participants.

Subjective reports of pain were collected from the participants using a verbal numeric pain rating scale (VNPRS). Participants were asked verbally "on a scale of 0 to 10, zero being no pain and 10 being the worst pain imaginable, how would you rate your pain?" The Nottingham Health Profile (Appendix B) was

administered to all participants previous to acceptance into the study to screen for psychological distress that potentially could have affected ANS function. Participants were also required to answer a pre-trial questionnaire (Appendix A) preceding their participation in each trial in an attempt to better control factors which may affect ANS function, such as activity level, dietary intake, and sleep patterns previous to trial participation.

Protocol

All 10 participants completed the three trials in a randomized order. The study utilized a repeated measures design. All experiments took place in an isolated temperature controlled room (21°C ± 1°C) with minimal distractions/stimulation. Participants were reclined into a semi-supine position in an attempt to control for baroreflex activity across HRV recordings.

Three trials took place within the study: 1) *Effects of TENS on ANS*, 2) *Effects of nociception on ANS* and 3) *Effects of TENS following nociception on ANS.* The purpose of the *Effects of TENS on ANS* trial was to test the effects of TENS in the absence of any experimental ANS perturbations. The *Effects of nociception on ANS* trial examined the effects of experimental nociception on the ANS. The *Effects of TENS following nociception on ANS* trial focused on changes in ANS activity upon TENS application while the ANS responded to experimental nociception. Table 3 illustrates the trials and their respective periods.

1) *Effects of TENS on ANS*

Participants entered the isolated experiment room individually and had the heart rate monitor chest strap, skin conductance electrodes, and TENS electrodes physically attached. Participants were allowed 10 minutes to attain a baseline heart rate in the semi-supine position. Uninterrupted recording of HRV and skin conductance data then began for 60 minutes. The *effects of TENS on ANS* trial consisted of 4 periods; baseline, null, TENS, and post-TENS periods. The first 15 minutes (baseline period) was collected as baseline data. The following 5 minutes represents the null period which exists to compensate for the nociceptive period in subsequent trials. There are no perturbations during the null period. Following the null period, TENS was applied for a 20 minute duration (TENS period). Following the TENS application, recording continued for 20 minutes (post-TENS period). The investigator remained in the experiment room for the duration of the experiment. There was no communication between subject and investigator with the exception of the TENS amplitude set-up.

2) *Effects of nociception on ANS*

The *effects of nociception on ANS* trial consisted of 3 periods; baseline, nociception, and post-nociception periods. The first 15 minutes was collected as baseline data. The following 5 minutes represented the nociception period during which the battery clip was applied. Following the nociception period, the battery clip was removed and recording continued for 40 minutes (post-nociception

period). The TENS unit remained inoperable throughout this trial. Verbal numeric pain rating scale (VNPRS) scores were collected at the one and four minute marks within the nociception period, and at 5 minute intervals throughout the post-nociceptive period. There was no communication between subject and investigator with the exception of VNRPS score collection.

3) *Effects of TENS following nociception on ANS*

The *effects of TENS following nociception on ANS* trial consisted of 4 periods; baseline, nociception, TENS following nociception, and post-TENS following nociception periods. The first 15 minutes was collected as baseline data. The following 5 minute period represented the nociception period where the clip was applied as described previously. Immediately after the nociception period, TENS was applied for 20 minutes (TENS following nociception period). TENS was then stopped and recording continued for 20 minutes (post-TENS following nociception period). VNPRS scores were collected at the one and four minute marks within the nociceptive period, and at five minute intervals throughout the TENS following nociception period and the post-TENS following nociception period. There was no communication between subject and investigator with the exception of collecting VNPRS scores and the setting of TENS amplitude.

Data Analysis

All HRV data was processed using Polar Precision Performance software. An error correction filter was first applied to remove artifact due to gapping errors and/or interruptions in communication between the chest strap and the receiver. Very few errors were detected. Each 60 minute trial was divided into 5 minute blocks where a standard deviation (SD) value (ms) representing the variability of the R-R intervals within each respective five minute block was computed. SD values were exported to a spread sheet where they were normalized to 100% baseline value (15 minute block) within each trial and expressed as a percent of baseline. Power values (ms^2) within each frequency oscillation (VLF, LF, HF) were also computed. Power values were normalized to 100% total power within each 5 minute block. HF, LF, and VLF were expressed as a percentage of the total power within their respective 5 minute block. Power spectral analysis is performed through autoregressive modeling (maximum 18 coefficients) within the Polar Precision Performance software.

Skin conductance data was collected and saved within Biograph Infiniti software. Raw sensor recordings in μS were exported into a spreadsheet where data was divided into 5 minute blocks. Skin conductance data from the TENS period and the TENS following nociception period was found to show mild artifact due to TENS unit operation. Data within these periods were smoothed (16 point one side maximum smoothing width) to remove artifact using Diadem software (National Instruments, USA). Spreadsheet functions were used to determine the mean μ-Siemens value within each 5 minute block and then

normalized to 100% baseline value (15 minute block) within each trial and expressed as a percent of baseline.

Statistical Analysis

All statistical calculations were performed in Sigma Stat software (SPSS, USA). Two-way repeated measures of analysis of variance (ANOVA) were used to analyze time and frequency domain data for HRV, ipsilateral and contralateral skin conductance data and comparison between athlete versus non-athlete groups. Significant differences were accepted at $p<0.05$. When indicated, post-hoc Tukey's tests were used to locate significant differences between time blocks.

Results

Figure 1 presents time domain HRV data from all participants across the three trials. HRV is expressed as a standard deviation computed from the R-R intervals during each five minute block of time. All blocks are normalized to baseline (15 minute block) respective to each trial and presented as an average percent change of the SD. No significant changes to the HRV (SD) measure as an indicator of time domain HRV are seen within the three trials. Although there are fluctuations, HRV does not significantly change in response to the nociceptive stimulus or TENS used in this experiment. Figure 2 illustrates changes to heart rate over the different periods within the experiment. Data was divided into baseline, nociception, TENS following nociception, and recovery epochs from the *Effects of TENS following nociception on ANS* trial. No significant changes are

seen between any of the above epochs. Changes in average heart rate of the participants between epochs are less then 6 beats per minute.

Figure 3 demonstrates skin conductance activity recorded during the *Effects of nociception on ANS* trial to quantify SNS response to the noxious stimuli. Significant increases in skin conductance are seen within the nociception period (20 minute block) both ipsilaterally (226.2% ± 55.72 $p<0.001$) and contralaterally (213.5% ± 35.56 $p<0.001$) compared to baseline. Skin conductance remains significantly higher than baseline following the nociception period for 5 minutes (ipsilateral (177.4% ± 38.4 $p<0.040$), contralateral (159.4% ± 23.75 $p<0.012$) before gradually returning to baseline measures. There are no significant changes in skin conductance compared to baseline throughout the remainder of the experimental period. Note the mirror-like response of ipsilateral and contralateral skin conductance in response to clip application which would suggest application of the clip is a stimulus sufficient for activation of the SNS.

Figure 4 illustrates the analysis of HRV data in the frequency domain from the *Effects of nociception on ANS* trial. The three frequency components studied within HRV power spectral analysis are presented: Figure 4A HF (>0.15 Hz), Figure 4B LF (0.04 – 0.15 Hz) and Figure 4C VLF (<0.04 Hz). Power (ms^2) within each frequency range is normalized to the percent of total power within each five minute block throughout the trial. Baseline Period (5, 10, 15 minute blocks is compared to the five minute blocks over the nociception period (20

minute block) and the post-nociception period (25 to 60 minute blocks). Note that VLF (Fig. 4C) represents the bulk of the power spectrum in that over 88% of the total power falls within the VLF. Although there are no significant changes in percentages of the HF and VLF power spectrum with the application of the clip (Figs. 4A and 4C), a pronounced but not statistically significant decrease in HF power is observable in the nociceptive period (Fig. 4A, 30 minute block). There are however, significant LF changes within the *Effects of nociception on ANS* trial shown in Figure 4B. There is a decrease in the percentage of LF between the baseline period (4.91% ± 1.1), versus the post-nociception period 30 minute block (2.95% ± 0.61) $p<0.05$). In the attempt to further refine these results, post-hoc analysis was performed by way of a two-group comparison T-test's were used to compare mean normalized LF power between baseline and nociceptive perturbation epochs. Normalized LF power in each 5 minute block was averaged into two epochs; baseline epoch (5-15 minute blocks) and nociceptive perturbation epoch (20-60 minute blocks). Figure 5 illustrates a significant decrease in mean LF power in the nociceptive perturbation period (3.89% ± 0.69) compared to baseline (4.83% ± 0.12; $p<0.045$).

Figure 6 compares the three trials of the experiment in terms of HF, LF, and VLF behaviour. Figure 6A HF (>0.15 Hz), Figure 6B LF (0.04 – 0.15 Hz) and Figure 6C VLF (<0.04 Hz) show the comparison of each trial. Power (ms^2) within each frequency range is normalized to the percent of total power within each five minute block throughout the trial. Baseline Period (5, 10, 15 minute

blocks is compared to the five minute blocks over the nociception period (20 minute block) and the post-nociception period (25 to 60 minute blocks). Percent of total power within each 5 minute block for each frequency is averaged across the 10 participants. With the exception of LF in the *Effects of nociception on ANS* trial, neither the application of the mechanical cutaneous nociceptive stimulus nor TENS appears to affect HRV in the frequency domain.

Athlete and non-athlete groups were compared with respect to differences in HRV behaviour over the three trials in both the time and frequency domains. Comparison showed no significant differences between athlete and non-athlete groups in HRV time domain, HRV frequency domain, or skin conductance measures (repeated measures ANOVA). Figure 7 demonstrates time domain HRV (SD) data comparing athlete and non-athlete groups within each trial. Further post-hoc investigation was performed to allow a more thorough interpretation of athlete versus non-athlete differences in response to nociception. Figure 8 illustrates a two-group comparison approach used to compare baseline epochs and perturbation epochs. Normalized HRV (SD) data was averaged into a baseline epoch (5 -15 minutes blocks) and a perturbation epoch (20 – 60 minute blocks) within each trial respective to athlete and non-athlete groups. T-test's were used to identify differences between the epochs. No significant differences were seen between athletes versus non-athletes or baseline versus perturbation epochs.

Discussion

The primary objective of this study was to use HRV measures to evaluate the ANS response to mechanical cutaneous nociception and TENS. ANS behavior in response to nociception and TENS was also considered between athlete and non-athlete groups. HRV data was obtained via the Polar S810i heart rate monitor and analyzed in both time and frequency domains. Skin conductance data was recorded concurrently as a SNS indicator. The results of this study show the following: 1) There are no significant changes in time domain HRV (SD) in response to nociception. TENS failed to affect HRV (SD) both when applied to the unperturbed ANS and, when applied to the perturbed ANS following noxious stimuli. 2) A significant decrease in LF power is seen following mechanical cutaneous nociception. 3) No significant differences are seen between athlete and non-athlete groups when ANS response to TENS, nociception, and TENS following nociception are compared.

Upon review of time domain HRV data in Figure 1, no significant changes in HRV (SD) are observed throughout any of the three trials. Figure 1A shows HRV (SD) in response to TENS applied to an unperturbed ANS in healthy participants. Buonocore, Mortara, La Rovere, and Casale, (1992) performed a very similar experiment to the *Effects of TENS on ANS* trial. They tested the effects of TENS on 10 healthy participants while monitoring HRV (SD). Their results showed no significant changes in HRV parameters in response to TENS, however there was a small non-significant increase in HRV (SD) following TENS application. This same observation is also apparent within the *Effects of TENS on*

ANS trial in the present study. Figure 1A shows a slight but non-significant increase in HRV (SD) value throughout the TENS and post-TENS periods. Thus, our findings from the *Effects of TENS on ANS* trial coincide with the work of Buonocore et al. (1992) where it appears that TENS does not affect autonomic neural control of the heart in the unperturbed ANS.

Figures 1B and 1C illustrate the *Effects of nociception on ANS* and the *Effects of TENS following nociception on ANS* trials respectively. Within these trials the participants are exposed to a mechanical cutaneous nociceptive stimulus (nociception period). It has been well documented in the literature that a noxious stimulus will evoke dominant sympathetic activity from the ANS (Janig, 1985). Dominant SNS activity is characterized in the time domain by a decrease in HRV (SD), and would be the expected observation following exposure to a noxious stimulus. Few studies have looked at HRV in response to nociception. Terkelsen et al. (2004) studied HRV in the presence of experimental nociception in the form of electrical shock. They report no significant changes to HRV (SD) in response to electrical shock. Our findings illustrated in Figures 1B and 1C show no significant changes within the nociception or post-nocieption periods, similar to results reported by Terkelsen et al. (2004). It appears that experimental nociception, both in terms of electrical shock and mechanical cutaneous stimuli, fail to affect the HRV (SD) measure. In contrast, HRV (SD) has been shown to reliably decrease with increasing sympathetic activity upon psychological stress, orthostatic stress and exercise stress (Martinelli et al. 2005; Terkelsen et al. 2005;

Raczek et al. 2005; Lucini, Norbiato, Clerici, & Pagani, 2002). This contrast suggests that the HRV (SD) value may not be an appropriate measure for observing nociceptive perturbations. HRV (SD) may lack the necessary sensitivity to accurately characterize nociceptive perturbations of the ANS. This paradox may also indicate that autonomic cardiovascular modulations due to psychological, orthostatic and nociceptive stressors may differ depending on the specific perturbation.

Having said that, small increases in HRV (SD) are seen within the nociception period consistently in both trials (Figures 1B, 1C) suggesting perturbation due to the noxious stimulus, despite the non-significance. This observation is in contrast to previous research showing that dominant sympathetic activity produces a decrease in HRV (SD). When considered with data indicating the significant increase in skin conductance presented in Figure 3, the relationship between HRV (SD) and autonomic activity in response to mechanical cutaneous nociception should be considered unique. Figure 3 clearly shows that the SNS was activated by the nociceptive stimulus in that skin conductance, the gold standard measure increased over 200% from baseline values, substantially higher then other studies that have used skin conductance as a marker of SNS activity in pain (Sterling, Jull, & Wright (2001). The significant increase in SNS activity in this case is not seen within the HRV (SD) measure, in that there was no decrease in HRV (SD) but rather a small increase. This inconsistency adds to the argument that HRV (SD) may not be sensitive to ANS function in response to nociceptive

perturbations. HRV is affected by many factors including baroreceptor and chemoreceptor activity, muscle afferents, local tissue metabolism, circulating hormones, circadian variations, and environmental factors. Although research specific to nociceptive effects on HRV (SD) is sparse, it is likely that nociception has different effects on these factors. Thus, the way in which nociception affects the ANS and ultimately HRV, are unique when compared to other ANS stressors and their respective HRV responses.

Our findings support others' recommendations for HRV research that optimal data collection conditions exist for specific phenomena, and investigators should bear this in mind during research design (Sandercock, Bromley, and Brodie, 2005). Based on our results, it is recommended that future studies investigating time domain HRV responses to nociception use other measures of HRV in addition to the standard deviation measure. Further research is required to interpret the significance of HRV (SD) in response to nociception and determine other time domain values that may better represent cardiac autonomic modulations in response to nociception. The emergence of different algorithms designed to examine dynamic threshold crossings to robustly classify various perturbations / conditions may provide a more sensitive and valid approach to studying HRV changes to nociception (Arif & Aziz, 2005).

Very few studies have analyzed the effects of nociception on HRV in the frequency domain. It has been proposed that specific sympathetic and

parasympathetic modulations can be identified by measuring the power of specific frequency oscillations through power spectral analysis. One study examined LF oscillations in response to nociception via electrical shock and demonstrated significant increases in LF power following nociception (Terkelsen et al., 2005). This finding is in contrast to our results where we show a decrease in LF in response to mechanical cutaneous nociception. In comparison, both studies agree that there are no significant HF changes in response to nociception. The interpretation of these results remains difficult due to a great deal of controversy surrounding the neural mechanisms underlying the three frequency oscillations, particularly that of the LF. HF oscillations have been shown to be a good indicator of parasympathetic activity in that there is significant attenuation of HF oscillations following pharmacological atropine administration (Camm et al. 1996; Selman, McDonald, Kitney, 1982). LF oscillations are thought to be dependant on sympathetic control by several authors (Weise, Heydenreich, & Runge, 1987; Pagani, Lombardi, Guzzetti, Rimoldi, Furlan, Pizzinelli, Sandrone, Malfatto, Dell'Orto, Piccaluga, Turiel, Baselli, Cerutti, & Malliani, 1986). Recent findings have shown that in humans, beta-blocking agents only slightly reduced LF power and therefore only a small percentage of LF can be attributed to direct sympathetic drive (Jokkel, Bonyhay, & Kollai, 1995; Weise et al. 1987; Pomeranz et al., 1985). Karemaker (1999) reports that atropine also blocks the majority of LF demonstrating that both HF and LF oscillations are due to mainly vagal (parasympathetic) activity. Others have shown that the LF arises from the control of peripheral resistance of probable sympathetic origin that is conveyed to the

heart through efferent vagal activity which is modulated by the baroreflex, thus, sympathetic influence in the LF is indirect (Grasso et al., 1997). Current research suggests that both sympathetic and parasympathetic activity influences the LF spectrum of oscillations. Studies are beginning to specifically identify how the sympathetic and parasympathetic modulations affect the LF but this topic remains rather vague. Due to the fact that the LF encompasses both SNS and PNS processes, it becomes extremely difficult to explain LF behavior in response to nociception in terms of specific sympathetic and parasympathetic responses. Increases and/or decreases in LF can potentially be caused by either SNS or PNS activity. Previous models of sympatho-vagal balance fail to explain these dynamics.

In the present study, it appears that nociception does affect the LF. Our findings support a decrease in LF. The frequency domain data was presented as a percentage of total power (see Figure 4). Although this method allows simple monitoring of the frequency bands, more detailed information may be obtained by observing the power peaks within each frequency. The specific location of the peak may change whereas overall power within the frequency band can remain the same. This type of analysis may assist in identifying significant changes within frequency bands in response to perturbations. Differences in data collection procedures may in part explain contrasting results. Different nociceptive perturbations resulting in different cardiovascular autonomic modulations is also a possibility. Future experiments looking at HRV and

nociception should standardize the nociceptive modality for meaningful comparison.

The athlete versus non-athlete comparison revealed no significant differences between the two groups (two-way repeated measures ANOVA). However, it does appear that a slight, yet non-significant difference is seen between the athlete and non-athlete group in response to nociception and TENS following nociception (Figure 7B and 7C). No other studies have examined HRV response to nociception in athletes versus non-athletes. The stratification procedure of the two groups may be a limitation of the study. The use of resting heart rate to determine the presence of a training bradycardia may not be sensitive enough to differentiate between highly trained athletes and normal participants in terms of ANS behavior. Metabolic testing may be required to accurately classify these two groups and study their respective autonomic behavior. The use of five athletes versus five non-athletes may not provide enough statistical power to identify small yet significant differences between the two groups. Including a greater sample size when comparing these two groups may overcome this potential drawback.

Conclusions

The low frequency power component of HRV is affected by the application of a mechanical cutaneous nociceptive stimulus. Further research is required to examine time domain measures of HRV as indices of autonomic

behavior. Ongoing research is required to interpret the significance of HRV (SD) and determine other time domain values that may better represent cardiac autonomic modulations in response to nociception. The ability to utilize other measures of autonomic activity may also assist in the interpretation of HRV findings, especially those that manifest within the LF (arterial pressure control / baroreflex). Further research is required to clarify the mechanisms mediating the LF change in response to nociception. In light of these findings, indices of autonomic control and sympatho-vagal balance should be used with caution. It is unknown what neural mechanism (i.e. SNS or PNS) mediates change in the frequency domain in response to nociception.

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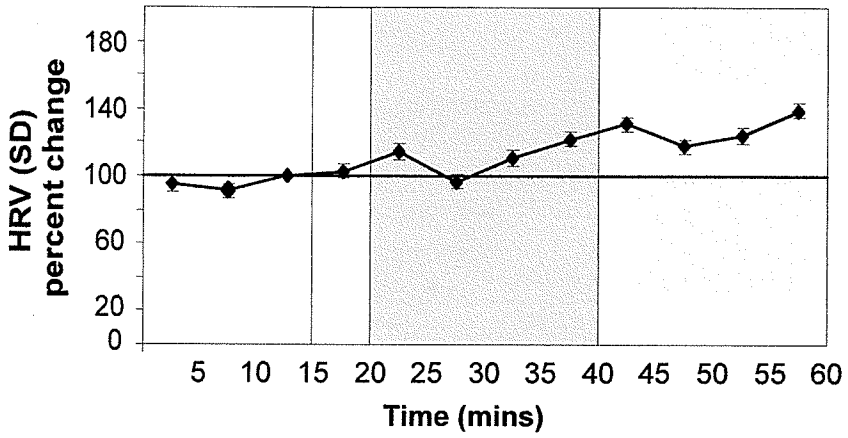
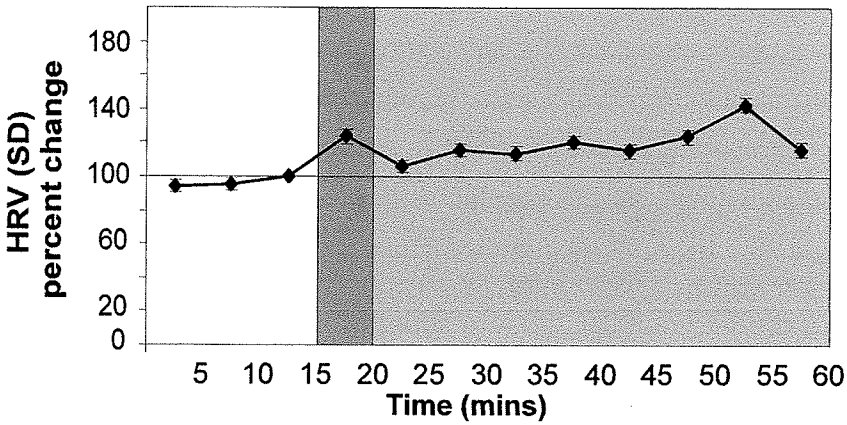
Figure 1) Time Domain Analysis of HRV

1A) Effects of TENS on ANS



1B) Effects of nociception on ANS



1C) Effects of TENS following nociception on ANS

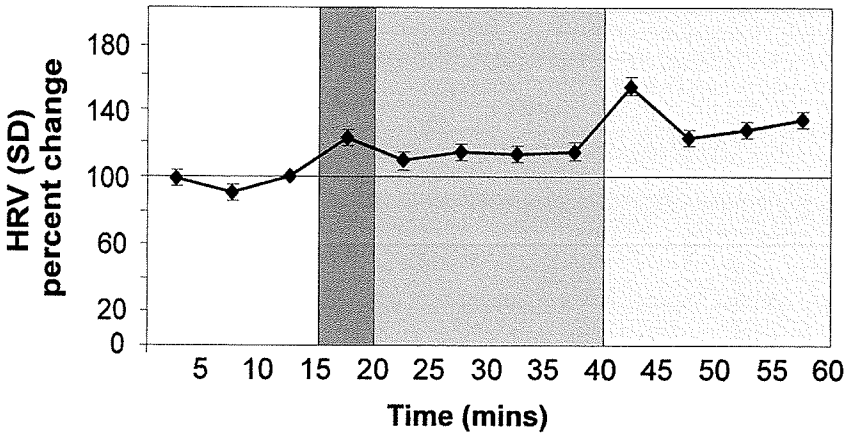


Figure 2) Changes in heart rate to experimental periods

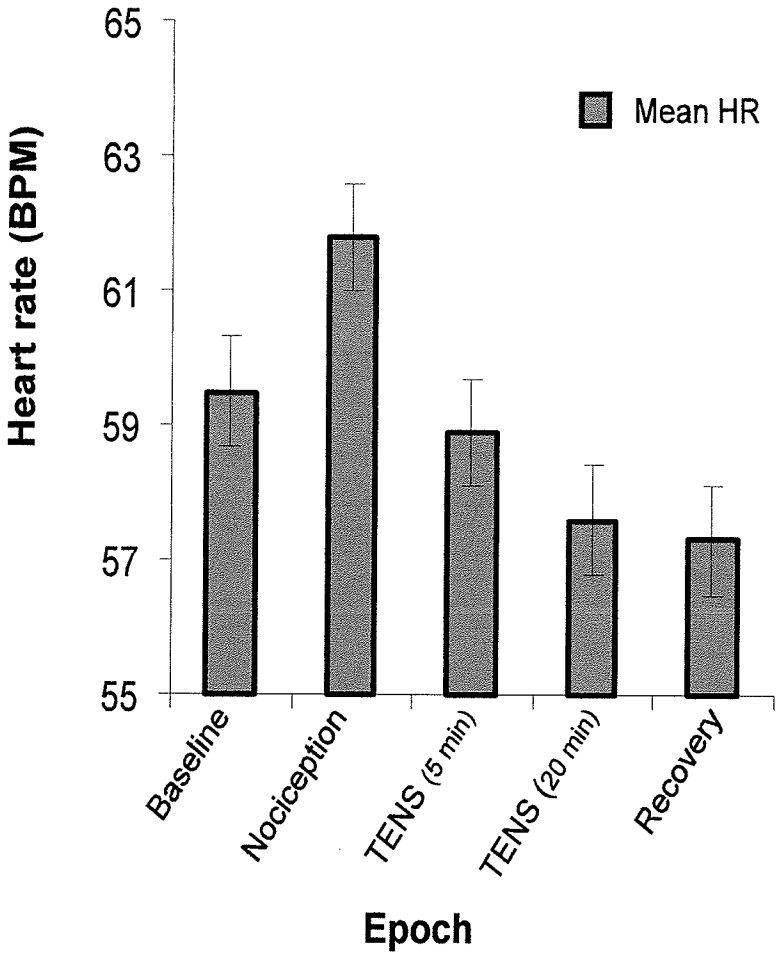


Figure 3) Effects of nociception on skin conductance

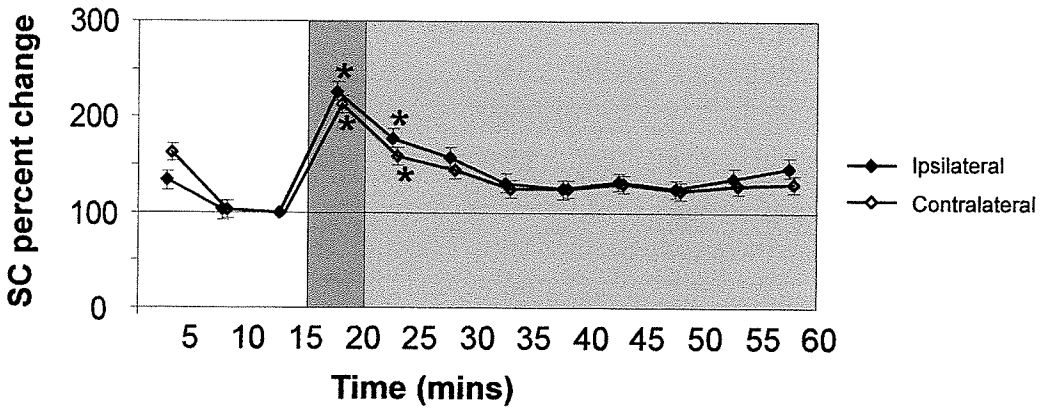
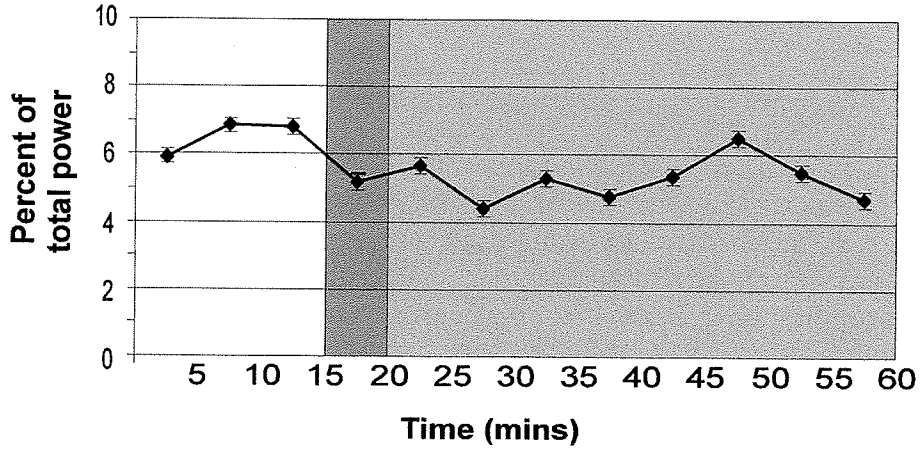
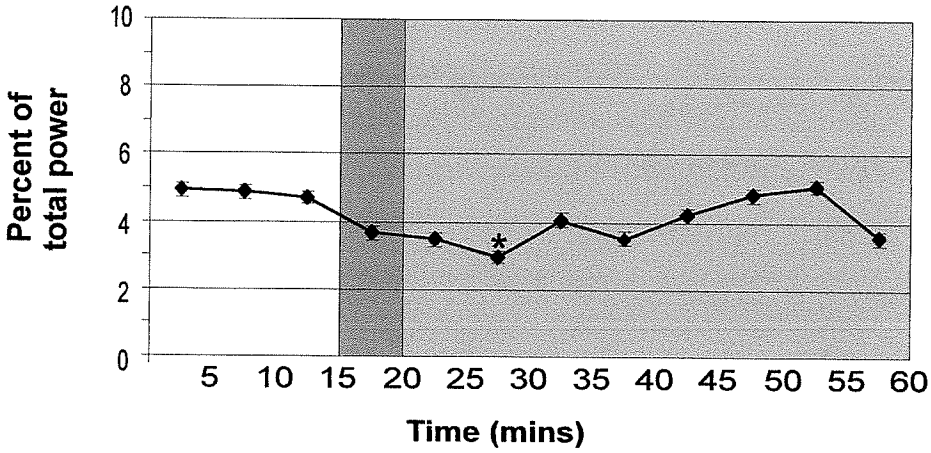


Figure 4) Frequency domain analysis of HRV

4A) HF



4B) LF



4C) VLF

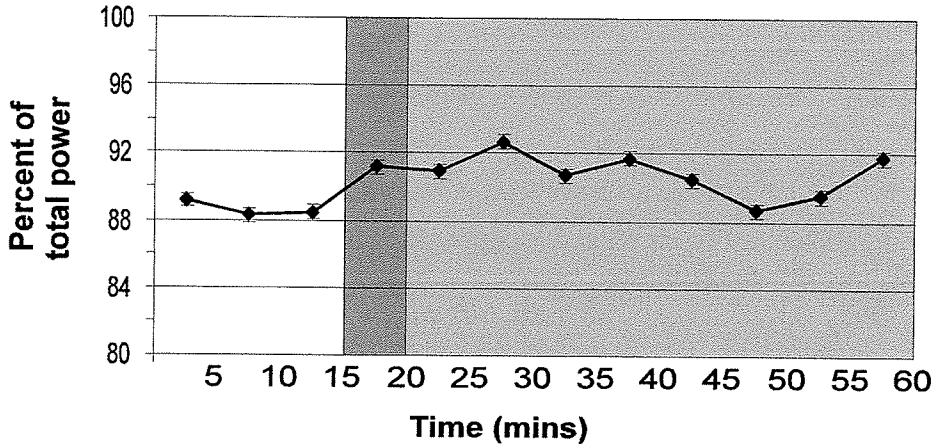


Figure 5) Mean LF power; two-group comparison

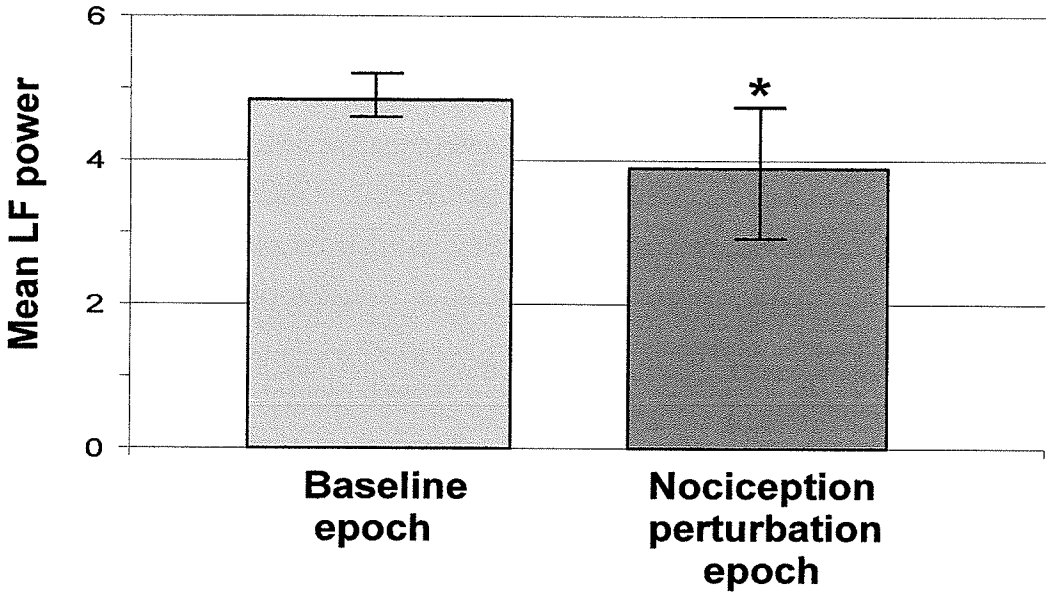
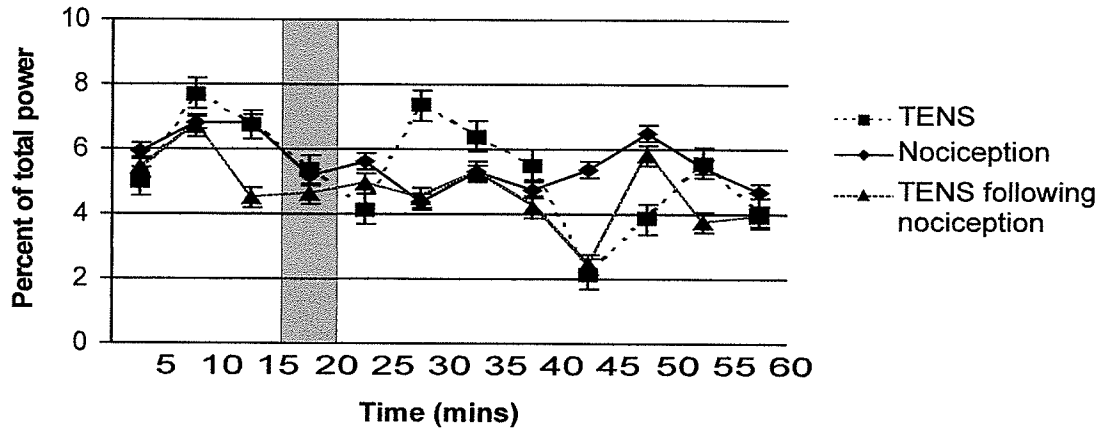
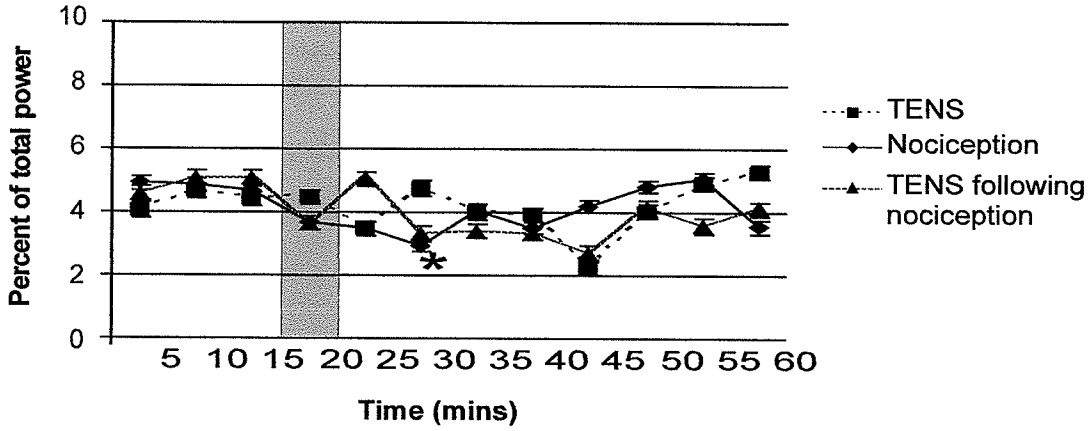


Figure 6) Comparison of the three trials within HF, LF, and VLF power

6A) HF



6B) LF



6C) VLF

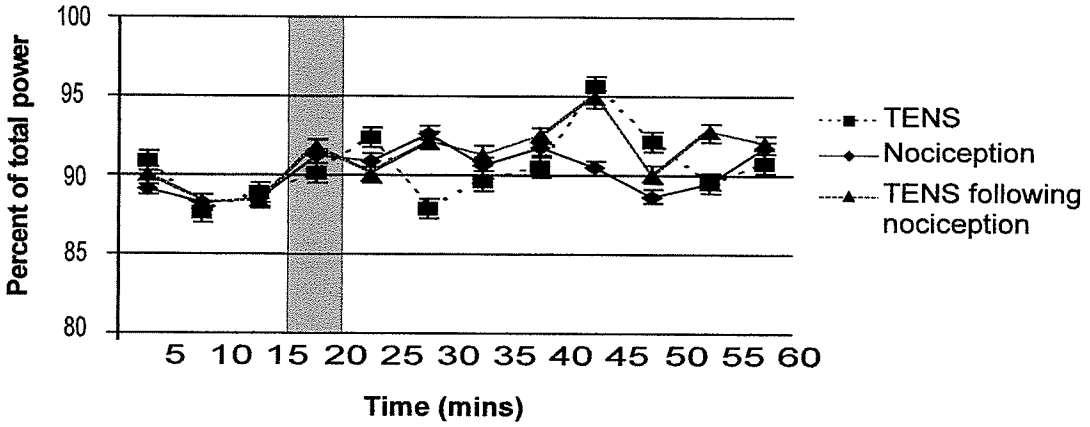
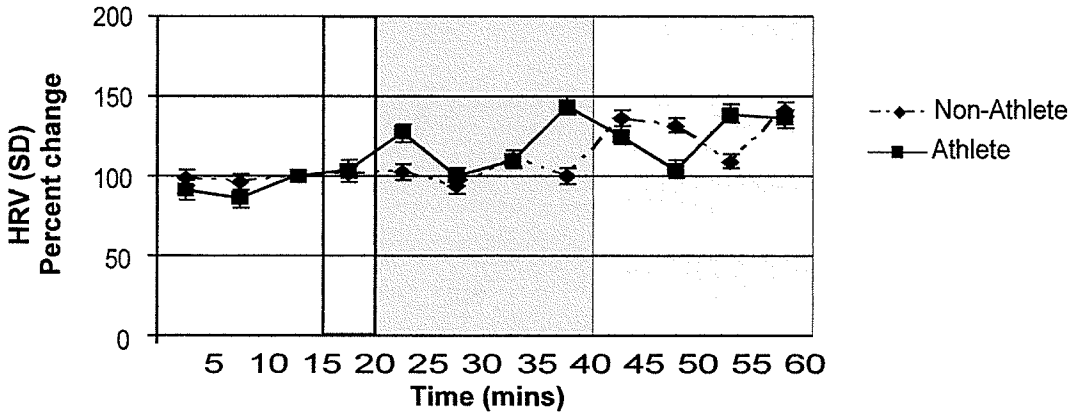
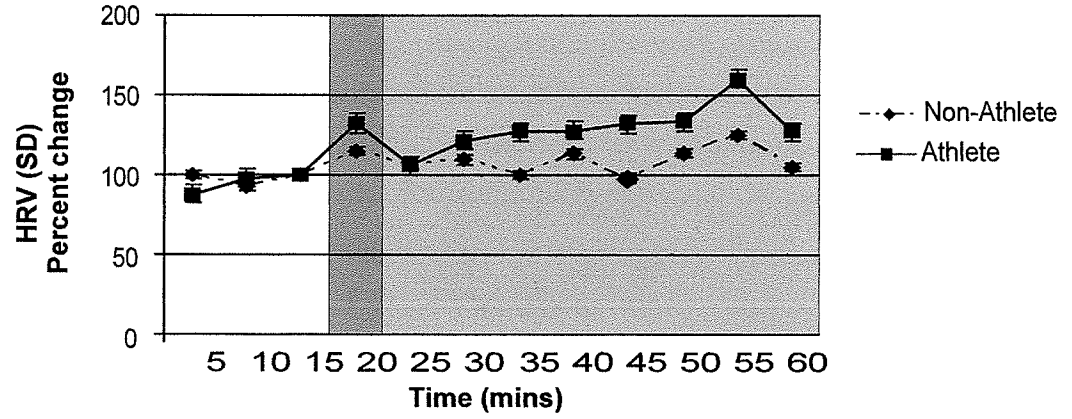


Figure 7) Comparison of HRV (SD) between athlete and non-athlete groups

7A) Effects of TENS on ANS



7B) Effects of nociception on ANS



7C) Effects of TENS following nociception on ANS

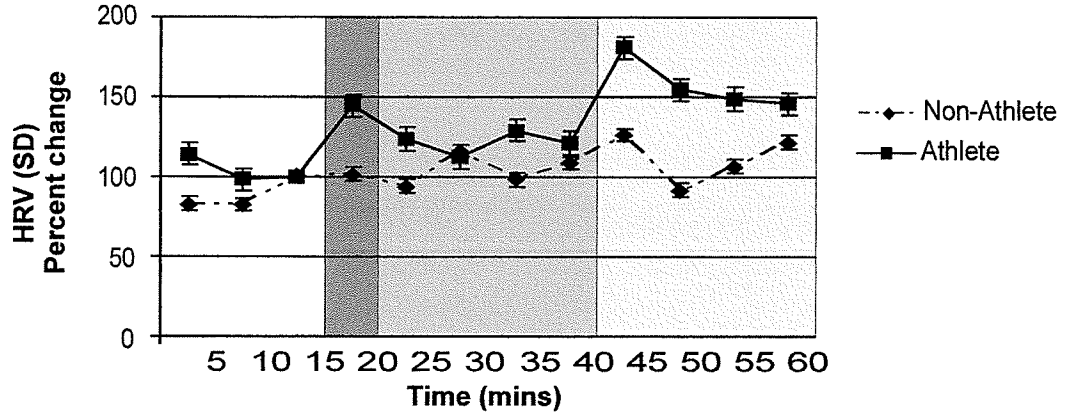
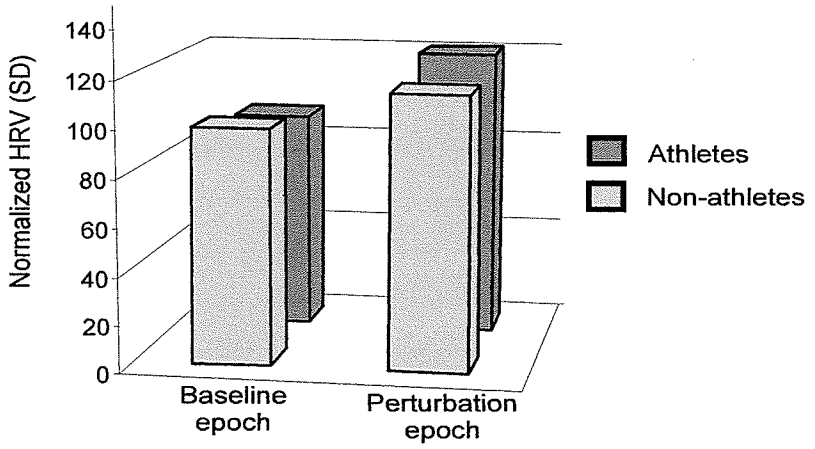
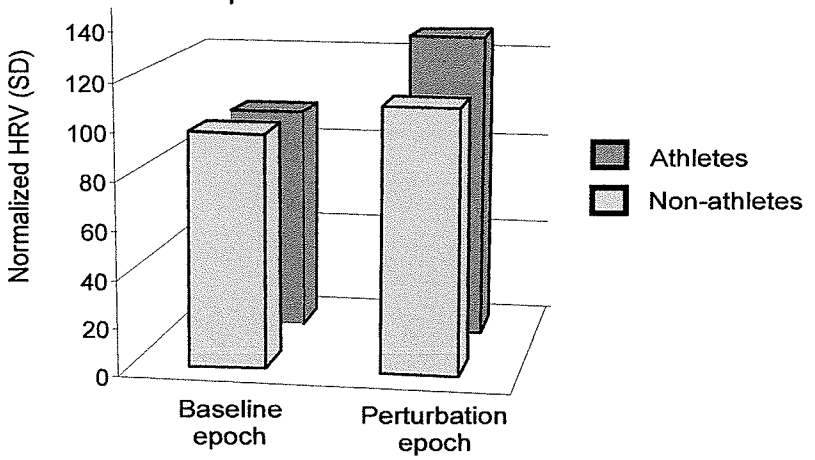


Figure 8) Athlete versus non-athlete; two-group comparison

8A) The effects of TENS on ANS



8B) The effects of nociception on ANS



8C) The effects of TENS following nociception on ANS

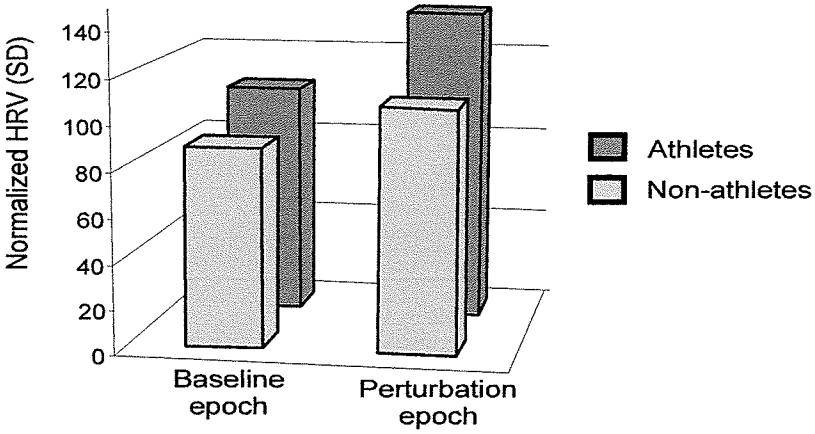


Figure Legends

Figure 1) Time Domain Analysis of HRV
Graphs illustrate the analysis of HRV in the time domain. Standard Deviation (SD) values of the R-R intervals were computed within five minute blocks and normalized to 100% baseline (15 minute block) respective to each trial and presented as an average percent change of the SD ± standard error for all participants, n = 10. Percent change is shown on the vertical axis while time and trial periods are displayed on the horizontal axis. The three trials are represented accordingly; 1A) *Effects of TENS on ANS* (shaded baseline, TENS, post-TENS trials), 1B) *Effects of Nociception on ANS* (shaded baseline, null, nociception, post-nociception trials), 1C) *Effects of TENS following nociception on ANS* (shaded baseline, nociception, TENS following nociception, Post-TENS following nociception trials). Two-way repeated measures ANOVA were used to identify significant changes throughout the experiment compared to baseline. No statistically significant differences were seen within the three trials.

Figure 2) Changes in heart rate to experimental periods
The vertical axis represents heart rate (HR) while the horizontal axis displays various epochs created from the experimental periods within the *Effects of TENS following nociception on ANS* trial ± SEM (baseline; 15 min. block, nociception; 20 min. block TENS; 25 min. block, TENS; 40 min. block, recovery; 60 min. block). Heart rates were averaged within the 5 minute epoch for each participant. Average HR over the epoch was then averaged over the 10 participants. No significant changes are seen between any of the above epochs. Changes in average heart rate of the participants between epochs are less then 6 beats per minute.

Figure 3) Effects of nociception on skin conductance
The figure compares the effects of nociception on skin conductance bilaterally. Normalized mean skin conductance values ± standard error are presented from the *Effects of nociception on ANS*. Percent change is shown on the vertical axis while time is displayed on the horizontal axis. The respective periods within the trial are shaded for comparison. A significant increase in skin conductance is observed during the nociceptive period (20 minute block) (ipsilateral 226.2% ± 55.72 $p<0.001$, contralateral 213.5% ± 35.56 $p<0.001$) and 5 minutes post-nociceptive period (25 minute block), (ipsilateral 177.4% ± 38.4 $p<0.040$ contralateral 159.4% ± 23.75 $p<0.012$). Significant differences are indicated by asterisks.

Figure 4) Frequency domain analysis of HRV

The graphs present HF, LF, and VLF data from the *Effects of nociception on ANS* trial in figures 4A, 4B, and 4C respectively. Each frequency component is expressed as a percent of total power averaged for all 10 participants within each five minute block. Values shown are mean ± SEM. Percent of total power is shown on the vertical axis while time (minutes) is displayed on the horizontal axis. Trial periods are shaded white (baseline), dark grey (nociception) and light grey (post-nociception). Repeated measures ANOVA were used to identify significant differences between baseline values, nociception and post-nociception period values. Figure 4B demonstrates significant LF changes between the baseline period (5 minute block) (4.91% ± 1.1) and the post-nociception period (30 minute block) (2.95% ± 0.61; $p<0.05$). Significant differences are indicated by asterisks.

Figure 5) Mean LF power; two-group comparison
The graph illustrates the comparison of mean low frequency power between two epochs. Normalized LF power in each 5 minute block was averaged in two epochs (baseline epoch (5-15 minute blocks) mean power 4.83% ± 0.12; nocicepive perturbation epoch (20-60 minute blocks) mean power 3.89% ± 0.69) as indicated on the horizontal axis. The vertical axis represents mean LF power. Values shown are mean ± SEM. A significant decrease in mean LF power in the nociceptive perturbation epoch is seen compared to baseline (t-test; $p<0.045$). Significant differences are indicated by asterisks.

Figure 6) Comparison of the three trials within HF, LF, and VLF power
The graphs compare the three trials for each of the power frequencies. HF, LF, and VLF normalized power compared among the three trials is shown in figures 6A, 6B, and 6C respectively. Trials are represented as the TENS only (squares), nociception (diamonds) and TENS following nociception (triangles). Each frequency component is expressed as a percent of total power averaged for all 10 participants within each five minute block. Values shown are mean ± SEM. Percent of total power is shown on the vertical axis while time (minutes) is displayed on the horizontal axis. The dark grey shading highlights the nociception/null period. Repeated measures ANOVA were used to identify significant differences between baseline values, nociception and post-nociception period values. LF significantly decreased from the baseline period during the nociception trial from 4.91% ± 1.1 to 2.95% ± 0.61; $p<0.05$ for 5 minutes following clip removal. Significant differences are indicated by asterisks.

Figure 7) Comparison of HRV (SD) between athlete and non-athlete groups
Graphs illustrate the analysis of HRV in the time domain comparing athletes vs non-athetes. Standard Deviation (SD) values of the R-R intervals were computed within five minute blocks and normalized to 100% baseline (15 minute block) respective to each trial and presented as an average percent change of the SD ±

standard error during each 5 minute block for both athletes (squares) and non-athletes (diamonds); n=5 in each group. Figures 7A, 7B, and 7C represent the three trials of the experiment respectively with trial periods shaded. Percent change from baseline is shown on the vertical axis while time (minutes) is shown on the horizontal axis. No significant differences between the two groups are appreciated.

Figure 8) Athlete versus non-athlete; two-group comparison
The three graphs are representative of the three trials within the experiment. The vertical axis represents normalized HRV (SD) and the horizontal axis represents the baseline and perturbation epochs. HRV (SD) data is averaged into a baseline epoch (5-15 minute blocks) and a perturbation epoch (20-60 minute blocks) and presented in athlete and non-athlete groups (5 participants per group). T-test's were used to identify differences between the epochs. No significant differences were seen between athletes versus non-athletes or baseline versus perturbation epochs.

Table 2) Subject demographics

	Non-athlete	Athlete
Age (years)	25.8 (2.4)	24.6 (1.5)
Resting heart rate (bpm)	69.9 (5.3)	54.2 (3.2)
R-R interval at rest (ms)	999.32 (112.5)	1104.88 (188.8)
HRV (SD) at rest (ms)	62.7 (11.7)	85.5 (16.4)
VNPRS		
clip exposure (1 min)	5.2 (0.3)	5.5 (0.2)
clip exposure (4 min)	4.1 (0.5)	4.3 (0.5)
post-clip removal (5 mins)	0	.3 (0.1)
post-clip removal (10 mins)	0	.2 (0.1)

All categories are represented by averages. Standard deviations appear in brackets. VNPRS = verbal numeric pain rating scale; bpm = beats per minute; HRV (SD) = heart rate variability standard deviation in the time domain; min = minute; ms = millisceond

Table 3) Trial protocols; manuscript 1

	Time (mins)									
Effects of TENS on ANS	15	20	25	30	35	40	45	50	55	60
Period	Baseline	Null	TENS				Post-TENS			
Athlete (Normalized HRV (SD)										
Non-Athlete (Normalized HRV (SD)										

	Time (mins)									
Effects of Nociception on ANS	15	20	25	30	35	40	45	50	55	60
Period	Baseline	Clip	Post-nociception							
Athlete (Normalized HRV (SD)										
Non-Athlete (Normalized HRV (SD)										

	Time (mins)									
Effects of TENS following Nociception on ANS	15	20	25	30	35	40	45	50	55	60
Period	Baseline	Clip	TENS following nocieption				Post-TENS following nociception			
Athlete (Normalized HRV (SD)										
Non-Athlete (Normalized HRV (SD)										

Shaded areas represent the different periods throughout the trial. The clip (battery clip) is applied in the nociception period.

Manuscript 2

The Effects of Transcutaneous Electrical Nerve Stimulation (TENS) on the Autonomic Nervous System Following Exposure to Acute Mechanical Cutaneous Nociception

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Keywords: TENS, ANS, Skin conductance, Nociception

Abstract

Transcutaneous electrical nerve stimulation (TENS) is an electrophysiological modality used for pain control. Current paradigms regarding the neurophysiological mechanisms in which TENS exerts its effects arise from the gate control theory and endogenous opioid release. It is well known that the autonomic nervous system (ANS) is involved in painful states usually through increases of sympathetic activity, yet the interaction between TENS and the ANS is not well understood. Whether TENS affects the SNS, or produces analgesia through its effects on the SNS is unclear. Research investigating the effects of TENS on the ANS in the presence of nociception is very limited. The objective of this study is to test the effects of TENS on the ANS responding to a sympathetic perturbation caused by a nociceptive stimulus. METHODS: 10 participants completed three separate trials in random order. The three trials studied the effects of TENS on ANS, the effects of nociception on the ANS, and the effects of TENS following nociception on ANS. Skin conductance measures were recorded from the upper extremities as an index of autonomic (sympathetic) activity, and a small battery clip was used to pinch the participant's skin ipsilaterally, which produced the mechanical cutaneous nociceptive stimuli. RESULTS: The mechanical cutaneous nociceptive stimulus produced significant increases in SNS activity (ipsilateral $p<0.001$, contralateral ($p<0.001$)). Application of TENS following exposure to nociception resulted in a prolonged duration of increased SNS activity when compared to SNS activity of nociception alone (ipsilateral; $p<0.023$, contralateral; $p<0.004$) and, a significant increase in

the magnitude of SNS activity was seen on the contralateral side ($p<0.026$) when TENS followed nociception versus nociception alone. Higher values of SNS activity where seen ipsilaterally but not statistically significant ($p<0.067$). CONCLUSIONS: TENS appears to increase and prolong SNS activity in response to acute mechanical cutaneous nociception. The effects of TENS on the ANS may differ depending on the presence of autonomic perturbations.

Introduction

Transcutaneous electrical nerve stimulation (TENS) is an electrophysical modality used for pain control. Current paradigms regarding the neurophysiological mechanisms in which TENS exerts its effects arise from the gate control theory and endogenous opioid release (Sluka & Walsh, 2003). It is widely used in all types of health care facilities to treat both acute and chronic pain arising from many different conditions. The efficacy of TENS remains controversial with evidence indicating conflicting results with respect to the effects and mechanisms of analgesia in clinical studies. Further, interpretation of the results among different studies remains complicated. Comparison of results across different studies is difficult due to variations in methodology, lack of appropriate controls, differences in electrode placement, difference in TENS stimulation parameters, positioning of, and type of physiological recording devices, and differences in treatment, stabilization, and baseline times (Cramp, Gilsenan, Lowe, & Walsh, 2000).

It is well known that the autonomic nervous system (ANS) is involved in painful states usually through increases of sympathetic activity (Janig, 1985, Janig, Levine, & Michaelis, 1996, Devor, 1995). Sympathetic dysfunction has been implicated in acute and chronic pain conditions. Changes in autonomic function have been documented in peripheral and central sensitization (Wright, 1999). Links between the experience of pain and alterations of sympathetic function suggest that sympathetic outflow may influence or maintain afferent

activity in nociceptive neurons, thus, abnormal functioning of the sympathetic nervous system (SNS) can create and/or contribute to pathological pain (Campbell, Meyer, Davis, & Raja, 1992; Roberts, 1986).

The interaction between TENS and the ANS is not well understood. The SNS plays an important role in mediating pain, and whether TENS produces analgesia through its effects on the SNS is unclear (Reeves, Graff-Radford, & Shipman, 2004). Anatomically, relationships exist between sensory afferent pain fibers and sympathetic fibers. The nerves are distributed in similar regions in the viscera and cutaneous tissue and travel similar paths in the CNS. Pain fibers may course through sympathetic ganglia en route to the spinal cord (Stanton-Hicks, 1990). Thus, a physiological/anatomical basis for TENS affecting the SNS may exist, and an increasing amount of research has focused on elucidating the effects of TENS on the SNS.

It has been reported that high frequency TENS, at intensities just above or below motor threshold, does not affect local blood flow, and thus concluded that TENS shows no effect on the SNS in healthy volunteers (Indergrand and Morgan, 1994). Several other studies show similar findings where TENS fails to exert an inhibitory effect on the SNS (Nolan, Hartsfield, Witters, & Watson, 1993; Johnson, Hajela, Ashton, & Thompson, 1991; Tracy, Currier, & Threlkeld, 1988). Conversely, several researchers have reported that TENS does have an affect on the SNS. Multiple investigators have shown that TENS decreases sympathetic

tone demonstrated through decreases in skin conductance measures, and increases in skin temperatures and cutaneous blood flow (Cramp, et al., 2000; Noble, Henderson, Cramp, Lowe & Walsh, 2000; Currier, Petrilli, & Threlkeld, 1986). Olyaei, Talebian, Hadian, Bagheri, and Momadjed (2004) present results from an experiment examining the effects of TENS on skin conductance. A significant decrease in both the amplitude and latency of the skin conductance measure was found after 20 minutes of TENS applied to the right wrist. They concluded that TENS has an inhibitory effect on the SNS which leads to a decrease in overall sympathetic tone in healthy participants. Electrical stimulation at intensities that could activate Aδ fibers was found to decrease SNS activity by increasing local blood flow and decreasing vascular resistance (Schaible & Grubb, 1993). Transient increases in blood flow with low frequency TENS were observed at the area of stimulation if the intensity exceeded the motor threshold (Sherry, Oehrlein, Hegge, & Morgan, 2001). Laser doppler imaging studies show increases in blood flow in response to both high and low frequency TENS set at intensities reported to be non-painful (Wilkstrom, Svedmen, Svensson, & Tanweer, 1999). Thus there appears to be support for the suggestion that TENS decreases SNS activity.

When studying the effects of TENS on the ANS, several possibilities must be considered. First, TENS may have little effect on the ANS in healthy participants. It could be that the action may only become evident in a system in which the ANS activity is non-typical. Second, in situations where the ANS is

perturbed, such as in response to noxious stimuli (sympathetic perturbation), it is possible that the application of TENS may have different and distinctive effects on the ANS when compared to its effects on a typical, non-perturbed ANS. Research investigating the effects of TENS on the ANS in the presence of nociception is limited. This direction of research would provide clinically relevant findings with regards to the use of TENS for pain control and its effect on ANS perturbations that occur in response to acute noxious stimuli. The objective of this study is to test the effects of TENS on the ANS responding to a sympathetic perturbation caused by a nociceptive stimulus. The study also introduces a non-invasive acute model of pain for human participants which allows for controlled manipulation of variables. It is hypothesized that a brief acute mechanical noxious stimulation will result in a recruitment of sympathetic activity which would be reflected in increased skin conductance and TENS will have no effect on sympathetic activity alone but will decrease the heightened sympathetic activity in response to the noxious stimulus, assuming TENS acts via decreasing the sympathetic response that could augment the pain.

Methods

Participants

Ten male university students aged 19-29 years were recruited for the study. Participants were asked to maintain their normal daily routines for the duration of the study period. They were in good health and free of illness on testing days. Exclusion criteria included reports of any painful conditions, tobacco

and/or alcohol addiction, drug or medication use, and, any contraindications to TENS. Written informed consent was obtained and the study protocol was approved by the Human Research Ethics Board, Faculty of Medicine, University of Manitoba (H2005:136).

Equipment

A small battery clip applied to a pinch of skin on the dominant upper extremity within the C_7 dermatome (lateral elbow) provided the mechanical cutaneous nociceptive stimuli. The pressure exerted by the clip on the skin produced significant local nociception. The clip used in this experiment produced 2.4 Kg of force on a pinch dynamometer (Therapeutic Instruments, USA). Pressure exerted by the battery clip was monitored for consistency between participants.

Skin conductance was measured using the Flexcomp Infiniti and SC-Flex/Pro skin conductance sensors (Thought Technology, Canada). Skin conductance was sampled at 32 Hz and recorded in micro-siemens (μS) through two electrodes placed on the participants' 2nd and 5th digits of the hand bilaterally. The Flexcomp Infiniti encoder was linked to a PC where data was stored in Biograph Infiniti software (Thought Technology, Canada) for off-line analysis.

The Dynex V (Empi, USA) was the TENS unit used in the experiment. Using hypoallergenic conductive gel (Empi, USA), standard 2" x 2" carbon

rubber electrodes were attached to the skin with surgical tape (3M, USA). Electrodes were positioned in a 2-channel cross-over configuration within the C_7 dermatome with positive polarity electrodes proximal. The battery clip position was centered in the electrode configuration. The Dynex V produces a pulsed current with a balanced, asymmetrical, biphasic waveform. TENS parameters were as follows: Normal stimulation mode, 100 μs pulse duration and 100 Hz frequency/rate. The amplitude was 'sensory plus' threshold (defined by increasing intensity until a motor response is visualized, then lowering intensity to the level where the motor response is no longer present). This method allows standardization between participants.

Participants were screened for psychological distress that could potentially affect ANS function using The Nottingham Health Profile (Appendix B). They were also required to answer a pre-trial questionnaire (Appendix A) preceding participation in each trial in an attempt to better control factors which may affect ANS function, such as activity level, dietary intake, and sleep patterns previous to trial participation. Subjective reports of pain were collected from the participants using a verbal numeric pain rating scale (VNPRS). Participants were asked verbally "on a scale of 0 to 10, zero being no pain and 10 being the worst pain imaginable, how would you rate your pain?"

Protocol

All experiments took place in an isolated temperature controlled room (21°C ± 1°C) with minimal distractions/stimulation. Participants were reclined into a semi-supine position. The study utilized a repeated measures design. Three trials took place within the study; *1) Effects of TENS on ANS, 2) Effects of nociception on ANS,* and *3) Effects of TENS following nociception on ANS.* All 10 participants completed the three trials in a randomized order. The purpose of the *Effects of TENS on ANS* trial was to test the effects of TENS on the ANS in the absence of any experimental ANS perturbations. The *Effects of nociception on ANS* trial examined the effects of experimentally induced mechanical cutaneous nociception on the ANS. The *Effects of TENS following nociception on ANS* trial focused on the changes within the ANS upon TENS application while the ANS responds to experimental nociception. Table 4 illustrates the trial protocols and their respective periods.

1) *Effects of TENS on ANS*

Participants entered the isolated experiment room individually and had the skin conductance and TENS electrodes physically attached. Participants assumed a semi-supine position and were allowed 10 minutes of rest to attain a baseline heart rate. Uninterrupted recording of skin conductance data then began for 60 minutes (trial duration). The *effects of TENS on ANS* trial consists of 4 periods; baseline, null, TENS, and post-TENS periods. The first 15 minutes (baseline period) was collected as baseline data. The following 5 minutes represented the null period. The null period existed to compensate for the nociceptive period in

the other trials. TENS was then applied for a 20 minute duration (TENS period) followed by the 20 minute post-TENS period. The investigator remained in the experiment room for the duration of the experiment. There was no communication between subject and investigator with the exception of the adjustment of the TENS amplitude.

2) *Effects of nociception on ANS*

Participants were prepared in a similar fashion as described in the *effects of TENS on ANS*. The *effects of nociception on ANS* trial consists of 3 periods; baseline, nociception, and post-nociception periods. The first 15 minutes were collected as baseline data. The next 5 minutes represented the nociception period during which the battery clip was applied. Subsequent to the nociception period, the battery clip was removed for the 40 minute post-nociception period. Verbal numeric pain rating scale (VNPRS) scores were collected at the one and four minute marks within the nociception period, and at 5 minute intervals throughout the post-nociceptive period. There was no communication between subject and investigator with the exception of collecting VNPRS scores. The TENS unit remained inoperable throughout this experiment.

3) *Effects of TENS following nociception on ANS*

Participants were prepared in a similar fashion as described in the *effects of TENS on ANS*. The *effects of TENS following nociception on ANS* trial consists of 4 periods; baseline, nociception, TENS following nociception, and post-TENS

following nociception periods. The first 15 minutes were collected as baseline data. The following 5 minute period represented the nociception period as described above. Immediately after the nociception period, TENS was applied for 20 minutes (TENS following nociception period). TENS was then stopped and recording continued for 20 minutes (post-TENS following nociception period). VNPRS scores were collected at the one and four minute marks within the nociceptive period, and at five minute intervals throughout the TENS following nociception period and the post-TENS following nociception period. There was no communication between subject and investigator with the exception of collecting VNPRS scores and the setting of TENS amplitude.

Data Analysis

Skin conductance data was collected and saved within Biograph Infiniti software to a PC for offline analysis. Raw sensor recordings were exported into a spreadsheet where data was divided into 5 minute blocks. Skin conductance data from the TENS period and the TENS following nociception period was found to show mild artifact due to TENS unit operation. Data within these periods was smoothed (16 point one side maximum smoothing width) to remove artifact using Diadem software (National Instruments, USA). The mean micro-Siemens (μS) value within each 5 minute block was determined and values were then normalized to the baseline value (15 minute block) within each trial and expressed as a percent of baseline.

Statistical Analysis

All statistical calculations were performed in Sigma Stat software (SPSS, USA). One-way repeated measures analysis of variance (ANOVA) was used to analyze normalized ipsilateral and contralateral skin conductance data. Significant differences were accepted at $p<0.05$. When indicated, post-hoc Tukey's tests were used to locate significant differences between time blocks. Two-way repeated measures ANOVA was used to study the differences between the *Effects of nociception on ANS* trial and the *Effects of TENS following nociception on ANS* trial. Significance was accepted as $p<0.05$. Tukey's tests were used to further study significant differences between trials.

VNPRS scores collected throughout the experiment were not analyzed statistically. Statistical testing of ordinal data limits the interpretation of this data within this experimental design. The primary purpose of this data is to relate subjective reports of pain/discomfort to autonomic modulations seen through objective measures. Studying the data in this manner provides useful insight towards underlying ANS activity and its relation to subjective reports of pain.

Results

Figure 9A shows skin conductance in response to TENS treatment. Both ipsilateral and contralateral recordings demonstrate a significant increase in skin conductance during the first 5 minutes of the TENS period (25 minute block) compared to baseline (ipsilateral 131.2% ± 11.51 $p<0.041$, contralateral 131.6% ±

7.94 $p<0.033$). Skin conductance promptly returned to baseline following the initial increase and no other significant changes were noted throughout the TENS period. Significant increases in skin conductance were noted at the end of the post-TENS period within the 60 minute block both on ipsilateral (134.9% ± 19.62 $p<0.016$) and contralateral (139.5% ± 18.18 $p<0.003$) measures.

Figure 9B demonstrates the effects of mechanical cutaneous nociception on skin conductance. Significant increases in skin conductance were seen within the nociception period (20 minute block) both ipsilaterally (226.2% ± 55.72 $p<0.001$) and contralaterally (213.5% ± 35.56 $p<0.001$) compared to baseline. Skin conductance remained significantly higher than baseline following the nociception period for 5 minutes (25 minute block) bilaterally; ipsilateral (177.4% ± 38.4 $p<0.040$), contralateral (159.4% ± 23.75 $p<0.012$). There were no significant changes in skin conductance compared to baseline throughout the remainder of the post-nociception period.

Figure 9C demonstrates the effects of TENS application following a nociceptive stimulus. Again, significant increases in skin conductance compared to baseline were seen with the application of the clip (20 minute block) both ipsilaterally (214.1% ± 35.62 $p<0.001$) and contralaterally (230.1% ± 37.42 $p<0.001$). Skin conductance remained significantly elevated bilaterally for ten minutes of TENS intervention (25 and 30 minute blocks) following clip application. Skin conductance remained increased ipsilaterally at 211% ± 31.73

($p<0.001$), and 176.2 ± 25.84 ($p<0.023$) and contralaterally at 226.5% ± 36.51 ($p<0.001$) and 186.4 ± 27.74 ($p<0.004$) at the 25 and 30 minute blocks respectively. There were no significant changes in skin conductance compared to baseline throughout the remainder of the TENS following nociception, and post-TENS following nociception periods.

Table 5 summarizes VNPRS scores in response to the battery clip. Comparisons of the two trials indicate reproducible levels of pain produced by the battery clip. VNPRS scores were reported at zero for nine out of the ten participants once the clip was removed, thus the application of TENS, VNPRS scores remained at zero showing no subjective change in pain levels with TENS intervention.

Figure 10 illustrates the comparison between the two trials used to evaluate the effects of TENS on skin conductance following a nociceptive stimulus. Prolonged increases in SNS activity were seen upon application of TENS following nociception. Significant increases in skin conductance from baseline were recognized into the 30 minute block in the TENS following nociception trial versus the 25 minute block of the nociception trial. This finding indicates a maintanence of increased SNS activity in response to TENS. Higher values of skin conductance were observed bilaterally when TENS was applied (squares) compared with the clip alone (diamonds). Contralaterally, skin conductance is significantly greater in the TENS following nociception trial when

compared to the nociception trial ($p<0.026$), while ipsilaterally, skin conductance values are greater but non-significant ($p<0.067$).

Discussion

The primary objective of the study was to test the effects of TENS on the ANS responding to nociception. Information regarding the effects of TENS on the unperturbed ANS, and the effects of a mechanical cutaneous nociceptive stimulus on the ANS in healthy individuals was also obtained. Skin conductance was utilized as a measure of peripheral SNS activity in both upper extremities. The results of the study indicate that the mechanical cutaneous nociceptive stimulus produced significant increases in SNS activity, and application of TENS following exposure to nociception resulted in a prolonged increase of SNS activity of greater magnitude. This was contrary to the hypothesis that TENS would reduce the sympathetic response to the noxious stimulus.

The *Effects of TENS on ANS* trial (figure 9A) demonstrated the effects of TENS on the unperturbed, ANS in healthy participants. We observed a transient increase in SNS activity upon initial application of TENS. Following this initial increase no other changes in SNS activity were noted over the duration of the TENS treatment. It appears that this initial increase in SNS activity may be a non-analgesic effect of TENS due to a sympathetic reflex / axon reflex (Cramp, et al. 2000). As participants became accustomed to the TENS stimulus, SNS activity promptly returned to baseline and remained unchanged for the duration of TENS.

Besides the initial sympathetic reflex seen with TENS application, TENS appears to have no effects on the unperturbed ANS in healthy participants. The cause of the significant increase in skin conductance seen in the 60 minute block remains unclear. It may be attributed to a decrease in patient comfort due to the sustained position held for the experiment. Levels of discomfort may manifest as an increase in SNS activity.

It is possible that in order for TENS to affect the sympathetic nervous system, there needs to be a painful stimulus. In this study we show significant increases in SNS activity upon application of the nociceptive perturbation (Figure 9B). SNS activity remains significantly higher than baseline for 5 minutes following the removal of the nociceptive stimulus. The increased SNS activity following the removal of the nociception may be mediated by peripheral sensitization mechanisms including release of several inflammatory and chemical mediators and peripheral nociceptor sensitization (Dray, 1995). SNS activity remains slightly heightened following the 25 minute block for the remainder of the trial although this is not statistically significant from baseline. Attenuation of the peripheral sensitization mechanisms are most likely the factors responsible for the long lasting, non-significant increase in SNS activity.

The use of the battery clip as a mechanical cutaneous nociceptive stimulus is a novel approach to creating experimental pain. The battery clip appears to perturb the ANS in a similar fashion when compared to other modalities of experimental pain such as capsaicin and electrical shock (Terkelson et al. 2005; Reeves et al.

2004). The use of the battery clip provides a safe and effective way to perturb the ANS with a noxious stimulus. Comparison of VNPRS scores between the two trials indicate reproducible levels of pain produced by the battery clip (Table 5). Pilot testing demonstrated significant attenuation of the pain with the application of TENS at these stimulation parameters (VNPRS scores decrease from approximately $^{6}/_{10}$ to $^{1}/_{10}$; data not shown).

The ability to evaluate the effects of TENS on the perturbed ANS presents a very clinically relevant scenario where the effects of TENS can be explored as it is used in a clinical setting, that is, in situations where pain is experienced. This study is unique in the sense that it allowed the effects of TENS to be studied on the ANS in a perturbed state. Very few studies exist where the effects of TENS on the ANS have been studied in this fashion, thus, this approach adds important and clinically relevant findings to this body of knowledge.

The *Effects of TENS following nociception on ANS* trial permits the testing of the effects of TENS on the ANS responding to nociception. Upon application of TENS following a nociceptive perturbation, the increase in SNS activity due to the perturbation is not only increased in magnitude, but the SNS response is also prolonged (Figure 9C) by 5 minutes compared to the *Effects of nociception on ANS* trial (Figure 9B). Figure 10 highlights the significant differences between the SNS response to nociception and SNS response to nociception with TENS intervention. Significant increases in SNS activity are appreciated until the 30

minute block, one five minute block longer then significant SNS increases from nociception alone, and significanly greater values of skin conductance are seen with TENS intervention following nociception versus nociception alone contralaterally, while ipsilaterally, higher values of skin conductance are apparent but not quite significant ($p<0.067$).

Only one previous experiment was found which used a form of acute experimental pain to test the effects of TENS on the perturbed ANS (Reeves et al., 2004). A psychophysiological perturbation (anticipation of electrical shock) was used to achieve a SNS response and perturb the ANS. Measures of heart rate, digital pulse volume, and skin conductance were used to measure SNS activity in response to TENS treatment. Within the limitation of the experimental paradigm, no support was found for TENS affecting SNS function. Since we could find no other studies to date testing the effects of TENS on a sympathetically perturbed ANS in healthy participants, this experiment stands as the only basis for meaningful comparison. Our findings disagree with those reported by Reeves et al. (2004). The differences are likely due to the particular perturbation used to elicit a SNS response.

Other studies performed on various patient populations have suggested TENS has an inhibitory response on the SNS (Olyaei, et al., 2004; Sherry, et al., 2001; Cramp et al., 2000; Noble, et al., 2000; Wilkstrom, et al., 1999; Schaible, et al., 1993; Currier, et al., 1986). In contrast, others have shown that TENS fails to

affect the SNS (Nolan, et al., 1993; Johnson, et al., 1991; Tracy, et al., 1988). Our findings from the *Effects of TENS on ANS* trial are in agreement with these studies demonstrating that TENS fails to affect the SNS in the unperturbed ANS, although when TENS is applied to a sympathetically perturbed ANS, we show prolonged and increased SNS activity. Autonomic perturbations may be specific to the cause of the perturbation. Further research looking at different ANS responses to various models of experimental pain is required to better understand ANS dynamics in response to nociception and ultimately therapeutic treatments. The use of the mechanical cutaneous nociceptive stimuli (battery clip) appears to better replicate a clinically relevant ANS perturbation and may in fact provide more relevant results using this paradigm versus other modalities of experimental pain.

Upon review of TENS literature, two separate mechanisms are thought to mediate the analgesic effects of TENS; segmental inhibition through the gate control theory and stimulation of the release of endogenous opioids. The original gate control theory hypothesized that descending inhibitory pathways exist and modulate activity of the neurons within these pathways (Melzack & Wall, 1965). Further research in the area of descending pain control systems implicated the periaqueductal gray area (PAG) as a key area for endogenous analgesic mechanisms. Two distinct areas within the PAG appear capable of eliciting two different forms of analgesia (Faneslow, 1991; Lovick 1991; Morgan, 1991). Analgesia produced from vPAG stimulation is associated with sympathoinhibition

(Lovick, 1991). This has been described as an opioid form of analgesia (Cannon, Prieto, Lee, & Liebeskind, 1982). In contrast, analgesia produced from stimulation of the dPAG is associated with sympathoexcitation (Lovik & Li, 1989) and has been described as being a non-opioid form of analgesia (Cannon et al. 1982). Anatomic links from the vPAG and dPAG to two distinct spinal cord projection sites that utilize two different neurotransmitters have been identified (Fields and Bausbaum, 1989). Projections from the dPAG via nucleus gigantocellularis, paragigantocellularis and paragigantocellularis lateralis utilize noradernaline as a neurotransmitter, and are described as noradrenergic. Projections from the vPAG via nucleus raphe magnus use serotonin as a neurotransmitter and are described as serotonergic. The descending noradrenergic system acts at the spinal cord level to inhibit the release of substance P evoked by peripheral noxious mechanical stimuli where the descending serotonergic system acts to inhibit somatostatin evoked by peripheral noxious thermal stimuli (Kuraishi, 1990; Kuraishi, Harada, Aratani, Satoh, & Takagi, 1983).

Several experiments have convincingly shown that the effects of TENS may be mediated by endogenous opioid release involving serotonin as the neurotransmitter (Sluka, Deacon, Stibal, Strissel, and Terpstra, 1999, Kalra, Urban and Sluka, 2001). The results suggest the endogenous opioid release is mediated through descending pathways involving projections from the PAG to the nucleus raphe magnus to dorsal horn neurons. The possibility exists that the effects of TENS can also be mediated through the dPAG via noradrenergic

descending pathways. One experiment using systemic phentolamine to block alpha-adrenergic receptors while testing the effects of TENS on mechanical hyperalgesia and cold allodynia suggests that activation of sympathetic noradrenergic receptors may mediate TENS effects (Nam, Choi, Yeon, Leem, and Paik, 2001). Thus, TENS may be mediated at least in part, through a noradrenergic descending pathway from the dPAG. This mechanism would produce a non-opioid mediated analgesia and be associated with a sympathoexcitation. Activation of the noradrenergic descending control system plays a key role in mediating analgesia produced by peripheral noxious mechanical stimulation by inhibiting the release of substance P at the spinal cord level. The results of our experiment demonstrate an increased SNS response (sympathoexcitation) with TENS in response to mechanical nociception. Our results suggest that the noradrenergic descending control system may contribute to the neurophysiological mechanisms of TENS effectiveness when the ANS is perturbed with a mechanical cutaneous noxious stimulus.

Although our study demonstrated increased SNS activity with TENS that suggests involvement of a descending noradrenergic pathway as a possible mechanism of TENS efficacy, we are unable to report on the subjective effects of TENS treatment. In future studies, a different scale, such as a 10 cm visual analogue scale for pain intensity, to measure subjective changes in perceived pain with TENS treatment should be used to provide feedback in regards to treatment effectiveness. Further suggestions include applying TENS during exposure to

acute nociception and monitor pain ratings for TENS effectiveness. Animal experiments using pharmacological blockades directed at various receptors have been successful at identifying some of the neuroanatomical substrates responsible for mediating the effects of TENS (Kalra et al., 2001; Nam et al., 2001). This method has worked extremely well in characterizing the contribution of endogenous opioids in TENS analgesia. Further studies utilizing this approach have the potential to specifically test the involvement of the dPAG and the descending noradrenergic pathway as a potential mechanism for mediating the effects of TENS.

Conclusion

Further research into the effects of TENS on the ANS is needed to fully understand the mechanisms of TENS and its effects on the SNS. TENS appears to increase and prolong SNS activity in response to acute mechanical cutaneous nociception. The experimental pain used within this study offers a clinically relevant acute pain model. The findings within this study are limited to acute pain conditions, more specifically, those involving peripheral noxious stimulation. The effects of TENS on the ANS may vary depending on the presence of ANS perturbations, and the nature of the specific perturbation, thus our results may be specific to our model of experimental pain.

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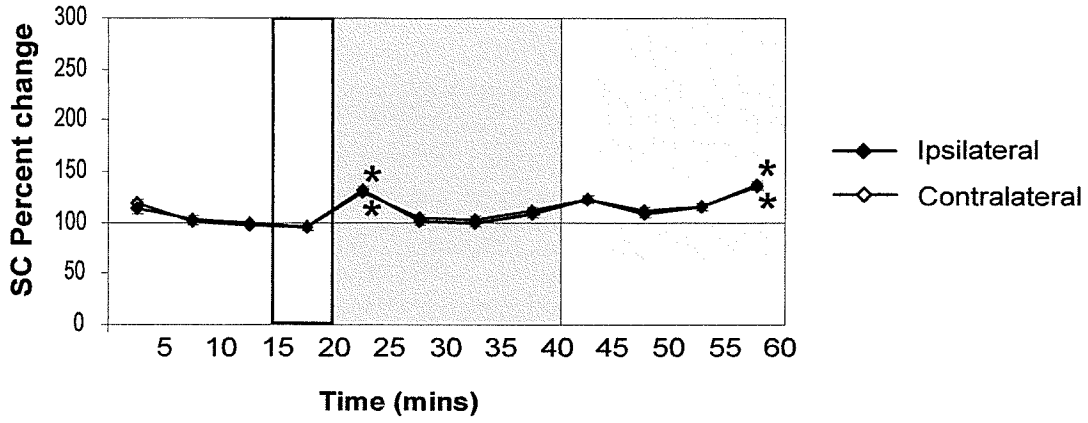
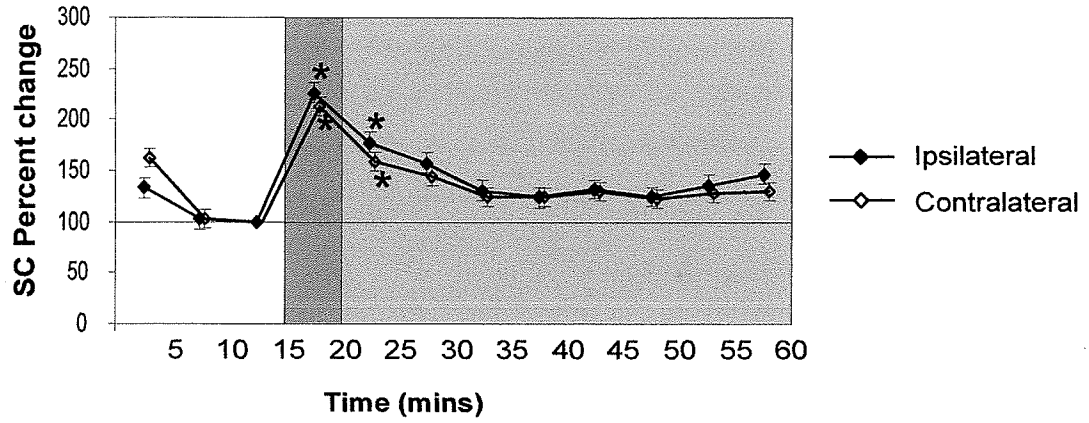
Figure 9) Ipsilateral and Contralateral Skin Conductance Responses

9A) Effects of TENS on ANS



9B) Effects of nociception on ANS



9C)Effects of TENS following nociception on ANS

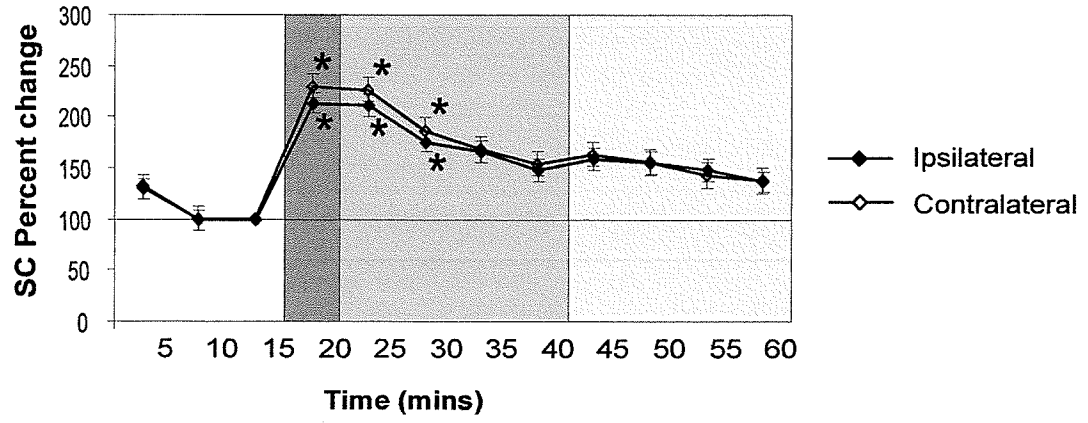
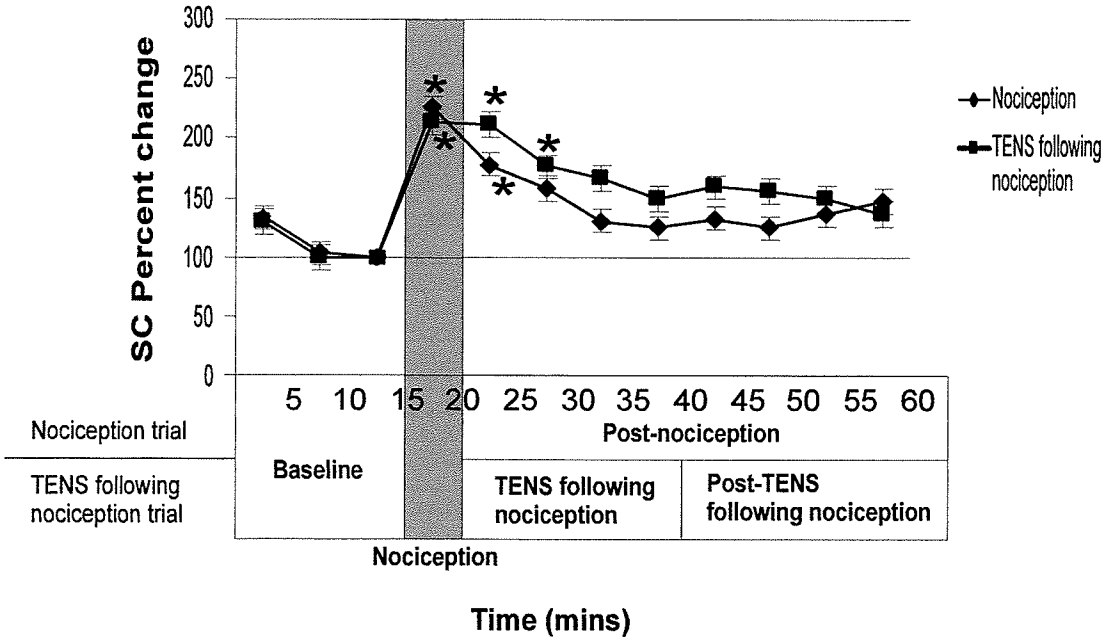


Figure 10) Effects of TENS on Skin Conductance Following Nociception

10A) Ipsilateral skin conductance



10B) Contralateral skin conductance

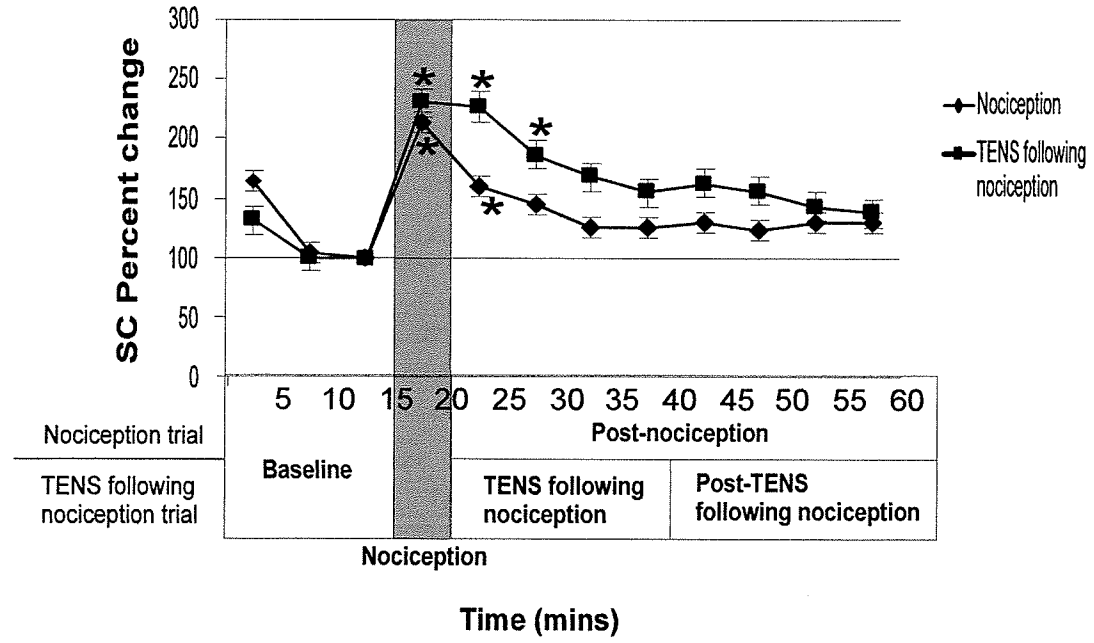


Figure Legends

Figure 9) Ipsilateral and Contralateral Skin Conductance Responses
Graphs illustrate skin conductance (SC) responses bilaterally to pertubations. Ipsilateral SC response is shown in solid and contralateral SC response is shown in no-fill. Figure 1A shows SC response to TENS, Figure 1B shows SC response to nociception and Figure 1C show SC response to TENS following nociception. Mean skin conductance values for all 10 participants are computed within each 5 minute block and normalized to baseline (15 minute block) ± SEM. Percent change from baseline is displayed on the vertical axis. Time (mins) is displayed on the horizontal axis. Trial periods are shaded respectively. One-way repeated measures ANOVA was used to identify significant changes throughout the experiment compared to baseline. Significant differences are indicated by asterisks.

Figure 9A) Effects of TENS on ANS: Significant increases in skin conductance are observed within the 25 minute block (TENS period) both ipsilaterally (131.2% ± 11.15 $p<0.041$) and contralaterally (131.6% ± 7.94 $p<0.033$) and the 60 minute block (post-TENS period) both ipsilaterally (134.9% ± 19.62 $p<0.016$) and contralaterally (139.5% ± 18.18 $p<0.003$).

Figure 9B) Effects of nociception on ANS: Significant increases in skin conductance are observed within the 20 minute block (nociceptive period); ipsilateral 226.2% ± 55.72 $p<0.001$, contralateral 213.5% ± 35.56 $p<0.001$, and maintained during the 25 minute block (post-nociceptive period); ipsilateral 177.4% ± 38.4 $p<0.040$ contralateral 159.4% ± 23.75 $p<0.012$.

Figure 9C) Effects of TENS following nociception on ANS: Significant increases in skin conductance are observed within the 20, 25, and 30 minute blocks. 20 minute block (nociceptive period); ipsilateral 214.1% ± 35.62 $p<0.001$ contralateral 230.1% ± 37.42 $p<0.001$, 25 minute block (TENS/nociception period); ipsilateral 211% ± 31.73 $p<0.001$ contralateral 226.5% ± 36.51 $p<0.001$, and 30 minute block (TENS/nociception period); ipsilateral 176.2% ± 25.84 $p<0.023$ contralateral 186.4% ± 27.74 $p<0.004$). Note that the addition of 100 Hz TENS following nociception maintains the increased SC response noted with clip application and continues to be significantly increased for and additional five minute period.

Figure 10) Effects of TENS on Skin Conductance Following Nociception
Graphs illustrate comparison of data from the *Effects of Nociception on ANS* and *Effects of TENS following Nociception on ANS* trials. Figure 2A shows the ipsilateral skin conductance and Figure 2B shows the contralateral skin conductance. Values represent mean ± SEM for 10 participants. Percent change compared to baseline is displayed on the vertical axis while time and trial period

is displayed on the horizontal axis. Significant changes from baseline as reported in figure 1B and 1C are shown by asterisks. Note the prolonged increase in skin conductance within the *Effects of TENS following nociception on ANS* trial (squares) compared to the *Effects of nociception on ANS* trial (diamonds). Skin conductance remains significantly increased for an additional 5 minutes (30 minute block) when TENS follows nociception versus nociception alone. Contralaterally, skin conductance is significantly greater in the TENS following nociception trial when compared to the nociception trial ($p<0.026$), while ipsilaterally, skin conductance values are greater but non-significant ($p<0.067$).

Table 4) Trial protocols; manuscript 2

	Time									
Effects of TENS on ANS	15	20	25	30	35	40	45	50	55	60
Period	Baseline	Null	TENS				Post-TENS			
Participants (Normalized SC)										

	Time									
Effects of nociception on ANS	15	20	25	30	35	40	45	50	55	60
Period	Baseline	Nociception	Post-nociception							
Participants (Normalized SC)										

	Time									
Effects of TENS following nociception on ANS	15	20	25	30	35	40	45	50	55	60
Period	Baseline	Nociception	TENS following nociception				Post-TENS following nociception			
Participants (Normalized SC)										

Shaded areas represent the different periods throughout the trial.

Table 5) VNPRS score summary

Time	VNPRS scores Nociception trial	VNPRS scores TENS following nociception trial
1 minute clip exposure	5.3 ± 1.57	5.0 ± 1.41
4 minutes clip exposure	4.3 ± 1.95	3.8 ± 1.81
5 minutes post-clip removal	0.0	0.3 ± 0.67
10 minutes post-clip removal	0.0	0.2 ± 0.63
15 minutes post-clip removal	0.0	0.0

VNPRS scores in response to battery clip application are taken from the *Effects of nociception on ANS* and the *Effects of TENS following nociception on ANS* trials. VNPRS scores are expressed as an average representing the 10 participants ± standard deviation.

General Discussion

The underlying purpose of this thesis was to put forward a clinically relevant approach to pain research in a physical rehabilitation paradigm. This direction is felt to be extremely necessary in current practice, especially with the rapidly growing interest in evidence-based practice. In the past, anecdotal, subjective, and clinically observed benefits of physical therapy have been appreciated without question towards scientific evidence to support different treatment outcomes. As research advances and public awareness grows, it is critical to present solid, scientific rationale to support all clinical decisions. This "more recent" form of clinical practice is now expected from both private and public insurance companies, employers, government, and most importantly the patient's seeking intervention. This subject presents unique challenges to clinicians and researchers alike. Very few scientific studies exist that probe the efficacy of various rehabilitation approaches, and attempt to explain why such approaches are advantageous or ineffective in terms of physiology, pathology, and anatomy.

The discussion of pain in rehabilitation can be very confusing and complex. This situation arises from the fact that pain is a multi-factorial sensation with physical, emotional, and psychological attributes. Due to the multi-factorial nature of pain, the ability to control experiments involving pain responses in human research is difficult. Knowing this, we looked to the ANS to assist in providing an objective, measurable response to a controlled nociceptive stimulus.

Exploiting the intimate relationship between pain and the ANS there may be an ability to provide an objective pain measure. HRV appears to have the potential for this application. Although our findings are preliminary, our experiments present promising results that further research with HRV may indeed lead to an objective pain measure. It was our intention to introduce the Polar S810i as a valid means of studying HRV. The Polar heart rate monitor is very simple to integrate into clinical practice. Its operation is straight forward, it is very portable and compact, the data analysis is uncomplicated, and it is financially attractive, however further research remains a priority.

The ability to objectify pain in response to various treatment interventions would allow clinicians to scientifically reason their overall treatment approach. The experimental method we introduced can be utilized to examine almost any treatment intervention. Within our study we looked at the effects of TENS on the ANS, but TENS could have been substituted with interferential current therapy, spinal or peripheral joint mobilization / manipulation, acupuncture, and so forth. Not only can the effects of individual modalities be tested on the ANS and monitored, but the ability to test different parameters (i.e. pulse width, frequency, various manual techniques, needle location, depth, etc.) truly allows clinicians to employ the most efficacious form of the selected treatment. It is strongly recommended that further research continue to look at a variety of different measures in attempt to objectively quantify pain. Within our study, the focus lies heavily on ANS behavior in response to pain. ANS measurements, especially

HRV, offer valuable insight to physiological responses to pain, but concurrent physiological measurements should be used as this area in pain research develops. Subjective measurements and demographic data should also be used in attempt to further the interpretation of the objective data due to the multi-factorial nature of pain.

Our experiment introduced a unique and novel approach to creating experimental pain. Further research is definitely required in order to develop this model of experimental pain. The mechanical nature of the nociception is believed to more so resemble the pain that is experienced clinically in acute states of musculoskeletal injury. Previous models of experimental pain in humans, including electrical shock and topical capsaicin application do not appear to represent the nociception associated with injuries commonly seen in the rehabilitation paradigm. It is well known that descending pain control systems within the central nervous system respond differently to various types of nociception, and therefore, testing interventions on nociceptive perturbations that behave similarly to the nociception associated with musculoskeletal injury is imperative in order to present valid findings. The use of the clip is effective at producing mechanical nociception. It is considered ethical in its use. The clip is tolerated well by most individuals, and by looking at our VNPRS scores and objective skin conductance data, the clip appears quite reliable. The use of experimental nociception and testing the effects of various interventions on this state of experimental pain offer a powerful experimental paradigm. The ability to

study individuals undergoing pain and treatment concurrently is essentially what happens in the clinical setting, thus research using this approach will become prominent in rehabilitation.

The notion we present with regards to differences in ANS behavior in response to nociception in athletically trained individuals versus sedentary individuals was rather interesting. It is well known that athletes tolerate physical stressors better than non-athletes. No studies to date that look at the differences between athletes and non-athletes in terms of their responses to nociception and treatment intervention were found. It is possible that ANS adaptations that occur with physical conditioning may be beneficial in decreasing the deleterious effects of nociception in terms of ANS response, and these ANS adaptations may also render these individuals to enhanced treatment efficacy to interventions that effect the ANS. The significance of such findings could potentially affect the way we approach rehabilitation. The traditional focus of rehabilitation has always been to treat the area / structure responsible for the pain. Knowing that the pain due to the effects of injury may be decreased by adaptations of the ANS with physical conditioning will place a huge priority on overall fitness development and physical conditioning. This knowledge will empower the patient to uphold fitness and conditioning responsibilities throughout their rehabilitation and also in their day to day lifestyle. Although our study did not reveal any significant findings in this area, given our limitations, further research is definitely warranted in this

area. This direction of research remains in its infancy, but as it progresses, significant and interseting results are expected.

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Appendix A

Pre-experiment questionnaire

The Effects of Transcutaneous Electrical Nerve Stimulation on Autonomic Cardiovascular Modulations in Response to Mechanical Cutaneous Nociception

Principal Investigator: Garrett Morrison
Supervisor: Dr. Barbara Shay

Name:_____

D.O.B.:_____
Age:_____

Sport /
Activities:_____

Date / Time of Exp.:_____
RHR:_____

---

1) Are you feeling well today?

2) Did you walk to the lab? (yes/no) if yes, did you walk for greater then 10 minutes or less then 10 minutes?

3) Did you take the stairs or the elevator to the lab?

4) When was your last meal? When was your last snack?

5) How many caffeinated beverages do you consume in a typical day?

- How many have you had today?

- When was your last one consumed?

6) Have you exercised today?

- When did you exercise last?

7) Have you performed any strenuous activities today? If yes, please list.

8) Do you currently feel any pain?

9) Are you currently recovering from any type of injury?

10) Have you had any alcoholic beverages today?

11) Do you smoke?

12) Are you currently using any drugs or medication?

13) Have you received TENS treatment before? (yes/no) if yes, what for?

- Was the TENS treatment effective? (yes/no)

14) How many hours per night do you sleep normally?

- How many hours did you sleep last night?

15) Do you participate in any type of physical activity? (yes/no), if so, what kind of activities do you participate in?

- How many times per week do you partake in the activities listed above?

- How would you rate the intensity of your participation in the above activities?
(low, moderate, high, very high)

Appendix B

Nottingham Health Profile

Nottingham Health Profile

Overview:

The Nottingham Health Profile is intended for primary health care, to provide a brief indication of a patient's perceived emotional, social and physical health problems.

Breakdown of questionaire

(1) Part I: 38 questions in 6 subareas, with each question assigned a weighted value; the sum of all weighted values in a given subarea adds up to 100

- energy level (EL): 3
- pain (P): 8
- emotional reaction (ER): 9
- sleep (S): 5
- social isolation (SI): 5
- physical abilities (PA): 8

(2) Part II: 7 life areas affected

Completing questionaire

- each question answered "Yes" or "No"
- important that all questions are answered
- if the patient is not sure whether to say "yes" or "no" to a problem, s/he are instructed to answer the one more true at that time.

Part I

Question	Yes	No	Section	Weight
I'm tired all the time.			EL	39.20
I have pain at night.			P	12.91
Things are getting me down.			ER	10.47
I have unbearable pain.			P	19.74
I take pills to help me sleep.			S	22.37
I've forgotten what it's like to enjoy myself.			ER	9.31
I'm feeling on edge.			ER	7.22
I find it painful to change position.			P	9.99
I feel lonely.			SI	22.01

I can walk about only indoors.			PA	11.54
I find it hard to bend.			PA	10.57
Everything is an effort.			EL	36.80
I'm waking up in the early hours of the morning.			S	12.57
I'm unable to walk at all.			PA	21.30
I'm finding it hard to make contact with people.			SI	19.36

Question	**Yes**	**No**	**Section**	**Weight**
The days seem to drag.			ER	7.08
I have trouble getting up and down stairs and steps.			PA	10.79
I find it hard to reach for things.			PA	9.30
I'm in pain when I walk.			P	11.22
I lose my temper easily these days.			ER	9.76
I feel there is nobody that I am close to.			SI	20.13
I lie awake for most of the night.			S	27.26
I feel as if I'm losing control.			ER	13.99
I'm in pain when I'm standing.			P	8.96
I find it hard to get dressed by myself.			PA	12.61
I soon run out of energy.			EL	24.00
I find it hard to stand for long (e.g., at the kitchen sink, waiting in a line).			PA	11.20
I'm in constant pain			P	20.86
It takes me a long time to get to sleep.			S	16.10
I feel I am a burden to people.			SI	22.53
Worry is keeping me awake at night.			ER	13.95
I feel that life is not worth living.			ER	16.21

I sleep badly at night.			S	21.70
I'm finding it hard to get along with people.			SI	15.97
I need help to walk about outside (e.g., a walking aid or someone to support me).			PA	12.69
I'm in pain when going up or down stairs.			P	5.83
I wake up feeling depressed.			ER	12.01
I'm in pain when I'm sitting.			P	10.49

Part II

Is your persent state of health causing problems with your:	**Yes**	**No**
Work? (that is, paid employment)		
Looking after the home? (cleaning & cooking, repairs, odd jobs around the home, etc.)		
Social life? (going out, seeing friends, going to the movies, etc.)		
Home life? (that is, relationships with other people in your home)		
Sex life?		
Interests and hobbies? (sports, arts and crafts, do-it-yourself, etc.)		
Vacations? (summer or winter vacations, weekends away, etc.)		

Interpretation

- number of questions in each section affected
- relative level affected, in which the sum of the relative weights are subtracted from 100%, giving values between 0 and 1, with 0 indicating poor and 1 good health

Appendix C

Informed Consent form

RESEARCH PARTICIPANT INFORMATION AND CONSENT FORM

Title of Study: The Effects of Transcutaneous Electrical Nerve Stimulation on Autonomic Cardiovascular Modulations in Response to Mechanical Cutaneous Nociception

Principal Investigators:

Garrett Morrison

Barbara Shay

You are being asked to participate in a research study. Please take your time to review this consent form and discuss any questions you may have with the study staff. You may take your time to make your decision about participating in this study and you may discuss it with your friends, family or (if applicable) your doctor before you make your decision. This consent form may contain words that you do not understand. Please ask the study staff to explain any words or information that you do not clearly understand.

Purpose of Study

This research study is being conducted to study the effects of pain and a treatment intervention for pain on the autonomic nervous system.

Objectives

1. To look at the response of the autonomic nervous system when an individual is experiencing pain
2. To study the effects of transcutaneous electrical nerve stimulation (TENS) on the autonomic nervous system after an individual has experienced pain
3. To examine the above situations in two (2) separate groups of individuals made up of either athletic or sedentary individuals

A total of twenty (20) participants will participate in this study

Study Procedures

Once you decide to participate, you will be asked a few questions regarding your participation in physical activity and have your resting heart rate recorded. This information will allow us to place you in to one of two groups, either the athletic group or the sedentary group. Once you are placed into a group, you will then participate in a preliminary trial. In this preliminary trial we will attach a heart rate monitor chest strap around your chest, two skin conductance electrodes on your index and little fingers of each arm, and four TENS electrodes on the forearm of your dominant arm. You will be asked to lie comfortably in a recliner chair on your back for the duration of the preliminary trial. We will take five minutes of baseline measurements while you remain at rest, then we will turn on the TENS machine. The TENS machine will produce a "tingling-like" sensation in the area of the four electrodes on your forearm. The tingling-like sensation will slowly increase in strength until we see a nearby muscle twitch. Upon seeing the muscle twitch we slowly turn down the strength until the muscle relaxes, you will feel a comfortable but strong tingling-like sensation at this point. At no point when the TENS unit is on should you feel any pain, if you sense any discomfort please alert the research staff immediately. The TENS unit will be on for 20 minutes while we continue our recording. The TENS unit is then turned off and recording continues for another 15 minutes. The preliminary trial will take approximately 65 minutes to complete.

After the preliminary trial is complete, you will be randomly selected to another set of groups, a No TENS group, or a TENS group and participate in a second trial. The second trial will take place roughly two to seven days after the preliminary trial.

All Participants: you will be asked to wear a comfortable short sleeve T-shirt to the laboratory on your testing day. You will have all of the devices physically attached to you as you did in the

preliminary trial. You will be asked to lie comfortably in a recliner chair on your back for the duration of the experiment. The experiments start with five minutes of baseline measurements while you remain at rest. For the next measurement, a battery clip will be physically attached in the centre or the four TENS electrodes on your arm. The battery clip will be attached to the skin and will produce an uncomfortable pinching sensation. The battery clip is kept in place for five minutes and then removed.

No TENS Participants: measurement continues for 50 minutes after battery clip removal. The TENS unit is not turned on in this experiment. Throughout the experiment the research staff will ask you to rate your pain/discomfort based on a 10 point scale (0 = no pain/discomfort, 10 = worst possible pain/discomfort), you will respond to the question with a single number. The control group experiment will take approximately 85 minutes to complete.

TENS group: once the battery clip is removed the TENS unit is turned on in the same manner explained in the preliminary trail. The TENS will be applied for 20 minutes and then it will be turned off. Measurement continues for 30 minutes beginning immediately after the TENS is turned off. Throughout the experiment the research staff will ask you to rate your pain/discomfort based on a 10 point scale (0 = no pain/discomfort, 10 = worst possible pain/discomfort), you will respond to the question with a single number. The experimental group experiment will take approximately 85 minutes to complete.

All trials and experiments will take place in the School of Medical Rehabilitation Pain Research Laboratory located at RR355-800 Sherbrook Street, Winnipeg, Manitoba

Participation in the study will consist of two (2) Laboratory sessions that will occur on two separate days. The two laboratory sessions will be roughly two to seven days apart. The first session will be approximately 65 minutes while the second session will be approximately 85 minutes.

The investigators may decide to take you out of the study if they feel that you may have problems tolerating the battery clip for the five minute duration or if they feel that the battery clip poses a harm above and beyond that expected in normal situations.

Risks and Discomforts

The battery clip used in this experiment will produce a painful pinching sensation at the site of application. In most people, the pain/discomfort is rated as moderately painful. Most people can tolerate the five minute duration required in this experiment. Pressure markings will be left on the skin as well as local redness seen at the area of application. Pain/discomfort usually disappear within 15 to 30 minutes after the battery clip is removed. Sensitivity to the area may be noticed for another 24 to 48 hours. Any physical effects due to battery clip application should disappear by 72 hours after experimentation. Normal use of the arm should not be affected at any time after the experiment.

Benefits

There may or may not be direct benefit to you from participating in this study. We hope the information learned from this study will improve our understanding of pain and our treatment approach to people with painful conditions.

Costs

All the procedures, which will be performed as part of this study, are provided at no cost to you.

Payment for Participation

You will receive no payment of reimbursement for any expenses related to taking part in this study.

Confidentially

Information gathered in this research study may be published or presented in public forums, however your name and other identifying information will not be used or revealed. Despite efforts to keep your personal information confidential, absolute confidentiality cannot be guaranteed. Your personal information may be disclosed if required by law.

The University of Manitoba Health Research Ethics Board may review records related to the study for quality assurance purposes.

All records will be kept in a locked secure area and only those persons identified will have access to these records. If any of your medical/research records need to be copied to any of the above, your name and all identifying information will be removed. No information revealing any personal information such as your name, address or telephone number will leave *the University of Manitoba.*

Participants wishing to view results of the experiments once it is finished can contact the principle investigators for further details.

Voluntary Participation/Withdrawal from the Study

Your decision to take part in this study is voluntary. You may refuse to participate or you may withdraw from the study at any time. Your decision not to participate or to withdraw from the study will not affect your care at this centre. If the study staff feel that it is in your best interest to withdraw you from the study, they will remove you without your consent.

We will tell you about any new information that may affect your health, welfare, or willingness to stay in this study. Participants who are students or employees of the University of Manitoba or associated professionally with any of the investigators can be assured that a decision not to participate will in no way affect any performance evaluation of potential participants.

Medical Care for Injury Related to the Study

You are not waiving any of your legal rights by signing this consent form nor releasing the investigator(s) or the sponsor(s) from their legal and professional responsibilities.

Questions

You are free to ask any questions that you may have about your treatment and your rights as a research participant. If any questions come up during or after the study or if you have a research-related injury, contact the principle investigator: Garrett Morrison at [redacted]

For questions about your rights as a research participant, you may contact The University of Manitoba, Bannatyne Campus Research Ethics Board Office at (204) 789-3389

Do not sign this consent form unless you have had a chance to ask questions and have received satisfactory answers to all of your questions.

Statement of Consent

I have read this consent form. I have had the opportunity to discuss this research study with Garrett Morrison and Dr. Barbara Shay or his/her research staff. I have had my questions answered by them in language I understand. The risks and benefits have been explained to me. I believe that I have not been unduly influenced by any study team member to participate in the research study by any statements or implied statements. Any relationship (such as employer, supervisor or family member) I may have with the study team has not affected my decision to participate. I understand that I will be given a copy of this consent form after signing it. I understand that my participation in this study is voluntary and that I may choose to withdraw at any time. I freely agree to participate in this research study.

I understand that information regarding my personal identity will be kept confidential, but that confidentiality is not guaranteed. I authorize the inspection of any of my records that relate to this study by The University of Manitoba Research Ethics Board for quality assurance purposes.

By signing this consent form, I have not waived any of the legal rights that I have as a participant in a research study.

Participant signature_____

Date _____

Participant printed name: _____

I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has knowingly given their consent

Printed Name: _____

Date _____

Signature: _____

Role in the study:_____

Relationship (if any) to study team members_____

APPENDIX

Relevant Definitions

Battery Clip: Similar to a clothes pin but made of metal. The clip will be used to pinch the skin to produce a sensation of pain/discomfort.

Heart Rate Variability (HRV): A specific way of studying how the heart beats. HRV is concerned with the timing of each heart beat. Necessary information is collected through a sensor that is attached to a strap that is placed around an individual's chest. HRV data provides information about the autonomic nervous system.

Skin Conductance: A measure of autonomic nervous system activity. Skin Conductance uses two sensors attached typically to the index and little fingers of the hand. The two sensors measure properties of electrical current flow over the skin.

Transcutaneous Electrical Nerve Stimulation (TENS): A device used for the treatment of pain. TENS treatment consists of usually 2 or 4 electrodes attached to the skin by a coupling gel. An electrical current is passed through the skin in attempt to stimulate various nerves which can thereby decrease pain. The TENS unit produces a "tingling" sensation when it is turned on.