Conditioned Pain Modulation (CPM) Test:

Reliability, Feasibility, and Normal Values

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Abstract

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Oral Medicine

Pain is the common characteristic associated with conditions such as chronic headache, arthritis, herpes zoster, diabetic neuropathy, and temporomandibular joint disorders (TMD). Typically, nerve impulses produced peripherally by a stimulus travel centrally to the spinal cord and the brain stem where they are elaborated before they reach various regions in the cortex, resulting in pain perception. Impulses may go through a modulation process -through the endogenous pain inhibitory pathways in humans- where alterations of the pain signals lead to either their inhibition or enhancement before they reach the higher centers of the brain. Currently, the best way to assess the efficiency of endogenous pain inhibition in humans is by performing a test that assesses pain modulation. Pain modulation can occur by several mechanisms and previous work has shown that the application of a noxious stimulus in one part of the body can inhibit pain in another part of the body, presumably by activating the descending inhibitory pain system. This phenomenon was previously termed 'Diffuse Noxious Inhibitory Control' (DNIC), but recently the term Conditioned Pain Modulation (CPM) is recommended for humans. The laboratory assessment of CPM involves a subject rating the pain intensity of a "test stimulus" (TS), during and after the application of a second painful "conditioning stimulus" (CS)

to a distant region of the body. However, the reliability of this test has been investigated only by a few studies, and questions remain unanswered about its reproducibility, as well as its feasibility. Different types of conditioning and test stimuli have been employed. Our systematic review has shown that using cold pressor test (CPT) as a CS is one of most efficient methods to induce CPM when combined with pressure pain threshold (PPT) test stimulus. Previous studies have shown that chronic pain is associated with decreased endogenous analgesia which is indicated by less efficient CPM. Only a few studies have explored the reliability of the CPM test to assess the alterations in pain modulation in chronic pain patients. As a diagnostic test, it is essential that any CPM assessment yields consistent and reliable results. Therefore, in this dissertation we aimed to:

1-Dedicate the 1st chapter to provide a background about suggested pain modulation mechanisms including CPM, and factors that may affect this phenomenon.

2-Report the reliability of the CPM assessment paradigm by performing a systematic review of published studies on this topic. One assessor searched multiple electronic databases including: PubMed, EMbase and Web of Science, from inception to December 2015 inclusive. Inclusion criteria were comprised of original research, human subjects, and CPM tested identically at least twice with at least a 60 minutes interval between testing sessions. The main outcome was reliability. Reliability of CPM was reported as the Intra-class Correlation Coefficient (ICC), Coefficient of Variation (CV), and Coefficient of Repeatability (CR).

3-Assess the reliability of CPM through intra-session reliability and inter-session reliability. Healthy volunteers were tested three times (three visits) within a two-week period; each visit included two test sessions. In each session, PPT was performed identically on the test sites of the dominant side of the body in a non-overlapping fashion: the masseter muscle (side of the

face), hand (thenar eminence), and foot (dorsum of the foot) three times. The PPT (the TS) was applied during and before the CPT (the CS) to calculate the CPM value. The CPM test stability was assessed by assessing the change in CPM scores from the baseline visit to the end of the follow-up visit.

4- The development of CPM normal values and its relationship at three body sites by age/gender. The difference between PPT values before and during the CPT is the magnitude of CPM. We aimed to determine the relationships between CPM at three body sites and age/gender in a healthy population, and second, to begin the development of a normative database for CPM. Specifically, the mean, median, lowest and highest pain inhibition scores were described separately for males, females, younger, middle and older age individuals, and by the anatomical sites of the pain stimulus – foot, hand and face.

5-Measure feasibility of clinical CPM testing: patient experience, and any adverse events. Although subjects were tested multiple times during each testing session, the proposed actual test procedure in the clinic is the PPT at one of the three tested sites during and before the CPT. Subjects answered a brief post-testing questionnaire that included questions on their tolerance for the test, the perceived safety of the test, as well as any experienced side effects.

From the totality of our findings, our final recommendation for a reliable and feasible clinical CPM testing protocol includes performing the test on the hand, employing PPT using a manual algometer as a TS applied to the dominant hand, and CPT as a CS applied to the non-dominant hand. We recommend performing the test in the form of three rounds of PPT with 20s in between before CPT on the dominant hand, followed by immersion of non-dominant hand in cold water (CPT) at temperature of 5-7^{o}C. When pain from CPT reaches 7 out of 10 according to numerical pain scale- where 0 is no pain and 10 is severe pain, the PPT should be applied

simultaneously during the CS at this point while the non-dominant hand remaining in the cold water. At the beginning of the visit, we recommend performing a training session of the pain tests (CPT and PPT) on the volunteer's hand 5 minutes before starting the testing session.

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Finally, I thank **my God**, I will keep on trusting You for my future. Thank you.

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CHAPTER 1: BACKGROUND

INTRODUCTION

PAIN:

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

Pain indicates that tissue damage has either occurred or is about to occur if the stimulus persists. A normal or “physiologic pain” occurs as a result of tissue injury or intense noxious (tissue-damaging) stimulus, and it is associated with an inflammatory response that is part of the healing process. Normally it subsides with tissue healing. “Pathophysiologic pain”, on the other hand, persists after tissue healing, and is believed to result from alterations in the peripheral and/or central nervous system, which leads to chronic pain. Pain is the common characteristic associated with conditions such as chronic-recurrent pain, arthritis, herpes zoster, diabetic neuropathy, and temporomandibular joint disorders (TMD)[1].

According to the origin, pain is classified into nociceptive and non-nociceptive pain. The nociceptive type is caused by detection of a noxious stimulus such as mechanical, thermal or chemical, while non-nociceptive pain is caused by a functional or structural abnormality within the nervous system itself, such as neuropathic pain.

MECHANISMS:

Mechanism of pain

Typically, nerve impulses -produced peripherally by a stimulus- travel centrally to the spinal cord and the brain stem, where it is altered before finally reaching to the cortex (parietal lobe), resulting in pain perception.

a) Transduction:

The noxious stimulus is transduced through the conversion of various physical and chemical stimuli into an electrical nerve impulse –known as an action potential. An action potential is created through a cycle of resting potential state, depolarization, and repolarization, which is modulated by the electrical charge of the nerve cell membrane. A noxious stimulus must be of a sufficient strength (threshold potential) to initiate the cycle of the action potential. In case of trauma, this threshold decreases, resulting in a condition known as allodynia where non-painful stimuli provoke pain[2].

b) Transmission:

In the transmission process, the action potential travels to the synapse between the first order (peripheral) and second order central nervous system (CNS) neurons. When second order neurons are activated by painful stimuli, they may become sensitized (central sensitization)[3] resulting in amplification and spreading of pain. The transmission of the impulse is affected by the axon diameter (increasing the diameter will increase the velocity) and the myelin wrapping (myelinated axon allow the signal to travel at a higher velocity than un-myelinated axons). Another action potential is initiated at the second order neuron synapse, and at the nodes of Ranvier –gaps in the myelin wrapping – along the axon.

1-The trigeminal system: Pain signals from the intraoral and extraoral structures of the head and face are carried to the CNS by the trigeminal nerve through all three divisions the ophthalmic, maxillary and mandibular. The first-order neurons of the trigeminal nerve are located in the trigeminal ganglion and the trigeminal mesencephalic nucleus of the CNS. Most sensory fibers in the trigeminal nerve have their cell bodies in the trigeminal ganglion. This is located in the lateral wall of the cavernous sinus,

immediately lateral to the pituitary gland. There is one very unusual exception, sensory cell bodies of proprioceptive nerve fibers are located inside the CNS, in the mesencephalic nucleus of the trigeminal. The trigeminal nerve enters the brain stem through the middle cerebellar peduncle of the pons. These neurons synapse with second-order neurons in the trigeminal brain stem complex where modulation takes place; an impulse can either continue to higher centers in the CNS or be altered by a descending modulating system, specifically the trigeminal spinal tract nucleus[4]. The spinal tract nucleus is composed of three separate nuclei: subnucleus oralis, subnucleus interpolaris, and subnucleus caudalis. The caudalis is continuous with and structurally similar to the spinal dorsal horn. Both incoming and outgoing nociceptive signals to and from the subnucleus caudalis can be modified by the modulating system. The second-order nociceptive neurons in the subnucleus caudalis are mainly nociceptive specific (NS) neurons, and wide dynamic range (WDR) neurons that have large receptive fields; these neurons receive impulses from A-delta (small lightly myelinated fibers stimulated by pain and cold stimuli) and C-fibers (small un-myelinated fibers stimulated by Pain, thermal, and mechanical stimuli)[5]. Most of the second-order neurons carrying pain information cross the midline to the opposite side and ascend to the thalamus via the anterolateral trigeminothalamic tract, where those neurons synapses with the third-order neurons in the thalamus[1]. Finally, signals travel from the thalamus to higher centers in the brain specifically the parietal lobe of the cerebral cortex.

2-Pain Pathways in the Spinal Dorsal Horn: Below the level of the head, pain signals are carried to the higher centers of the brain through the dorsal horn of the spinal cord that consists of sensory afferent fibers. The first-order neurons synapse directly on second-

order neurons in the dorsal horn. These second-order neurons, NS and WDR neurons, are similar to those found in the subnucleus caudalis of the trigeminal system. Then, signals follow a similar pathway as for the trigeminal system, i.e. they cross the midline in the spinal cord to ascend through the anterolateral spinothalamic tract to synapse with the third order neurons in the thalamus, and from there they follow the same pathways as signals from the trigeminal system.

c) Perception:

Pain perception is often quantified by static pain parameters, such as pain threshold and pain tolerance; however, these measures do not specifically assess pain modulation. The dynamic psychophysical pain parameters include two lab tests that reflect inhibition and excitation of nerve impulses: conditioned pain modulation (CPM) that represents the inhibition, and temporal summation (TeS) that represents the excitation. Previous studies show that the application of a noxious stimulus in one part of the body can inhibit pain in another part of the body, by activating the descending inhibitory system. This phenomenon was initially termed 'diffuse noxious inhibitory control' (DNIC)[6], but is currently called CPM[7,8]. In a normally functioning inhibitory system, the amount of pain will be decreased in the presence of the noxious stimulus. In altered systems -such as in chronic pain- patients may experience hypoalgesia (abnormally decreased sensitivity to pain).

d) Modulation:

The modulation phase is the phenomenon of interest in this study. Nerve impulses may go through the modulation process and are subjected to various modulatory influences that alter pain perception by either inhibition or enhancement of the signals before reaching the higher centers of the brain.

Mechanism of modulation

a) Melzack and Wall:

Melzack and Wall proposed the gate control theory of pain in 1965[9]. Their theory proposed the concept of a non-noxious stimulus signals closes the "gates" to noxious stimulus signals, and stopes its transmission to the higher centers of the brain, which results in pain inhibition. The gate as understood currently is the subnucleus reticularis dorsalis (SRD); a brain-stem structure. It responds to nerve impulses from the periphery to the brain, as it is the origin of descending fibers to the spinal cord. The SRD is under dual influences: one of them is the ascending nociceptive neural impulses that induce descending neuronal signals from the SRD reaching down to the entry zone at the dorsal horn of the spinal cord. This activity can either increase or decrease the signal, which is conveyed by small fibers through the dorsal horn. The second influence is the interaction of large and small fibers in the dorsal horn. While the A-delta and C-fibers (small afferent fibers) convey the nociceptive data - to go through the “gate”- to eventually cause perception of pain, data conveyed by the A-beta (large afferent fibers) act to inhibit the latter, thus "closing" the gate[10].

In 2016, other authors suggested one variation from the gate control theory regarding the excitatory and inhibitory axon terminal such "that afferent nociceptors produce an excitatory stimulus on first central transmission (CT) neurons and, simultaneously, an inhibitory stimulus on neurons in the SG", substantia gelatinosa[11]. Other authors suggested a modulatory effect of multiple neurotransmitters serotonin (5-HT), and endogenous opioids (EOs) on structures of the rostral ventromedial medulla (RVM) and periaqueductal grey (PAG)[12,13].

b) Descending Pain Modulating System:

The brain itself is involved in pain modulation through various pathways known as the descending pain modulating system. It includes three parts: the periaqueductal gray (PAG) area located in the midbrain, the rostral ventral medulla (RVM), and an endogenous opioid system. As the name indicates, neurons in the brain exert their effect though their descending signals to the subnucleus caudalis and the dorsal horn of the spinal cord.

c) Endogenous Opioid Inhibitory System:

Various pharmacological agents and non-pharmacological approaches can activate endogenous pain inhibitory systems mediated by opioids. Opioid peptides and their receptors are found presynaptically on first-order neurons and postsynaptically on second-order neurons, and in the rostral ventromedial medulla (RVM), and periaqueductal grey (PAG) PAG-RVM networks.

CONDITIONED PAIN MODULATION (CPM)

In 2010, Yarnitsky and colleges stated "DNIC, a form of supra-spinal descending endogenous analgesia, requires a noxious conditioning stimulus for pain attenuation"; in other words, pain inhibits pain[6].

CPM -previously known as DNIC- is thought to represents the activity of endogenous analgesia system, where descending pathways modulate the incoming spinal nerve impulses. This phenomenon is based on a spino-bulbar-spinal loop under cerebral control[6] (first investigated in rats by Le Bars et al. in 1979[14]; branches of this loop move through the ventrolateral and dorsolateral funiculi - the white matter in the spinal cord.

It is thought that DNIC is triggered by noxious stimulation of widespread areas of the body that exert an inhibitory influence on wide-dynamic-range (WDR) neurons in animals, however

similar mechanism was suggested in human[15,16]; those neurons in the spinal cord responsive to a broad range of intensity of stimulation by peripheral nerves[17]. The majority of WDR neurons are inhibited by noxious stimulation of any part of the body as long as it was not applied to the same neuron's excitatory receptive field. CPM is typically tested in the lab using the 'pain inhibits pain' theory; two noxious stimuli are applied, one presented as the 'conditioning' pain inhibits another one presented as the 'test' pain[18]. This phenomenon has been questioned as whether the inhibitory effect was due to cognitive attention or it is a physiological mechanism, in other words, whether distraction and application of noxious stimulus inhibit pain through the same or different mechanisms. In 2010, Yarnitsky et al. looked whether there is similar or even an additive effect on pain reduction, and he confirmed that"… further pain inhibition when CPM and distraction were combined compared to CPM alone"[6].

Inefficient CPM has been found in several chronic pain conditions when compared to healthy volunteers, such as fibromyalgia, chronic TMD, irritable bowel syndrome, migraine, tension type headache[15], both acute and chronic LBP (lower back pain)[19], Achilles tendinopathy[20], and acute and chronic whiplash-associated disorders (WAD)[21].

TYPES OF STIMULI:

As mentioned previously CPM requires the application of two stimuli to achieve the "pain inhibits pain" effect. The first pain is induced by the test stimulus (TS) such as a pressure, electrical, or thermal stimulus. The second pain is known as the conditioning pain (CS) and is induced by stimuli such as cold water, heat, or ischemia[7]. In 2012, a systematic review reported that immersion of the hand in cold water -cold pressor test (CPT)- is the most common CS employed, followed by ischemia, heat, and finally capsaicin. Mechanical pressure was reported as the most common test stimulus employed, followed by the electrical stimulus, and finally the

thermal stimulus[22].

Several authors explored the effect of the CS 'intensity' on the magnitude of the CPM, however their findings were inconsistent. Nir in 2011[8] stated that a significant CPM effect is initiated with a CS that produces moderate to intense pain levels, not mild pain. His findings are consistent with Calvino's statement that, "Stronger stimuli trigger more potent inhibition and longer-lasting post-stimulation effects, which may persist for several minutes"[17]. Other studies suggested that CPM magnitude is not affected by the intensity of the CS; once endogenous analgesia has been stimulated[8,23], even a non-painful CS was reported to induce CPM as well[18]. In addition, different stimulus modalities such as pressure based CPM response are associated with different psychological factors, such as anxiety[24].

FACTORS AFFECTING CPM:

It has been well established in the literature that CPM is affected by several factors that are modifiable, while others are not[25]. Therefore, CPM may be possibly enhanced, which have the potential to improve chronic pain experience.

Non-Modifiable

Some factors cannot be altered in human subjects and hence are considered non-modifiable. These factors are listed below.

a) CPM and Age:

Chronic pain conditions are common among the elderly, and the prevalence of pain for some conditions increases by age[26], possibly as a result of an altered endogenous analgesic system. In 2003, Edwards investigated the age-related differences in endogenous pain inhibition, by testing CPM of two groups of healthy individuals (younger and older) twice. His findings showed thermal pain facilitation and not inhibition in the older group, as opposed to the younger

group[16]. The next question would be at what time point pain modulation efficiency changes. In 2007, Larivie` re-investigated the pain thresholds of young, middle-aged, and elderly healthy volunteers to answer this question. Even though the results showed increased pain threshold by middle age than older participants, there was a negative correlation between CPM response and age. These findings suggest that CPM efficacy may decrease starting at middle age, which is earlier than expected which is older age[27].

b) Sex difference in CPM:

Sex differences in pain perception have been supported in the literature[69]. Several studies have reported that females are more sensitive to pain than males either to experimental pain such as mechanical pressure pain, or to clinical pain such as lower back pain[28,29,30,31]. Females have less efficient CPM effect than males [32,33,29,34,35], and that CPM in females, lasts for a shorter duration; i.e., is less maintained than in males[34]. Also, one study found that "contribution of stress to pain-evoked hypoalgesia differed by sex, with greater perceived stress associated with greater hypoalgesia in men and the opposite trend in women"[36]. This concept is supported in the literature by several pieces of evidence, such as nociceptive flexion reflex at low intensity stimulus, more pupil dilatation, and more brain activity in females than males during experimental pain. In 2003 Karibe tested the effect of physiologically relevant exercise (chewing bubble gum for 6 min) on pain level on male and female TMD patients as well as controls. As expected, pain increased in both genders in the patient group; however, it lasted longer in female patients than male patients[37]. In 2005, Ge investigated almost the same concept. Healthy age-matched subjects were recruited from both genders. Each subject was injected with two glutamate injections with a 5-minute interval between injections. Females reported more pain with the second injection as compared to males, which goes in line with the results of other

studies[38]. Biological differences between the sexes play a role in their different response to pain as well. Several studies support the concept that the difference in pain response between males and females is due at least in part to gonadal hormones, which affects basal pain sensitivity and response to analgesics[39,40,41]. Pain inhibition is different in females and males; endogenous opioid receptor binds differently in the brain in males and females, females show greater mu-opioid binding in cortical and subcortical areas[42]. On the other hand, in a different study there was no sex differences found for TeS or pain inhibition, even though females were significantly more sensitive to pressure pain[8].

c) CPM and Genetics:

Although underexplored, there are a few studies that link inefficient CPM to certain genotypes (such as lower 5-HTT gene-expression[44]) that are associated with chronic pain conditions such as fibromyalgia[44,45,46].

Modifiable

a) CPM, anxiety and stress:

Multiple studies found that a reduced state of anxiety reduces pain sensitivity[47] such as pressure pain sensitivity[48]. Also, it was found that dysfunctional pain modulation is associated with higher trait-anxiety in patients with myofascial pain syndrome[49], as well as in individuals with high catastrophizing personality trait[50]. Acute stress was found to stimulate analgesia[51], but other studies found that it decreases CPM efficiency[52].

b) CPM and sleep:

Sleep deprivation is common in chronic pain conditions. Noxious stimuli interfere with sleep and lack of sleep increases pain sensitivity. Thus noxious stimuli contribute to the experience of pain by increasing pain sensitivity, and significantly reducing pain inhibition;

inefficient CPM occurs in subjects who suffer primary from insomnia[53]. Authors have concluded, “There is a reciprocal relationship between sleep quality and pain, and the recognition of disturbed or un-refreshing sleep influences the management of painful medical disorders”[54]. However, in 2015 Matre concluded that “Sleep restriction leads to increased pain perception but not reduced inhibitory CPM”[55].

c) CPM and Exercise:

Several studies during the past few years investigated exercise as one of the factors that affect CPM efficiency[19]. Although endogenous analgesia decreases with age, exercise can enhance CPM in young as well as adult healthy subjects "adults with greater CPM were more likely to report greater EIH "i.e., exercise-induced hypoalgesia[56]. Others proposed that the CPM mechanism might be a potential explanation for EIH. Even though this hypothesis was not confirmed, it was found that hypoalgesia was greater especially after painful exercise as opposed to non-painful exercise[57], that there was greater EIH in the exercising body part as opposed to non-exercising body parts[58], that more efficient CPM was associated with more physical activity[59]. Vigorous intensity physical activity doesn’t further improve the pain modulation ability[60]. On the other hand, a few studies reported there was no significant change in CPM with exercise[61, 62].

d) CPM and pharmacological agents:

In 2015 Martini suggested that morphine affects CPM efficacy, while tapentadol does not[63]. Also, it was found that patients with less efficient CPM could benefit from serotonin–norepinephrine reuptake inhibitors (SNRIs) that enhance descending inhibition, improve their CPM[15].

INNOVATION

The experience of pain is a dynamic and homeostatic process that protects individuals from injury, while chronic pain is now seen as a disorder of this system. Endogenous pain modulation has heretofore not been measured in the clinic, and is generally ignored; yet it can be modified and enhanced. CPM testing-a psychophysical evaluation of endogenous pain modulation- represents a potentially valuable new metric in pain assessment for researchers. Although the reliability of certain CPM tests has been measured[64,65], there is no available clinical test that is known to be reliable and feasible for clinicians to use to assess patients' endogenous inhibition.

We believe that a clinically useful CPM test should be able to: 1) allow reliable assessment of endogenous pain modulation, 2) help tailor management strategies, and 3) possibly help predict the risk of developing chronic pain in some individuals. In short, this new metric may play an important role in developing personalized pain medicine[66].

SIGNIFICANCE: SHORT COMINGS, RELIABILITY AND FEASIBILITY OF CPM TEST

In spite of altered CPM being a common feature in chronic pain patients, the extent of its impairment is variable. This variability may possibly be explained by different measurement techniques, different CS and TS, different sites of testing, and different pain scales across different studies, in addition to individual differences such as age and gender. The four-step process for measuring knowledge utilization proposed by Dunn places great emphasis on the fourth step; reliability and validity of the test[67, 68]. As the CPM paradigm is used to assess pain modulation in chronic pain patients, it could become a primary functional outcome measure in

pain clinical trials. As a diagnostic test, it is essential that the CPM paradigm show consistent and repeatable measurements.

Despite increasing numbers of new trials every year, only a few studies have explored the test-retest reliability of the CPM for assessing pain modulation in chronic pain patients. Therefore, the utility of the test remains unconfirmed. The study described in the following chapters was designed to address questions about reliability, to develop a normal data set for CPM, as well as to design a feasible paradigm that can be easily incorporated as a diagnostic test in clinical assessments of chronic pain patients. Among the four classes of reliability, the test-retest reliability seems to be the most appropriate measure for this test, because it is used to assess the consistency of a measure from one time to another.

The clinical significance of the CPM test lies mainly in three domains: treatment of chronic pain conditions as chronic pain patient may benefit more from different pharmacological agents that target different altered pain modulation mechanisms, prediction of chronic pain development, and, modifying CPM -such as by exercise- to help with chronic pain symptoms. The reliability and feasibility of the CPM test is a necessary condition for significant substantial and meaningful results, which in turn is essential for the validity of the research findings.

HYPOTHESES

1. Employing the cold pressor as a conditioning stimulus, and pressure pain threshold as a test stimulus; the Conditioned Pain Modulation test shows:
    a. Good intra-session reliability, ICC $\geq$ 0.75.
    b. Good inter-session reliability, ICC $\geq$ 0.75.
2. Normal values of conditioned pain modulation in healthy individual range from 20 to 40%.

3. A single session of conditioned pain modulation test that consists of cold pressor as a conditioning stimulus, and PPT as a test stimulus applied to the hand is a feasible test.

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CHAPTER 2: RELIABILITY OF CONDITIONED PAIN MODULATION PARADIGM: SYSTEMATIC REVIEW

INTRODUCTION

Typically pain perception is quantified by static pain parameters, such as pain threshold and tolerance; however, they do not reflect all the psychophysical measures of pain or dynamic pain modulation. Dynamic psychophysical pain parameters include two lab tests that reflect inhibition and sensitization of nerve impulses: condition pain modulation (CPM) and temporal summation (TeS). Previous studies show that the application of a noxious stimulus to one part of the body can inhibit pain in another part of the body by activating the descending inhibitory system. This phenomenon was initially termed 'diffuse noxious inhibitory control' (DNIC) but is now called Conditioned Pain Modulation (CPM). TS is believed to be the "psychophysical correlate of wind-up of second/third order neurons reflecting central sensitization; excessive activation of NMDA receptors in response to high levels of repetitive input, clinically manifested by allodynia and hyperalgesia"[1,2]. The four-step process for measuring knowledge utilization proposed by Dunn emphasized greatly the fourth step: reliability and validity. As CPM and TeS paradigms are used to assess pain modulation in chronic pain patients, they are an outcome measures in many clinical studies[3,4,5,6,7]. Therefore, reliability of CPM and TeS assessment is a necessary condition for valid results. Reliability is the overall consistency of a measure. A test is reliable when its results are reproducible and consistent. There are several general classes of reliability estimates, such as inter-rater reliability, and test-retest reliability. Despite increased numbers of new studies using these tests every year, their reliability remains unconfirmed. Among the four classes of reliability, inter-rater reliability and test-retest reliability are the most

important classes for a clinical trial where multiple observers will be conducting the test. The aim of this systematic review is to gather published evidence describing the reliability of the CPM assessment paradigm, and to combine existing information and available data to assist us in decision making for designing a reliable clinical CPM test.

METHODS

This review was conducted following the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) 2009-check list[23].

Eligibility Criteria

One investigator/reviewer performed all eligibility assessments. We included all clinical studies, where study populations were human, using either healthy individuals or patients, or both, of any age or race, and any numbers of participants. Only studies that measured pain modulation, specifically DNIC/CPM, in two or more identical sessions with at least 60 minutes between sessions or studies that measured the DNIC/CPM reliability were included. There were no specific restrictions on the study design (except that cross over studies were excluded because the testing sessions are not identical), intervention, or language, CPM assessment methodology, or whether CPM was a primary or secondary outcome. We excluded animal studies, studies that did not assess DNIC/CPM, weren't related to pain modulation, or assessed CPM once only.

Search Strategy

The primary question posed in this review was whether the literature provides sufficient evidence for reliability of CPM in healthy subjects or patients. A comprehensive search of electronic databases was performed from inception to December 2015 including: EMBASE (OVID), Web of Science (Thomson Reuters), and NCBI (Pubmed). The broad keywords, created with the help of a librarian, used to meet our aim were: (1) Pain Modulation, (2)

Conditioned Pain Modulation, (3) Diffuse Noxious Inhibitory Control, and (4) Dynamic sensory testing.

In addition, the following Mesh terms were used to search NCBI (Pubmed):

1. Pain/physiopathology[Mesh] NOT conditioned pain modulation
2. Pain/physiopathology[Mesh] AND "pain measurement"[Mesh] NOT conditioned pain modulation
3. Pain/physiopathology[Mesh] AND "pain measurement"[Mesh] AND "pain threshold/physiology"[mesh]
4. Pain/physiopathology[Mesh] AND "pain measurement"[Mesh] AND "pain threshold/physiology"[mesh] NOT conditioned pain modulation
5. Pain/physiopathology[Mesh] AND "pain measurement"[Mesh] AND "pain threshold/physiology"[mesh] NOT diffuse noxious inhibitory controls

With each database search we only included human studies. All titles were reviewed to extract only the papers most relevant to our study question. This was followed by abstract and study design review of the selected articles for more refined search. Only studies that met our inclusion criteria were included.

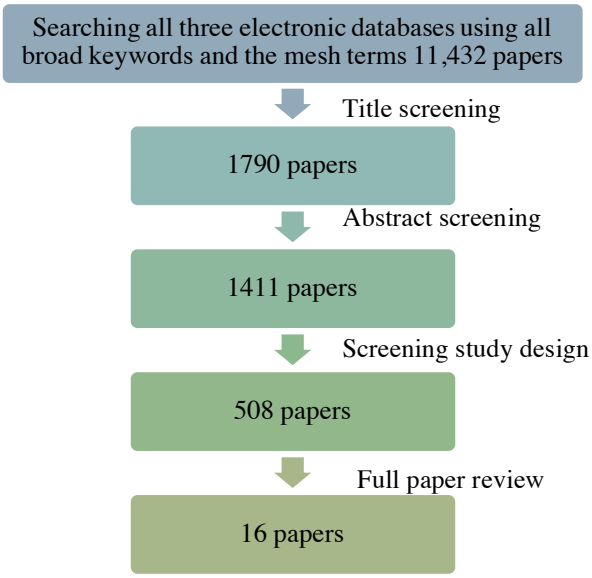

Figure 1: Study Design

All titles and abstracts were screened initially to determine their relevance to our question (Fig 1). Next, further detailed investigation of the retrieved records was performed according to the eligibility criteria mentioned previously.

Outcomes, collected data, and statistical consideration

The following data on CPM assessment, relating to primary or secondary outcome were extracted from each article: study design, randomization, blindness, subject numbers and gender of subjects, health status, age range, ethnicity, whether the study was relevant to pain modulation or not, number of visits, whether CPM & TeS were assessed or not, CPM reliability, the test stimulus (TS) and the conditioning stimulus (CS), available interval time either between sessions or visits. Reliability presented as: overall, inter-session, and intra-session intra-class correlation coefficient (ICC), overall, inter-session, and intra-session coefficient of repeatability (CR), and coefficient of variation (CV).

Risk of Bias

Methodological quality data were collected by one independent researcher (RN). This assessment was repeated three times. In case of disagreements, a second opinion was provided by a second researcher (MD). Both researchers were trained in assessing methodological quality as well as in CPM assessment and pain research while MD was experienced in writing systematic reviews.

RESULTS

Search Strategy

Our search strategy identified 11,432 papers as human studies; only 1790 titles were conditioned pain modulation-related. Abstracts were reviewed, and 1411 studies were eligible for full paper review. After screening each study's research design resulted in 508 studies,

however, only 16 were included (Fig 1). Excluded studies weren't related to pain modulation, animal studies, did not explore DNIC/ CPM, explored DNIC/CPM in one session only, or explored DNIC/CPM in more than one non-identical session. Data from the 16 papers were used to explore the reliability estimates across all available studies. Available extracted data from these 16 studies were used to calculate reliability estimates across all available studies. The assessment of methodological quality for all 16 studies included showed 60% agreement across the three repeated search rounds by RN. After a second opinion from MD, full agreement was achieved for all included studies. Included papers were published between 2009 and 2015, and randomization was considered in 11 out of the 16 papers (68.75%), while only 4 papers used blinding procedures for either the subjects or investigators (25%) (Table 1). All included articles tested healthy subjects as well as patients (total number of subjects in all included studies n=908); and included 467 males, and 441 females. Across all studies, age ranged from 21.6±2.1 to 63.1±4.6 years old (Table 2.1). Ethnicity of subjects were mainly non-hispanic (n=278) Hispanic (n=22), and unknown ethnic background (n=14). Also, the majority of participants were white (n=224), followed by black (n=21), and 39 were of other races (Table 2.2).

Methodological design

According to data availability, all included studies assessed CPM, however only 8 studies out if the 16 studies we included investigated CPM test reliability (Table 3.1). In the 8 studies, the number of visits ranged from 2 to 8, with one or two sessions per visit, and time interval ranging from 15 minutes between sessions during a visit up to 12 months between visits. Various methodological designs were used to evaluate the CPM; the most frequent modalities of TS used were thermal stimulation (50%), pressure pain threshold (37.5%), electrical stimulation (6.2%), and chemical stimulation (6.2%) (Table 3.2). The cold pressor test (CPT) was the most

frequently CS used in 75% of studies, followed by ischemic (15%), and hot water immersion and electrical stimulation (10%) (Table 3.2). The TS was applied to a number of different sites, however thenar eminence (palm of the hand at the base of the thumb) was the most tested site. In addition to thenar eminence, the forearm, ankle, thigh quadriceps muscle, shoulder, finger, knee, masseter muscle, flexor carpi radialis muscle, lower leg, nose, temporomandibular joint, trapezius, and lower arm were tested as well. Regarding the CS, the hand is the most tested site, followed by arm, craniofacial region, cheeks, forearm, and foot (Table 3.2).

The assessed CPM test reliability was quantified using various measures: Intra-class correlation coefficient (ICC), and coefficient of repeatability (CR), inter-individual coefficient of variation (Inter-In CV), and intra-individual coefficient of variation (Intra-In CV). The (ICC) and (CR) were the most frequently used measures. Overall ICC values ranged from ranged from 0.1 to 0.94, intra-session reliability ICC ranged from 0.39 in one group of male patients to 0.94 in a sample of healthy subjects of both sexes, inter-session reliability ICC ranged from -0.4 in another group of male patients to 0.82 in healthy subjects, CR ranged from 0.3 to 0.35 but was measured in only one study. Inter-In CV ranged from 11.5 to 103.2, Intra-In CV ranged from 27.0 to 99.6. In patients the intra-session reliability ICC ranged from 0.39 (males) to 0.73 (Table 4).

Table 1: Eligible articles identified by first authors, publication year, publishing journal, order of testing: counterbalanced order or randomized tested site, randomization of test battery or other forms of randomization, and blindness

	Author	Year	Journal	Random/ Counter-balance	Site random.	Test battery randomized	Other forms of randomization	Blindness
				Y/N	Y/N	Y/N	Y/N	Y/N
1	Edwards et al.[3]	2003	Pain	Y	Y	N	Temperature	N
2	Valencia et al.[8]	2012	J.Pain	N	N	N	N	N
3	Olesen et al.[9]	2012	RA.Pain Med	N	N	N	N	Y
4	Cathcart et al.[10]	2009	Pain.Res Manage	Y	Y	N	N	N
5	Lewis et al.[11]	2012	Pain.Res Manage	Y	N	Y	N	N
6	Oono et al.[12]	2011	J.SjPain	Y	N	Y	N	N
7	Granovsky et al.[13]	2013	E.J.Neur	Y	Y	Y	N	N
8	Kunz et al.[14]	2014	Somatosens Mot Res	Y	Y	N	N	N
9	Nahman-Averbuch et al.[15]	2014	Pain	Y	N	Y	N	N
10	Kothari et al.[16]	2014	J. Oral Maxillofac. Surg	Y	N	Y	N	N
11	Manresa et al.[17]	2014	PLOS ONE	N	N	N	N	Y
12	Moisset et al.[18]	2015	J.Brs	Y	N	Y	Y	Y
13	Martel et al.[19]	2013	Pain Med	N	N	N	N	N
14	Valencia et al.[20]	2013	BMC Musculoskelet Disord.	Y	N	N	Y	N
15	Niesters et al.[21]	2014	Bja	Y	N	N	Tapentadol Tablets	Y
16	Wilson et al.[22]	2013	Pain	N	N	N	N	N

Table 2.1: Demographic data of participants including age and gender: Total: total subject number, M: Male, F: Female. The number of participant, and mean age are mainly reported as published in the original paper, otherwise numbers were calculated if possible for standardized presentation.

	Total	M	Healthy	Patient	F	Healthy	Patient	Mean age Males	Mean age Females	Mean age Healthy	Mean age Patient	Mean age Young	Mean age Old
1[3]	93	Y:20 O:16	36	-	Y:25 O:32	57	-	-	-	-	-	21.6±2.1	63.1±4.6
2[8]	114	81	40	41	33	16	17	-	-	28.71±8.44	32.34±11.55	-	-
3[9]	62	38	-	38	24	-	24	-	-	-	53±11.0	-	-
4[10]	20	9	9	-	11	11	-	27±6.4	23±3.6	-	-	-	-
5[11]	20	7	7	-	13	13	-	-	-	25±8.0	-	-	-
6[12]	12	12	12	-	-	-	-	25.6±1.5	-	-	-	-	-
7[13]	45	24	9	15	21	10	11	-	-	59.3 ±6.0	61.5±7.0	-	-
8[14]	17	8	8	-	9	9	-	-	-	27.3±2.7	-	-	-
9[15]	13	5	5	-	8	8	-	-	-	25.6± 2.8	-	-	-
10[16]	27	11	9	2	16	11	5	-	-	32	47	-	-
11[17]	34	34	34	-	-	-	-	27.5±6.8	-	-	-	-	-
12[18]	14	7	7	-	7	7	-	-	-	26.9±5.5	-	-	-
13[19]	55	20	-	20	35	-	35	48.9±10.5	49.5±8.9	-	-	-	-
14[20]	324	161	74	87	163	116	47	-	-	23.02±6.04	43.83±17.80	-	-
15[21]	24	14	7	7	10	5	5	-	-	62.75	62.75	-	-
16[22]	34	-	-	-	34	34	-	-	27±7.0	-	-	-	-

Table 2.2: Demographic data of participants including Ethnicity or Race: Total: Total: total subject number, E: Ethnicity, R: Race, H: Healthy, P: Patients. Data are mainly reported as published in the original paper, otherwise numbers were calculated if possible for standardized presentation.

	Total	E. Hispanic		E. Non-Hispanic		E. Unknown /other		R. White		R. Black		R. Asian		R. Non-Hispanic-Pacific		R. Am.Indian /Alaskan Native	R. Hispanic	R. Mixed		R. Unknown/ Others	
1[3]	93	-		83		10		83		-		-		-		-	-	-		10	
2[8]	114	H 7	P 5	H 48	P 50	H 1	P 3	H 44	P 51	H 2	P 1	H 4	P 1	H 1	P 0	-	-	H 3	P 5	H 1	P 3
3[9]	62	-		-		-		-		-		-		-		-	-	-		-	
4[10]	20	-		-		-		-		-		-		-		-	-	-		-	
5[11]	20	-		-		-		-		-		-		-		-	-	-		-	
6[12]	12	-		-		-		-		-		-		-		-	-	-		-	
7[13]	45	-		-		-		-		-		-		-		-	-	-		-	
8[14]	17	-		-		-		-		-		-		-		-	-	-		-	
9[15]	13	1		12				10		1		1		-		-	1	-		-	
10[16]	27	-		-		-		-		-		-		-		-	-	-		-	
11[17]	34	-		-		-		-		-		-		-		-	-	-		-	
12[18]	14	-		-		-		-		-		-		-		-	-	-		-	
13[19]	55	3		52				36		16		-		-		-	3	-		-	
14[20]	324	-		-		-		-		-		-		-		-	-	-		-	
15[21]	24	-		-		-		-		-		-		-		-	-	-		-	
16[22]	34	1		33		-		-		1		10		-		-	1	-		-	

Table 3.1: Study Design: relevant to PM: pain modulation, CPM: Conditioned pain modulation assessed, TeS: Temporal Summation, CPM Rel/ Stab: Conditioned Pain Modulation Reliability/stability, number of visits, number of sessions, Interval minutes, Interval days, and Interval months.

	Relevant to PM	CPM	TeS	CPM Rel/ Stab	Number of visits	Number of sessions	Interval time in minutes	Interval time in days	Interval time in months	Number of examiners
	Y/N	Y/N	Y/N	Y/N						
1[3]	Y	Y	Y	N	2	1 per visit	Non-consecutive days.			Unknown
2[8]	Y	Y	N	N	Patient grp 2	1 per visit	-	0-2-4	-	Unknown
					Healthy grp 3	1 per visit	-	0-3 to 1	3	
3[9]	Y	Y	N	Y	2	1 per visit	-	-	-	Unknown
4[10]	Y	Y	Y	Y	1	2	60	-	-	Unknown
5[11]	Y	Y	N	Y	1st Visit	2	15	-	-	Unknown
					2nd Visit	1	-	3	-	
6[12]	Y	Y	N	Y	3	1 per visit	-	2	-	Unknown
7[13]	Y	Y	Y	N	1	2	60	-	-	Unknown
8[14]	Y	Y	N	N	2	1 per visit	-	3	-	Unknown
9[15]	Y	Y	N	N	2	1 per visit	-	-	-	Unknown
10[16]	Y	Y	N	N	4 patient grp	1 per visit	-	7	1,3,12	Unknown
					4 patient grp	1 per visit	-	7	1,3,12	
					4 Healty grp	1 per visit	-	7	1,3,12	
					4 Healty grp	1 per visit	-	7	1,3,12	
11[17]	Y	Y	N	Y	2	1 per visit	-	7 to 21	-	Unknown

	Relevant to PM	CPM	TeS	CPM Rel/ Stab	Number of visits	Number of sessions	Interval time in minutes	Interval time in days	Interval time in months	Number of examiners
	Y/N	Y/N	Y/N	Y/N						
12[18]	Y	Y	Y	N	4	1 per visit	-	14	-	Unknown
13[19]	Y	Y	N	Y	2	1 per visit	-	7 to 10	-	Same
14[20]	Y	Y	N	Y	Patient grp 2	1 per visit	-	-	3	Unknown
		Y	N	Y	healthy grp 3	1 per visit	-	2	-	
15[21]	Y	Y	N	N	2	1 per visit	-	28	-	Unknown
16[22]	Y	Y	N	Y	8	1 per visit	-	14	-	Unknown

Table 3.2: Study Design: Test Stimulus used in CPM assessment and its site as well as the Conditioned Stimulus used and its site.

	Test stimulus		Test stimulus site		Conditioning stimulus		Conditioning stimulus site	
1[3]	Thermal Pulses		L.D.forearm /L. Ankle		Cold water 5°C		R. Hand	
	Thermal Pulses		L.D.forearm /L. Ankle		water 22°C		R. Hand	
2[8]	Patient	Heat	Patient	Thenar eminence of nonsurgical	Patient	Cold water 8°C	Patient	Surgical side Hand
	Healthy	Heat	Healthy	ND-Thenar eminence	Healthy	Cold water 8°C	Healthy	ND- Hand
3[9]	PPT		Quadriceps m (L4)		Cold water 2°C		R. Hand	
4[10]	PPT		R. Shoulder trapezius, Dorsal of R mid finger		Ischemic		L. Arm	
5[11]	PPT		R. Knee		Ischemic		L. Arm	
	PPT		R. Knee		Cold water 12°C		L. Hand	
6[12]	PPT/PPTol		R.masseter.m & L.flexor carpi radialis m.		Cold water 2-4°C		R. Hand	
	PPT/PPTol		R.masseter.m & L.flexor carpi radialis m.		Ischemic		R Up arm	
	PPT/PPTol		R.masseter.m & L.flexor carpi radialis m.		Mechanical Press		Craniofacial region	
7[13]	Heat		Low leg		Hot water 46.5°C		Ipsilateral Hand	
8[14]	CO2		R & L of Nose		Heat		R & L cheek & volar forearm	
9[15]	Heat		low L leg		Cold water 10-12°C		R.foot	
10[16]	PPT		TMJ & thenar m. of surg.		Cold water 2- 5°C		Non-surg side leg	
	PPT		TMJ & thenar m. of surg.		neutral water 26-28°C		Non-surg side leg	
	PPT		D-TMJ & thenar m.		Cold water 2- 5°C		ND- leg	
	PPT		D-TMJ & thenar m.		neutral water 26-28°C		ND- leg	
11[17]	Electrical		D. Ankle		Cold water 2°C		ND- Hand	
12[18]	Heat		R.thenar eminence		Cold water 4-8°C		L.foot	
13[19]	PPT		R. Up Trapezius		Cold water 4°C		L.Hand	
14[20]	Heat		Thenar eminence of nonsurg.		Cold water 8°C		Surg.side Hand	
			ND -Thenar eminence		Cold water 8°C		D-Hand	

	Test stimulus	Test stimulus site	Conditioning stimulus	Conditioning stimulus site
15[21]	Heat	ND-low arm	Cold water 6-18°C	Low foot and leg
16[22]	Heat	D-forearm	Hot water 46.5°C	ND-hand

Table 4: CPM values (mainly reported as percent of change, otherwise it was presented as the original paper format when calculating the percent of change was not possible) CPM related test sites/parameter/or group of participants, ICC: Intra-class Correlations (ICC) inter-session intersession or overall ICC reported as between session or between visits, coefficient of repeatability (CR), Inter-In CV: inter-individual coefficient of variation, Intra-In CV: intra-individual coefficient of variation.

	Test parameter/Grp	CPM Value		Test sites	Intra-S-ICC	Inter-S-ICC	Over. ICC	Over. ICC F	Over. ICC M	CR	Inter-In CV %	Intra-In CV %
1[3]		**Young**	**Old**	-	-	-	-	-	-	-	-	-
	Immersion 1	21.27	16.78	-	-	-	-	-	-	-	-	-
	Immersion 2	18.06	20.36	-	-	-	-	-	-	-	-	-
	Immersion 3	17.77	12.08	-	-	-	-	-	-	-	-	-
	Immersion 4	17.5	12.93	-	-	-	-	-	-	-	-	-
2[8]		**Healthy**	**Patients**	-	-	-	-	-	-	-	-	-
	Percent change CPM baseline	54.12	17.08	-	-	-	-	-	-	-	-	-
	Post-48 hours	35.51		-	-	-	-	-	-	-	-	-
	Post-96 hours	49.91		-	-	-	-	-	-	-	-	-
	3m after surgery		35.67	-	-	-	-	-	-	-	-	-
3[9]	1st session	Average 19		Quadriceps m (L4)	-	-	0.1	-	-	-	-	-
	2nd session	Average 9										
4[10]		**Time 1**	**Time 2**	Finger	-	-	0.57	-	-	0.35	-	-
		0.63±1.4 (29%)	0.98±1.7 (38%)									
		0.80±1.2 (38%)	0.10±1.3 (46%)	Shoulder	-	-	0.69	-	-	0.3	-	-

	Test parameter/Grp	CPM Value		Test sites	Intra-S-ICC	Inter-S-ICC	Over. ICC	Over. ICC F	Over. ICC M	CR	Inter-In CV %	Intra-In CV %
5[11]		-		Ischemic L. arm(N)	0.75	-0.4	-	-	-	-	-	-
		-		Cold pressor L. hand (N)	0.85	0.66	-	-	-	-	-	-
		-		Ischemic L arm (NPS)	0.60	0.82	-	-	-	-	-	-
		-		Cold pressor L. hand (NPS)	0.94	0.80	-	-	-	-	-	-
6[12]	**PPT 1**	Cold	66.3±10	TA	-	-	-	-	-		54	40.1
	2	Tourniquet	43.4±5.8	TA	-	-	-	-	-		54.3	40.9
	3	Head Band	29.1±5.7	TA	-	-	-	-	-		57.4	54.5
	4	Cold	23.3±4.3	Masseter	-	-	-	-	-		61.2	65.9
	5	Tourniquet	20.7±3.4	Masseter	-	-	-	-	-		62.7	64.3
	6	Cold	16.7±2.8	Forearm	-	-	-	-	-		74.1	66.6
	7	Tourniquet	15.1±2.6	Forearm	-	-	-	-	-		80.5	72.9
	8	Head Band	13.8±4.7	Forearm	-	-	-	-	-		100.6	84.1
	9	Head Band	10.1±2.7	Masseter	-	-	-	-	-		103.2	84.1
	PPTol 1	Cold	32.6 ± 4.6	Masseter	-	-	-	-	-		41.4	35.2
	2	Tourniquet	24.7±4.9	Forearm	-	-	-	-	-		45.1	38.6
	3	Cold	24.6±2.3	TA	-	-	-	-	-		46.9	42.4
	4	Head Band	24.4±4.9	Masseter	-	-	-	-	-		52.1	59
	5	Tourniquet	20.5±3.7	Masseter	-	-	-	-	-		57.5	66.7
	6	Tourniquet	20.2±2.7	TA	-	-	-	-	-		58.9	70.9
	7	Cold	19.8±2.4	Forearm	-	-	-	-	-		66.0	72.2

	Test parameter/Grp	CPM Value		Test sites	Intra-S-ICC	Inter-S-ICC	Over. ICC	Over. ICC F	Over. ICC M	CR	Inter -In CV %	Intra -In CV %
6[12]	**8**	Head Band	18.7±4.8	TA	-	-	-	-	-		68.7	81.5
	9	Forearm	15.0±3.4	Head Band	-	-	-	-	-		101.7	99.6
	VAS1.4 1	TA	41.5±5.3	Cold	-	-	-	-	-		60.1	27
	2	TA	26.7±5.1	Tourniquet	-	-	-	-	-		64.8	42.4
	3	TA	24.0±3.8	Head Band	-	-	-	-	-	-	86.2	70.3
	VAS1.6 1	TA	37.2±4	Cold	-	-	-	-	-	-	56.8	36.9
	2	TA	33.2±5.4	Tourniquet	-	-	-	-	-	-	67.7	55.5
	3	TA	21.3±3.2	Head Band	-	-	-	-	-	-	74.3	66.3
7[13]		-		-	-	-	-	-	-	-	-	-
8[14]		-		-	-	-	-	-	-	-	-	-
9[15]	**1st Repetition**	3.25± 1.28		-	-	-	-	-	-	-	-	-
	2nd Repetition	2.78± 1.15		-	-	-	-	-	-	-	-	-
	3rd Repetition	2.96±1.16		-	-	-	-	-	-	-	-	-
10[16]		-		-	-	-	-	-	-	-	-	-
11[17]		***1st session***	***2nd session***	-	-	-	-	-	-	-	-	-
	NWR threshold (mA)	3.5±1.9 (23.81%)	3.3±2.7 (23.2%)	-	-	-	0.94	-	-	-	11.5	-
	Electrical pain detection threshold (mA)	2.3±1.3 (30.7%)	2.1±1.1 (25.9%)	-	-	-	0.69	-	-	-	14.9	-
	Pain intensity rating to Supra-threshold electrical stimulation	1.5±1.2 (28.3%)	1.4±1.2 (26.4%)	-	-	-	0.74	-	-	-	35.3	-

	Test parameter/Grp	CPM Value		Test sites	Intra-S-ICC		Inter-S-ICC		Over. ICC	Over. ICC F	Over. ICC M	CR	Inter -In CV %	Intra -In CV %
12[18]		-		-	-		-		-	-	-	-	-	-
13[19]	***M***	142.2		-	-		-		0.59	0.75	0.33	-	-	-
	F	127.1		-	-		-		-	-	-	-	-	-
	Overall	-		-	-		-		-	-	-	-	-	-
14[20]		***F***	***M***	-	***F***	***M***	***F***	***M***	-	-	-	-	-	-
	Patients: Trial 1 Pre surgery	34.1%	18.9%	-	0.57	0.39	-	-	-	-	-	-	-	-
	Trial 2 Pre surgery	21.1%	23.8%	-			-	-	-	-	-	-	-	-
	Trial 1 (3 M)	40.3%	35.6%	-	0.73	0.41	-	-	-	-	-	-	-	-
	Trial 2 (3 M)	1.2%	12.3%	-			-	-	-	-	-	-	-	-
	Healthy: Trial 1 Day 1	44.7%	54.0%	-	0.55	0.66	Trial 1 D1-5	Trial 1 D1-5	-	-	-	-	-	-
	Trial 2 Day 1	41.5%	54.0%	-					-	-	-	-	-	-
	Trial 1 Day 3	50.9%	39.6%	-	0.61	0.52	0.62	0.60	-	-	-	-	-	-
	Trial 2 Day 3	41.9%	39.7%	-			Trial 2 D1-5	Trial 2 D1-5	-	-	-	-	-	-
	Trial 1 Day 5	37.8%	47.8%	-	0.74	0.48			-	-	-	-	-	-
	Trial 2 Day 5	38.6%	41.2%	-			0.61	0.58	-	-	-	-	-	-
15[21]	**Before treatment**	5.4%		-	-		-		-	-	-	-	-	-
	After treatment placebo	7.2%		-	-		-		-	-	-	-	-	-
	After treatment Tapentadol	7.7%		-	-		-		-	-	-	-	-	-
16[22]		-1.3±0.3 (mean)		-	-		-		0.39	-	-	-	-	-

DISCUSSION

CPM has been increasingly used to investigate pain modulation in healthy subjects as well as patients, in experimental settings[3,23,24,25,26]. The assessment of CPM phenomenon has been demonstrated using different modalities. The test-retest reliability of these techniques is underexplored in the literature, and findings are inconsistent. The present study reviewed the scientific literature regarding the reliability of CPM assessment in both healthy subjects and patients. We combined existing information and published data to design a reliable clinical CPM test. The literature was searched for Peer-reviewed studies published prior to December 2015 using EMBASE (OVID), Web of Science (Thomson Reuters), and NCBI (Pubmed). Only 16 studies were included, however reliability estimates and measures where available in 8 studies only, which we used to estimate reliability across all included studies[9, 10,11,12,17,19,20,22]. Shrout et al. stated that ICC parameter ranges from 0 to 1, with values closest to 1 indicating the highest reproducibility, and greater than 0.75 indicates excellent agreement[28]. Three out of the 8 studies reported an excellent reliability: Lewis et al.[11] intra-session reliability (ICC 0.94), Manresa et al.[17] overall reliability (ICC 0.94), and Martel et al.[19] overall reliability in females (ICC 0.75). The Lewis and Manresa studies included healthy young subjects 25±8 years old and 27.5±6.8 years old respectively, while Martel et al. included middle aged healthy females 49.5±8.9 years which might be the cause for the slightly lower ICC value. The nature of the CS and the tested site was the same among the three studies, it was CPT temperature 2, 8, and 12°C and the site was the hand. As it has been stated in the literature, the segmental nociceptive counter-stimulation significantly inhibits the pain intensity[30], therefore performing the test on the hand may play role in the reliability of the test, because of the counter stimulation.

Further CPM test-retest reliability assessment:

Currently, the lack of standardized CPM paradigm protocols is a major issue which compromises researchers' ability to gather data from various studies and draw a conclusion regarding the clinical application of such a test. Due to the altered nature of CPM in several chronic conditions, healthy subject population seems to be a suitable population to investigate the test-retest reliability. Among the different types of conditioning that have been employed, CPT was the most frequently used CS. Also, it was proposed as one of the most efficient conditioning stimuli to induce CPM when combined with PPT as a test stimulus using manual algometry because it demonstrated good test-retest reliability and repeatability[27,29].

Conclusion:

These findings are important in the general context of designing a reliable CPM test. From this review, a potentially reliable clinical CPM test includes PPT as test stimulus performed on the dominant hand, and CPT as a conditioning stimulus on the non-dominant hand.

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CHAPTER 3: RELIABILITY OF A CLINICAL CONDITIONED PAIN MODULATION (CPM) TEST

INTRODUCTION

A noxious conditioning stimulus that is applied to one region of the body facilitates the inhibition of an original pain activity in a remote region; this phenomenon is known as conditioned pain modulation[1,2,3]. The diffuse noxious inhibitory control (DNIC), describes the same phenomena in animals[4,5], where "inhibitory and facilitatory descending systems can be assessed individually. In humans, only the net sum between inhibition and facilitation can be measured"[6]. Nowadays there is growing evidence indicating that inefficient pain inhibition is associated with several chronic pain conditions, such as fibromyalgia, chronic temporomandibular disorders (TMD), irritable bowel syndrome (IBS), migraine, tension-type headache[1], and chronic whiplash-associated disorders (WAD)[7]. The extent of this impairment is variable, which may be due to employing different techniques, different conditioning stimuli (CS) and test stimuli (TS), different sites of testing, and pain scales; in addition to the individual differences like age and gender. Different types of conditioning and test stimuli have been employed; cold pressor test (CPT) is one of most common CS used to induce CPM effect when combined with pressure pain thresholds (PPT) as a TS[8].

The significance of the CPM test lies mainly in three conceptual domains: 1) Chronic pain patients may benefit more from tailored pharmacological agents that target specific altered pain modulation mechanisms such as Serotonin–Norepinephrine Reuptake Inhibitor (SNRIs), which augment descending inhibition[10]. 2) Prediction of chronic pain development in some individuals could help in the prevention of post-surgical pain by pre-emptive analgesics[9]. Finally, 3)

Modifying CPM could help diminish chronic pain symptoms. Thus, clinical CPM test may play a role in developing "personalized" pain medicine.

In order for CPM to be developed into a diagnostic test, it is essential that the CPM paradigm show consistent and repeatable measures. The reliability of the CPM test is a necessary condition for useful results, which in turn is essential for the validity and the effectiveness of the research findings. The four-step process for measuring knowledge utilization proposed by Dunn emphasized greatly the fourth step: reliability and validity of the test[11]. Only a few studies have explored the reliability of CPM test measure in relation to alteration in pain modulation in chronic pain patients, and the level of reliability remains unconfirmed. Although the reliability of CPM has been measured[6,12,13], there is no clinical test that is known to be reliable, and feasible for clinicians to assess patients' endogenous pain inhibition. This study was designed to address questions about CPM reliability in order to develop a test that can easily be incorporated as a diagnostic test in research and clinical settings. Among the four classes of reliability, test-retest reliability class seems to be the most appropriate measure for this test. Therefore, our primary aim was to measure the reliability of a CPM protocol that could be used in a clinical setting, measuring both within and between sessions (intrasession and intersession), and between different days of testing, all by the same examiner.

METHODS

This study was performed at the Department of Oral Medicine, University of Washington (UW), Seattle, (USA) between November 2014 and April 2016. It was approved by Institutional board review (IRB) number 47524-D. The primary endpoint was the reliability of the CPM with CPT as a CS and PPT as TS.

Design:

CPM and PPT were assessed by repeated measurements on the same volunteer in three different visits that took place in the same clinic, with a minimum interval time of 2 days to a maximum of 7 days between visits. All three visits for the same volunteer were carried out at the same time of the day. Each visit included two experimental sessions, with 15 minutes between sessions, for a total of six sessions per volunteer. CPT was used as a CS in 5 sessions, and one session out of the six sessions was randomly assigned as a control session where lukewarm water was used as a CS instead of CPT. The control session was not used in calculating reliability. At the beginning of the first visit, a training session of the pain tests (CPM and PPT) was performed on the hand 'H" before starting the experiment, until the subjects were familiar with the testing procedures. CPT was chosen as CS, as it was found to be an efficient CPM-inducing stimulus, and PPT was used as a test stimulus. Tests were performed on three sites of the dominant side of the body in a non-overlapping fashion: side of the face "M" (masseter muscle), hand "H"(thenar eminence), and foot "F"(dorsum of the foot) (Fig.1, Fig.2). Brain hemispheres process incoming signals differently; the dominant side is the more active hemisphere. Therefore, testing the dominant side of the body yields a more accurate interpretation of pain.

Volunteers answered a short post-test feasibility questionnaire twice, once at the end of the 1st session during the first visit, and once at the end of the 2nd session at the third visit.

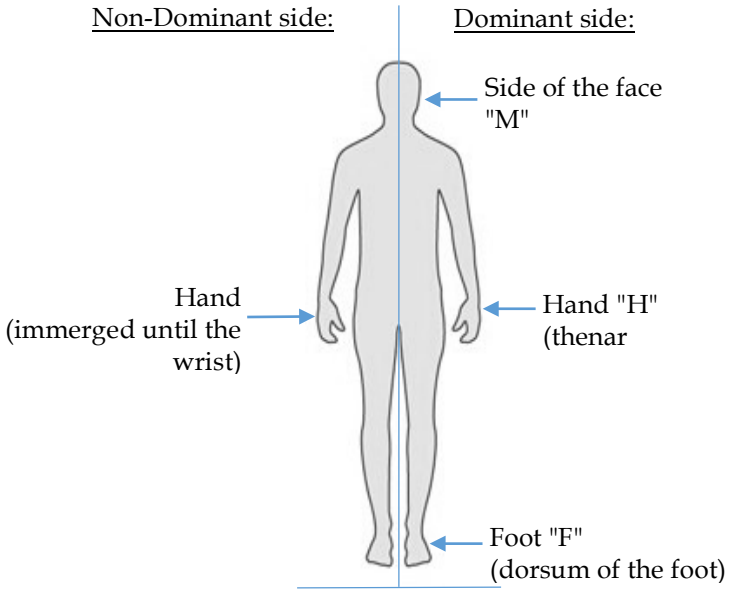

Figure 1: tested sites

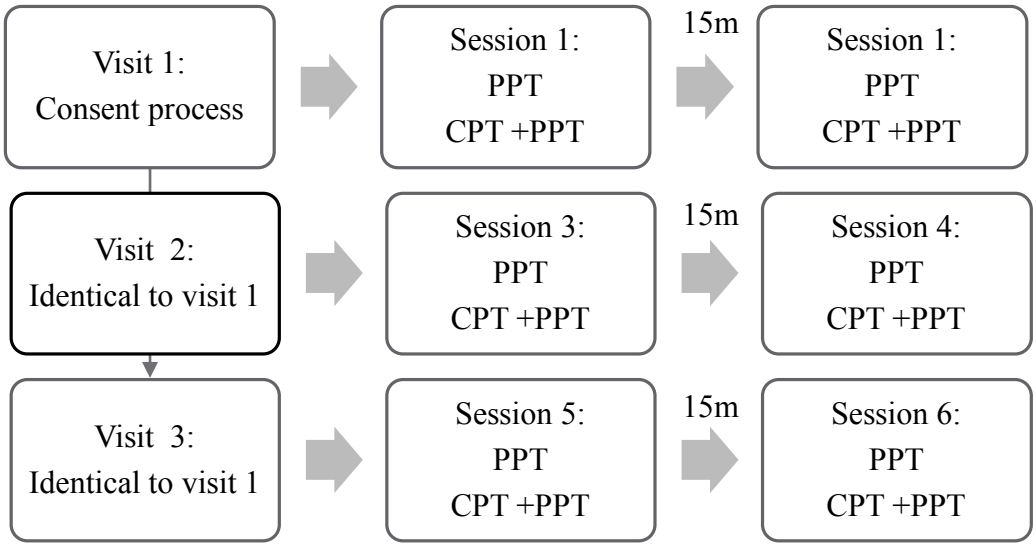

Figure 2: Study flow chart: Volunteers were tested in three visits, each visit consisted of two sessions in each session two tests were performed, the baseline test PPT, and the CPM assessment test PPT +CPT.

Volunteers

Sixty-one volunteers were recruited by advertisements posted in the University of Washington School of Dentistry, and received $150 as compensation for their participation, however 60 volunteers completed the study, because one volunteer dropped out. Inclusion criteria were: 18-80 years old, healthy females and males, able to provide informed consent. Exclusion criteria were*:* individuals who reported current spontaneous pain or injuries in the lower or upper limbs or face, unable to stop their analgesics 24 hours before their visit, pregnant females, and the presence of current or previous major medical conditions such as severe heart disease or respiratory conditions, or a psychiatric condition including depression. Potential volunteers, who expressed interest or contacted a study team member in response to the flyer or study handout, were recruited using a standard script as a guide over the phone or in person. Volunteers were asked not to consume any pain medication for 24 hours prior to their appointment.

Tests

1- Pressure pain threshold test PPT (TS): is a test that involves applying increasing pressure on a body site, PPT is defined as the amount of pressure that results in the first sensation perceived by volunteers as painful, it was assessed three times with a Somedic Pressure Algometer with 20 seconds in between (PPT is defined in unites of pressure kPs). Among the three Algometer probes available, the $2cm^2$ was used as it exerts up to 1000 kPs. The PPT was performed on each of the tested sites in a non-overlapping fashion; a template was used to repeatedly identify the three testing sites, and the algometer was calibrated between visits, and between volunteers as well.

2-Conditioned Pain Modulation test (PPT+CPT): is the CPM test was repeated three times at each of the three sites following the PPT. The non-dominant hand was immersed in 41°F (5°C) cold water up to the wrist as a CS (a container filled with water and ice), the temperature of the cold water was monitored by a thermometer placed in the water bath, to ensure water temperature stability. Volunteers were asked to report their non-dominant hand pain level when it reached 7 out of 10 according to a numerical pain scale- where 0 is no pain and 10 is severe pain. The PPT was applied simultaneously with the CS at this point with the non-dominant hand remaining in the water. One session was randomly assigned as a control session where lukewarm water –temperature of 80°F (26.6°C) as previous studies-[34] was used instead of CPT, and the PPT was performed at the 30th second instead of when the subject reported the pain level as 7 out of 10 as occurs in the CPT.

Quantification of the CPM

Following current recommendations, the magnitude of CPM was quantified as the difference in PPT during CPT minus PPT before CPT[14].

Randomization

To avoid potential bias that could result from an effect of testing order, the order of testing the three sites in each visit was randomly assigned while maintaining counterbalancing of the possible orders. The three sites were tested in specific order in both sessions of a visit for a given subject. Therefore, with using a sample size of 60 subjects with three visits per subject, yields 180 total visits. The order was fixed for each subject during both sessions of each visit. Evaluating three test sites during each session, i.e., the masseter (M), hand (H), and foot (F), yields 6 possible orders for each visit MHF, MFH, FMH, FHM, HFM, and HMF. These 6 groups were randomly assigned to each of the 180 visits; such that, there were 30 sessions of

each order. Of the total 6 sessions (i.e., 3 visits) for each subject, the conditioning stimulus in one of the sessions was lukewarm water bath instead of the CPT. With 60 subjects, 10 subjects were randomly selected to have the control session occur in one of the six possible sessions, i.e., 10 subjects had the control session occur in Session 1, another 10 subjects had it occur in Session 2, and so on for all six sessions.

STATISTICAL ANALYSIS

All data are presented as mean (SD), unless otherwise indicated. Statistical analysis was conducted using SPSS statistical software (SPSS Inc, USA). The CPM value was calculated as the difference between PPT value during CPT and before CPT, which represents the efficacy of the pain inhibition. A positive value indicated an increase in threshold, while a negative value indicates a decrease in threshold.

$$\frac{\text{average of the three PPT during CPT - average of the three PPT before CPT}}{\text{average of the three PPT before CPT}} \times 100$$

Most reliability studies employ measure of relative reliability; for example, the intraclass correlation coefficient (ICC). The intrasession reliability values were analyzed (ICC) between the 1st and 2nd sessions of each visit, and intersession reliability were analyzed using the ICC between 1st visit and 2nd visit, between 2nd visit and 3rd visit, and between the 1st and 3rd visit. The ICC parameter ranges from 0 to 1, with values closest to 1 indicating the highest reproducibility. An ICC less than 0.4 was considered poor agreement; 0.4 to 0.59, fair agreement; 0.6 to 0.75, good agreement; and greater than 0.75, excellent agreement[15,16].

ICC's (relative measure) were estimated using a two-factor random effects model, with person as one factor and visit nested within person as the other. Variance components were estimated using REML (restricted maximum likelihood) in the statistical programming package SPSS v19.0 (IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 19.0.

Armonk, NY: IBM Corp.). The within-person ICC was estimated by var_{person}/var_{total}. The within-visit ICC was estimated as $(var_{person}+var_{visit})/ var_{total}$.

The coefficient of repeatability was computed as the square root of the mean square error from the two-factor random effects model. Coefficient of variation (CV) (absolute measure) is the coefficient of repeatability divided by the mean of the outcome being evaluated. It is often expressed as a percentage, and it shows the extent of variability in relation to the mean of a given population. The higher the CV, the greater the dispersion in the variable, and the lower the CV, the smaller the residuals relative to the predicted value; which suggest higher reliability.

RESULTS

Sixty healthy volunteers completed the study, 38 females (age 37.6±15.1 years) and 22 males (age 34.2±14.7ys) Table 1. The mean number of days between visits was 3.5±1.4 days between the 1st and 2nd visit and 3.1±2.8 days between the 2nd and 3rd visit, the mean session duration was 22±4.6 minutes, and one visit (which included two sessions) lasted an average of 62.4±9.2 minutes. All participants reached a pain level of 7 out of 10 before the PPT was applied to the tested site. The mean CPT temperature of the ice cold water was 41.1±0.08°F (5.2±0.05°C). Results of the CPM magnitude using CPT and lukewarm water (control sessions) will be further be presented in Chapter 4.

Table 1:Demographics: n(%)		
	Females	**Males**
	N=38	N=22
Age (mean, SD)	37.6±15.1	34.2±14.7
Race		
American/Indian	0(0)	0(0)
Asian	10 (26.3)	10(45.5)
Native Hawaiian	1(2.6)	0(0)
Black	5(13.1)	2(9.1)
White	15 (39.5)	10(45.5)
Others/Mixed	3 (7.8)	0(0)
Ethnicity:		
Hispanic	3 (7.8)	1(4.5)
Non-Hispanic	35 (92.1)	20(91)
Level of education:		
Eighth grade or less	0(0)	0(0)
High school	2(5.3)	3(13.6)
Collage/more than 18 ys.	36(94.7)	19(86.4)

Overall CPM reliability

The overall CPM test intersession and intrasession reliability was fair when the test was performed on the hand with an ICC of 0.44 and 0.58, respectively, and CV of 0.61. However, the test intersession and intrasession reliability was poor when performed on the foot, giving ICC of 0.19 and 0.22, respectively, and a CV of 1.07, as well as on the side of the face, with an ICC of 0.14 and 0.20, respectively, and CV of 0.99. To assess the temporal stability of the test, we investigated the intersession and intrasession reliability from 1st to 2nd visit excluding the 3rd visit, from 2nd to 3rd excluding the 1st visit, and from 1st to 3rd visit excluding the 2nd visit. The

intersession and intrasession reliability of the test from 1st to 2nd visit, from 2nd to 3rd visit, and from 1st to 3rd visit. The intersession reliability from 1st to 2nd visit was fair, giving an ICC of 0.59 and intrasession reliability was good with an ICC of 0.66 when the test was performed on the hand, and CV of 0.56. Also the intersession and intrasession reliability from 2nd to 3rd visit was fair when the test was performed on the hand, with an ICC of 0.49, and 0.57, and CV of 0.64, and from 1st to 3rd visit the intersession reliability was poor and intrasession reliability was fair, with an ICC of 0.24 and 0.48 when the test was performed on the hand, and CV of 0.64 (Table 2.A-B-C-D). The test continues to be reliable the most when performed on the hand, mostly at early sessions from 1st to 2nd visit with even higher intrasession reliability, then it becomes less reliable over time from 1st to 3rd visit. Those findings suggest that the test may lack its temporal stability over time, either between visits or even between sessions especially when performed multiple time in short time frame.

Table 2.A: Over all Intersession and Intrasession ICC (relative measure), and CV (absolute measure) values for three tested sites. CV value is within-visit sd/mean.

Reliability Estimates ICC			**mean**	**within-visit sd (repeatability measure)**	**CV**
Tested site	**Intersession**	**Intrasession**			
Hand (H)	0.44	0.58	23.0	14.1	0.61
Foot (F)	0.19	0.22	19.1	20.5	1.07
Face (M)	0.14	0.20	19.2	19.0	0.99

Table2.B: Intersession and Intrasession ICC (relative measure), and CV (absolute measure) values between 1st and 2nd visit. CV value is within-visit sd/mean.

Reliability Estimates: 1st and 2nd visit ICC			Repeatability Metrics:1st and 2nd visit CV		
Tested site	**Intersession**	**Intrasession**	**mean**	**within-visit sd (coefficient of repeatability)**	**CV**
Hand (H)	0.59	0.66	23.9	13.4	0.56
Foot (F)	0.23	0.23	20.4	22.0	1.08
Face (M)	0.09	0.14	20.6	20.8	1.01

Table2.C: Intersession and Intrasession ICC (relative measure), and CV (absolute measure) values between 2nd and 3rd visit. CV value is within-visit sd/mean.

Reliability Estimates: 2nd and 3rd visit ICC			Repeatability Metrics:2nd and 3rd visit CV		
Tested site	**Intersession**	**Intrasession**	**mean**	**within-visit sd (coefficient of repeatability)**	**CV**
Hand (H)	0.49	0.57	21.0	13.4	0.64
Foot (F)	0.17	0.17	18.9	21.0	1.11
Face (M)	0.19	0.28	17.2	16.4	0.95

Table2.D: Intersession and Intrasession ICC (relative measure), and CV (absolute measure) values between 1st and 3rd visit. CV value is within-visit sd/mean.

Reliability Estimates: 1st and 3rd visit ICC			Repeatability Metrics:1st and 3rd visit CV		
Tested site	**Intersession**	**Intrasession**	**mean**	**within-visit sd (coefficient of repeatability)**	**CV**
Hand (H)	0.24	0.48	24.1	15.5	0.64
Foot (F)	0.15	0.32	17.8	18.2	1.02
Face (M)	0.15	0.19	19.7	19.6	0.99

Conditioned pain Modulation CPM Reliability by age

In younger volunteers aged 18-30 years (n=27), the CPM test intersession and intrasession reliability on the hand was fair, with an ICC of 0.42 and 0.55, and CV of 0.54. However, when the test was performed on the side of the face and the foot, both the intersession and intrasession reliability were poor, with the ICC of 0.04 and 0.04, and CV of 0.93 for the foot and 0.97 for the side of the face. In older volunteers aged 31-80 (n=33), when the test was performed on the hand, the intersession reliability was fair and intrasession reliability of was good, with an ICC of 0.45 and 0.60, respectively, and CV of 0.69. However, both intersession and intrasession reliability were poor when applied to the foot, with an ICC of 0.27 and 0.31, and CV of 1.21, and these are poor intersession reliability and fair intrasession reliability when the test was performed on the side of the face, with an ICC of 0.19 and 0.41, and CV of 0.99. (Table 3).

Table 3: Intersession and intrasession ICC (relative measure), and CV (absolute measure) values in two age groups. CV value is within-visit sd/mean.

Reliability Estimates: Age group 18-30yrs. (n=27) ICC			**Repeatability Metrics: Age group 18-30yrs. (n=27) CV**		
Tested site	**Intersession**	**Intrasession**	**mean**	**within-visit sd (coefficient of repeatability)**	**CV**
Hand (H)	0.42	0.55	25.0	13.5	0.54
Foot (F)	0.04	0.04	20.7	19.2	0.93
Side of the face (M)	0.04	0.04	22.7	22.0	0.97
Reliability Estimates: Age group 31-80yrs. (n=33) ICC			**Repeatability Metrics: Age group 18-30yrs. (n=27) CV**		
Tested site	**Intersession**	**Intrasession**	**mean**	**within-visit sd (coefficient of repeatability)**	**CV**
Hand (H)	0.45	0.60	21.4	14.7	0.69
Foot (F)	0.27	0.31	17.7	21.4	1.21
Side of the face (M)	0.19	0.41	16.3	16.2	0.99

Conditioned Pain Modulation CPM Reliability by gender

CPM test intersession reliability was fair and intrasession reliability was good, with an ICC of 0.45 and 0.65, respectively, and CV of 0.68, when the test was performed on the hand in females'(n=38), when compared with males (n=22) who showed poorer intersession and intrasession reliability, with an ICC of 0.36 and 0.39, and CV of 0.53, when the test was performed on the hand. The intersession and intrasession reliability was poor when applied to the feet in both females with an ICC of 0.23 and 0.23, and CV of 1.25, and males, with an ICC of 0.11 and 0.28, and CV of 0.84. Finally, intrasession and intersession reliability was also poor in both genders when applied to the side of the face, females with an ICC of 0.11 and 0.16, and CV of 1.19 and males with an ICC of 0.16 and 0.23, and CV of 0.75. (Table 4.A-B).

Table 4.A: Intersession and intrasession ICC (relative measure), and CV (absolute measure) values in Females. CV value is within-visit sd/mean.

Reliability Estimates: Females (n=38) ICC			**Repeatability Metrics: Females (n=38) CV**		
Tested site	**Intersession**	**Intrasession**	**mean**	**within-visit sd (coefficient of repeatability)**	**CV**
Hand (H)	0.45	0.65	19.5	13.3	0.68
Foot (F)	0.23	0.23	17.2	21.4	1.25
Side of the face (M)	0.11	0.16	16.7	19.8	1.19

Table 4.B: Intersession and intrasession ICC (relative measure), and CV (absolute measure) values in Males. CV value is within-visit sd/mean.

Reliability Estimates: Males (n=22) ICC			**Repeatability Metrics: Males (n=22) CV**		
Tested site	**Intersession**	**Intrasession**	**mean**	**within-visit sd (coefficient of repeatability)**	**CV**
Hand (H)	0.36	0.39	29.0	15.4	0.53
Foot (F)	0.11	0.28	22.3	18.7	0.84
Side of the face (M)	0.16	0.23	23.5	17.6	0.75

DISCUSSION

The primary aim of the present study was to measure the reliability of a CPM protocol that could be used in a clinical setting, measuring both within and between sessions (intrasession and intersession), and between different days of testing, all by the same examiner. We found that one of the anatomical test sites, the hand, did show acceptable intra- and intersession reliability given our protocol of using a CS on the contralateral hand, while the face and foot sites showed substantially poorer reliability. We believe that CPT on the hand was an efficient activator of the descending inhibitory pathway due to the CPT nature of conducting a focal type of pain that results in more consistent activation of descending inhibitory pathways[3,12]. In addition, the manual pressure-pain method has previously demonstrated reliable values for PPT[17]. In our study, sixty healthy volunteers participated in three visits with two sessions per visit. Each visit was separated by an average of 3 days. In most other studies the test was performed in one or two visits only. Our study gave additional information about the temporal stability of CPM, a critical finding if this protocol is to be used in the clinic, where patients return to the clinic over repeated visits. In line with previous studies[15,13,26], we combined the CPT as a CS with the PPT as TS on multiple sites as well as testing multiple sites; hand, side of the face, and foot.

Among the few published CPM reliability findings, previous results were inconsistent,[15,6,12,13,18,19,20,21,27] which may be due to the considerable variation in the methodological approaches. Our CPM test results showed the best intrasession reliability when applied to the hand; this finding may reflect the fact that segmental nociceptive counter-stimulation significantly inhibits the pain intensity[22]. Also different testing areas may have different pain thresholds when

subjected to fixed stimulus intensity. However, the test was less reliable when applied to the side of the face and foot, non-segmental regions to the conditioning stimulus.

Several factors were taken in attempt to reduce measurement variability. Subjects did not consume any analgesics 24 hours before each visit because analgesic medication might inhibit the CPM response, and subjects consuming psychiatric medications were excluded. Also, subjects were tested at the same time of the day and in the same clinic at each visit, to avoid any circadian influences on pain sensitivity. They were asked to use a button switch to indicate PPT, and the conditioning stimulus CPT was applied until a specific numerical pain scale rating had been reached (7/10). The 5-minute interval between testing sites was intended to avoid the carry over analgesic CPM effect, which can last even several minutes after the removal of the conditioned stimulus[23]. All experiments were performed by the same researcher (R.N.) in order to avoid inter-rater variation.

CPM is generally thought to reflect the activation of endogenous inhibitory pathways in the form of supra-spinal descending endogenous analgesia. The descending pathways modulate the incoming spinal nerve impulses. This phenomenon is based on a spino-bulbar-spinal loop under cerebral control[3,33,24], where the branches of this loop move through the ventrolateral and dorsolateral funiculi - the white matter in the spinal cord. The top-down processes in this spinobulbospinal loop have been shown to be less involved in the modulation responses than the bottom-up processes[25]. This might explain why the hand test is the most reliable among the all three tested sites, since the bottom-up processes of the spinobulbospinal loop are more involved in the inhibition pathway, in addition to the fact that segmental nociceptive counter-stimulation significantly inhibits the pain intensity[22].

It has been previously reported that some volunteers could leave their hand in cold water of 1°C for 3 to 5 minutes, while 60% took their hand out within 90 seconds[30]. Therefore, we chose the water temperature to be 41.1±0.08°F (5.2±0.05°C), which is similar to other studies [19,18,28,26,29], however, it was barely tolerated by our volunteers. Other studies have used even warmer temperatures of 8°C[31], 12°C[13], or 10°C[32]; as the endogenous analgesia processes require some degree of conditioning painfulness, and its efficiency was not found to be further affected by increased conditioning pain levels (i.e.it reaches a ceiling effect), a warmer temperature may be used in the future. Conclusion: In the present study, we measured the reliability of a CPM paradigm that could be used in a clinical setting. Based upon our findings, we recommend performing the CPM test on the dominant hand with an average of three measurements of PPT (TS) alone with 20s in between stimuli, followed by PPT (3 measurements 20 seconds apart as well) during CPT (CS) on the non-dominant hand. These findings are important in the general context of understanding the reliability of the test and possibly incorporating the CPM paradigm as a diagnostic test in clinical assessments of chronic pain patients well as its use in clinical research studies.

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CHAPTER 4: THE DEVELOPMENT OF CPM NORMAL VALUES AND ITS RELATIONSHIPS AT THREE BODY SITES BY AGE/GENDER.

INTRODUCTION

Transmission of noxious stimuli is modulated by several excitatory and inhibitory endogenous mechanisms[1]. The inhibitory ones are collectively known as endogenous analgesia[4]. One of the most explored pain inhibitory pathways is the diffuse noxious inhibitory control (DNIC), which has been widely studied in animals. In humans, the psychophysical paradigm term ‘‘conditioned pain modulation” (CPM) is used, although it was indicated recently that the phenomena observed as CPM is an interaction between physiological pathways and psychological-cognitive ones[4]. CPM reflects the efficiency of ‘pain-inhibits-pain’ phenomena, which is likely mediated through the spino-bulbo-spinal loops, and involves the spinal dorsal horn and the trigeminal nuclei[2]. The involved tracts of this loop in the pain modulation process - either inhibition or facilitation- are influenced by both cerebral the (top-down) effect, and up-going painful stimuli (bottom-up).

Due to the subjective nature of pain, the difficulty in measuring it, and its complexity, researchers use experimental pain to explore CPM in what is referred to as dynamic stimulation protocols. Multiple studies have discovered less efficacious CPM in patients with chronic pain, which is possibly due to an alteration in the balance between the facilitation and the inhibition pathways[3]. A few studies show that less efficient CPM in a pre-operational pain-free state may be a risk factor for the development of chronic pain[5,9]. Less efficient pain inhibition was found to be associated with pain disorders, particularly fibromyalgia, irritable bowel syndrome, migraine, tension-type headache, and temporomandibular joint (TMJ) disorders[4]. The intensity of the administered conditioning stimulus (CS) affects the degree of CPM; however; the CPM reaches

a ceiling effect once endogenous analgesia provoked by a "required degree of conditioning painfulness"[4] occurs.

CPM magnitude may vary greatly among individuals, and although there are several suggestions regarding a standard protocol for the induction of endogenous pain inhibition, the normative values for the magnitude of CPM, and a standard measurement of the extent of CPM effect in healthy individuals are less explored. Incorporating a pain modulation CPM test in the patient's treatment plan as a diagnostic tool may play an important role in developing personalized pain medicine. Multiple trial and error cycles of medications, especially in treating neuropathic pain, may be due to a lack of understanding of the role a patient's CPM plays in their pain problem. It was found that patients with less efficient CPM are more likely to benefit from serotonin– norepinephrine reuptake inhibitors (SNRIs) such as duloxetine, which augments the descending inhibition[11,34] as CPM can be modified in response to treatment[34]. In addition, increasing physical activity can also enhance CPM, so that is known to be a modifiable metric of health. However, the relationship between age, gender and other factors and CPM values is not well characterized. Therefore, this study aimed first, to determine the relationships between CPM at three body sites and age/gender in a healthy population; second, to begin the development of a normative database for CPM. Specifically, the mean, median, lowest and highest pain inhibition scores were described separately for males, females, younger, middle and older age individuals, and by the anatomical sites of the pain stimulus – foot, hand and face.

METHODS

This study was performed at the Department of Oral Medicine, University of Washington (UW), Seattle, (USA) between November 2014 and April 2016. It was approved by Institutional board review (IRB) number 47524-D.

Design:

The protocol of this chapter was explained in details previously in chapter 3. In short, CPM was assessed by repeated measures on the same volunteer over three visits. Each visit included two experimental sessions, with 15 minutes between sessions; for a total of six sessions. CPT was used as a CS in 5 sessions, and one session out of the six was randomly assigned as a control session where lukewarm water was used CS instead of CPT. The most efficient endogenous pain inhibition appears to be induced by CPT as the CS, combined with use of PPT as TS[6].

Tests were performed on three sites on the dominant side of the body in a non-overlapping fashion: side of the face (masseter muscle), hand (thenar eminence), and foot (dorsum of the foot). Brain hemispheres process incoming signals differently; the dominant side is the more active hemisphere. Therefore, testing the dominant side of the body yields a more accurate interpretation of pain. All sessions took place in the same clinic, with an interval time of 2-7 days between visits.

Volunteers:

Sixty-one volunteers were recruited by advertisements posted in the University of Washington School of Dentistry, and received $150 as compensation for their participation, however 60 volunteers completed the study, because one volunteer dropped out. Inclusion criteria were: 18-80 years old, healthy females and males, able to provide informed consent. Exclusion criteria were: individuals who reported current spontaneous pain or injuries in the lower or upper limbs or face, unable to stop their analgesics 24 hours before their visit, pregnant females, and the presence of current or previous major medical conditions such as severe heart disease or respiratory conditions, or a psychiatric condition including depression. Potential

volunteers, who expressed interest or contacted a study team member in response to the flyer or study handout, were recruited using a standard script as a guide over the phone or in person. Volunteers were asked not to consume any pain medication for 24 hours prior to their appointment.

Tests:

1- Pressure pain threshold test PPT (TS): In a test that involves applying increasing pressure on a body site, PPT is defined as the amount of pressure that results in the first sensation perceived by volunteers as painful, it was assessed three times with a Somedic Pressure Algometer with 20 seconds in between (PPT is defined in unites of pressure kPs). Among the three Algometer probes available, the $2cm^2$ was used as it exerts up to 1000 kPs. The PPT was performed on each of the tested sites in a non-overlapping fashion; a template was used to repeatedly identify the three testing sites, and the algometer was calibrated between visits, and between volunteers as well.

2-Conditioned Pain Modulation test (PPT+CPT): the CPM test was repeated three times at each of the three sites following the PPT. The non-dominant hand was immersed in 41°F (5°C) cold water up to the wrist as a CS (a container filled with water and ice), the temperature of the cold water was monitored by a thermometer placed in the water bath, to ensure water temperature stability. Volunteers were asked to report their non-dominant hand pain level when it reached 7 out of 10 according to a numerical pain scale- where 0 is no pain and 10 is severe pain. The PPT was applied simultaneously with the CS at this point with the non-dominant hand remaining in the water. One session was randomly assigned as a control session where lukewarm water –temperature of 80°F (26.6°C) as previous studies[34] was used instead of CPT,

and the PPT was performed at the 30th second instead of when the subject reported the pain level as 7 out of 10 as occurs in the CPT.

Randomization:

The three tested sites were tested in random order in the first session of each visit and the same order was followed in the second session. One of the six sessions was randomly assigned as a control session where lukewarm water was used instead of CPT.

STATISTICAL ANALYSIS

All data are presented as mean (SD), unless otherwise indicated. Current recommendations also suggest the quantification of CPM as a percent change[33]. A positive value indicates an increase in threshold, while a negative value indicates a decrease in threshold. The magnitude of the CPM was calculated as the following:

$$\text{CPM magnitude} = \frac{\text{PPT value during CPT - PPT value before CPT}}{\text{PPT value before CPT}}$$

The dataset for all subjects was stratified by anatomical site, foot, hand, face. The mean, median, minimum and maximum values were first computed for PPT, and then done for the CPM value. The sample of subjects was then stratified by age – younger, middle and older subjects – and the PPT and CPM reported for each strata. Figures were also constructed to better depict the differences between these subgroups.

Means were calculated for the active CPM (CPT, not controls) by gender, along with associated 95% confidence intervals. The 95% confidence intervals were computed using a robust sandwich variance estimator (Generalized Estimating Equations) to account for correlation between the multiple observations for each individual study participant. Means were compared between genders using the Generalized Estimating Equations approach, which

basically a t-test is accounting for the correlation between observations from the same study participant.

The cohort was then split into two groups by gender, and for each gender the means of the CPM values (active CPM only) were computed for each age group, along with associated 95% confidence intervals using methods detailed above. Hypothesis tests evaluating whether there are any differences in means between age groups (with gender) were computed using the Generalized Estimating Equations approach (null hypothesis: all group means are the same).

RESULTS

The baseline PPT for a given site was virtually identical whether the visit used the CS as CPT or the control (Table 1.a; Figure 1a). Overall, the side of the face was the most sensitive to PPT, while the least sensitive site was the foot. The CPM effect was detected among all three tested sites; face, hand, and foot as the CPT induced endogenous pain inhibition and CPM effect. Overall, the mean of CPM magnitude among the three sites in CPT session was (20.9%±2.5), and (10%±4.3) in control sessions. The CPM magnitude was the highest (23.7%) when the test was performed on the hand compared to control sessions (8%) however it was not significant (p=0.3) followed by the face (20.3%) which was not significantly higher than control sessions (7%) (p=0.6), and finally the lowest CPM magnitude was in the foot (18.7%) vs control sessions (15%), which was also not significantly different (p=0.4) (Table1.a-b; Fig.1.a-b).

Table 1: Overall value of PPT and CPM magnitude A) PPT: Pressure Pain Threshold baseline before conditioning in both testing sessions and control sessions, values in kPs. B) CPM magnitude among all three sites. All mean, median maximum and minimum values of CPM magnitude are presented in the form of percent of change, in both CPT sessions and control sessions.

A) PPT	Before CPT			Control			B) CPM	During CPT			Control		
	Face	Hand	Foot	Face	Hand	Foot		Face	Hand	Foot	Face	Hand	Foot
Mean	243.4	432.7	500.9	243.0	443.0	503.0	**Mean**	20.3	23.7	18.7	7.0	8.0	15.0
Med	226	417	489	225	426	491	**Med**	18	22	16	7	6	7
Min	68	78	142	83	106	169	**Min**	-42	-30	-46	-36	-49	-19
Max	529	953	1050	476	828	878	**Max**	107	108	97	56	78	288

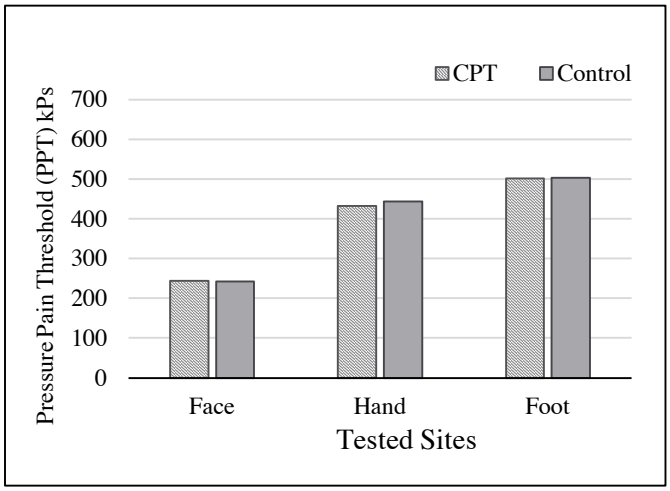


Figure 1.A: PPT: Pressure Pain Threshold baseline in both CPT sessions and control sessions, before the CPT. CPT: indicates the baseline value of PPT in sessions that CPT was used as conditioning, and control is the baseline PPT in control sessions where lukewarm water was used as a conditioning stimulus, values in kPs.

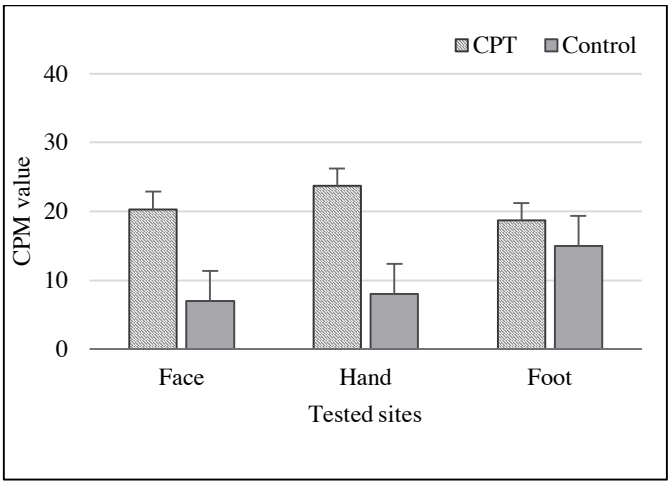


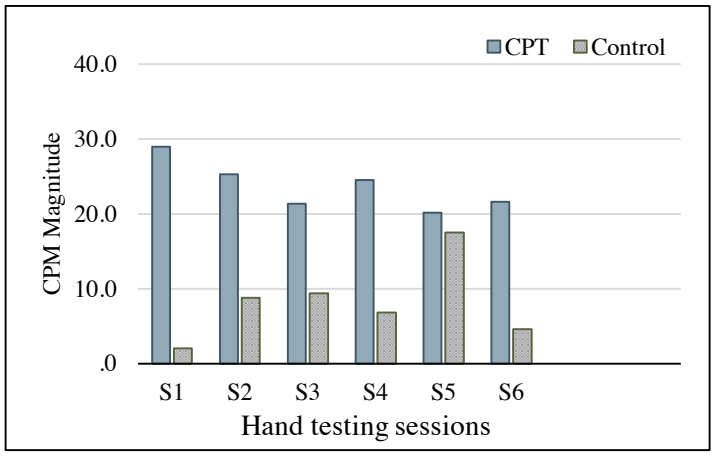
Figure 1.B: CPM magnitude among all three sites. All means of CPM magnitude are presented in the form of percent of change ±SD, in both CPT and control. CPT: indicates the PPT value in sessions that CPT was used as conditioning, and control is the PPT in control sessions where lukewarm water was used as a conditioning stimulus, values in kPs. Hand p=0.3, face p=0.6, and foot p=0.4.

Figure 2.a-b-c shows the mean of CPM magnitude in each session. When using CPT as a CS the mean was highest when the test was performed on the hand in session 1 (29%), followed by session 2, session 4, session 6, session 3 and finally the lowest mean was in session 5 (20.2%). The highest CPM mean when the test was performed on the side of the face was in session 1 (28.3%), followed by session 3, session 2, session 5, session 6, and the lowest was in session 4 (15.4%). The highest CPM mean when the test was performed on the foot was in session 3 (25.6%), then session 5, session 1, session 2, session 4, and the lowest mean was in session 6 (10.7%). When using lukewarm water as CS the CPM mean was the highest when the test was performed on the hand in session 5 (17.5%), then session 3, session 2, session 4, session 6, and finally the lowest was in session 1 (2.0%). When the test was performed on the on the side of the face, the highest mean was in session 6 (14.6%), then session 2, session4, session 5, session 3, and the lowest was in session 1 (0.1%). When the test was performed on the foot, the

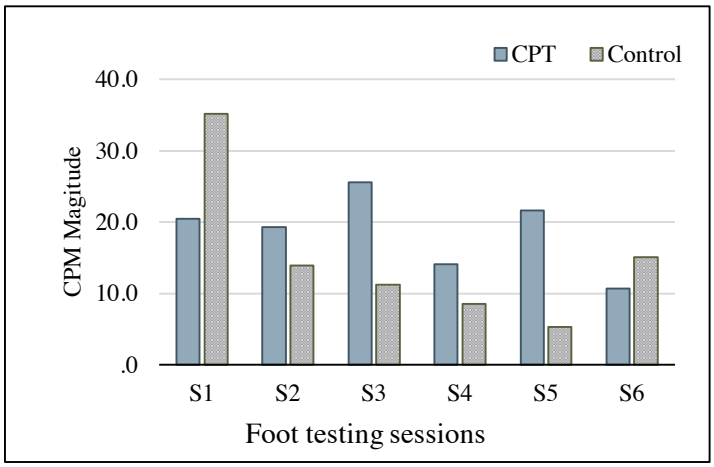
CPM mean was the highest in session 1 (35.2%), session 6, session 2, session 3, session 4, and finally session 5 (5.3%). When using CPT, the CPM mean decreased over time from session 1 to session 6 more in the face than in the foot and hand; when using lukewarm water as a CS, the decrease in CPM over the sessions was the most in the foot than the hand and face.

To test whether the cold pain vs control CPM changed over the six test sessions, the slope of CPM over these six sessions was fit (Figure2.1a, b, c & 2.2.a, b, c). The interaction of CPT/control and session was statistically significant (p = 0.018) by generalized score test when the test was performed to the side of the face, but not significant when performed to the hand (p=0.13) and not significant when performed to the foot (p= 0.65) (Table 2). This decreased CPM over multiple tests in a short time frame may suggest pain adaptation with prolonged inhibition.

A)



B)



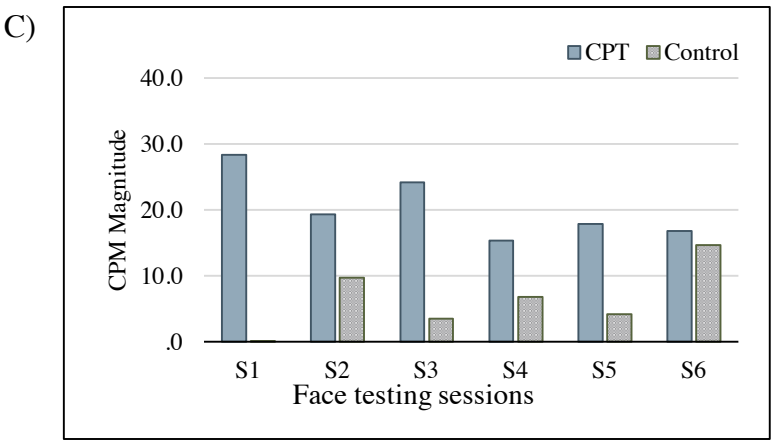


Figure 2.1: CPM magnitude among all 6 testing sessions S1 to S6 in both CPT and Control A) Hand. B) Foot and C) face. All mean values of CPM magnitude are presented in the form of percent of change in CPT (Cold Pressor Test) and control. Hand p=0.13, face p=0.018, and foot p=0.65.

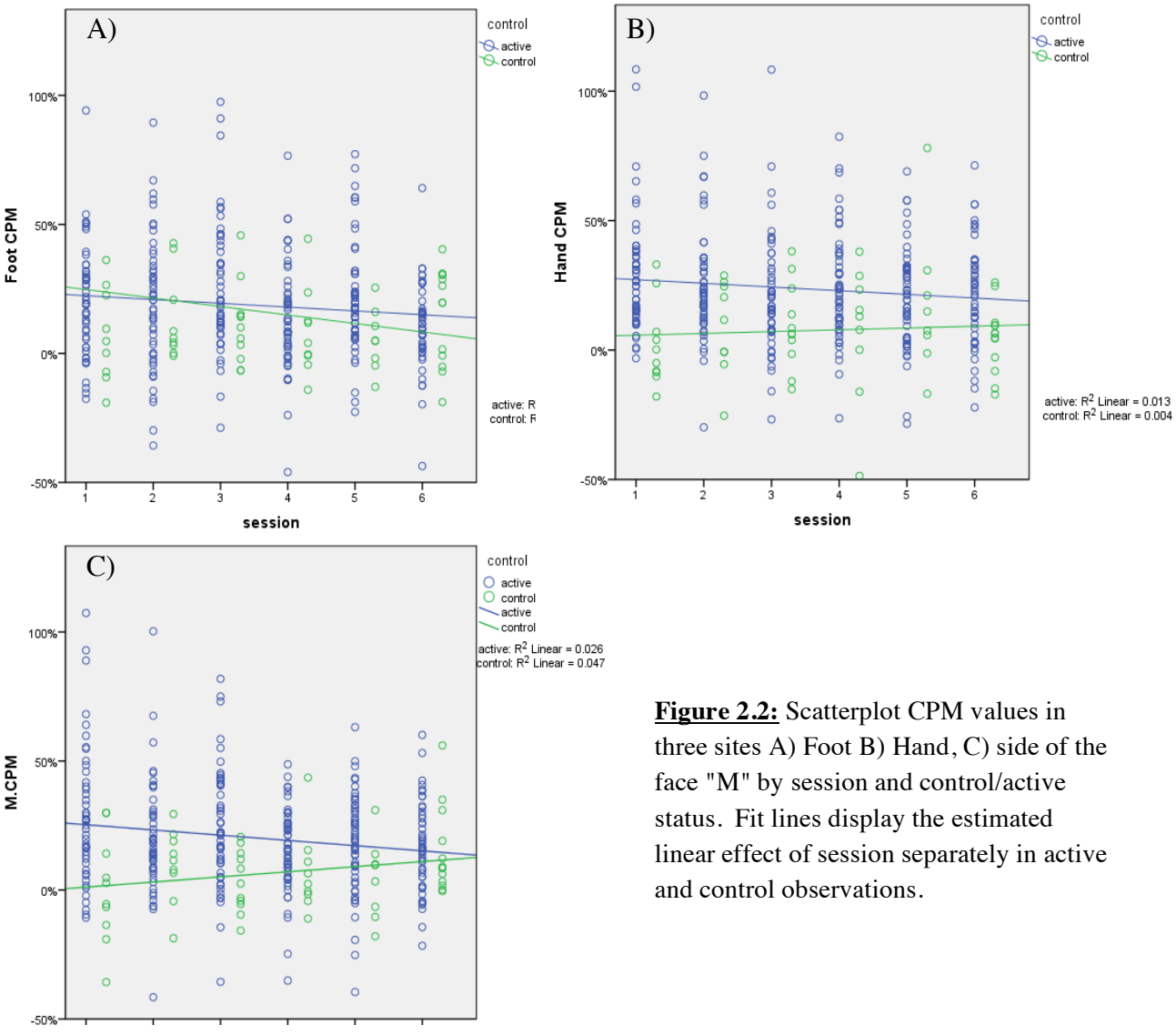


Figure 2.2: Scatterplot CPM values in three sites A) Foot B) Hand, C) side of the face "M" by session and control/active status. Fit lines display the estimated linear effect of session separately in active and control observations.

Table 2: Side of the Face (M), hand, and foot CPM session and CPT vs. control effects over six sessions.

Table for **Side of the Face CPM** session and active vs. control effects*					
	coef. (%)	Std. Error	95% conf. int.		Sig.
Effect of active treatment					
at session 1	23.59	4.62	14.54	32.64	.000
at session 6	3.55	4.46	-5.20	12.30	.426
Effect of session**					
when tx =control	1.98	1.27	-.51	4.47	.118
when tx = active	-2.03	.69	-3.38	-.67	.003
* in original model interaction of active/control and session was statistically significant (p = 0.018 by generalized score test)					
* session modelled as linear effect, so this is effect of one later session (e.g. session 4 compared to session 3)					

Model for Hand CPM					
Parameter	coeff. (%)	std. error	95% conf. int.		Sig.
Active CPM*	15.8	3.0	9.9	21.7	0.000
Session**	-1.0	0.6	-2.2	0.1	0.086
* effect of active CPM compared to control					
** session modelled as linear effect, so this is effect of one later session (e.g. session 4 compared to session 3)					
*** interaction of active/control and session was evaluated and found to not be statistically significant (p = 0.13 by generalized score test)					

Model for Foot CPM					
Parameter	coeff. (%)	std. error	95% conf. int.		Sig.
Active CPM*	3.3	5.4	-7.3	13.8	0.55
Session**	-1.8	0.9	-3.6	0.0	0.055
* effect of active CPM compared to control					
** session modelled as linear effect, so this is effect of one later session (e.g. session 4 compared to session 3)					
*** interaction of active/control and session was evaluated and found to not be statistically significant (p = 0.65 by generalized score test)					

Next, the pain-modulatory capacity was measured in comparison to age. Table 3.a-b and Figure 3.a-b shows the PPT and CPM magnitude among three age groups: young adults (18-30 y) (n= 30), middle age adults (31-50 y) (n=17), and older adults (51-80 y) (n=13). The point estimates show that the most sensitive site to PPT was the side of the face in all three age groups, while the least sensitive site was the foot.

Among all three age groups the CPM magnitude was highest when the test was performed on the hand in the middle age group (28%) (p=0.16). It was significantly higher when the test was performed on side of the face in the middle age group (23.4%) (p=0.018), and high when performed on the foot in young age group (20.0%) although this difference was not significant (p=0.73)

Since the CPM magnitude was the highest when performed on the hand, we compared the hand to the other two sites in each age group. In each age group, the CPM magnitude was significantly higher in young (24.4%) (p=0.0002), and middle age group 28% (p=0.03); and, although it was higher in old age groups (16.3%), the difference was not significant (p=0.19).

Table 3: PPT readings and CPM magnitude among the three age groups A) PPT: Pressure Pain Threshold baseline before conditioning, values in kPs. B) CPM magnitude among all three sites. All mean, median maximum and minimum values of CPM magnitude are presented in the form of percent of change in CPT sessions only.

A) PPT	Young age			Middle age			Old age		
	Face	Hand	Foot	Face	Hand	Foot	Face	Hand	Foot
Mean	234.5	397.7	496.1	234.5	447.2	474.7	275.6	494.3	546.4
Median	223	392	496	217	396	431	267	493	544
Min	68	78	142	108	204	213	105	219	190
Max	420	697	897	512	953	1050	529	806	896

B) CPM	Young age			Middle age			Old age		
	Face	Hand	Foot	Face	Hand	Foot	Face	Hand	Foot
Mean	22.2	24.4	20.0	23.4	28.0	18.7	11.7	16.3	15.7
Median	18	22	17	22	23	13	11	16	18
Min	-25	-16	-15	-25	-15	-24	-42	-30	-46
Max	107	102	91	82	108	97	73	67	94

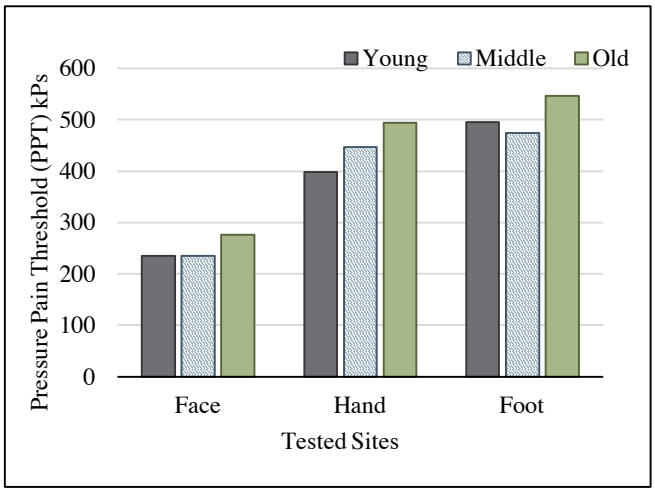


Figure 3.A: PPT: Pressure Pain Threshold baseline in CPT sessions, before the CPT in three age groups. CPT: indicates the baseline value of PPT in sessions that CPT was used as conditioning, values in kPs.

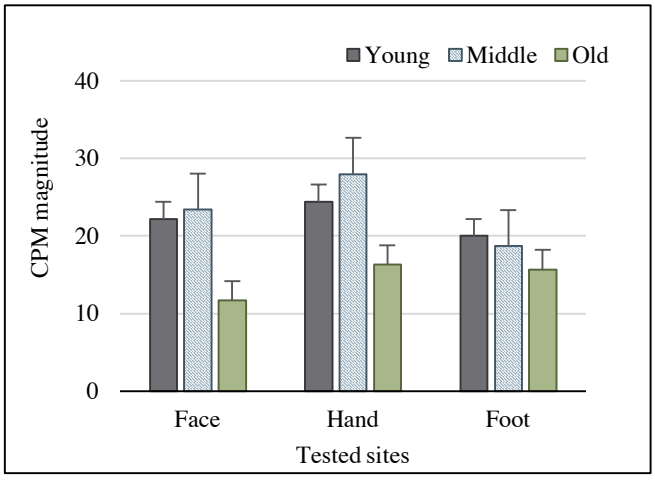


Figure 3.B: CPM magnitude among all three age groups. All means of CPM magnitude are presented in the form of percent of change ±SD, in both CPT and control. Hand and Face in middle age group p=0.16 and 0.018, foot in young age group p= 0.73.

Differences between males and females in PPT and CPM were assessed next. The most sensitive site to PPT was the side of the face in both females and males; however, PPT was 23% lower for females than males (Table 4). The least sensitive site was the foot among the three sites in both males and females, however, females demonstrated more sensitivity (27%) to PPTs than males. An overall mean CPM magnitude in males (n=22) was 24.9% and 18.5% in females (n=38). Table 4.A-B-C and Figure 4.A-B shows the highest CPM magnitude in females when applied to the hand (20.3%) when compared with males (29.5%) which was significantly different (p=0.019), followed by face (17.9%), compared to males (24.3%) which was also significantly different (p=0.038) as well, then CPM magnitude in the foot was (17.3%) in females and (21.1%) in males which was not significantly different (p=0.26).

Table 4: PPT and CPM magnitude among both gender groups A) PPT: Pressure Pain Threshold baseline before conditioning, values in kPas. b) CPM magnitude among all three sites. C) Mean CPM values by gender and site.

A) PPT	Females (n=38)			Males (n=22)			B) CPM	Females (n=38)			Males (n=22)		
	Face	Hand	Foot	Face	Hand	Foot		Face	Hand	Foot	Face	Hand	Foot
Mean	224.1	407.3	455.1	276.7	476.4	580.1	**Mean**	17.9	20.3	17.3	24.3	29.5	21.1
Med	212	398	430	260	452	568	**Med**	17	17	15	22	27	19
Min	68	78	142	125	204	213	**Min**	-42	-30	-46	-14	-4	-24
Max	529	953	897	512	817	1050	**Max**	107	108	91	93	108	97

C) Mean CPM values by gender and site					
Site	Gender	Mean	95% confidence interval		p-value
Hand	Female	20.3	14.9	25.7	0.019
	Male	29.5	24.0	35.0	
Foot	Female	17.3	12.8	21.8	0.26
	Male	21.1	16.2	26.1	
Face	Female	17.9	14.2	21.7	0.038
	Male	24.3	19.6	29.0	

Figure 4.A: PPT: Pressure Pain Threshold baseline in CPT sessions, before the CPT in both genders. CPT: indicates the baseline value of PPT in sessions that CPT was used as conditioning, values in kPs.

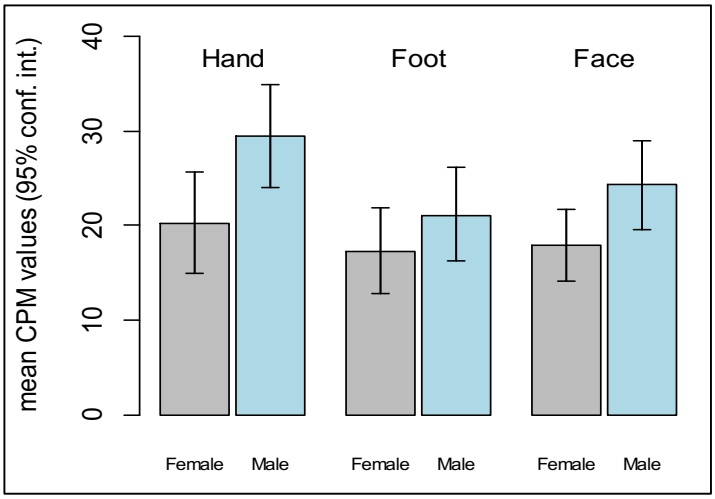


Figure 4.B: CPM magnitude among both genders. All means of CPM magnitude are presented in the form of percent of change ±SD, in both CPT. CPT: indicates the PPT value in sessions that CPT was used as conditioning values in kPa's. Hand p=0.19. face p=0.038, and foot p=0.26.

The overall mean of PPT values among the three tested sites is (346.3±124) in the young females group (n=17), (356.6±121.57) in middle age group (n=12), and (399.6±123.6) in older age group (n=9). The overall mean of CPM magnitude among the three tested sites is (21.2 ±1.4) in young females group, (21.2±2.63) in middle age group, and (10±1.1) in older age group. The overall mean of PPT values among the three tested sites is (415±143) in young males group (n=13), (454.7±159.1) in middle age group (n=5), and (526.91±188.83) in older age group (n=4). The overall mean of CPM magnitude among the three tested sites is (23.6±3.3) in young males group, (28.6±11.5) in middle age group, and (24.9±6.7) in older age group.

The side of the face in young females was the most sensitive area to PPT (211.2±67.9), while the least sensitive area was the foot in older females (491.7±159). The same trend was found in males, side of the face in young males was the most sensitive area to PPT (265±74.8), while the least sensitive area was the foot in older males (669.4±160.6) (Table 5a).

The highest CPM magnitude among the three age groups in females was in the middle age group when the test was performed on the hand (24.2%) and the least CPM magnitude was (9.5%) when the test was performed on the foot in older females. Similar results were found in males as well, the highest CPM magnitude was in the middle age group when the test was performed on the hand (37%) and the least was (15.5%) also in the middle age males (Table 5b). The only significant findings were in young male groups, who CPM magnitude was (23.1%) compared to other older (17.3%) and middle (33.2%) age groups when the test was performed on the face $p=0.016$.

Table 5: Values of PPT and CPM magnitude in both genders among three age group. A) PPT: Pressure Pain Threshold baseline before conditioning in both genders, values in kPs B) The CPM magnitude among both genders. All CPM mean, median maximum and minimum values are presented in the form of percent of change in CPT sessions only. C) Mean CPM values by Gender Age, and Site.

A) PPT	Females									Males								
	Young age n=17			Middle age n=12			Old age n=9			Young age n=13			Middle age n=5			Old age n=4		
	Face	Hand	Foot	Face	Hand	Foot	Face	Hand	Foot	Face	Hand	Foot	Face	Hand	Foot	Face	Hand	Foot
M.	211.2	372.7	455.0	216.2	425.8	427.8	259.0	447.9	491.7	265.0	430.3	549.8	278.3	498.4	587.4	312.7	598.6	669.4
Med	210	364.3	425.7	204	378.3	410.2	232	448.7	508	248.7	408.7	528.7	250.3	483	543.7	312.2	596.3	718.7
Min	68.0	78.3	142	108.3	209.3	279.3	105	218.7	189.7	141.3	214.7	284	125	204	212.7	208	421.3	348.3
Max	412	697	897.3	333.3	952.7	696.3	529	715.7	797	420.0	668.3	861.3	511.7	817.3	1050.3	435.3	806.3	895.7

B) CPM	Females									Males								
	Young age n=17			Middle age n=12			Old age n=9			Young age n=13			Middle age n=5			Old age n=4		
	Face	Hand	Foot	Face	Hand	Foot	Face	Hand	Foot	Face	Hand	Foot	Face	Hand	Foot	Face	Hand	Foot
M.	21.6	22.3	19.6	19.3	24.2	20.0	9.2	11.3	9.5	23.1	27.1	20.6	33.2	37.2	15.5	17.3	27.6	29.9
Med.	18.7	18.8	16.9	17.5	20.4	13	10.6	14.6	9.5	18.3	26.7	18.8	31.7	30.5	11.5	12.0	17.3	19.5
Min	-24.8	-15.9	-15.2	-25.2	-14.8	-19.7	-41.5	-29.9	-46	-3.0	-3.5	-13.5	-6.1	3.3	-23.9	-14.4	2.2	6.4
Max	107.4	101.7	91.1	81.8	108.4	84.4	73.1	67.1	62	92.9	70.9	60.3	64.0	108.3	97.5	59.8	58.4	94.1

C) Mean CPM values by Gender Age, and site						
Site	Gender	Age group	mean	95% confidence interval		p-value
Hand	Female	Young	22.3	14.6	30.1	0.14
		Middle	24.2	15.0	33.3	
		Old	11.3	1.1	21.5	
	Male	Young	27.1	22.6	31.6	0.57
		Middle	37.2	19.3	55.1	
		Old	27.6	17.4	37.8	
Foot	Female	Young	19.6	15.5	23.6	0.31
		Middle	20.0	12.1	27.8	
		Old	9.5	-3.0	21.9	
	Male	Young	20.6	15.6	25.6	0.29
		Middle	15.5	5.4	25.7	
		Old	29.9	15.3	44.4	
Face	Female	Young	21.6	17.1	26.2	0.064
		Middle	19.3	14.3	24.4	
		Old	9.2	-0.2	18.5	
	Male	Young	23.1	17.4	28.7	0.016
		Middle	33.2	23.8	42.6	
		Old	17.3	11.7	22.8	

DISCUSSION

The main findings of this study were: 1) CPM measured at the same segment as the conditioning stimulus – the hand – showed the highest values, compared to the foot and face; 2) males showed higher CPM than females; 3) people in the middle age groups – aged 31 to 50 - had higher CPM than those in younger or older than this age group in years. In addition, a pilot normative database for the CPM protocol was created, allowing the goal of a clinical CPM test to be realized in the near future.

We also showed in this study that 98.3% of healthy adult volunteers exhibited a CPM effect (increased PPT from pretest baseline) during CPT, which is consistent with previous studies using the same CPM induction methodology[21,22,23]. In 2012, a systematic review reported that immersion of the hand in cold water (the CPT) was the most common conditioning stimulus employed followed by ischemia, heat, and finally capsaicin[14]. In 2014, it was reported that CPT is the most efficient endogenous pain inhibition CS when combined with PPT[6,10] using an algometer, which is a reliable and responsive technique to assess measures of pressure pain threshold[12,13].

Our overall CPM magnitude was 20.9%, compared to other studies finding 29%[7,8] and 43.6%[10]. Several studies reported that older adults experience greater pain than younger adults[18]: frequency of pain increased with age[15], and self-report of chronic pain seems to increase up to the seventh decade of life[16]. This might be due to an increase in musculoskeletal degeneration with aging[17,29], or an increased sensitivity to painful stimuli[17]. Also, older adults demonstrated less inhibition of cold stimulation[19,20], and it was suggested that "some pain inhibitory mechanisms start declining at middle-age"[20]. Our findings show that the middle age group demonstrates the highest CPM magnitude, which then decreases in older adults. These results are consistent with

previous studies, however, the CPM magnitude from our results in the young adults was lower than the middle age group. The foot was the least sensitive site to pain in older adults, which might due to lower extremities in older adults being generally less sensitive to pain in older adults[17,30] especially to PPT test[31,32].

Females were more sensitive to PPT than males. Even in the least sensitive site which was the foot, females demonstrated more sensitivity to PPT, 27% lower than males. Our findings reinforce previous studies' results that show increased PPT sensitivity at baseline in healthy females compared to males[24,25]. However, the CPM magnitude in males was (6.4%) higher than females. Across the literature findings remains inconsistent regarding CPM effect in both genders[26,27,28].

The CPM mean was not stable across sessions either in with CPT or control CS, which suggests that pain adaptation may have occurred due to repetitive testing to prolonged inhibition, especially since pain adaptation has been suggested in the literature before. A previous study found that 40% of their participants were able leave their hands in cold water of 1°C for 3 to 5 minutes, whereas 60% took their hands out within 90 seconds[35], others results suggest that individual sensitivity and adaptability to pain does not correlate with the potency of CPM, but might be associated with longer-lasting local pain inhibition[36].

Several authors explored the effect of the CS 'intensity' on the magnitude of the CPM, however their findings were inconsistent. Some stated that a significant CPM effect is initiated with a CS that produces moderate to intense pain levels. In other words, stronger stimuli trigger more potent inhibition and longer-lasting post-stimulation effects[37]. Other studies suggested that CPM magnitude is not affected by the intensity of the CS even a non-painful one was reported to induce CPM as well. The later finding supports our results regarding developing small CPM

magnitude with lukewarm water[38,39].

Some of the strengths of this study are 1) 6 testing sessions completed over 9 days although that might have caused pain adaptation; 2) a control session included for all subjects; 3) blinding of the subjects to the study hypotheses; 4) randomized order of the testing; 5) a single examiner with a standardized protocol.

The main weakness of this study was the smaller sample size of 60 subjects, so that some of the subgroups of age and gender were small in size and the relationships noted are somewhat unstable. In addition, we deem this database construction as a pilot of a larger one that is to be completed in the near future. Finally, our findings show how the CPM values can vary substantially over the 6 testing sessions over 3 different days, raising questions as to the temporal stability of the measure when it is repeatedly measured. Nevertheless, these valuable data help pave the way for the potential development of a clinical test for CPM.

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CHAPTER 5: FEASIBILITY AND CONSTRUCTION OF A CLINICAL CONDITIONED PAIN MODULATION (CPM) TEST

INTRODUCTION

The significance of the CPM test lies mainly in three domains; treatment of chronic pain conditions, prediction of chronic pain development, and modifying CPM to help with chronic pain symptoms. The reliability and feasibility of the CPM test is a necessary condition for useful results, which in turn is essential for the validity and the effectiveness of the research findings. Therefore, improving the performance of this test is an essential step to incorporating this measure as a diagnostic test in clinical assessments of chronic pain patients. Few standardization measures have been suggested to clinically assess CPM[1,2]. Since this test's reliability has been explored previously[3,4,5], we created a short feasibility questionnaire that focused essentially on our volunteers' ability to tolerate, complete, and repeat the test more than once, and we inquired about any potential lasting side effects, as well, to gauge the safety of the test. In addition, we measured the reliability, the elapsed time of the tests, and made qualitative observations about the test in terms of tolerability and feasibility. The aim of this chapter is to summarize, analyze and combine the results from our prior CPM studies to present the case for constructing the most reliable, feasible CPM test that could be used potentially as a test in a clinical setting. Subtopics of safety, tolerability, reliability, total elapsed test time, cost of test equipment, scoring and interpretation of the CPM test, are also discussed enroute to this recommendation.

METHODS

This research was performed at the Department of Oral Medicine, University of Washington (UW), Seattle, (USA) between November 2014 and April 2016. It was approved by Institutional Board Review (IRB) number 47524-D. The primary endpoint was the reliability of

the CPM using the cold pressor test (CPT) as conditioning stimulus (CS) and PPT as a test stimulus (TS). Other aims were to assess the feasibility, tolerability and safety of the test. A questionnaire was administered to participants to evaluate these aspects. The total elapsed time was measured for each test, session and visit. The sole examiner observed 60 x 6 tests for a total of 360 tests and made notes as to the overall functioning of the test.

Design:

This study was discussed in previous Chapters 3 and 4. In short, CPM and PPT were assessed by repeated measures on the same volunteer in three different visits that took place in the same clinic, with a minimum interval of 2 days to a maximum of 7 days between visits. All three visits for the same volunteer were carried at the same time of the day. Each visit included two experimental sessions, with 15 minutes between sessions, which is, a total of six sessions. CPT was chosen as CS as it was found to be an efficient CPM inducing stimulus, and pressure pain threshold PPT was used as a TS. Tests were performed on three sites of the dominant side of the body in a non-overlapping fashion: side of the face (masseter muscle), hand (thenar eminence), and foot (dorsum of the foot). We used the dominant side of the body since brain hemispheres process incoming signals differently, with the dominant side as the more active hemisphere. Therefore, testing the dominant side of the body is theorized to yield a more accurate interpretation of pain. Volunteers answered a short post-test feasibility questionnaire twice, once at the end of the 1st session during the first visit, and once at the end of the 2nd session at the end of the third visit. It was explained to each subject that although they have been tested multiple times, the actual proposed test procedure in the clinic is only one set which is the PPT followed by PPT together with CPT to either their face, hand or feet, and all the questions are

related to just that set of tests. The post-test feasibility questionnaire included the following questions:

> Although you have been tested multiple times today and on other days, the actual test procedure in the clinic will only consist of the pressure pain test to you face, hand and feet and then repeating it while your other hand is in cold water. The next set of questions are related to just that set of tests.
>
> 1. Do you think you could, as a patient in a clinic:
>   a) Easily tolerate such a test?
>   b) Easily complete such a test
>   c) Have it done more than once at future visits in that clinic?
> 2. Do you believe the test is safe and not harmful to people?
> 3. Did you suffer any lasting side effects from the testing?

STATISTICAL ANALYSES

Results from the survey were tallied and the proportion answering yes or no are reported. Qualitative observations from the single examiner are also reported. Previous reliability data and systematic review data are discussed and compared.

RESULTS:

Review of CPM protocols:

In laboratory assessment of experimental CPM, subjects rate the test stimulus pain during and before having a second painful sensation which is the CS applied to a distant region of the body. Different types of stimuli have been employed; the TS previously used have included pressure, electrical, and thermal stimulus, and the conditioning pain used has been cold water, heat, ischemia, and capsaicin. In 2012, a systematic review reported that immersion of the hand in cold water is the most common CS used, followed by ischemia, heat stimulus, and finally capsaicin[6]. Mechanical pressure was reported as the most common test stimulus employed, followed by the electrical stimulus, and finally the thermal stimulus[6]. Both stimuli TS and CS were applied to different sites of the body (mostly upper and lower extremities) and both

ipsilateral and contralateral. The testing order of anatomically distinct locations varied, but the hand was the most frequently used site. Lastly, heterotopic stimulation – meaning stimulation in the same dermatome, but on the opposite side - resulted in a more efficient CPM response[7].

Our findings from this review aided us in designing a potentially clinically applicable CPM assessment test. From this review, we learned that the CPT as a CS is one of most efficient methods to induce CPM when combined with PPT as a test stimulus, which has been supported by other authors as well[8]. A potentially reliable clinical CPM test included PPT as test stimulus performed on the dominant hand, and CPT as a CS on the non-dominant hand.

Systematic Review of CPM reliability:

We initially collected methodological design data from our review regarding: number of visits (ranged from 2 to 8 visits) number of sessions (one to two sessions per visit), time interval (range15 minutes to 12 months), test stimulus application site (mainly the thenar eminence) CS application site (mainly the hand), and test reliability measures used (Intra-class correlation coefficient (ICC), coefficient of repeatability (CR), and coefficient of variation (CV) (Table 1). The systematic review revealed a wide variation in reliability for the different types of CPM test paradigms, although most were acceptable in reliability for healthy controls.

Table 1: Data extracted from included papers in our systematic review, ICC: Intra-class Correlations (ICC) inter-session intersession or overall ICC, coefficient of repeatability (CR), Inter-In CV: inter-individual coefficient of variation, and Intra-In CV: intra-individual coefficient of variation.

Measure	Participants	Values
Overall ICC		Ranged from 0.10 to 0.79
intra-session reliability ICC	Healthy	0.55 (both genders) to 0.94 (females)
intra-session reliability ICC	Patients	0.39 (males) to 0.73 (females)
coefficient of repeatability (CR)	Healthy	Ranged from 0.30 to 0.35
inter-individual CV	Healthy	Ranged from 11.5 to 103
intra-individual CV	Healthy	Ranged from 27 to 100

In general, the reliability of CPM was in the acceptable range for healthy controls, but often times somewhat lower in patients.

Overall Study design - Elapsed time

As mentioned in previous chapters, sixty healthy volunteers completed the study, 38 females and 22 males. Their mean age was 37.6 years for females, and 34.2 years for males. The average number of days between first and second visit was 3.5 days and between second and third visit 3.1 days, and the average session duration was 21.9 minutes, the mean time for one visit was 62.0 minutes. All participants reached a pain level of 7 before the PPT was applied to the tested site. The mean temperature of the CS cold water was 5.2°C (41.14°F). Thus, a single test session that evaluated all three anatomical sites – hand, face and foot – took about 22 minutes.

Ability of the CPM test protocol to elicit pain inhibition

After evaluating what others had done, a CPM test paradigm using CPT on the opposite hand was adopted. The mean temperature of the cold water we used was 5.2°C (41.14°F) which is similar to what was used in previous studies[9,10,11,12,13]. CPM was exhibited in 59 out of 60 of the subjects at least once. The mean CPM magnitude was the highest (23.7%) when the test was performed on the hand.

Summary of CPM test reliability study

The reliability and feasibility of the CPM test is a necessary condition for repeatable results, which in turn are essential for the validity and the utility of the research findings. The reliability study (Chapters 3 and 4) clearly showed better and likely acceptable test retest reliability when the dominant hand was tested and the opposite hand was placed in the cold water as the CS. Our findings show that the test was overall most reliable when applied to the hand

(intrasession ICC= 0.58 and intersession ICC= 0.44) and was substantially better than for the foot and face.

In addition, completing only testing on the hand would cut the total test time by almost two thirds, and, since most evidence shows that CPM is a whole body vs a regional test, any testing site will likely indicate the underlying endogenous inhibition of the person.

Survey of tolerability, ease of completion, ease of multiple repeated testing sessions:

Overall, fifty-six (93.3%) subjects agreed in both sessions 1st and 3rd that our proposed test is tolerable, one (1.7%) subject thought the test was intolerable in both sessions, two (3.3%) subjects indicated it was intolerable in the 1st session but that it was tolerable in the 3rd session, and only one (1.7%) subject felt it was tolerable in the 1st session and intolerable in the 3rd session. Fifty-eight subjects (96.7%) agreed in both sessions that the test is easy to complete, and only two (3.3%) subjects disagreed in the 1st session and agreed in the 3rd session (Table 2). When were asked about the volunteers' ability to repeat the test, fifty-two (86.7%) subjects agreed in both sessions that the test is repeatable, two (3.3%) subjects disagreed in both sessions, three (5%) subjects disagreed in the 1st session and agreed in the 3rd session, and three (5%) subjects agreed in the 1st session and disagreed in the 3rd session that the test can be repeatable.

Survey regarding test safety, lasting side effects:

All but one of the subjects (59/60, 98.3%) agreed in both sessions that the test is safe and not harmful and only one (1.7%) subject disagreed in both sessions. Fifty-six subjects (93.3%) did not suffer any lasting side effects, two (3.3%) subjects reported skin rash on the non-dominant hand that lasted for maximum 10 minutes after the end of the session in the 3rd visit, and only two (3.3%) subjects reported skin redness after the 1st session (Table 2).

Survey differences by gender:

Gender difference: More females (94.74%) than males (90.9%) agreed in both sessions that this test is tolerable; slightly more females (97.4%) than males (95.45%) reported that it can be easily completed; substantially more females (92.1%) than males (77.3%) said they could have it done more than once, and slightly more females (94.7%) than males (90.9%) reported no lasting side effects. All males (100%) on the other hand thought this test was safe and not harmful to people, when compared to females (97.37%), (Table 3, 4).

Table 2: Overall volunteers' responses in both sessions 1st and 3rd to feasibility questionnaire: Y yes, N: no.

	Questions	Y-Y n(%)	N-N n(%)	N-Y n(%)	Y-N n(%)
1A	Easily tolerate such a test?	56(93.3)	1(1.7)	2(3.3)	1(1.7)
1B	Easily complete such a test	58(96.7)	0(0)	2(3.3)	0(0)
1C	Have it done more than once at future visits in that clinic?	52(86.7)	2(3.3)	3(5)	3(5)
2	Do you believe the test is safe and not harmful to people?	59(98.3)	1(1.7)	0(0)	0(0)
3	Did you suffer any lasting side effects from the testing?	0(0)	56(93.3)	2(3.3)	2(3.3)

Table 3: Females' responses in both sessions 1st and 3rd to feasibility questionnaire: Y yes, N: no.

F	Questions	Y-Y n(%)	N-N n(%)	N-Y n(%)	Y-N n(%)
1A	Easily tolerate such a test?	36(94.74)	0(0)	1(2.63)	1(2.63)
1B	Easily complete such a test	37(97.4)	0(0)	1(2.6)	0(0)
1C	Have it done more than once at future visits in that clinic?	35(92.1)	0(0)	1(2.6)	2(5.3)
2	Do you believe the test is safe and not harmful to people?	37(97.37)	1(2.63)	0(0)	0(0)
3	Did you suffer any lasting side effects from the testing?	0(0)	36(94.74)	1(2.63)	1(2.63)

Table 4: Males' responses in both sessions 1st and 3rd to feasibility questionnaire: Y yes, N: no.

M	Questions	Y-Y n(%)	N-N n(%)	N-Y n(%)	Y-N n(%)
1A	Easily tolerate such a test?	20(90.9)	1(4.55)	1(4.55)	0(0)
1B	Easily complete such a test	21(95.45)	0(0)	1(4.55)	0(0)
1C	Have it done more than once at future visits in that clinic?	17(77.27)	2(9.09)	2(9.09)	1(4.55)
2	Do you believe the test is safe and not harmful to people?	22(100)	0(0)	0(0)	0(0)
3	Did you suffer any lasting side effects from the testing?	0(0)	20(90.9)	1(4.55)	1(4.55)

Examiner qualitative observations:

The examiner (RN) performed a total of 60 x 6 or 360 CPM tests, not including the additional training and calibration sessions that preceded the study. The most significant examiner observation was that many subjects struggled to keep their non-dominant hand in the cold water during the conditioning stimulus until they attained the 7 of 10 pain score. Some chronic pain patients may not be able to complete the test at this cold temperature, and a slightly warmer temperature along with lowering the pain requirement to 6 of 10 may still provide an adequate CS but allow more patients to complete the test.

Scoring and interpretation of the CPM test:

We previously indicated that the construction of a normative database for this CPM protocol has been initiated with data from 5 tests over 3 days for 60 people entered. As more subjects are added to the data base we will be better able to indicate standardized abnormal cutoffs of one or two standard deviations from the mean score for each age-gender category. Thus, given the CPM score, any clinical subject could be quickly determined to be within or above/below 67% ((~1 SD) or 95% ((~2 SD) of the population, and could be identified as having high, low or abnormally high/low CPM.

Cost of equipment, expertise of personnel:

The CPM test we used required relatively inexpensive equipment, including a commercially available insulated ice-bucket, ice, and portable temperature gauge, and digital timer and an algometer. Although we used the more expensive Somedic algometer, use of a less expensive algometer such as the Wagner pressure meter may provide similarly reliable data. After using the inexpensive ice-bucket, we believe that the use of a temperature controlled water bath would be worth the additional cost to keep the conditioning water temperature in an acceptable range and allow easier use in a busy clinic. In addition, it appears feasible that personnel such as medical or dental assistants could quickly learn the straightforward protocol.

DISCUSSION

Final Recommendation for Clinical Applications of a Reliable and Feasible Clinical CPM Test

Given the review of the literature, systematic review, and our own preceding studies, we are able to better guide the construction of a reliable and feasible test to evaluate CPM in the clinic. From the totality of our findings, listed above, our final recommendation for a reliable and feasible clinical CPM testing protocol includes performing the test on the hand, employing PPT using a manual algometer as a TS applied to the dominant hand, and CPT as a CS applied to the non-dominant hand. We recommend performing the test in the form of three rounds of PPT with 20s in between before CPT on the dominant hand, followed by immersion of non-dominant hand in cold water (CPT) at temperature of 5-7ºC. When pain from CPT reaches 7 out of 10 according to numerical pain scale- where 0 is no pain and 10 is severe pain, the PPT should be applied simultaneously during the CS at this point while the non-dominant hand remaining in the cold water. At the beginning of the visit, we recommend performing a training session of the pain tests (CPT and PPT) on the volunteer's hand 5 minutes before starting the testing session.

A CPM magnitude value is then calculated as the percent of change in the average of the three PPT during CPT and before CPT, which represents the efficacy of the pain inhibition:

$$\frac{\text{average of the three PPT during CPT - average of the three PPT before CPT}}{\text{average of the three PPT before CPT}} \times 100$$

The equipment needed for this test consisted of two inexpensive devices: a temperature controlled water bath to provide superior temperature stability throughout the duration of the test and a manual Algometer with a built-in pressure transducer and electronics. With the growing understanding of this test application, our proposed diagnostic test is relatively simple and may be clinically applicable, and can be mastered by different types of clinical personnel and practitioners with different backgrounds, as well as by laboratory experimental researchers.

CPM Test Utility:

CPM represents a valuable new metric in pain assessment for researchers, and also for clinicians. To our knowledge, no clinical CPM test is currently available that is known to be reliable and feasible for clinicians to use to assess patients' endogenous inhibition. The clinical CPM test we propose 1) appears to be clinically feasible; 2) allows reliable assessment of endogenous pain modulation; 3) may help to tailor management strategies; and 4) may possibly help to predict the risk of developing chronic pain in some individuals. In summary, this new metric may help play an important role in developing one of the next steps towards personalized pain medicine.

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SUMMARY

Measurement of pain and modifiers of pain in the clinic has historically been challenging. Due to the subjective nature of pain, responses can vary greatly from one individual to the other, including their response in laboratory experimental settings. Individual variation may possibly be explained by different measurement techniques, conditioning and test stimuli, sites of testing, and pain scales across different studies, in addition to individual differences such as age and gender. Also, despite a growing number of animal and imaging studies conducted in effort to explain the underlining CPM mechanism, the underlying mechanisms are still not fully understood. For example, in addition to the involvement of subnucleus reticularis dorsalis (SRD) in spino-bulbar-spinal loop, recently, Youssef et al. suggested the possibility of cortex involvement in the modulation process, particularly anterior, mid-cingulate, and dorsolateral prefrontal cortices[1]. Given these variables and mechanisms, designing a method to evaluate pain modulation through a clinical CPM test is difficult, but it is not impossible, and is a worthwhile endeavor.

Despite major problems in assessment of pain modulation, such as the lack of knowledge about reliability, feasibility and accuracy of the CPM test, we aimed to address some of these concerns, and our attempt initiated guarded but promising results. We assessed the reliability of CPM, measured its feasibility, and began to develop initial CPM magnitude, to design a potentially clinically applicable CPM diagnostic test. The test showed acceptable reliability when test stimulus (PPT) was performed on the dominant hand, and conditioning stimulus (CPT) performed on the non-dominant hand. From our findings, a rapid form of a clinical CPM test which includes a short training session, and then a test session, consisting of two identical 2-minutes sessions with 5 minutes in between, all performed on the hand (PPT on dominant, and

CPT on non-dominant) is likely to be feasible, tolerable and reliable. The test will be in the form of three rounds of PPT with 20s in between before CPT on the dominant hand, followed by immersion of non-dominant hand in cold water (CPT) at temperature of 5-7ºC. When pain from CPT reaches 7 out of 10 according to numerical pain scale- where 0 is no pain and 10 is severe pain, the PPT should be applied simultaneously during the CS at this point while the non-dominant hand remaining in the cold water. Given this test form, the next step would be to test the reliability of the shortened clinical test on healthy controls and patients with chronic orofacial pain.

The reliability of CPM has been reported to be good in healthy volunteers, as we found, but fair to poor in chronic pain patients in prior studies, partly because patients may have less inefficient CPM and thus lower value of CPM. Olesen SS, et al. suggested that the "poor reliability may be due to the nature of descending pain modulation, the variability of CPM over time, particularly for chronic pain patients"[2]. In his study, suprathreshold pressure was used as TS which demonstrated high variability, and pain detection thresholds were recommended instead. Therefore, we suppose our protocol may yield higher reliability in patients, because we utilized pain detection method as TS.

As mentioned previously, we began to develop an initial normative database of the magnitude of CPM that ranged from a mean of 23.7% when the test was performed on the hand to 18.7% when performed on the foot, but the main weakness of our study was the small sample size of 60 subjects. A larger number is required for to obtain a normative set of CPM values that is stable and accounts for differences by age and gender. For example, if six cells of younger, middle and older age males and females was constructed of 20 subjects in each cell, 120 healthy subjects would be required. Once such a database is completed, any given subject in the clinic

could have an average of their three CPM values compared to the appropriate normative database to determine if their values were normal, abnormally high or abnormally low.

Measuring the accuracy of the CPM test is required for understanding whether the test will be useful or not. As with any test of pain, evaluating the CPM validity or accuracy is a difficult task and needs novel approaches. We measured CPM over one week, at three visits, with two sessions each visit. We found that the stability of CPM measure varied by person, and the mean values decreased at the third visit. Finding the best method to evaluate the temporal stability of CPM as a strategy to find the true magnitude of CPM for any individual is an important next step. If that could be attained, then a reference standard for each person could be approximated, and the accuracy of a CPM test could be assessed. Given such a reference standard of CPM magnitude, assessing the CPM test accuracy would be possible, using standard sensitivity and specificity statistical measures.

All of our studies were performed by the same researcher (R.N.) in order to avoid inter-rater variation. These next efforts should be directed toward evaluating multiple trained raters using the rapid form of the CPM test where inter-rater reliability may be a measured; raters can be trained by following instructions and perform calibration procedures. Not only will it further evaluate the reliability of the CPM test, it will also help in understanding the possible sources of error variance or disagreement between raters, minimizing experimenter's bias,

In conclusion, a series of studies measuring factors related to pain modulation have been conducted on healthy controls, yielding a reliable subset of CPM tests that could be used clinically to assess CPM. We have outlined the next steps that are needed to bring the goal of a CPM test into clinical use. Finally, we believe that the measurement of endogenous pain inhibition may yield important gains in the diagnosis, management and prognosis of humans.

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ABBREVIATIONS

5-HT	5-Hydroxytryptamine (serotonin)
bja	British Journal of Anaesthesia
BMC Musculoskelet Disord.	BioMedical Center Musculoskeletal Disorder
CNS	Central Nervous System
CPM	Conditioned Pain Modulation
CPT	Cold Pressor Test
CR	Coefficient of Repeatability
CS	Conditioning Stimulus
CT	Central Transmission
DNIC	Diffuse Noxious Inhibitory Control
E.J.Neur	European Journal of Neurology
EIH	Exercise-Induced Hypoalgesia
EOs	Endogenous Opioids
ICC	Intraclass Correlation Coefficient
Inter-In CV	Inter-individual coefficient of variation
Inter-rater R	Inter-rater reliability
Intra-In CV	intra-individual coefficient of variation
Intra-rater R	Intra-rater Reliability
IRB	Institutional Review Board
J. Oral Maxillofac. Surg	Journal of Oral & Maxillofacial Surgery
j.brs	Brain Stimulation Journal
j.pain	The Journal of Pain

j.sjpain	Scandinavian Journal of Pain
LBP	Lower Back Pain
MD	Mark Drangsholt
NCBI	National Center for Biotechnology Information
NMDA receptor	*N*-methyl-D-aspartate receptor
NS	Nociceptive Specific neurons
PAG	Periaqueductal Grey
Pain Med	Pain Medicine
Pain.res manage	Pain Research and Management Journal
PM	Pain Modulation
PPT	Pressure Pain Threshold
RA.Pain Med	Regional Anesthesia and Pain Medicine
RN	Rania Nuwailati
RVM	Rostral Ventromedial Medulla
SG	Substantia Gelatinosa
SNRI	Serotonin–Norepinephrine Reuptake Inhibitor
Somatosens Mot Res	Somatosensory & Motor Research
SRD	Subnucleus Reticularis Dorsalis
TMD	Temporomandibular Joint Disorders
TeS	Temporal Summation
TS	Test Stimulus

USA	United States of America
UW	University of Washington
WAD	Whiplash-Associated Disorders
WDR	Wide Dynamic Range