Effect of High Frequency TENS on Cold Hyperalgesia Induced by Topical Menthol in Healthy Subjects

By

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Abstract

The primary aim of this study was to determine the effect of high frequency Transcutaneous Electrical Nerve Stimulation (TENS) on cold sensory function following topical application of menthol. Quantitative sensory testing was used to determine cold sensation and cold pain thresholds before and after topical application of a 40% menthol solution in 9 male and 11 female subjects. In a separate session the effect of TENS (100 HZ, constant pulse, 100μs, 20 minutes) was determined on menthol-induced cold sensation. Menthol produced a distinct cold hyperalgesia which was significantly reduced during the application of high frequency TENS. The analgesic effect of TENS persisted beyond the application period for at least 20 minutes. Menthol also reduced cold detection thresholds but TENS had no effect on this aspect of cold sensation. These data support the use of TENS as a means of treating cold hyperalgesia such as that found in neuropathic pain states.

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Thanks for making this a filling and wonderful experience.

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Introduction

Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage (International Association for the Study of Pain, 2011).

An inferred purpose of pain is to prevent injury. If injury does occur, a further purpose of pain is to prevent further injury during the healing phase. This intent is expressed in the form of hyperalgesia and allodynia. These conditions are characterised by an increased sensitivity to a painful stimulus or to a non painful stimulus, respectively. When pain persists after tissue healing or the initial cause for pain has disappeared, pain is no longer a symptom but rather a disease and is referred to as chronic pain.

Neuropathic pain can arise from injury to the nervous system and is characterized by chronic pain in addition to other sensory abnormalities. Cold hyperalgesia is common to a number of neuropathic pain conditions and is defined as increased sensitivity to a cold stimulus. Verdugo et al. established that 8% of patients with peripheral nerve disorders experience pure cold hyperalgesia which is often combined with cold hypoesthesia, especially in those with Complex Regional Pain Syndrome (CRPS) (Kemler, et al., 2000; Rommel, Malin, Jänig, & Zenz, 2004; Rommel, Malin, Zenz, & Jänig, 2001; Verdugo & Ochoa, 1992).

Many patients with neuropathic disorders receive little benefit from current treatment options which are mainly pharmacological, and in most cases cause undesired side effects. Medication only reduced pain severity by 30-40% and significant pain relief was achieved in less than 50% of patients (Freynhagen, Baron, Gockel, & Tölle, 2006;

Haanpää, et al., 2009). Medications do not produce concomitant improvement of physical and emotional functioning (Pérez, Saldaña, Navarro, Vilardaga, & Rejas, 2009).

Non pharmacological treatments are available as well. They are used as a complement or as an alternative to the pharmacological treatment. A multimodal approach is common in neuropathic conditions. A study in patients with postherpetic neuralgia showed that the use of Transcutaneus Electrical Nerve Stimulation (TENS) with Pregabalin resulted in a significant reduction in pain and sleep interference compared to Pregabalin alone (Barbarisi, et al., 2010). In particular TENS is one of the commonly used treatments by physiotherapists. It is safe with unlikely side effects and only a few contraindications. Additionally, TENS devices are inexpensive and simple to operate which makes ownership and operation by patients a reality. TENS has been shown to be effective in a variety of animal and human models of pain. Among others, these models include thermal heat pain in healthy subjects and those with neuropathic pain, however the effects of TENS on cold hyperalgesia has not been studied. The purpose of the present study is to evaluate the effectiveness of TENS in reducing cold hyperalgesia as a first step in the assessment of TENS as a therapeutic tool in the treatment of cold hyperalgesia in neuropathic pain patients.

Literature review

Receptors involved in thermal sensation and cold pain

The detection of any sensation requires transduction of the sensory stimulus into an action potential. Different transducers are responsible for this essential function. In the case of thermal sensation, this role is carried out by the transient receptor potential (TRP) family of ion channels. A variety of subsets of TRPs have been identified with separate receptor subtypes mediating warm and cold sensations. Warm and hot pain ($\geq 42^{o}$C) sensations are generated through activation of the TRPV1 receptor expressed on the free nerve endings of C fibre primary afferents (Story, 2006). Much of what is known about the TRPV1 receptor has been revealed through the use of capsaicin which is a specific agonist of the TRPV1 receptor (Carlton & Coggeshall, 2001). Another receptor responsible for warm detection in ranges from 27 to 42^{o}C is the TRPV4, which declines in activity as temperature increases further (Story, 2006).

In contrast, cool and cold sensations are mediated by two other members of the TRP family of ion channels. The cold and menthol sensitive TRP melastatin 8 (TRPM8) channel is activated within the range of 8–28°C (Bautista, et al., 2007; Colburn, et al., 2007; Dhaka, et al., 2007; Knowlton, Bifolck-Fisher, Bautista, & McKemy, 2010). In other instances with temperatures lower than 17^{o}C, TRPA1 is activated which also responds to pungent natural compounds like icilin and to bradykinin (Bandell, et al., 2004).

As mentioned above, TRPM8 has been reported to mediate thermal sensations of cold (Bautista, et al., 2007; Colburn, et al., 2007; Dhaka, et al., 2007; Knowlton, et al., 2010). Several studies have examined the physiological roles of TRPM8 using genetically altered

mice. Bautista et al. performed electrophysiology studies combined with behavioural assessment of sensory function. Cultured sensory neurons and intact sensory nerve fibres from TRPM8-deficient mice both showed a significantly reduced response to cold. Their ability to respond to evaporative cooling was significantly decreased as well. In addition the response rate of neurons to menthol was profoundly diminished. The animals were still able to avoid noxious cold under 10^{o}C, although less efficiently (Bautista, et al., 2007). The preservation of cold avoidance was suggested to be due to the intact function of TRPA1 receptors, which also respond to noxious cold. In contrast, response to warm temperatures or capsaicin was not affected. It was concluded that TRPM8 is the principal detector of environmental cold stimuli. Similar to the previous report, Colburn et al. reported that the number of neurons responding to cold (<18^{o}C) or menthol was attenuated in TRPM8 null mice (Colburn, et al., 2007).

Additional studies have examined if increased pain due to cold stimulation is linked to this receptor. A recent study examined the role of TRPM8 and TRPA1 in cold-evoked pain. Knowlton et al. used mice lacking both channels and examined behaviours and neural activity in response to painful cold and noxious cooling compounds. They showed that mice that normally have a strong preference for warm temperature no longer avoid cold until it reaches noxious ranges. Additionally, icilin, an agonist for TRPM8 that also activates TRPA1 receptors, was used to assess nocifencive responses, which were not seen in mice lacking TRPM8 but were maintained in TRPA1 knockouts. These findings support the conclusion that TRPM8 is essential for mediating responses to cold and noxious cold (Knowlton, et al., 2010).

Another study also shows behavioural deficits in the response of neurons to cold and menthol, as well as reduced cold avoidance in TRPM8-null mice. These animals had normal nocifencive responses to temperatures below 0C. For this reason they suggest that at least one other cold receptor should be responsible for noxious cold sensation below 0C (Dhaka, et al., 2007).

In addition to the above findings Macpherson et al. reported that menthol is not specific to TRPM8 receptors, since it cross activates other TRP ion channels such as TRPA1 and TRPV3 (Macpherson, et al., 2006; Sherkheli, Gisselmann, Vogt-Eisele, Doerner, & Hatt, 2008; Sherkheli, et al., 2010).

Aside from mediating cool sensations and cold pain, evidence also suggests that TRP ion channels play an important role in pain states where thermal sensitivity is an issue. Up regulation of TRPs has been reported by different authors in neuropathic conditions. Xing et al. used a chronic constriction injury in rats as a model of neuropathic pain to assess the role of TRPM8 in cold allodynia. They showed that a non-selective antagonist for TRPM8 and TRPV1 was able to attenuate cold allodynia, but not a selective blocker for TRPV1. They also found an increase in immunoreactive TRPM8 neurons in the dorsal root ganglion neurons of L5 as well as an increase in the percentage of menthol and cold sensitive neurons. The responsiveness to menthol and innocuous cold was clearly enhanced in these neurons and TRPM8 was expressed in sensory fibers innervating the skin of the hind paw. These findings show that TRPM8 expression was increased centrally on the cell bodies as well as on the peripheral nerve fibers. The neurons and nerve fibers that displayed increased TRPM8 expression were also capsaicin sensitive neurons (TPRV1-positive) which represent a subtype of nociceptive C fibres indicating

the presence of TRPM8 on nociceptive afferents (Xing, Chen, Ling, Tan, & Gu, 2007). These findings indicate that TRPM8 receptors are co-labeled with TRPV1 and contribute to cold allodynia in neuropathic pain states.

Serra et al. reported some interesting facts regarding a patient with small fibre neuropathy of unknown origin. The patient's main complaint was burning pain and cold allodynia. Thermal perception thresholds in the feet and hands were increased for cold sensation (cold hypoesthesia) and markedly reduced for cold pain (cold allodynia). Microneurographic recordings of subtypes of C nociceptors showed ongoing spontaneous activity in almost 50% of recordings and an increased responsiveness to menthol and cold was also observed. These two observations may account for the burning sensations and the cold allodynia, respectively. Further, menthol was able to activate C fibres and further sensitize neurons to cold (Serra, et al., 2009). Although data regarding receptor subtype was not available, this case report supports the use of menthol to investigate cold sensory function in humans.

Neurons responsible for mediating cold sensation and cold pain

The issue of which sensory afferent nerve fibers express TRP receptors has been the focus of considerable research (Campero, Baumann, Bostock, & Ochoa, 2009; Campero & Bostock, 2010; Carr, Pianova, McKemy, & Brock, 2009; Kobayashi, et al., 2005; McKemy, Neuhausser, & Julius, 2002; Takashima, et al., 2007; Xing, et al., 2007; Xing, Ling, Chen, & Gu, 2006). Studies concur that both A-fibre and C-fibre neurons express TRPM8, suggesting that the TRPM8 receptor serves non-nociceptive and nociceptive functions.

Xing et al characterized TRPM8 expression on rat dorsal root ganglion (DRG) neurons. They observed TRPM8 in two subpopulations of neurons. The first subpopulation was menthol sensitive, capsaicin insensitive, and showed non-nociceptive properties. The second was sensitive to menthol and capsaicin, which is representative of C nociceptors and therefore indicates that some menthol-activated neurons have nociceptor properties (Xing, et al., 2006). This supports the idea that the menthol activated cold receptor is localized on afferents with different sensory functions.

Co-expression of TRPM8 and TRPV1 was initially reported by McKemy et al. in 2002 and subsequently confirmed by several groups (Broad, et al., 2009; Story, 2006; Stucky, et al., 2009). Takashima et al. created mice expressing a genetically encoded axonal tracer in TRPM8 neurons. Identifying the expression of the tracer they determined that TRPM8 was expressed on Aδ as well as C fibres assuming a non-nociceptive and nociceptive function. Their data showed that approximately 39% and 24% of trigeminal and DRG cultured sensory neurons showed co-expression of both TRPV1 and TRPM8 receptors (Takashima, et al., 2007).

Consistent with these findings McKemy et al. characterized and cloned menthol receptors from trigeminal sensory neurons. They found that 55% of these neurons were activated by menthol and cold as well as by capsaicin. This functional data was corroborated with immunohistochemistry which showed that menthol and cold activated neurons were overlapping in a vast subset of neurons, 54.5% of which were also activated by capsaicin. These data indicate that TRPV1 and TRPM8 are co-expressed on the same neurons. These authors also inferred that because capsaicin is a feature of nociceptive neurons, half of the menthol sensitive neurons should be categorized as such (McKemy, et al., 2002).

Kobayashi et al. reported that TRPM8 was located on both A fibre and C fibre neurons, but additionally found one conflicting outcome. In contrast to the previous studies, when they analysed the TRPM8 mRNA in the DRG using immunohistochemistry, it was not expressed in the TRPV1 positive neuronal population; therefore they concluded that TRPM8 was not co expressed with TRPV1 (Kobayashi, et al., 2005).

Menthol or cold on the skin can not only be experienced as cold but is also usually described as burning. This burning sensation is further enhanced by blockade of Aδ fibres (Campero, et al., 2009; Wasner, Schattschneider, Binder, & Baron, 2004). Co-expression of TRPM8 with TRPV1 on nociceptors that are typically associated with hot pain sensations may account for the burning sensation that can accompany cold pain.

To explain this phenomenon Campero et al. used microneurography in humans to look for C fibres activated by cooling and menthol. He classified three types of C fibres and measured their response to cooling and heat before and after topical menthol. The first type was classified as a polymodal nociceptor Type 1A, the second a mechanically insensitive nociceptor Type 1B and the third a cold sensitive Type 2. Menthol was able to activate Type 2 C fibres, these fibres also had a strong response rate to cold applied to the skin in non-noxious and noxious ranges down to 0^{o}C. The same Type 2 fibres were also activated by heating, which suggests co-localization with the TRPV1 receptor (Campero, et al., 2009).

Transient receptor potential ion channels play a very complex role in thermal sensation. Evidence shows that one sensory neuron can respond to very different stimuli like cold and warm. This phenomenon is explained by the fact that a type C nociceptive afferent co-expresses two completely different receptors, TRPV1 and TRPM8, responsible for hot

and cold sensation, respectively. There is also strong evidence that TRPM8 activates distinct sensory afferent subtypes which produce differing responses, in ranges within noxious and innocuous cold. Stimuli in the non-painful range are mediated by Aδ sensory neurons whereas painful stimuli are mediated by smaller C fibre neurons. In conclusion, TRPM8 is an ion channel that plays a multidimensional role responding to a wide range of cold stimuli.

Pain mechanisms

Pain is a symptom which aims to protect the body. Suitably intense stimuli activate nociceptive sensory afferents that result in the experience of pain which produces responses intended to remove the offensive stimuli. It is now known that acute tissue and neural injuries can result in sensitization within the peripheral and central components of the pain system. Sensitization arises in two forms; hyperalgesia and allodynia. Hyperalgesia is mediated by nociceptive high threshold C fibres and allodynia is mediated by low threshold Aβ fibres. Each condition may be triggered by a characteristic set of changes within the central and peripheral nervous system. The consequences of these changes may be necessary or sufficient for induction of hyperalgesia or allodynia; others may facilitate or inhibit changes in pain sensitivity. Changes in the balance between excitatory and inhibitory function within the nervous system may be responsible for sensitization. In most clinical features, there is a complex interaction that involves the peripheral and central nervous systems rather than a single mechanism. Since numerous mechanisms are implicated, it is essential to understand them in order to be able to treat pain effectively.

Peripheral pain mechanisms

Peripheral sensitization is defined as "Increased responsiveness and reduced threshold of nociceptors to stimulation of their receptive fields"(Baron, 2009). This process is responsible for hyperalgesia in the area immediately surrounding the primary injury. Sensitized nociceptors present pathological spontaneous discharge, a lowered activation threshold for thermal and mechanical stimuli, and an enhanced discharge to supra-threshold stimulation (hyperalgesia).

A variety of mechanisms are involved in the sensitization of nociceptors and the consequent development of primary hyperalgesia. The main changes involve cell damage and the consequent release of aracadonic acid, potassium and hydrogen, upregulation of cyclooxygenase (COX)-2 followed by subsequent synthesis of prostaglandins. Consequently prostaglandins and bradykinin sensitize the nociceptor endings. Neurogenic peptides, including Substance P, result in vasodilation and edema as well as the release of histamine from mast cells and serotonin from platelets. Release of norepinephrine from sympathetic efferent fibers also affects inflammation and sensitizes peripheral nociceptors (Vadivelu & Sinatra, 2005).

In the presence of injury to the nervous system, the hyperalgesia is maintained in addition to other changes that include ectopic discharge of primary afferent sensory neurons and changes in expression of ion channels in nociceptive axons and dorsal root ganglia neurons. It has been hypothesised that novel expression of TRPV1and TRPM8 ion channels might be responsible for thermal hyperalgesia. The changes outlined can lead to prolonged noxious transmission into the dorsal horn which results in secondary hyperalgesia or central sensitization.

Central pain mechanisms

Central sensitization is defined as an "Increased responsiveness of nociceptive neurons in the central nervous system to their normal or subthreshold afferent input". It is manifested by at least three different modes: increase of neuronal activity to noxious stimuli, expansion of size of neuronal receptive fields, and spread of spinal hyperexcitability to other segments (Sandkuhler, 2009). The main mechanisms behind this phenomenon are changes in expression of neurotransmitters and their receptors, disregulation of inhibitory neurons and modulatory descending pathways, and the anatomical and functional reorganization of primary afferents in the dorsal horn (Casals-Diaz, Viva, & Navarro, 2009)

Physiological human model for cold hyperalgesia

A number of animal and human models of pain have been established. These models have permitted the study of the underlying mechanisms of pain. Pain models are also essential to evaluate the effect of new treatments. In the specific case of increased sensitivity to cold, which is a very common modality of pain in neuropathic patients, human models of cold hyperalgesia have been studied (Binder, Stengel, Klebe, Wasner, & Baron, 2011; Hatem, Attal, Willer, & Bouhassira, 2006; Namer, Seifert, Handwerker, & Maihöfner, 2005; Wasner, et al., 2004). As discussed above, the TRPM8 receptor is in part responsible for cold sensation and cold pain. This receptor is sensitive to both menthol and cold; therefore a human experimental model of cold hyperalgesia is topical menthol application. Different concentrations of menthol have been proposed with some variation in the obtained effects (Binder, et al., 2011; Hatem, et al., 2006; Namer, et al., 2005; Wasner, et al., 2004).

Wasner et al. and Namer et al. applied a 40% L-menthol solution to the forearm for 20 minutes to obtain marked changes in cold sensitivity as well as punctate hyperalgesia, an indication of central sensitization. Hatem et al. used a lower concentration of menthol (30%) for 10 minutes as spontaneous pain and possible skin reactions were undesired in this study. Despite the reduction in the strength and duration of menthol exposure, 67% of subjects reported cold hyperalgesia. Punctuate hyperalgesia was not achieved at this concentration.

These studies showed a significant increase in cold pain threshold after menthol application whereas cold sensation was not significantly affected. In all studies hyperalgesia was predominantly achieved in the primary area of hyperalgesia.

Wasner et al. added another series of experiments in which A fibre conduction was blocked. This was achieved by applying pressure through a rubber band loaded with a weight to the superficial radial nerve. The blockade of mechano-sensitive Aβ fibres was determined by anaesthesia to light touch with a cotton swab over the skin. Sufficient blockade of Aδ fibres was considered present when cold sensation required temperatures below 10^{o}C. This model of A fibre nerve block allowed for cold pain perception to continue, since cold-sensitive C fibers were unaffected. Complete A fibre blockade inhibited cold sensation and punctuate hyperalgesia, but did not affect induction of cold hyperalgesia, indicating that menthol induces its effect through nociceptive fibers. In all three studies pain was mostly reported as a burning sensation, including during A fibre conduction blockade. Blockade of A fibre also produced an increase in spontaneous pain sensation.

Binder et al assessed the 40% menthol model to determine if the model could be a suitable, reliable and stable model for cold hyperalgesia and mechanical hyperalgesia. They established that the menthol model is a useful model for psychophysical and pharmacological research (Binder, et al., 2011).

These models show clear evidence for the involvement of the menthol sensitive receptor in mediating cold pain. Researchers suggest that these mechanisms might be involved in cold hyperalgesia experienced by patients with neuropathic pain and that the topical menthol model is appropriate for the study of cold hyperalgesia in humans (Binder, et al., 2011; Hatem, et al., 2006; Wasner, et al., 2004).

Pharmacological effect on menthol induced cold hyperalgesia

Pain models are essential in the development of analgesic drugs and also to test the effects of drugs that are already being used on different pain modalities like inflammatory or neuropathic pain. The 40% topical menthol model has been used to test the effect of different drugs on cold hyperalgesia. Altis et al. tested three typical analgesic drugs that operate through completely different mechanisms on 20 healthy subjects with menthol induced cold hyperalgesia. Ibuprufen is an anti-inflammatory analgesic that works by inhibiting the production of prostaglandins, which are mediators of pain and inflammation. Additionally they tested the effect of pregabalin which is a voltage dependent calcium channel blocker, reducing the influx of calcium to the nerve terminal. The effect of tramadol, a centrally acting opioid analgesic, was also examined. Only tramadol showed a significant effect on cold pain thresholds when compared to placebo medication. Authors compared the data from this study to previous research regarding the effect of drugs on different neuropathic conditions. Based on these comparisons they

conclude that menthol-evoked cold hyperalgesia is a valid model for cold hyperalgesia (Altis, et al., 2009).

The evidence that an opioid analgesic is effective in treating cold hyperalgesia supports the idea of other opioid acting mechanisms being effective. Transcutaneous electric nerve stimulation (TENS) is a non-pharmacological agent that modulates pain responses through an action on opioid receptors indicating that cold hyperalgesia might be alleviated by treatment with TENS. Opioids are a second choice treatment in neuropathic patients because of the undesired side effects. Despite TENS acting through similar mechanisms, none of the side effects are seen with TENS treatment.

TENS as a treatment for pain control

TENS is effective in relieving pain in a variety of conditions. It is defined by the American Physical Therapy Association as the application of electrical stimulation to the skin for pain control. TENS can be applied using different frequencies, intensities or electrode placement which will affect the underlying mechanisms and treatment outcomes. Some mechanisms of TENS are frequency dependent (i.e., activated by high (>50 Hz) or low (<10 Hz) frequency stimulation). Intensity of stimulation also contributes to the physiological effect with low intensities defined as those that elicit only a sensory response while those that stimulate motor responses are considered high intensity.

Theories of TENS mechanisms

TENS is able to achieve an analgesic effect through peripheral and central mechanisms. Research has advanced greatly since the first widely accepted pain modulation theory was proposed. In 1965 Melzack and Wall, proposed that the stimulation of large diameter neurons (A fibres) are able to inhibit the response of nociceptive C fibres in the substantia gelatinosa of the dorsal horn. It was proposed that this segmental inhibition of noxious input to higher centers was responsible for reduced pain perception.

Much more evidence is available today. A vast amount of research has been done regarding the pain relief mechanisms of TENS. Several studies support activation of opioid receptors centrally as well as in the periphery as one of the main mechanisms (Cui, Zhao, Wu, Piao, & Xu, 2006; Kalra, Urban, & Sluka, 2001; Leonard, Goffaux, & Marchand, 2010; Sluka & Chandran, 2002; Sluka, Deacon, Stibal, Strissel, & Terpstra, 1999; Sluka, Vance, & Lisi, 2005). The use of specific opioid receptor antagonists has confirmed that the analgesic effect of TENS is mediated by specific opioid receptors. Naloxone a selective μ opioid antagonist is able to reverse the effect of low frequency TENS. In contrast naltrindole a selective δ antagonist reverses the effect of high frequency TENS (Kalra, et al., 2001; Sluka, et al., 1999). Leonard et al also showed that a high dose of naloxone is able to reverse the effect of both type of high frequency Tens, indicating a dose related response (Leonard, et al., 2010). There is evidence that this opioid mediated effect takes place at the rostroventromedial medulla (RVM) and periaqueductal grey (PAG) with either low and high frequency TENS (Ainsworth, et al., 2006; DeSantana, da Silva, de Resende, & Sluka, 2009).

Research also shows a reduction in aspartate and glutamate release in the spinal cord with high frequency TENS. However this reduction was prevented by administration of a δ opioid receptor antagonist (Sluka, et al., 2005).

Another mechanism responsible for the analgesic effect of high and low frequency TENS is the release of inhibitory neurotransmitters within the spinal cord dorsal horn. Spinal blockade of GABA receptors was able to prevent the analgesic effect of both, high and low frequency TENS in rats with joint inflammation. It was also observed that when animals without joint inflammation were treated with high frequency but not low TENS, concentrations of extracellular GABA increased. It was concluded that high and low frequency TENS reduce primary hyperalgesia by activation of $GABA_A$ receptors spinally (Maeda, Lisi, Vance, & Sluka, 2007). Another study investigated the role of spinal 5-HT and α_2-adrenoceptors in TENS analgesia as part of a descending inhibitory system. They used specific antagonists for each receptor to test their involvement in the TENS anti-hyperalgesic effect. They found that spinal 5-HT receptors only mediated the analgesic effect of low frequency TENS and that spinal noradrenergic receptors were not involved in TENS analgesia (Radhakrishnan, et al., 2003).

In addition to central processes, two additional peripheral mechanisms include a local role of α_{2A} adrenergic receptors in the anti-hyperalgesic effect of high and low frequency TENS and a local role of μ opioid receptors in the effect of low frequency TENS (DeSantana, Walsh, Vance, Rakel, & Sluka, 2008).

Table 1. Frequency specific analgesic mechanisms of TENS

Mechanism	TENS frequency
RVM and PAG	Low- High
Opioid receptor μ Opioid receptor δ	Low High
↓ in aspartate –glutamate release	High
↑GABA	High
GABA-mediated	Low- High
5-HT receptor-mediated	Low
local role of α_{2A}	Low - High

As summarized in Table 1, high frequency TENS is associated with most of the known analgesic mechanisms. It is also used at low intensities which are better tolerated by patients. Another important fact is that high frequency TENS targets δ opioid receptors, a different receptor than most opioid based analgesic drugs which normally target μ opioid receptors. Thus, TENS has the possibility of being used in combination with μ opioid agonists to optimize treatment effectiveness.

Effect of TENS on different pain modalities

Different authors have tested the effect of TENS on a variety experimental pain models such as inflammatory pain and blunt pressure, as well as on patients with chronic pain. (Ainsworth, et al., 2006; C. Chen & M. I. Johnson, 2010; C. C. Chen & M. I. Johnson, 2010; Francis, Marchant, & Johnson, 2011; Gopalkrishnan & Sluka, 2000; King & Sluka, 2001; Köke, et al., 2004; Sluka, Bailey, Bogush, Olson, & Ricketts, 1998; Vance, Radhakrishnan, Skyba, & Sluka, 2007). TENS has also been reported as an effective treatment modality for neuropathic pain (Cheing & Luk, 2005; Disselhoff, 2000; Inoue, et al., 2003; Jin, Xu, Geng, & Yan, 2010; Liu, et al., 2007; Norrbrink, 2009; Somers &

Clemente, 2003, 2006, 2009). The relationship between TENS and changes in thermal thresholds, specifically heat pain, has also been tested. High frequency TENS decreased heat pain thresholds in healthy subjects (Buonocore & Camuzzini, 2007; Cheing & Hui-Chan, 2003; Marchand, Bushnell, & Duncan, 1991). No changes in thermal perception has been reported for low frequency TENS (Palmer, Martin, Steedman, & Ravey, 2004). In contrast to the findings listed above there are few studies regarding the analgesic effect of TENS on cold pain. There is inconclusive data regarding the effect of TENS on cold using a cold pressor technique (C. Chen & M. I. Johnson, 2010; Francis, et al., 2011; Johnson, Ashton, & Thompson, 1992).These two types of thermal pain are different in a physiological aspect and are usually present in completely different pain conditions. Heat hyperalgesia is mostly present in inflammatory pain and cold hypersensitivity usually plays an important role in neuropathic conditions. It is therefore important to examine the role of a non pharmacological agent like TENS on cold sensitivity.

Research question

Does high frequency TENS reduce cold hyperalgesia induced by topical menthol?

Purpose

To determine the effect of high frequency TENS on cold pain threshold in cold hyperalgesia induced by topical menthol in healthy subjects.

Primary Objectives

- To assess the menthol effect on cold pain thresholds in healthy subjects.
- To assess if cold pain thresholds are increased during the application of high frequency TENS.
- To assess the menthol effect on cold sensation thresholds in healthy subjects.
- To assess if cold sensation thresholds are increased during the application of high frequency TENS.

Secondary Objectives

- To determine if the effects of high frequency TENS on cold pain thresholds persists after the cessation of the TENS treatment.
- To determine the consistency of the 40% menthol model of cold hyperalgesia within and between sessions.
- To determine the consistency of QST responses within and between sessions.

Hypotheses

It is hypothesised that this investigation is able to test that menthol will decrease cold pain thresholds, generating cold hyperalgesia in healthy subjects, with no effect on cold sensation. It is further hypothesised that TENS will have an anti-hyperalgesic effect, increasing cold pain thresholds, and will not affect cold sensation. The anti-hyperalgesic effect will continue after cessation of the TENS treatment.

Methods

All experimental procedures were approved by the Biomedical Research Ethics Board of the University of Manitoba.

Subjects

This study recruited 20 healthy volunteers. Inclusion criteria included age between 20 and 55 years with an ability to understand written and spoken English. Exclusion criteria included a history of peripheral vascular disease, diabetes mellitus, skin infection, neurological signs or symptoms, abnormal skin sensation or pacemaker. Also, individuals using analgesic medications within 24 hours before testing were also excluded.

Sample size

The data reported by Altis et al. was used to determine the sample size (Altis, et al., 2009). In this study the effect of three different drugs on cold hyperalgesia induced by topical menthol was examined. For the calculation of the sample size the data regarding the effect of tramadol was used as an estimate of the TENS effect. Cold pain threshold before tramadol had a mean of 12 +/- 5.9 ^{o}C and after tramadol was administered the mean was 8 +/- 4.4 ^{o}C. These data show an average tramadol effect of 4 degrees C on cold pain thresholds. The following formula was used to calculate the sample size.

$$n = 2\left(PI \frac{\sigma_d}{\mu_1 - \mu_2} \right)^2$$

where

PI is the Power index
σ is the anticipated standard deviation
μ is the anticipated mean
n is the number of pairs

PI = α error (type I) + β error (type II)

0.5 (1.64) + 0.20 (0.84)

PI = 2.48 $n = \left(2.48\frac{5.2}{4}\right)^2 = 10.3$

$\sigma_d = 5.2$

$\mu = 4$

The sample size generated was 10 participants necessary to demonstrate a significant difference. Thus, 20 subjects were sought as TENS may not be as effective as a specific opiate medication.

Experimental design

A randomized controlled within group study was used. The design included 20 subjects; all subjects participated in one experimental session and one control session with a one week interval.

On arrival to the testing room all subjects recruited signed an informed consent form to participate in the study. After consent was signed all subjects took part in a training session, where cold sensation and cold pain were assessed for three consecutive times. After 5 minutes, baseline measurements (B1) of cold sensation and pain thresholds were obtained prior to application of topical menthol. Menthol as then be applied for 20 minutes and cold sensation and pain thresholds were reassessed immediately following menthol treatment (+20) and every 10 minutes thereafter (+30, +40, +50, +60). The experimental group received Transcutaneous Electrical Nerve Stimulation (TENS) for 20 minutes immediately upon completion of sensory testing at the +20 time point. The control group did not receive TENS.

Subjects returned the following week for the second session. The order of testing for the control and experimental sessions was randomized.

Training session

Immediately prior to both testing session subjects were requested to practice the thermal testing procedures three times. Subjects were asked to report cold sensations followed by cold pain thresholds (each value for sensation or pain thresholds was the average of three reports). There was a 10 seconds interval between each individual testing. This was done for three consecutive trials 20 sec apart, generating three baseline triplicates. The cold stimulus was delivered through the thermode by the Quantitative Sensory Testing (QST) device.

Testing environment

All procedures were performed in a quiet location. Subjects were positioned in a chair with no visual access to the display screen of the computer or the TENS device. The forearm was placed in a mid supinated and comfortable position with the elbow in semi flexion. Pillows were used for comfort and support.

Menthol evoked cold pain

Topical menthol was utilized to induce cold hyperalgesia following published protocols (Wasner, Schattschneider et al. 2004; Namer, Seifert et al. 2005). A solution of 40% menthol dissolved in 95% ethanol was used to induce cold hyperalgesia. A 3x5 cm gauze pad soaked with 2ml of the prepared solution was applied for 20 minutes onto the skin of the right volar forearm (median nerve territory). The gauze was located at the point

midway between the lower crease of the wrist and the crease of the elbow. The gauze was covered with a plastic film to prevent evaporation. A Velcro band was fixed around the film to assure contact between the skin and gauze pad. Volunteers were also instructed to report any sensation of pain on a Numeric Rating Scale (NRS) every 5 minutes during the menthol application. The pain intensity was identified by a number from 0 to 10 with 0 representing no pain and 10 representing the worst imaginable pain. Quality of pain was also assessed through the Mac Gill pain descriptors every 5 minutes. After gauze removal the skin was wiped clean to remove any remaining solution.

Thermal testing

Perception thresholds were measured using a Madoc Neurosensory analyzer (Model TSA-II). Thermal stimuli were applied by a Peltier type thermode with an area of 3x3cm to the same skin site where menthol was applied. The thermode was held in place by a Velcro band tightened only enough to assure thermode contact with the skin. The thermode was removed after each recording. The thermode was maintained at a baseline temperature of 32°C and decreased in temperature at a rate of 1°C/s and returned to baseline at a rate of 3°C/s. A low temperature limit was set at 0°C to avoid injury to the skin. Each thermal stimulus was delivered 3 times with a rest interval of 10 seconds. The volunteer held a switch in the non-experimental hand and was asked to press the button as soon as the cold stimulus became painful. Standardized instructions including a description of cold pain was provided at each time point.

Description of TENS treatment

TENS (Model Cefar Tempo) was administered at a frequency of 100 Hz using a constant pulse duration of 100μs. Two adhesive (3.8 cm x 4.5cm) surface electrodes were fixed to the skin, 2 cm above and below the site of menthol application in the territory of the median nerve. The intensity of stimulation was adjusted for each patient. To adjust the intensity in a consistent manner, intensity was raised until a visible muscular contraction was achieved; subsequently it was lowered until the contraction disappeared. This was considered a strong sensory threshold. Paresthesia was clearly felt during the TENS treatment. The intensity of stimulation was adjusted after 10 minutes in the same manner as the starting intensity.

Statistical analysis

All data was analyzed for statistical significance using Statistica software version 5.1 (StatSoft Inc (1997), Tulsa, OK, USA.). Test of normality showed a bimodal distribution which indicated that nonparametric statistical analysis was required. The main effects of time on cold sensation and cold pain responses were assessed using Friedman ANOVA with Wilcoxon matched pairs tests used for pair-wise comparisons. A p value of < 0.05 was considered statistically significant for ANOVA tests. For multiple comparisons a Bonferroni correction was used.

Results

Baseline triplicates

In order to determine the consistency of sensory testing within and between sessions, participants completed 3 trials of cold sensation (Figure 1) and cold pain (Figure 2) testing at each session. Cold sensation testing showed significant differences during both the first (ANOVA X^2; n=17, df=2) = 25.5, p<0.0001) and second sessions (ANOVA X^2; n=14, df=2) = 19.9, p<0.0001). During both sessions the temperature at which cold sensation was first reported decreased significantly from the first to the second trial (p<0.05) but no significant difference was present between the second and third trials. Despite the initial change in cold sensation within each session, no significant differences were seen for any of the three trials when compared between sessions.

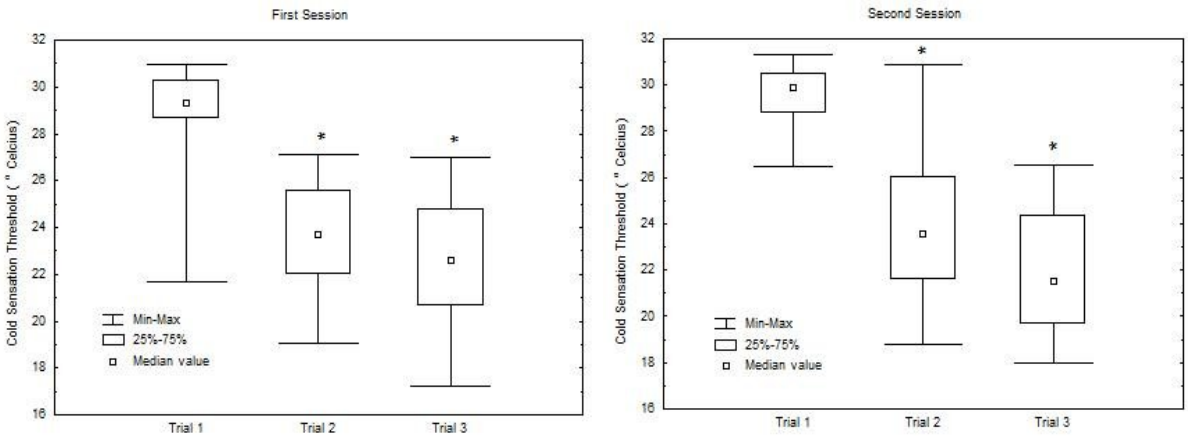

Figure 1. Cold sensation thresholds assessed during the first and second session. Cold sensation thresholds were assessed 3 times during each session (first session on the left, second session on the right). Medians, upper and lower quartiles and minimum and maximum values are provided for each trial.

*: significantly different from baseline (p<0.05).

In contrast to cold sensation, cold pain thresholds did not differ significantly within each session and no significant differences were found between sessions for each trial. Overall, these data indicate a high level of consistency for sensation and pain thresholds between and within sessions.

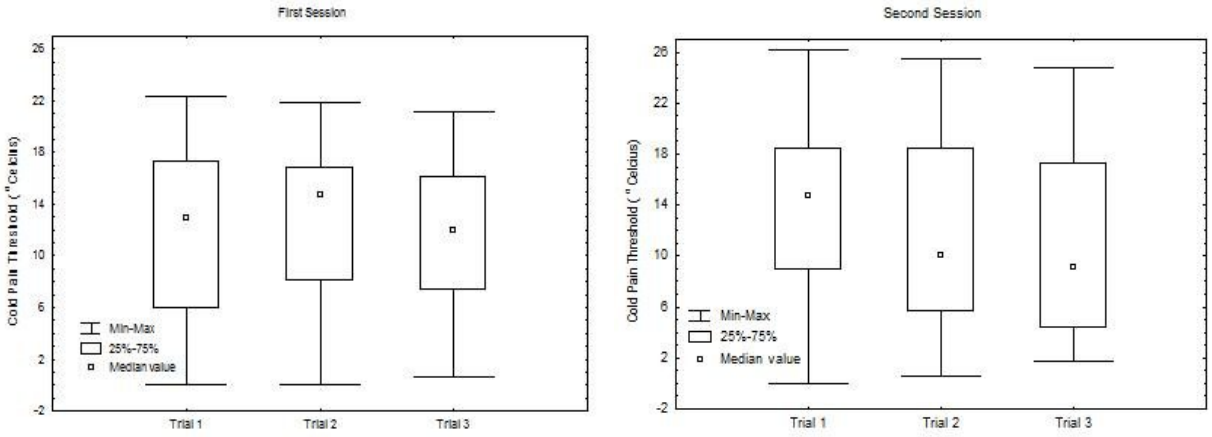

Figure 2. Cold pain thresholds assessed during the first and second session. Cold pain thresholds were assessed 3 times during each session (first session on the left, second session on the right). Medians, upper and lower quartiles and minimum and maximum values are provided for each trial.

Order effect by session

Aside from the overall consistency of QST, it was also important to determine whether subjects responded to menthol application in a similar manner between the two sessions. For this, the absolute and relative changes from baseline to the +20 time point were compared across the two sessions to test for an order effect. The magnitude of the menthol effect was significantly higher during the second session for both absolute and relative changes ($p<0.05$) for sensation thresholds (Table 1). Menthol induced larger absolute

changes in cold pain thresholds ($p<0.05$) whereas the same trend did not reach significance when expressed as relative values. The median percent increase of the effect for pain thresholds during the first session was 41% and 99% during the second session (Table 2). This indicates a stronger effect during the second exposure to menthol. Although an order effect was detected, the balanced design used in this study dispersed the effect equally throughout the control and TENS trials. Half of the subjects were assigned to the control trial first and the other half to the TENS trial first (Table 3).

Table 2. Absolute and relative changes in sensation and pain thresholds for the magnitude of the menthol effect between sessions

	Sensation		Pain	
	Session 1	Session 2	Session 1	Session 2
Absolute value*	3.38	4.5**	5.825	9.225**
Relative value#	14.35	18.58**	41.27	99.58541

*: values are expressed as median change from baseline to +20 minutes time point (^{o}C).

#: values are expressed as median percent change from baseline to +20 minutes time point.

**: significantly different from session 1.

Effect of menthol on cold sensation

There was a significant effect of time on cold sensation during the control trial (ANOVA X^2; n=20, df=5) = 38.8, $p<0.00000$). Compared to baseline, the temperature at which the first sensation of cold was reported increased immediately after menthol removal (baseline vs +20 minutes, $p<0.05$) and remained elevated throughout the following 40

minutes (baseline vs +30, +40, +50, and +60 minutes, $p<0.05$ for all). Further, cold sensation thresholds at later time points (+30, +40, +50, +60 minutes) did not differ from that measured immediately following menthol exposure (+20 minutes). The above results show that menthol increased the temperature at which cold sensation was reported and that the increase remained throughout the trial.

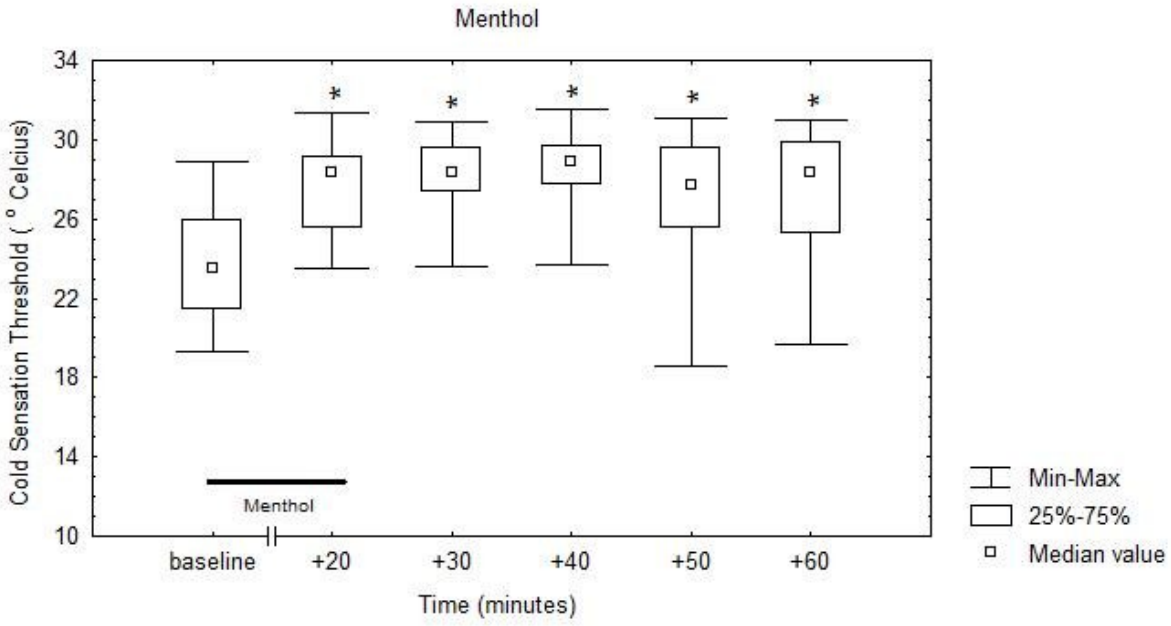

Figure 3. Cold sensation thresholds during the Control trial. Cold sensation thresholds were assessed at baseline followed immediately by a 20 minute topical menthol application (heavy bar). Cold sensation thresholds were re-assessed following menthol application (+20) and every 10 minutes thereafter. Medians, upper and lower quartiles and minimum and maximum values are provided for each time point. *: significantly different from baseline ($p<0.05$).

Pain Descriptors

The experience of pain during menthol exposure was indicated by subjects using McGill Pain Questionnaire descriptors. During the first 10 minutes of exposure the word cool and cold were mostly used. As the time of exposure increased the words hot, burning and tingling were most frequently reported.

Effect of TENS on menthol-induced changes in cold sensation

There was also a significant effect of time on cold sensation within the TENS trial (ANOVA X^2; n=20, df=5) = 36.8, p<0.00000). Similar to the Control session, cold sensation temperatures were increased immediately following menthol removal and remained elevated above baseline throughout the TENS session ($p<0.05$) except for the +50 time point which did not reach significance. Also in concurrence with the menthol trial, cold sensation temperatures measured at later time points did not differ from those measured immediately following menthol treatment. This outcome indicates that sensitivity to cold was increased throughout the trial with no observable effects of TENS treatment on cold sensation thresholds.

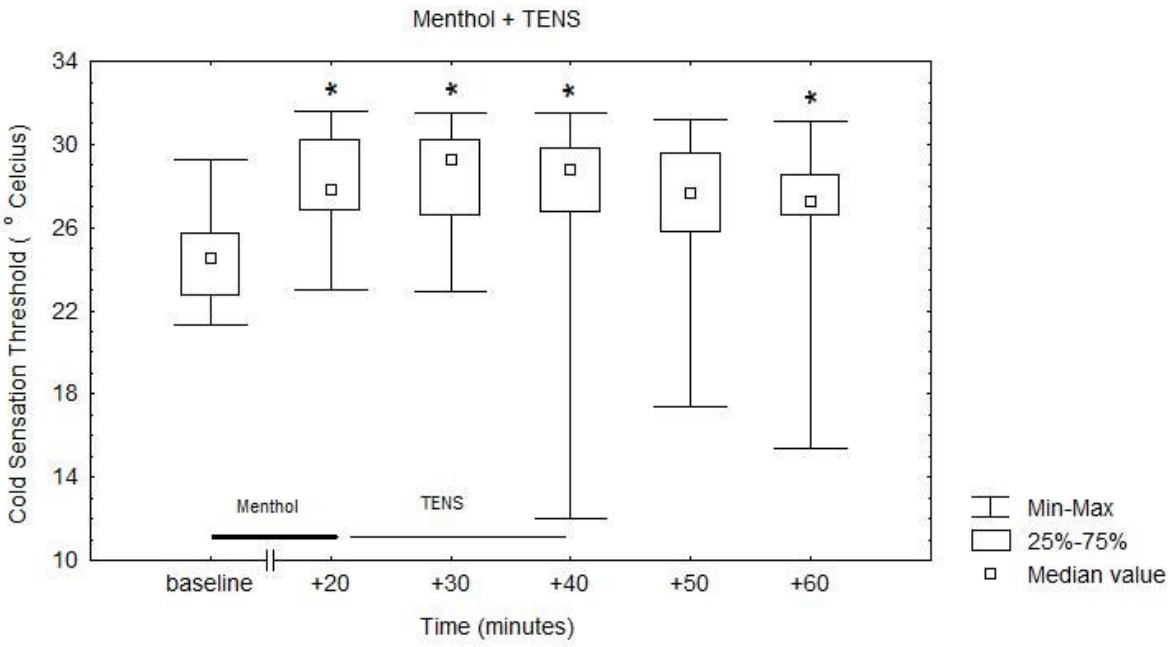

Figure 4. Cold sensation thresholds during the TENS trial. Cold sensation thresholds were assessed at baseline followed immediately by a 20 minute topical menthol application (heavy bar). Cold sensation thresholds were re-assessed following menthol application (+20) and every 10 minutes thereafter. TENS treatment (light line) was delivered over a twenty minute period following the menthol application. Medians, upper and lower quartiles and minimum and maximum values are provided for each time point.

*: significantly different from baseline ($p<0.05$).

Effect of Menthol on Cold Pain

The main effect of time was significant (ANOVA X^2; n=20, df=5) = 37.1, $p<0.00000$) during the Control trial for cold pain responses. The temperature, at which the first sensation of pain was reported, was significantly increased after menthol removal (+20) compared to baseline values ($p<0.05$). This temperature remained significantly increased

above baseline for 20 minutes after menthol removal (+30 and +40 minute time points, $p<0.05$ for both). The same time points were also not different from the +20 time point ($p>0.05$). While +50 and +60 time points were not significanty different from baseline ($p>0.05$), they were also not significatly decreased from the +20 time point. This indicates that cold pain temperatures thresholds were elevated by menthol although the response appeared to be waning 30minutes after removal of the menthol.

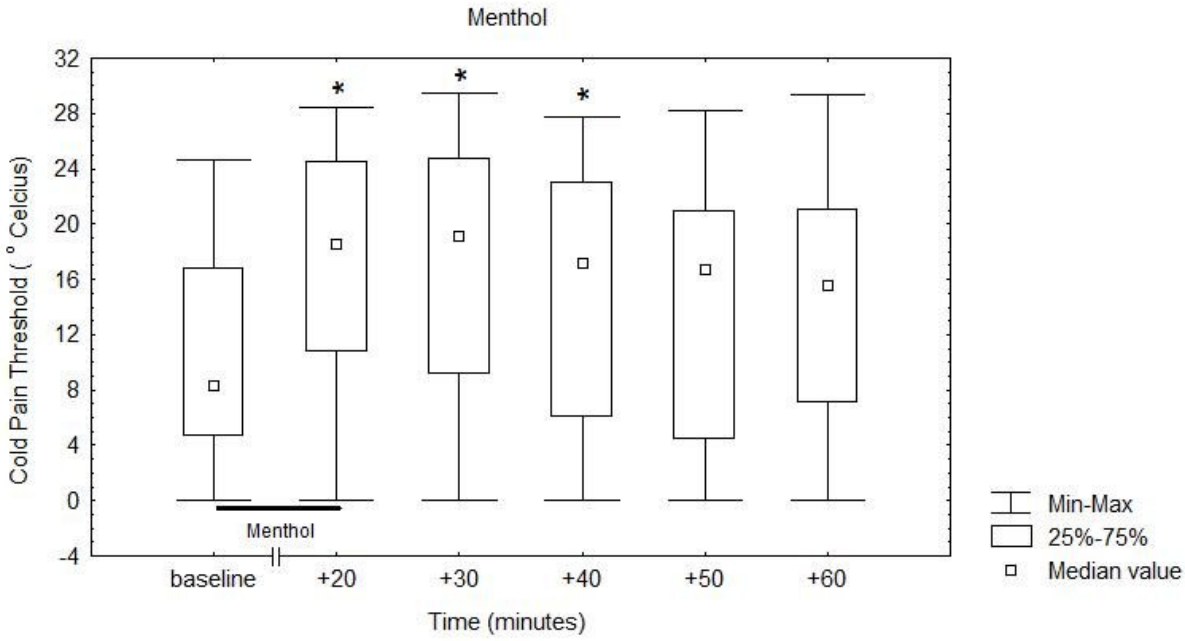

Figure 5. Cold pain thresholds during the Control trial. Cold pain thresholds were assessed at baseline followed immediately by a 20 minute topical menthol application (heavy bar). Cold sensation thresholds were re-assessed following menthol application (+20) and every 10 minutes thereafter. Medians, upper and lower quartiles and minimum and maximum values are provided for each time point. *: significantly different from baseline ($p<0.05$).

Effect of TENS on menthol-induced changes in cold pain responses

The effect of time on cold pain thresholds within the TENS trial was also significant (ANOVA X^2; n=20, df=5) = 34.2, p<0.00000). Relative to baseline, the temperature at which pain was reported was only significantly increased after menthol removal (p<0.05). The subsequent temperature thresholds (+30,+40,+50,+60) were not different from baseline (p>0.05). However, relative to the time point after menthol removal (+20), the temperature at which pain was reported was significantly different for all subsequent time points (+30,+40,+50,+60) throughout the TENS trial (p<0.05). This data shows that TENS is able to increase cold pain thresholds and therefore reduces cold hyperalgesia induced by menthol application.

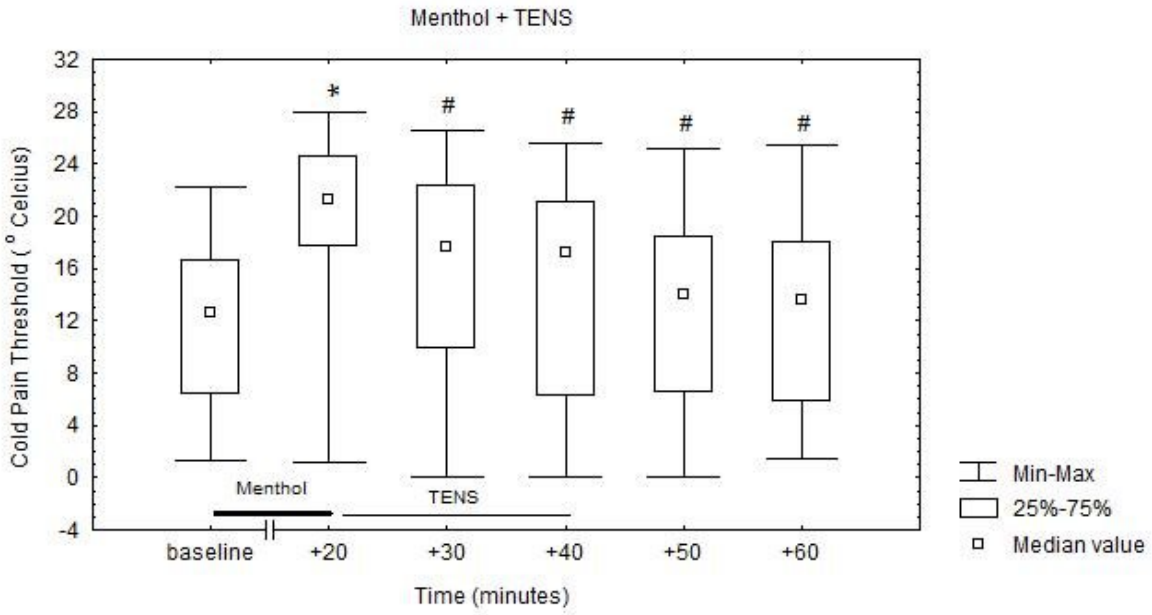

Figure 6. Cold pain thresholds during the TENS trial. Cold pain thresholds were assessed at baseline followed immediately by a 20 minute topical menthol application (heavy bar). Cold sensation thresholds were re-assessed following menthol application (+20) and every 10 minutes thereafter. TENS treatment (light line) was delivered over a twenty minute period following the menthol application. Medians, upper and lower quartiles and minimum and maximum values are provided for each time point.

*: significantly different from baseline ($p<0.05$).

#: significantly different from +20 time point ($p<0.05$).

Gender effect

It was also of interest to determine whether gender influenced either the effects of menthol on cold sensation and cold pain responses or the actions of TENS on these responses. The effect of gender was analysed by session due to the order effect described above. The magnitude of the menthol effect (baseline to +20 minutes) did not show any gender effects whether expressed as absolute or relative changes for either sensation or pain thresholds during either the first or second sessions. Further, the menthol effect, represented as the area under the curve (AUC) from the +30 time point to the +60 time point, was not influenced by gender for either cold sensation or cold pain responses at either session.

The effect of TENS was analysed over the same time period (AUC from +30 to +60) for gender differences. No differences between genders were seen for cold sensation or pain during the first session. In contrast, the AUC for cold pain threshold was significantly lower during TENS treatment in females ($p<0.05$). Lastly, the AUC for cold pain during the second session was portioned into periods when the TENS device was active (+30 and +40 time points) and when the TENS device was off (+50 and +60 time points). No gender differences were present during the TENS treatment, however, during the second session the AUC was smaller in females after the end of the active TENS period. This analysis indicates that TENS was equally effective in males and females during the active period of TENS. Although TENS was able to further decrease the temperature at which pain was reported in females compared to males after TENS was tuned off, this finding was only present in female subjects who received TENS treatment during the second

session. Similar to the order effect the balanced design of the study minimizes the impact on the main outcomes (Table 3).

Table 3. Distribution of subjects by gender and order of treatment

Gender	CT	TC	Total
Female	5	6	11
Male	5	4	9
Total	10	10	20

Control-TENS: CT TENS- control: TC

Correlation between magnitude of menthol effect and pain ratings

A correlation between pain ratings during menthol application and the magnitude of the menthol effect was tested for each session. The sum of ratings on the NRS was used as an indicator of total pain rating. As an indicator of menthol effect, the difference between baseline and the +20 time point threshold was calculated. There were no significant correlations between pain rating and the effects of menthol on cold sensation or cold pain thresholds. This indicates that the rating of pain during menthol application is not related to the magnitude of change on quantitative sensory testing.

Despite there being no correlation between pain rating and changes in quantitative sensory testing outcomes within the testing sessions, the total rating of pain during menthol application (NRS) was strongly correlated between sessions ($r=0.755$; $p=0.0018$). Furthermore, a correlation between sessions for the change in sensation thresholds was detected ($r=0.621$; $p=0.0034$). In contrast, there was no correlation between changes in

pain thresholds (r=-0.022; p=0.924). This indicates that subjects reported similar menthol experiences on both sessions for cold sensation, but not for cold pain.

Discussion

The purpose of this study was to determine the effect of high frequency TENS on cold hyperalgesia induced by topical application of menthol. Menthol reliably produced a distinct cold hyperalgesia as indicated by an increased temperature at which subjects reported the first sensations of cold pain. The menthol-induced cold hyperalgesia was significantly reduced during the application of high frequency TENS. Furthermore, the analgesic effect of TENS persisted beyond the application period for at least 20 minutes. This effect of TENS during the post-application window in the second session was stronger in females, indicating an effect of gender on post-stimulation analgesia. In contrast to the effects on cold pain, TENS had no effect on the menthol-induced changes in cold sensation.

Menthol is an agonist of the TRPM8 receptor, a member of the transient receptor potential family of ion channels (Bautista, et al., 2007; Colburn, et al., 2007; Dhaka, et al., 2007; Knowlton, et al., 2010). TRPM8 receptors respond to natural compounds such as menthol as well as cold stimuli and are located on two types of nerve fibers; thinly myelinated nociceptive neurons which transmit noxious cold and A delta fibers responsible for cold sensation (Campero, et al., 2009; Campero & Bostock, 2010; Carr, et al., 2009; Kobayashi, et al., 2005; McKemy, et al., 2002; Takashima, et al., 2007; Xing, et al., 2007; Xing, et al., 2006). Topical menthol, presumably acting on TRPM8 receptors, increases the temperature at which the onset of cold pain is reported, indicating a decrease in cold pain thresholds and thereby induction of cold hyperalgesia (Binder, et al., 2011; Hatem, et al., 2006; Namer, et al., 2005; Wasner, et al., 2004). A recent study has shown that mechanical pain thresholds are also lowered following menthol treatment, and, based on

the combination of cold and mechanical hyperalgesia, the authors suggested the menthol model was useful in the study of neuropathic pain (Binder, et al., 2011).

The results of this study are consistent with previous research in that menthol application resulted in a significant increase in the temperature at which individuals reported the first sensation of pain; i.e., cold hyperalgesia. However, previous studies have reported that menthol does not alter cold detection thresholds (Binder, et al., 2011; Hatem, et al., 2006; Namer, et al., 2005; Wasner, et al., 2004). In the present study, menthol treatment produced a significant increase in cold sensitivity as indicated by higher temperatures at which subjects reported the first sensation of cold. Although there is no clear explanation for these different outcomes, several steps were taken to assess the consistency of QST data. A practice session consisting of three separate data collection trials was completed before starting each experimental session. Analysis of the cold detection data showed that subjects responded differently between the first and second trials but no further change occurred between the second and third trials. This pattern suggests a brief learning process occurs at the start of each session. Importantly, this pattern was consistent across each session. Cold pain data did not show any significant differences across these practice trials. Furthermore, there were no differences in the baseline measurements completed immediately prior to menthol application and menthol induced clear changes in sensory function during each experimental session. Lastly, subject's cold sensation responses were highly correlated between sessions as were subject's verbal pain ratings during menthol exposure. Collectively, these findings indicate a high degree of consistency for cold sensation data within, and between, experimental sessions and adding support to the finding of altered cold detection thresholds in the present study.

The menthol model used in this study (40% solution) has been used previously to show the effectiveness of an opioid analgesic in reversing the cold hyperalgesia induced by topical application of menthol (Altis, et al., 2009). It was relevant to assess if TENS was able to achieve similar results. Obviously, the effect of TENS cannot be equally compared to an opioid drug, but the results of this investigation showed that TENS was able to significantly decrease the temperature at which cold pain thresholds were reported after the application of menthol. This indicates a clear anti-hyperalgesic effect on cold pain. This study is the first to examine the effects of TENS on cold hyperalgesia and the findings support the use of TENS as an alternative or complement to drug therapies in the treatment of cold hyperalgesia. In the absence of hyperalgesia, it is unclear whether TENS has hypoalgesic effects as conflicting results have been reported in studies using the cold pressor test (C. Chen & M. I. Johnson, 2010; Francis, et al., 2011; Johnson, et al., 1992). In contrast, a hypoalgesic effect of TENS has been reported for heat pain in a non-hyperalgesic state (Buonocore & Camuzzini, 2007; Cheing & Hui-Chan, 2003; Marchand, et al., 1991). Resolving whether TENS can impact sensory function in a non-hyperalgesic versus hyperalgesic state may provide clues to mechanisms which may be specific for certain sensory modes.

At present, there are several theories regarding the mechanisms responsible for the analgesic effects of TENS. The oldest and most commonly known theory is the gate control theory in which stimulation of large diameter fibers inhibit nociceptive fiber response in the dorsal horn. However, recent research suggests that endogenous opioid release may be the main mechanism behind TENS-induced analgesia given the ability of opioid antagonists to block the anti-hyperalgesic effects of TENS (Cui, et al., 2006; Kalra,

et al., 2001; Leonard, et al., 2010; Sluka & Chandran, 2002; Sluka, et al., 1999; Sluka, et al., 2005*)*. In the present study, the amplitude of TENS stimulation was increased until the first visible muscle twitch was observed, and then the amplitude was slowly decreased until the muscle twitch disappeared. This allowed a strong but non-painful sensation with clear paresthesia in the skin area of the peripheral nerve stimulated. Thus, assuming activation only of low threshold nerve fibres, the anti-hyperalgesic effects of TENS in this study may have been mediated through the gate control theory, at least during the period of TENS delivery. However, the effect of TENS persisted beyond the 20 minute treatment time for at least another 20 minutes, an effect reported previously for TENS in both humans and animals (Buonocore & Camuzzini, 2007; Sluka, et al., 1998). This after stimulation anti-hyperalgesic effect suggests an opioid mediated mechanism. Although an overlap of both mechanisms might as well be responsible for the anti-hyperalgesic effect of TENS.

It was essential for this investigation that a significant menthol effect was achieved during each session. The magnitude of the menthol effect was, therefore, assessed to determine if this was achieved and whether the magnitude of the menthol effect was similar between sessions or whether an order effect was present. In both sessions menthol produced a significant change in cold sensation and cold pain responses. A within group paired comparison of the magnitude of the effect between the first and second session showed a significant difference. Individuals showed a greater response on the second menthol exposure for both pain and sensation thresholds. Relative to baseline, the median percent increase in cold pain temperatures during the first session was 41% and 99% during the second visit. Additional studies would be needed to fully determine the reproducibility of

menthol-induced sensory changes although a loss of responsiveness does not appear to be a concern. Although the order effect was a potential limitation, the main outcomes of this study were not unduly impacted due to the balanced design regarding the order in which subjects received the experimental treatments.

The effect of menthol on cold sensation was correlated between sessions. This indicates that subjects respond in the same manner during both sessions meaning that an individual who showed a high response during the first session also showed a high response during the second session. Individuals displayed much higher variability in cold pain responses and no correlation was present between the responses during the first and second session. The higher variability likely arises from the difficulty in deciding at what point a sensation changes from cold to cold pain. These data expose a potential limitation of QST; individual responses can vary considerably between sessions. Nevertheless, the magnitude of the menthol and TENS effects on pain thresholds were strong enough to overcome this issue. Additionally, the impact of the between session variability was also diminished by the use of a repeated measures design that permits within session analysis.

Beyond the concern regarding an order effect, it was of interest to determine whether any gender differences were present within the menthol and TENS effects. Whether the menthol effect was analysed as the change seen immediately following application or by the AUC, no significant differences were found for gender. A limited gender difference was found for the overall TENS effect (AUC) in that this was only present in those that received the TENS treatment during the second experimental session. A further analysis showed that the difference was only present during the period after TENS was turned off. During this period, females showed a smaller AUC indicating that the TENS effect

seemed to persist to a greater degree in females during the second session. To assure that this difference was not due to the menthol effect wearing off faster in females, an analysis of gender on the magnitude of the menthol effect showed no differences between genders for the same time period. Similar to the order effect for menthol, this relatively minor gender difference would have minimal impact on the main outcomes of the study due the approximately equal distribution of gender within the ordering of the treatment groups.

An assumption within this study was that cold detection thresholds correlated to the onset of low-threshold, non-nociceptive fiber activation and that cold pain thresholds represented the onset of high-threshold, nociceptive fiber activation. These assumptions are supported by the fact that TENS was only able to affect pain threshold and not sensation. This indicates that TENS has an effect on the response of high-threshold nerve fibers without affecting low-threshold fibers. Definitive statements regarding which sensory afferents are stimulated during QST would require further studies such as electrophysiological recordings of cutaneous afferents.

It was also assumed that individuals would respond similarly to menthol during both sessions. Indeed, it was necessary that menthol, induced changes in sensory function during each session. Menthol reduced cold sensation and cold pain thresholds on both sessions, although a stronger effect was seen during the second exposure. This did not prevent the detection of significant TENS effects, however, future studies should formally address the reproducibility of the menthol model.

A further issue was the overall variability within the QST technique. This was addressed by including three separate data collection trials at each session prior to the baseline

measurements. This served two purposes; it provided the subjects with practice for the QST process and also permitted assessment of the consistency of the QST responses. Analysis of this data demonstrated that, as a group, the subjects were generally consistent both within and between the two experimental sessions. These are important considerations given the novel outcome of altered cold detection thresholds following menthol treatment; an outcome not present in other studies using a similar design.

In conclusion this study reveals that menthol affects cold sensation as well as cold pain responses. It also shows that the 40% menthol application model was consistent within and between sessions. Moreover TENS is able to reduce induced cold hyperalgesia without affecting the ability of sensing cold. The provided data suggest that TENS could be a useful tool in the treatment of pain syndromes involving cold hyperalgesia.

Conclusion

This investigation demonstrated that menthol was able to decrease cold pain thresholds, generating cold hyperalgesia in healthy subjects. Menthol was further able to also decrease cold sensation thresholds. The magnitude of the menthol effect showed a significant difference between sessions, individuals had a greater response on the second menthol exposure for pain and sensation thresholds. Additionally TENS was able to increase cold pain thresholds reversing menthol induced hyperalgesia, thus showing an anti-hyperalgesic effect. This effect also continued after cessation of the TENS treatment for at least 20 minutes. Interestingly, TENS had no effect on cold sensation thresholds. This data shows that TENS could be a valuable tool in the treatment of patients experiencing cold hyperalgesia. Current treatment options are mainly pharmacological; a non-pharmacological treatment such as TENS, with its very low likelihood of side effects and few contraindications, could be used as a complement or even as an alternative to the pharmacological treatment.

Further studies are needed to assess the effect of TENS on cold hyperalgesia in individuals with neuropathic pain. It would also be important to determine if there is a difference when comparing the effects on experimental pain versus clinical pain, although it is more difficult to control an experiment on clinical pain.

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Appendix 1

Data collection sheet
Quantitative sensory testing

Control session

Date:_____

Subject ID:_____ Gender:_____ Age:_____

Time of menthol application:_____ Time of TENS:_____

Cold sensation thresholds

	1	2	3	average
Baseline (0min)				
T1 (20 min, not)				
T2 (30 min)				
T3 (40 min)				
T4 (50min)				
T5 (60min)				

Cold pain thresholds

	1	2	3	average
Baseline (0min)				
T1 (20 min, not)				
T2 (30 min)				
T3 (40 min)				
T4 (50min)				
T5 (60min)				

Spontaneous pain (NRS)

5'_____ 10'_____ 15'_____ 20'_____

Descriptors McGill pain questionnaire

5'_____ TENS intensity: 1_____

10'_____ 2_____

15'_____

20'_____

Appendix 2

Data collection sheet
Quantitative sensory testing

Experimental session
Date:_____

Subject ID:_____ Gender:_____ Age:_____

Time of menthol application:_____ Time of TENS:_____

Cold sensation thresholds

	1	2	3	average
Baseline (0min)				
T1 (20 min, not)				
T2 (30 min)				
T3 (40 min)				
T4 (50min)				
T5 (60min)				

Cold pain thresholds

	1	2	3	average
Baseline (0min)				
T1 (20 min, not)				
T2 (30 min)				
T3 (40 min)				
T4 (50min)				
T5 (60min)				

Spontaneous pain (NRS)

5'_____ 10'_____ 15'_____ 20'_____

Descriptors McGill pain questionnaire

5'_____ TENS intensity: 1_____

10'_____ 2_____

15'_____

20'_____

Appendix 3

WANTED

HEALTHY PARTICIPANTS FOR A STUDY

INVESTIGATING THE EFFECT OF A ELECTRO THERAPY (TENS)

ON COLD SENSITIVITY

REQUIREMENTS:	20 to 50 years of age, able to speak and understand English.
	No Peripheral vascular disease,
	No diabetes mellitus,
	No skin conditions on forearm,
	No neurological signs or symptoms,
	No abnormal skin sensation
	No pacemaker
	Not taking pain medications
TIME COMMITMENT:	Two 1 hour sessions; one week apart
WHEN:	February- April 2011
LOCATION: Winnipeg	Room 355, 3rd floor, rehab hospital, 800 Sherbrook,
TO PARTICIPATE:	Contact Onae Iribarren
	Phone: (204) 804-9030
	Email: onairri@hotmail.com

Appendix 4

Participant Fact Sheet

Thank you for agreeing to participate in this study.

This study is part of a Master's degree at the University of Manitoba, School of Medical Rehabilitation in the Faculty of Medicine.

The purpose of the study is to determine the effect of an electrotherapy device called TENS on cold sensitivity in healthy participants. Cold sensitivity will be induced by the application of a topical menthol solution. TENS is a simple and safe device with without likely side effects. Additionally, TENS devices are inexpensive and simple to operate which makes ownership and operation by patients a reality. Studies have suggested that TENS is effective in a variety of pain conditions. Many patients with neuropathic pain (i.e. pain originating from nerves as opposed to other tissues like muscle or ligaments) suffer from pain due specifically to cold sensitivity which is similar to the cold sensitivity that will be induced in this study.

Your participation will provide important information about the effect of TENS in an experimental model of pain that is designed to simulate a type of pain normally experienced by patients.

This study will involve about two hours of your time (one hour on two consecutive weeks). Your involvement in the study will consist of:

Reading and signing an Informed Consent Form (10 minutes)

Menthol application (20 minutes)

- A gauze pad soaked in a menthol solution will be applied to a small area of skin on your forearm.

TENS treatment (20 minutes)

- An electrical non painful current will be applied to your skin through two electrodes.

Cold sensation and cold pain testing (15 minutes)

- Testing your ability to feel cold and cold pain applied to your forearm

The study will be conducted in Room 355 on the 3rd Floor of the Rehab Hospital. The address is 800 Sherbrook. Please try to arrive 15 minutes before your scheduled time for a training session. When you attend for the study, please be sure to bring your Subject Number. In addition please bring a T-shirt or some other loose fitting top. If you cannot attend or are sick on the day of your appointment please call us at 204-804-9030 and ask for Onae, or call Brian MacNeil at 977-5635 to reschedule your appointment. None of the components of the study are dangerous or harmful.

If you have any questions regarding the preceding information or anything else about our study please contact Onae Iribarren at 804-9030 or Brian MacNeil at 977-5635. Again, thank you for taking the time to participate in this important study.

Onae Iribarren,
MSc Rehab student