The effects of a conditioning stimulus provided as Cold Pressor Test on the Conditioned Pain Modulation of healthy persons based on the subjective experience of pain

Technical Medicine

MDO

Group 5

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Abstract

Chronic pain is burdening, both physically and psychologically. A special form is chronic post surgical pain. Since there are operations with up to 85% chance on chronic pain, research to a predictive value to generate chronic pain is important. The human body has its own mechanism for the prevention of pain perception, called Conditioned Pain Modulation (CPM). The performance of this mechanism is related to the development of chronic pain so further understanding of the subject is valuable. A painful conditioning stimulus (like a Cold Pressor Test, CPT) activates this modulation but there are also observations of inhibiting activity while the conditioning stimulus was not painful. Furthermore the behaviour in time of this effect and its reproducibility are not well known yet. The subjective pain experience is most important because this causes the inconvenience for the patient. This all leads to the question: What are the effects of a conditioning stimulus provided as Cold Pressor Test on the Conditioned Pain Modulation system of healthy persons on the basis of subjective experience of pain?

A group of 22 healthy volunteers (50% male, 50% female) between the ages of 17 and 54 participated in this study. The study consisted of one session. For five of the 22 participants it were two identical sessions on two separate days to research reproducibility. All participants endured four CPTs, all until a different pain level. The painful CPTs (NRS 10, 6 and 4) were in 0-2°C water and a painless one (NRS 0) in 33-34°C. The order of NRS 6, 4 and 0 was randomized. Each CPT was preceded and followed by three electrical stimuli to determine the EPDT (Electrical Pain Detection Threshold) with the NociTrack. The participant self administered this current until the feeling became unpleasant. The difference between these EPDT values shows the CPM effect and can be compared to understand the differences between achieving different pain levels. To research behaviour in time, after the first CPT each 5 minutes the EPDT is measured again.

For the main variable condition, NRS 10 differs significantly from NRS 6, 4 and 0. For the other main variable pre and post EPDT, the values differ significantly for all conditions except NRS 0. For the interaction effect between these variables, there is no significant value. By means of pairwise comparison is shown that the CPM effect is present but not deviating between the four conditions. From the second condition on, the CPTs were administered in randomised order. From this study can be concluded that the order is irrelevant. When separated for order the combined results for NRS 6, 4 and 0 are the same as when separated for condition. There seems to be a slight increase in relative difference in EPDT value in time, but it is pointed out that the change of EPDT in time is not significant. A factor that might play a role in this observation is habituation to the test stimulus. Earlier research mentions that there is still a CPM effect an one hour after the first conditioned stimulus is applied. This factor could also be a declaration that there is no significant difference in EPDT over time. Only one of the five participants shows reproducibility for the re-test at the four conditions with a deviation at NRS 10 and one participant shows a decrease when we compare the test and re-test to each other. Most participants show a decrease of the CPM effect when we compare the test and the re-test at the five different times after CPT 1. The group of five participants is to small in order to give reliable results. Further research is needed with at least 15 participants.

1 Introduction

Pain is an unpleasant experience. However, it is a mechanism that provides an important warning function when the body is damaged. In 1 out of 5 persons with acute pain symptoms these become chronic.[1] In case of chronic pain, unlike acute pain, the warning function is no longer directly related to the initial tissue damage. Therefore the pain is persisting longer than the normal process of healing (in case of postoperative pain longer than 3 to 6 months)[1][2]. The fact that pain is no longer useful makes it not only physically but also psychologically burdening. Like that, the negative effect on the quality of life is considerably large. The psychic burden has not only an emotional cause, research shows that chronic pain causes anatomic and functional changes in the brain[3][4]. While there are individual differences in severity and frequency, the consequences of chronic pain are large: 21% is diagnosed with a depression caused by pain and 61% cannot work or works less because of it. As the symptoms and therefore its consequences can endure for up to 15 years, the problem is not only burdening for patients but also for the society. In Europe, chronic pain accounts for almost 500 million sickness days and costs €34 billion a year.[1]

A specific form of chronic pain is chronic post surgical pain (CPSP). Also for CPSP applies that it starts as acute pain but it persists after the expected duration of healing. There are surgeries where the chance of chronification of pain is up to 85% (amputations). Other high-risk surgeries are thorax surgeries (35%), mammae surgeries (31%), total joint arthroplasty (20%) and groin rupture surgery.[5][6] Although increased knowledge about nociception accounted for improvement of treatment of acute pain throughout the years, this does not count for CPSP. It points out that chronic pain has an complex pathophysiology and therefore is difficult to treat. Researching the mechanism of chronification of pain is essential in preventing this tilting point. Therefore one of the goals of this study is evaluating a method which can be used in predicting the chronification of pain. This can be used in the prevention of chronic pain.[5][7]

The human body has its own mechanism for preventing the perception of pain, called Conditioned Pain Modulation (CPM). In older literature, this mechanism is also known as Diffuse Noxious Inhibitory Control (DNIC). The performance of this mechanism is related to the development of chronic pain, as will be elaborated later on. By means of a painful conditioning stimulus, for example in the shape of a Cold Pressor Test (CPT), the integrity of this endogenous modulating mechanism can be researched. However, there are observations of inhibiting activity while the conditioning stimulus was not painful[8]. In this study there were no differences found in inhibiting function as response on stimuli just under and above the pain detection threshold. At this moment it has been insufficient researched whether or not a subjective experience of pain is required to activate the inhibiting function of the CPM. If turns out that a conditioning stimulus does not have to be painful in order to activate the endogenous modulating system, this will lead to a less unpleasant stimulation for patients when trying to evoke this mechanism. Furthermore, the change in time of the CPM activity will also be researched.

Considering this information, the following research question is formulated: What are the effects of a conditioning stimulus provided as Cold Pressor Test on the Conditioned Pain Modulation system of healthy persons on the basis of subjective experience of pain? This study will provide answers to three subquestions which are formulated as follows:

- Is subjective experience of pain during a conditioning stimulus necessary in order to activate the inhibiting function of the CPM system?
- How does the inhibiting effect of the CPM behave in time after removal of the conditioning stimulus?
- Is the CPM effect quantitatively reproducible by administering the same stimuli?

By means of the "pain-inhibits-pain" principle, the initial pain has been inhibited by a second pain stimulus. That's why it is not expected that a non painful second stimulus will cause the same inhibiting effect. Logically thinking, there must be a minimum amount of stimulation necessary before inhibition takes place. Otherwise the slightest bit of perception would cause a form of analgesia. To place this limit on the tipping point of painful/non painful would make sense since from here, inhibition is favourable. From the same principle the expectation rises that the inhibiting effect of the CPM will decrease rapidly in time when the conditioning stimulus is removed. Surely, the pain responsible for the inhibiting effect is no longer present. This hypothesis is also plausible on the basis of the previously mentioned alarming

function of pain. A long lasting inhibition of pain signals is not favourable since this interferes with the warning function this pain fulfils.

In order to find out whether the CPM effect is quantitatively reproducible a re-test will be done several days after the first test. Small physiological changes caused by the first test will not influence the second one, because of the several days in between. The psychological influence this may have is hard to estimate. Furthermore, one can perceive pain more severe when he knows exactly what to expect. This might, during re-test, result in a measured CPM effect that is lower than reality. Realising this possibility enables the researchers to prevent its influence during the study so measured differences will tell something about the integrity of the CPM effect regarding its reproducibility.

This study is an assignment from the department of anesthesiology from the St. Antonius Hospital in Nieuwegein, Dr. H.P.A van Dongen and Drs.I.P.Krabbenbos, anesthesiologists - pain management. They proposed the idea of this study under the title "Dimensions of endogenous inhibitory effects after a cold pressor test using a conditioned pain modulation paradigm". During the study, guidance is provided by dr. ir. Jan R. Buitenweg (University of Twente) regarding technological subjects and drs. I.P. Krabbenbos on medical terrain.

2 Relevant scientific knowledge

2.1 Anatomy

The Neuron

There are two types of neurons, namely the afferent en efferent neurons. The afferent neurons, also called sensory neurons, pass information from the affector to the CNS. In contrary, efferent neurons, also called motor neurons, are passing information from te CNS to effectors. Effectors are muscle- and gland cells. Thus, the direction of signals in sensory neurons and motor neurons is opposite. In case of pain perception, the affectors are called nociceptors.

There is also another distribution for neurons, namely the projection neurons and the interneurons. The projection neurons transmit a signal over a long distance and therefore have a long axon with collateral ramifications. The interneurons release signals to other neurons, which are grouped and near to each other. Interneurons have short axons and are the connection between two neurons.

Many axons are surrounded by myelin sheaths. This results in a higher conduction of the signal. In neural tissue, grey matter and white matter can be distinguished. The grey matter consist of dendrites and somas, while the white matter is built up of axons with myelin sheaths. Somas are grouped together. When this grouping occurs in the CNS it is called a nucleus. When it occurs outside the CNS, and thus in the PNS, it is called a ganglion. A bundle neurons that is located between nuclei is named a nerve tract.[9] Such a nerve tract has the same origin, route and ending place and is located in the white matter of the spinal cord, which could be ventral, dorsal or lateral.[10]

Sensibility

Sensibility is defined as the perception of sensation of feelings. Sensibility is divided into two different categories; gnostic and vital sensibility. Gnostic sensibility includes neutral and fine touch, but also sense of position, movement and vibration. Vital sensibility consists of emotionally charged touch and rough touch. Also the perception of pain and temperature are part of vital sensibility.[11] The transduction of pain signals takes place via two different neurons, namely the Aδ-fibers and the C-fibers. The Aδ-fibers have a medium diameter and are myelinated. The pain signal that is released by these neurons is quick (almost directly after the pain stimulus) and well locatable. The pain is observed as a sharp pain. By contrast, the C-fibers have a small diameter and are not myelinated. The pain signal of these neurons is slower and that is why it mostly presents after the signal of the Aδ-fibers. The location of the pain signal is less locatable and is observed as nagging pain.[12]

Sensory signals originate in affectors. These affectors consist of terminal branches of axons or of specialized cells that transduce signals to nerve endings. There are different kind of affectors. The proprioceptors are located in muscles and connective tissue, the exteroreceptors provides information about the external environment and enteroreceptors are the receptors of the visceral organs.[9] The general tract of sensibility starts in the affectors, which are releasing a signal to first-order sensory neurons. These neurons are together with the first-order motor neurons in the anterior and posterior ramus. These rami congregate in the spinal nerve trunk. Here, the sensory and motor neurons are splitting apart. The sensory neurons form the posterior nerve root with the spinal ganglion within it. The posterior nerve root continues to the dorsal part of the spinal cord.[13]

2.2 Physiology

Pain stimulating pathway

The way of thinking about the location of the vital sensibility tract is changed during the years. At first it was thought that the sensation of pain and temperature proceeded via the lateral pathway of the spinothalamic tract (while the rough touch proceeds via the anterior spinothalamic tract). Nowadays it is thought that pain and temperature sensation runs via the ascending spinothalamic tract, which is located in the ventrolateral part of the spinal cord. This is largely at the contralateral side of where the afferents send signals into the vertebrae of the spinal cord.[10]

Pain sensation consists of a noxious stimulus (discriminative component), an emotional and an autonomous response on it and an increased attention (arousal-emotional component). The spinothalamic tract can be divided into the direct pathway and the indirect pathway. The direct pathway is also called the neospinothalamic tract en consists of the discriminative component. The arousal-emotional component proceeds via the indirect pathway, which consist of the paleospinothalamic, spinoreticular and the spinomesencephalic tract.[10]

The different components of the spinothalamic tract have the same course till the spinal cord. As mentioned before, the first-order sensory neuron gets into the spinal cord dorsally via the posterior nerve root. The first-order neurons reaches the gray matter of the vertebra through the Lissauers tract. The gray matter is divided into the ventral and the dorsal horn. From here there is a more specific divison of gray matter into different laminae. These laminae are classified based on their cytoarchitecture and function.[14] Figure 1 shows the vertebral laminae.

In the dorsal horn the switch from first-order neuron to second-order neuron takes place. In which lamina this happens, differs per component of the spinothalamic tract. Subsequently, the bulk of these second-order neurons cross via the ventral white commissure towards the contralateral side. In the dorsal horn different neurotransmitters transmit a signal. In case of a mild stimulus, glutamate is released, which engages with the postsynaptic AMPA[1] receptor, the metabotropic glutamate receptor (mGluR) and with the kainate ligand-gated ion channel. When the stimulus is stronger, tachykinins are released. This includes substance P, neurokinin A and neurokinin B. These tachykinins bind respectively with the NK-1, NK-2 and NK-3 receptor. This binding causes a more intense postsynaptic response. The response is extra amplified by the binding of glutamate with the NMDA (N-methyl-D-aspartate) receptor. There are also adrenoceptors which are located both in the PNS and the CNS. The adrenoceptors are divided into A1- and A2-receptors and bind with adenosine, AMP, ADP and ATP. Binding with the A1-receptor results in stimulation of the nociceptors. [15]

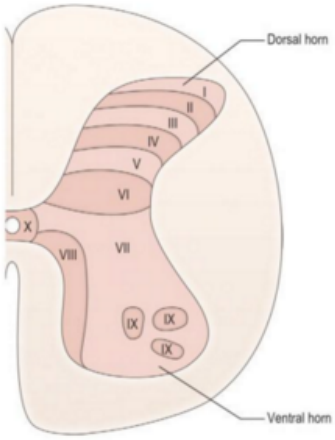

Figure 1: Vertebral Laminae.

Neospinothalamic tract

In this case, the transition to second-order neurons takes place in lamina III and IV. This transition is about two vertebrae higher than where the first-order neuron is reaching the spinal cord. The second-order neuron is travelling from the nucleus proprius to the white matter of the contralateral side. There is a somatotopic arrangement: second-order neurons from lumbar and sacral parts of the spinal cord ascend dorsolateral, while second-order neurons from cervical parts of the spinal cord ascend more ventromedial. The ascending neurons travel to the brain and in the brainstem it is called the spinal lemnicus[16]. The second-order neuron ends up in the ventral posterolateral nucleus of the thalamus and changes into third-order neurons. These are rising and end up in the primary sensory cortex in the postcentral gyrus. In

[1] Alfa-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid.

figure 2 the neospinothalamic tract is shown.[10]

Paleospinothalamic tract

For the paleospinothlamic tract, ther is in addition to the contralateral part of the ascending tract, also a part that stays at the ipsilateral side in the ventrolateral quadrant. The second-order neurons ascend to the intralaminar thalamic nuclei. On their way neurons synapse in the reticular formation. In the intralaminar thalamic nuclei second-order neurons merge into third-order neurons. After this, the third-order neurons are travelling to different cerebral corti, namely the limbic areas. In the left tract of figure 3 the paleospinothalamic tract is shown. However, the ipsilateral tract is not displayed.[10]

Spinoreticular tract

In this tract, the transition to second-order neurons takes place at the level of laminae V-VII. The second-order neurons ascend contralateral and ipsilateral of which a part terminates in the medullary reticular formation and the pontine reticular formation. The other part rises through to the intralaminar thalamic nuclei where the transition to third-order neurons is located. The third-order neurons project to the limbic areas and to the insula. The spinoreticular tract is shown in the middle part of figure 3. However, the ipsilateral tract is again not displayed.[10]

Spinomesencephalic tract

In this tract, second-order neurons travel from the anterior white commissure to the periaqueductal grey (PAG) and to the parabrachial nuclei. From the PAG neurons are stimulated that results in descending inhibition of pain, which will be discussed later on. Third-order neurons start in the the parabrachial nuclei and are ending in the amygdala. The spinomesencephalic tract is shown in the right part of figure 3.[10]

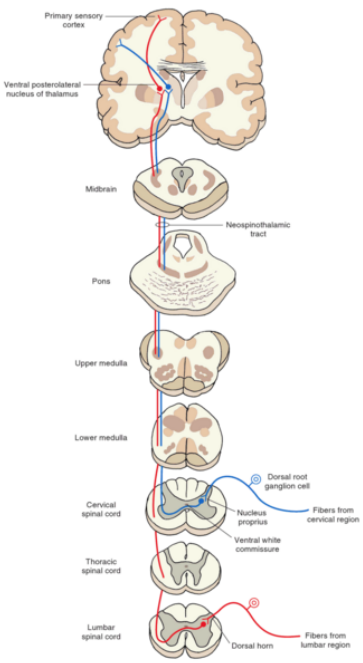

Figure 2: The neospinothalamic tract.

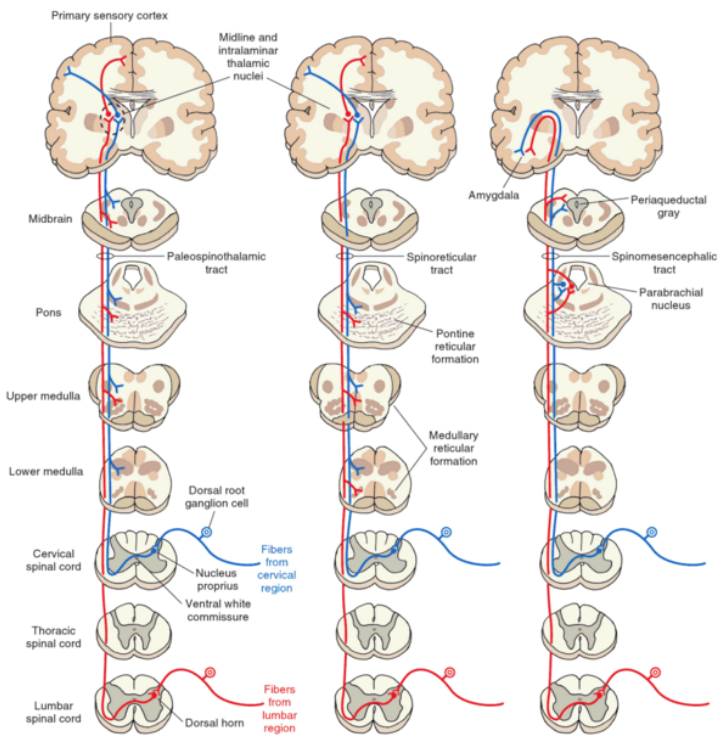

Figure 3: Vital tracts: left paleospinothalamisch; middle spinoreticular; right spinomesencephalic.

Descending pain inhibitory pathways

Inhibition of spinal nociceptive transmission takes place when the sensory cortex, ventrobasal thalamus, hypothalamus, mesencephalon, pons and medulla are activated by incoming pain signals. The PAG is a region in the mesencephalon which sends pain information to the rostral ventromedial medulla (RVM) and the dorsolateral pontine tegmentum (DLPT).[17] Depending on the class of neurons, inhibition or excitation will take place. Activation of glutamate or enkephalin neurons, which are derived from the PAG, result in activation of the descending pathways of the RVM. Activation of neurotensin-neurons derived from the PAG can both result in sending an inhibiting or facilitating signal to the descending RVM pathways. The nucleus raphe magnus (NRM) is a region of the RVM. Activated serotonergic-, cholecystokinergic, enkephalinergic and GABAergic pathways (which are activated by glutamate, enkephalin and neurotensin) from the NRM descend to the dorsal horn of the spinal cord in laminae I, II and V. Thus, descending pathways project on the same dorsal horn regions of the spinal cord as where periphery first-order afferent neurons synapse to second-order neurons. The following neurotransmitters are released by these pathways: serotonin, cholecystokinin, enkephalin and GABA. In exception of cholecystokinin, these neurotransmitters all have an inhibitory effect on the pain-information transport. Inhibition by enkephalin is induced through activation of opiate receptors such as mu (μ), delta (δ) and kappa (κ). μ-Receptors are mainly found at the supraspinal level and the δ- and κ-receptors at the spinal level. The release of substance P will be blocked when enkephalin binds to δ-receptors and κ-receptors, therefore second-order afferent neurons will not receive the pain information.[17] The RVM and DLPT are bound together by ascending enkephalin, substance P and GABA neurons. Unlike substance P and enkephalin neurons, which activate the DLPT, GABA neurons play a role in the inhibition of the DLPT.[18] Enkephalin activates the DLPT by inhibition of GABA neurons. Activated noradrenergic pathways descending from the locus coeruleus (LC), which is located in the DLPT, project to the dorsal horn of the spinal cord. The noradrenergic neurons release noradrenaline in the dorsal horn of the spinal cord. Noradrenaline blocks the transport of pain information to second-order afferent neurons by binding to presynaptic α2-adrenoceptors.[18][17] Thus, activation of the DLPT and the RVM play a role in the inhibition of pain. The descending pathways and their corresponding neurotransmitters are shown in figure 4.

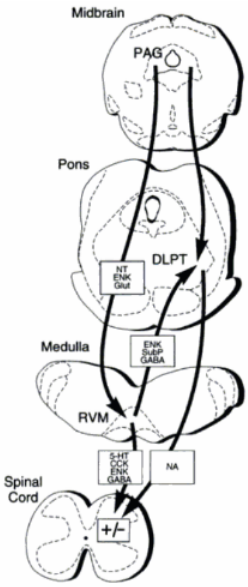

Figure 4: Descending pain pathways and the neurotransmitters that are involved[19].

Segmental pain inhibition

Another system involved in pain inhibition takes place on spinal cord level. Inhibitory interneurons receive information provided by mechanical (Aβ) and nociceptive neurons. The nociceptive neurons give off

their collateral branches to interneurons. These interneurons are inhibited by collateral branches of the nociceptive neurons when pain is induced. This leads to activation of second-order afferent neurons. When mechanical neurons send, in comparison to nociceptive neurons, a stronger signal to interneurons, these neurons will be activated. The activated interneurons send an inhibitory signal to the second-order afferent neurons located in the spinal cord. The mechanism of the interneurons is referred to as a so called gate, since the transport of pain information depends on inhibition or facilitation of these interneurons. This is also the reason why the segmental pain inhibition mechanism is called the gate theory. According to the gate theory, excitation of interneurons leads to closing the gate, therefore pain will not be sensed. Inhibition of interneurons leads to opening the gate which means that pain will be induced.[20] The inhibition and excitation of pain on spinal cord level is shown in figure 5.

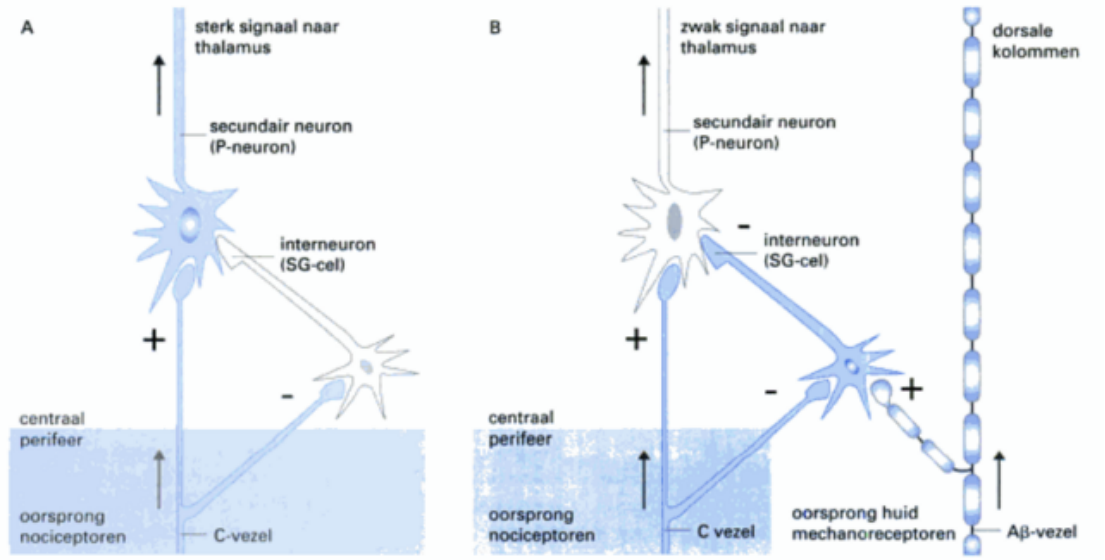

Figure 5: Control module for the "gate control theory of pain". A. Only the C-fibers are stimulated. B the Aβ-fibers are stimulated , and thereby also the SG-cells.[20]

Supraspinal pain inhibition

Multireceptive neurons in the dorsal horn and the trigeminal nuclei caudalis and oralis are found in both superficial and deeper layers of lamina V. They receive input from primary nociceptive neurons and non-nociceptive neurons. Multireceptive neurons are also called "wide-dynamic range" (WDR), "convergent", "lamina V type" or "class 2" neurons. These interneurons are involved in sending information to ascending pathways and to polysynaptic reflexes. A conditioning noxious stimulation of various areas of the body (unrelated to the receptive fields of the multireceptive neurons) can inhibit the activity of multireceptive neurons. This causes activation of the descending inhibitory mechanisms, which decreases pain responses to a second stimulus. Inhibition of one stimulus by the perception of a second stimulus, is called pain-inhibits-pain counterirritation. The pain relieving effect of counterirritation is provided by the "Diffuse Noxious Inhibitory Controls" (DNIC) mechanism. Only "noxious" is used in the term "DNIC" since it was originally thought that noxious stimuli could only inhibit second noxious stimuli. However, later studies suggested that non-noxious conditioning stimuli could also induce DNIC to some extent. Therefore the new term Conditioned Pain Modulation (CPM) was given. Thus according to those studies, multireceptive neurons are also activated by non-noxious stimuli.[21][22][23][24]

The ascending and descending pathway related to CPM travel through the dorsolateral and ventrolateral funiculi (supraspinal loop). Supraspinal structures (medulla oblongata, mesencephalon, diencephalon and cortex) are involved in this loop. Studies of Morton et al.(1987)[25] and Ossipov et al.(2014)[17] showed an involvement of the NRM in the CPM mechanism. However, other studies showed that NRM could not be directly involved in CPM mechanism since lesions of the RVM and PAG did not block the CPM.

Only lesions of the subnucleus reticularis dorsalis (SRD) blocked the CPM mechanism. Neurons of the SRD project to the PAG, RVM, thalamus, amygdala and other central areas. This means that the PAG, RVM and other central areas are indirectly involved in the CPM mechanism (Ossipov et al.(2014)[17] and Bouhassira et al.(1992)[22]).

Recent studies showed a lower CPM efficiency when patients had idiopathic pain disorders, such as migraine, fibromyalgia, tension headache and temporo-mandibular disorders. It has been also shown that a lower CPM efficiency was associated with an increase in pain among healthy subjects when exposed to a noxious stimulus. Based on these studies a connection could be made between CPM efficiency and prediction of chronic pain development. Thus, healthy patients with a lower CPM efficiency are expected to be more at risk for chronic post-operative pain in comparison to patients with a well working CPM mechanism. Lower CPM efficiency can be either ascribed to an enhancement of pain facilitation or loss of endogenous inhibitory control. Enhancement of pain facilitation can be explained by activation of ON-cells and inhibition of OFF-cells. These ON- and OFF-cells are nociceptive neurons found in the RVM and project to the spinal dorsal horn.[26][27]

Dermatomes

A dermatome is an area of the skin that belongs to the sensory distribution of a single dorsal nerve root.[28] Nerve roots arise from each level of the spinal cord. This means there are 31 nerve roots, which are cervical, thoracic, lumbar, sacral or coccygeal. Nerve roots intermingle in a plexus to form peripheral nerves. One nerve root can supply several peripheral nerves. Besides that, peripheral nerves can also exist from different nerve roots. A dermatome thus covers a larger area of the skin than a peripheral nerve does. There is made a general classification of dermatomes, called the ASIA (American Spinal Injury Association) classification. However, each person has slight differences in the location of dermatomes and there is also a large part of dermatomes that overlaps.[29] In this study electrodes are attached to dermatome C6. Dermatome C6 consist of the whole anterior forearm till just above the elbow, the radial side of the hand till thumb and index finger (in most cases).

2.3 Sensitization

There are three ways in which environmental stimuli produce changes in the nervous system. These are potentiation, habituation and sensitization. The first refers to the long-term strengthening of a nerve synapse because the pathways of together firing cells wire together. Habituation is the process of the body learning to not respond to a useless repeating stimulus. In case of chronic pain, especially sensitization is relevant because this refers to the process of increased reaction to a second stimulus.[30]

Mechanism

Central sensitization is a condition that results in a persistent state of high reactivity of the nervous system. The sensation of pain remains after the initial stimulus is removed[31]. It is called central because the central nervous system causes the pain sensation instead of the tissue at the pain location.[32] It is evoked by changes in the dorsal horn of the spinal cord and the brain on a cellular level such as receptor sites. These changes are caused by biological, psychological, and environmental predisposing factors but also onset of pain associated with depression, anxiety and other stressors. The essential thing to understand is that sensitization is not the cause of the pain, but the cause of its chronicity.[33] Effectively, central sensitization demonstrates unlike any pain model that pain may arise as a result of changes in the properties of neurons in the CNS. This makes pain also a dynamic reflection of central neuronal plasticity. Part of the plasticity of central sensitization is activity-dependent and is therefore reversible. This plasticity is triggered in the dorsal horn by the activity evoked neurons by input from C-nociceptors. To induce central sensitization, the noxious stimulus must be intense, repeated within a very short time, and sustained. Noxious stimuli causing tissue damage almost always suffice, but tissue damage is not required. When no new input is provided, the state of central sensitization lasts for tens of minutes to several hours.[31] Physiologically, the condition is based on increases in membrane excitability and synaptic efficacy, even as reduced inhibition. This causes sub-threshold synaptic inputs to create an (increased) action potential[34].

The membrane excitability depends partly on an increase in intracellular Ca^{2+}. But more factors play a role than change in membrane potential and that is where central sensitization differs from wind up, a state of increased excitability that lasts only a couple of seconds. These factors are summarised in figure 6. There is no specific pathway or transmitter devoted, each of the related transmitters can separately or together initiate the activation of those multiple intracellular signaling pathways that lead to the initiation of hyperexcitability in dorsal horn neurons.[31]

Now rests to discuss how central sensitization relates to chronic pain. It knows three increasing stages, namely activation (activity dependent), modulation (reversible functional changes), and modification. When modification occurs the pain becomes chronic because chronic structural and architectural alterations occur.[35] Central sensitization is characterised by both phenomenons hyperalgesia and allodynia[33][32].

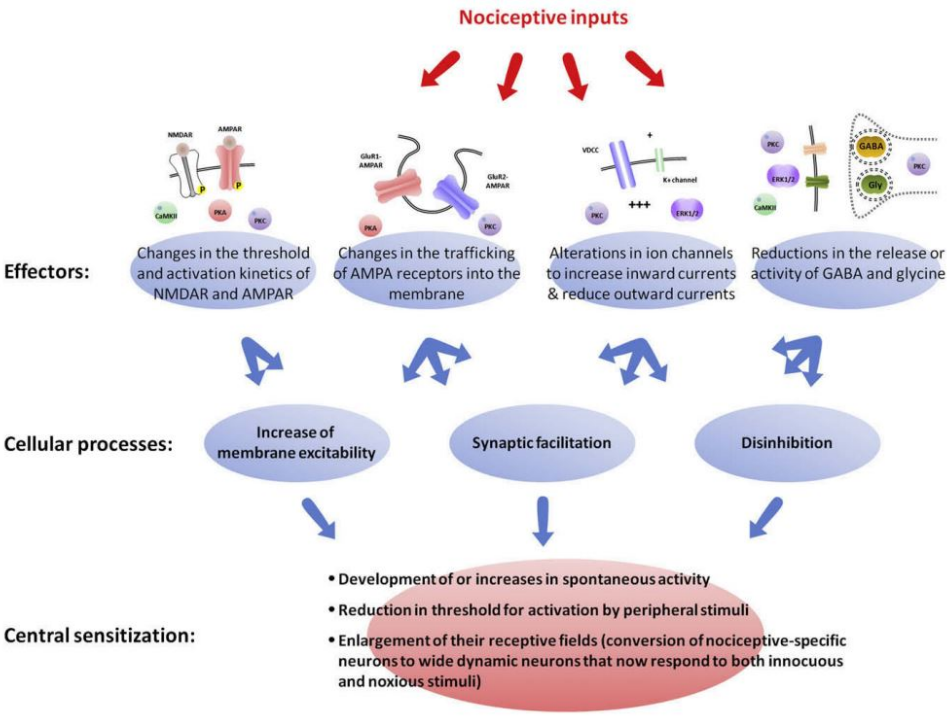

Figure 6: Cellular processes leading to central sensitization[31].

Hyperalgesia and Allodynia

The cause of chronic pain is not entirely known but it is commonly acknowledged that central sensitization and it characteristics play an important role[36][37]. Hyperalgesia is the increased sensitivity to pain. Therefore painful stimuli will be classified as more painful than they normally would. This should not be mistaken with allodynia, whereby things that should not hurt also become painful[31]. Hyperalgesia is often induced by the use of opiates. This is a process of habituation that causes patients to feel more and more pain or pain that spreads to other positions. This is a paradoxical effect that results in an ever increasing amount of medication.[38][39]

2.4 Technology

The NociTRACK

The NociTRACK is designed by researchers of the University of Twente (Nocitrack B.V. University of Twente, Enschede). The NociTRACK consists out of three parts: one small device to measure pain

sensitivity, one PDA[2] to collect data and a central database to compare data from individuals and other persons. The device contains two electrodes which are planted on the skin. The NociTRACK runs on batteries. When the participant presses the button of the device, weak electric pulses will be emitted through his arm (0,2 ms, 100 Hz). The pulses are increasing in current strength over time. The amplitude rises with 0.3 mA/s during the stimulation until a maximum of 14,0 mA. The participant will release the button when he finds the pulses uncomfortable so the generation of pulses will stop. This moment is called the pain detection threshold of the participant. By comparing this value with earlier values it can be concluded if a person has become more or less sensitive to pain. The NociTRACK can also be used to determine the detection threshold[3] of a participant. In theory the pain tolerance threshold[4] can also be determined with this mechanism, but this is not possible with a maximum of 14,0 mA, because with this level the pain tolerance threshold will not be reached in most cases. In this way the NociTRACK makes it possible to carry out electric quantitative assays and detect the pain threshold in an easy and not invasive way. The mobility and simpleness of the NociTRACK are some of the practical advantages of this device. Because of this the NociTRACK can be used on every location and patient can be checked at each moment of the day. The NociTRACK does not have a CE-marking, but is approved by the department of Medical Physics at the St. Antonius Hospital (Device number 66294). The safety of this device is described in the document: "MONDIAC project: Technical Proof of Principle(2008)". The electrodes which are planted on the skin, could cause irritation and local redness to the skin. This will be minimal based on previous studies.

Cold Pressor Test

A CPT is carried out by holding a hand of the participant in an ice water tub of 0-1 °C . The participant holds his hand in the tub for 30 seconds (or another time period of choice). During this time physiological changes can be measured in the body of the participant like blood pressure or heart rate. Other values, like the pain detection threshold and the pain tolerance threshold, can also be measured in combination with the use of the CPT. The participant will hold his hand in the tub as long as possible. He will warn the researcher when he experiences pain. The hand of the participant will be removed when this pain becomes unbearable. [40]

The CPT is not consistent in its method. There are a lot of differences between studies regarding CPTs. There are variations in methodology and equipment and specific values like water temperature. These variations affect the results of the tolerance time and pain scale. So these variations are making the studies incomparable. Therefore the reliability and validity of the cold pressure test can be doubted. In the study of Mitchell et al.(2004)[41] the objective was to come to a standard method in order to make different studies comparable. There was concluded that variations in temperature as small as 2 °C when using the Cold Pressor Test might result in significantly different pain experience in both men and women. It is therefore necessary to use equipment that ensures a precise and constant temperature to ensure replicable and reliable results. The comparison of different temperatures regarding CPT is relevant for the current study. It is therefore useful to look at the average outcomes of these studies. See figure 7 and 8.

At 1 °C is the VAS[5] score significant higher than at 3-7°C. This means that water of 1°C is more suitable for this study to produce an inhibitory effect. The tolerance time for women is with a temperature of 1 °C 25 seconds shorter than for men. This means that the duration of the test stimuli should be shorter. Furthermore the study of Mitchell et al.(2004)[41] stated that it is advisable to put the hand of a participant in a tub with water of 32 °C between two CPTs. This way the tolerance time will not be influenced by earlier measurements. [41]

There was also described that in only half the studies they examined, the researchers used circulating water to avoid heat building up near the hand. Therefore it is not odd that studies who did use circulated water have a lower average tolerance range (28.65-119.75 sec) than those using stationary water (37.11-190.3 sec).

[2]Personal Digital Assistant.
[3]The moment when the participant observes the pulses.
[4]The moment when the pain is not tolerable anymore for the participant.
[5]Visual analogue scale.

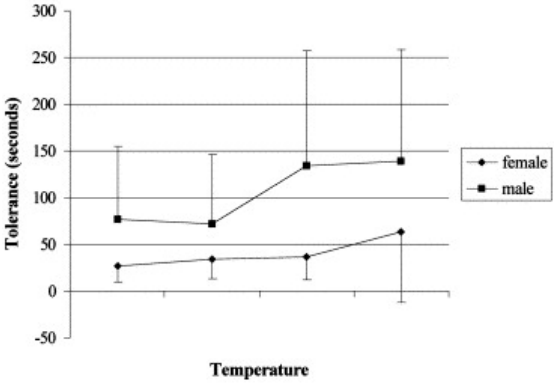

Figure 7: Overall tolerance time at resp. 1, 3, 5 and 7 °C[41].

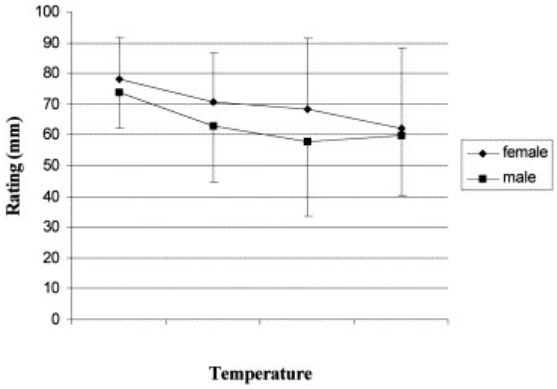

Figure 8: Average VAS/100mm rating at resp. 1, 3, 5 and 7 °C[41].

In a study of Fasano et al.(1996)[42] they tested the reproducibility of the Cold Pressor Test studies in healthy participants. The researchers worked with a test and two times a re-test which for group 1 fell on the same day and for group 2 on three consecutive days. Heart rate and blood pressure of the participants were measured to see if these values were similar during test and re-test. The values where in most cases at their highest point during the first test. Group 2 showed more difference between the three measurements, but these differences did not differ that much from group 1.[42]

2.5 Subjectivity of pain

Pain is a sensory modality and therefore everybody experiences it differently, just like taste for example. This subjectivity makes treatment of pain more complicated because another cannot totally understand how the patient experiences pain. That is the reason it is important to examine a possible objective factor of pain like activation of specific brain areas. When doing so, it appears that in persons who rate their pain higher on the VAS, a stronger activity in related brain areas[6] is measured. This observation underlines there are actual individual differences in perception of pain between persons. This is because pain is not just a sum of biological interactions but is being influenced by emotional and psychological factors. The effect of this influence is determined by genetic and environmental factors like stress and depression. [43][44][45] Furthermore, the psychological influence of awareness is large, by example whether the person is aware of the pain or his attention is directed at something else. The expectations of the patient are also very relevant. If the expectation of the kind of pain is unclear, this causes hypoalgesia[7] and when the pain to be expected is described very clearly this can emphasize the pain[43].

Next to the realisation of pain perception, a problem lies in sharing the perceived information. Other than its location, pain cannot be indicated quantitatively, so men is designated to metaphors for description. There are numerous questionnaires with the purpose of interpreting the otherwise elusive concept of pain. None of these covers solitary the essence of pain, the results must be combined in an overview in order to get closer to the reality.[45]

An often used pain scale is the Visual Analog Scale (VAS). This scale uses a line with a length of 100 mm. Hereby is pointed out that 0 equals no pain at all and 100 equals the worst imaginable pain. The participant is asked to draw a line perpendicular to the scale that represents the severity of the pain he experiences the best. In this way the severity of pain is represented by a number. The Numeric Rating Scale (NRS) is a similar scale used alongside the VAS to express pain in a number. The NRS is an 11-point scale existing of the numbers 0 to 10. Hereby 0 stands for no pain and 10 equals the worst imaginable pain. De patient names the number that reflects the severity of his pain the best.[46]

[6]Primary somatosensory cortex, anterior cingulate cortex and prefontal cortex.
[7]Decreased sensitivity to pain.

3 Previous research

3.1 Relevant studies with CPT

In an earlier study of Manresa et al.(2014)[47], the reliability of a conditioned pain modulation paradigm was tested. They only used healthy men as participants to avoid the possible influence of hormonal changes during menstrual cycle on pain. The test was done to detect a significant CPM effect and the reliability of that effect over time. The CPM effect was measured as the difference between the nociceptive withdrawal reflex (NWR)[8] threshold during CPT and the NWR threshold before CPT. Electrical stimuli were performed through bipolar Ag/AgCl-electrodes. These stimuli were the test stimuli and the CPT served as the conditioning stimulus. Besides the NWR threshold, also the pain detection threshold and the electrical pain ratings of the participants were measured. The electromyographic reflex responses were recorded through the electrodes when pulses were delivered. In 99% of the measurements the NWR threshold was increased during CPT. 1% resulted in a lower NWR threshold compared to measurements before CPT. During the study there was also an increased electrical pain detection threshold during CPT in 96% of the measurements. The remaining 4% showed no difference compared to the measurements before CPT. Regarding the pain intensity ratings to supra-threshold stimulation, 84% of the measurements resulted in lower ratings, 13% remained unchanged and 4% resulted in higher ratings during CPT. All results are shown in figure 9 .

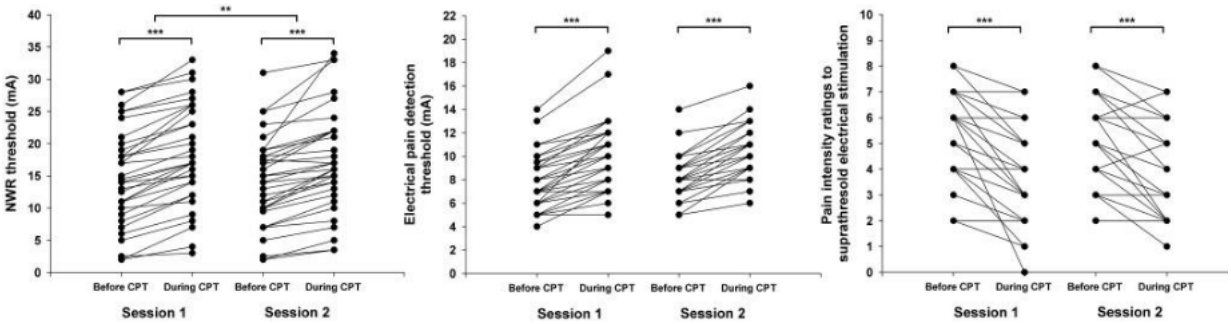

Figure 9: Results of measurements of the NWR threshold, the electrical pain detection threshold and the pain intensity ratings using CPT[47].

During this study a CPM reliability analysis was done with values for all performed tests. The psychophysical measures (electrical pain detection thresholds and pain intensity ratings after suprathreshold electrical stimulation) displayed significant lower ICC[9] values (i.e. worse relative reliability) than the electrophysiological assessment (with NWR threshold). Furthermore, the reliability of the electrical pain detection thresholds and the reliability of the NWR threshold did not substantially change during CPT, which implies that CPM does not introduce an additional source of variation to these measures. But that does not count for the pain ratings to suprathreshold electrical stimulation.[47]

During the study of Tsao et al.(2013)[40] stimuli were given at four moments. These moments of measurement were when participants held their hand in a cold water tub (5 °C). At first a stimulus was given to determine the baseline. At the second stimulus the participant held his hand in a cold water tub of (5 °C) . The third and fourth stimulus were given respectively 15 and 50 seconds after the participant held his hand in the cold water tub. It is remarkable that the pain rate of the second stimulus was notably lower than the pain rate of the first stimulus.[40]

In a study of O.H. Wilder Smith et al.(2010)[7] the relation between the presence of chronic pain and the endogenous descending inhibitory courts, nowadays called CPM, was observed. They researched the electrical pain tolerance threshold. During this study developing chronic pain six month after surgery with use of preoperative pain modulation, postoperative pain scores in rest and movement and determination of skin- and deep tissue hyperalgesia were related. The researchers expected that CPM works less preoperative

[8] A reflex intended to protect the body from damaging stimuli.
[9] Intraclass Correlation Coefficient.

and that postoperative pain scores and hyperalgesia were increased by patients with chronic pain six months after surgery. CPT was used as validated conditioned stimulus. Six months after the surgery six out of the twenty people had developed chronic pain. Figure 10 shows that these people had a higher score on the VAS scale at rest and movement at one, three or six months after surgery compared to the group who did not have chronic pain six months after surgery. It also showed that an impaired CPM has a direct connection with the holding of postoperative pain.[7]

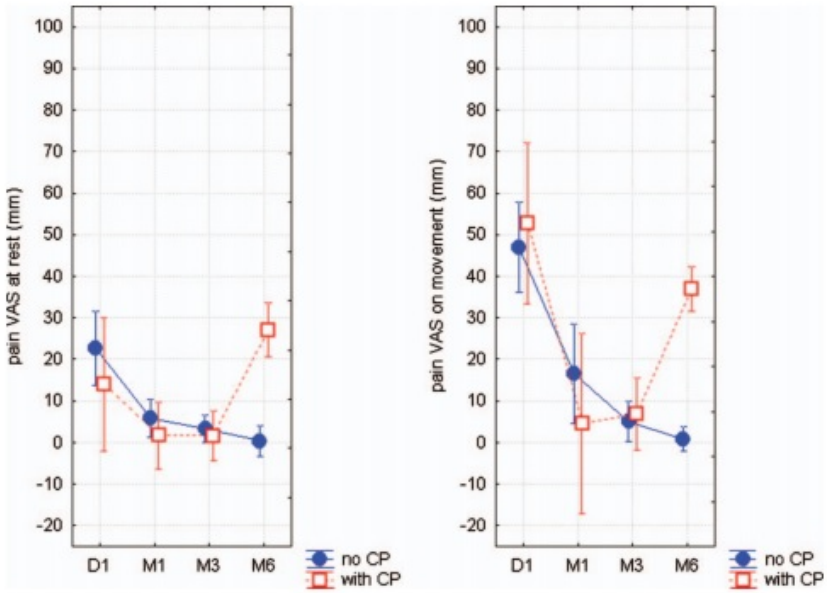

Figure 10: Postoperative time course of pain measured with VAS at rest and movement. D1 is day 5 after surgery. M1, M3 and M6 are respectively 1, 3 en 6 months after surgery[7].

The outcomes suggest that the progression to chronic pain involves the persistence of hyperalgesia as well as its spread to sites distant from the site of surgery. This seems particularly the case for skin hyperalgesia as tested by electric stimulation. But it may also apply to spreading deep tissue hyperalgesia as tested by pressure algometry during this study. This pilot study provides first evidence supporting links between the development of chronic pain after surgery, postoperative persistence and spread of hyperalgesia and poor preoperative inhibitory modulation of pain processing (CPM).The outcomes of this study suggest that preoperative testing of pain processing can be a useful clinical tool for the management and prevention of chronic pain after surgery.[7]

3.2 Gender, age and ethnicity

From a study of Tsao et al.(2013)[40] is shown that the gender of children does not affect the subjective experience of pain after a test stimuli but age does. Younger children (age 8 till 11) give by the same stimulus a higher pain rate compared to older children (age 12 till 17). From this it can be concluded that younger children have an lower CPM than older children. In these ages there are no differences regarding CPM when we look at the gender of the children.[40].

However, another study of Schmitz et al.(2013)[48] showed that there is only difference in outcome from the age of 14 when puberty is reached. This is a small difference, probably due to hormonal changes. The difference arises from the fact that the pain detection threshold and the pain tolerance threshold for girls decreases, while these stay the same by boys. It seems that the time of endurance at the tolerance pain detection threshold stayed the same for both genders. It can be concluded that the time of endurance is not dependent on the pain detection threshold.[48] When men looks at adults, clinical pain syndromes are more common in women than in men. Thereby in general, it seems that women have lower pain

thresholds and lower pain tolerance levels. This is demonstrated by experimental pain studies. Chronic pain conditions are also found more often in women, this could be explained by sex differences in the CPM system. Women could be higher sensitive to pain because of a less efficient CPM, which results in less effective pain inhibition. There are a lot of studies that investigated this paradigm. Many studies confirmed the fact that sex differences in pain perception are due to the efficiency of CPM. However a part of the studies did not demonstrate a sex difference in the CPM system. None of the studies had find a more efficient CPM effect in women. To draw a conclusion related to the sex differences in CPM, it is needed that consistent test protocols are used and the menstrual cycle phase is controlled.[48]

Since there are different outcomes considering the role of gender in CPM there cannot be drawn a probable conclusion. Earlier exposure to pain can be a reason why CPM is not the same by everyone[49].

Washington et al.(2000)[50] were first to notify an age dependant difference in endogenous analgesic responses as assessed by CPM. Older adults (mean age of 78 years) have decreased sensitivity to pain compared to young adults (mean age of 23 years). There was a significant increased pain threshold in both the groups after the CPT, although the increase was less with the older adults.[**?**] Edwards et al.(2003)[51] also found that there are age-related decrements in CPM. Young adults (average age of 21,6) were compared with elderly (average age of 63,1). It seems that young adults had a higher pain detection threshold when a cold stimulus was involved[51]. The older subjects were less sensitive to a thermal stimuli that was painful, but more sensitive to pain due to CPT. After the CPT, the younger subjects experienced the thermal pain stimulation as less painful which indicates an good working CPM response. By contrast, the older subjects experienced the thermal pain stimulation as more painful, so there may be no inhibition by CPM. The participants, 102 individuals in total consisting out of both men and women, were asked to put their hand in a circulating water bath of 5 or 22°C for 70 seconds. The order of water temperatures was randomized across sessions. Twenty seconds after the participants put his or her hand in the cold water bath a temporal summation sequence (ten thermal pulses) with target temperatures set at maximum temperature tolerated was delivered to either the left dorsal forearm or the left ankle. The maximum temperature tolerated was beforehand definite and the order of left dorsal forearm or the left ankle was randomized. There were no participants who withdrew their hand from the water bath before the 70 seconds at 22°C and there was also no difference between the younger and older group regarding pain ratings at 22°C. The measurements at a temperature of 5°C showed a difference between the two groups. A higher percentage of younger participants tolerated the 5°C water for the entire 70 seconds. These outcomes are probably due to potential decrements in a CPM endogenous system in the elderly participants. There were no age differences found when the intensity ratings were compared. The observed age differences in CPM were not attributable to attentional variables or differences in local skin temperature. Skin temperature among older adults returned to baseline more slowly than skin temperature among younger adults. But post-immersion local skin temperatures were not significantly correlated with the magnitude of CPM.[51]

Larivire et al.(2007)[52] did also research to the age-associated differences in CPM and discovered that middle-aged adults (age of 40 till 55 years) had a decreased CPM response compared to younger adults (age of 20 till 35 years). The CPM effect seemed to be absent in older adults (age of 60 till 75 years). Middle-aged and older adults did not have a different threshold for pain. The threshold of the young adults was significantly lower than the middle-aged and older adults. These results suggest that CPM starts to reduce at middle age and tamps out at older ages. There was no difference in expectation related to pain relief of the conditioning stimulus between the different groups, so the expectations did not influence the results.[52]

Overall, there are a little studies researching age differences in pain inhibition by CPM. The CPM seems to be absent, decreased or more variable in advancing age, but it depends on which paradigm was investigated. From the middle age, CPM appears to reduce. Alteration in the CPM system in elderly could be the result of enhanced clinical pain or reduced physical functioning. Besides that, it is mentioned that other mechanisms may influence the CPM in advancing age, like neuronal loss in key brain structures, a modification in facilitatory and inhibitory processes and the reduced regulation of the opioid systems. More research is needed to clarify the relation between advancing age and the alterations in CPM effects.[48]

Ethnic differences in both clinical and experimental pain studies have been consistently demonstrated, with African Americans being most sensitive to experimental pain and reporting higher levels of clinical pain. This 1 study precludes drawing any firm conclusions on the role endogenous pain mechanisms in explaining alterations in pain sensitivity between ethnic groups, but some ethnic differences in CPM appear to be present.[53]

3.3 Different stimuli and its effects

In general, the effects of CPM are only found in contralateral or extra-segmental areas and not in ipsilateral areas. However there are some studies that incidentally reported pain reduction after ipsilateral stimulation. A study of Pud et al.(2009)[54] reviewed on the diversity of methods used to evoke CPM effects in experiments. It is concluded that findings related to studies about CPM are hard to generalize because of the methodological variations. There are several factors that influence the magnitude of the CPM effect like the strength of stimulation, the duration of stimulation and the stimulated body region. These factors induce different modalities of stimulation.[54] Willer et al.(1984)[55] did research to the painfulness of the conditioning stimulus and the CPM effect. They have shown that a conditioning stimulus consisting of increasing painful water temperatures ($\geq$ 45°C) correlated with an increased CPM effect. It has been demonstrated that a cold conditioning stimulus is rated most painful and induces the greatest inhibitory effects comparing it to muscle pain and pressure pain as conditioning stimulus.[55]

Other studies have used stimuli of different thermal intensities, gastric and rectal distension or muscle pain by saline injection to show that a stronger conditioning stimulus leads to greater inhibitory effects[53]. So the strength of the conditioning stimulus seems to affect CPM. By contrast, the intensity of the perceived pain of a conditioning stimulus appears to have no effect on the magnitude of the CPM effect. The Van Wijk et al.(2010)[53] state that two contemporary stimuli can counteract each other, which leads to decreased CPM effects. Patients with clinical pain usually have more than one pain area, which could act the same as contemporary stimuli. This could be a reason for the less effective CPM in patients.[53]

There are different opinions about whether a stimulus needs to be painful to activate the CPM system. Lautenbacher et al.(2002)[23] did research the effect of a strong but non-painful conditioning stimulus on the test stimulus, which consisted of water with a temperature of 42°C. After application, the test stimulus was rated as less painful. So the non-painful conditioning stimulus has elicited CPM. The same outcomes were shown in different studies of the same authors. However, Granot et al.(2008)[56] found pain-suppressive effects when water stimuli of 12°C and 46°C were applied. 15°C, 18°C and 44°C did not activate inhibition, so there was concluded that an experience of mild pain was required to induce CPM effects. Willer et al.(1984)[55] found out that there was an increase in RIII reflex threshold when a water temperature of 44°C or higher was applicated as conditioning stimulus. The subjective pain threshold was increased when a temperature of 45°C or higher was applicated. The results correspond with the results of Granot et al, who found out that, as noted before, a temperature of 46.5°C induces inhibitory effects and 44°C do not. A possible explanation why a non-painful stimulus is activating the CPM system is the difference in time of application of the conditioning stimuli. Granot et al.(2008)[56] used a 60 seconds lasting immersion time, while Lautenbacher et al.(2002)[23] used immersion times of 6 and 10 minutes in water of 42°C. A longer immersion time could increase the number of nociceptive signals sent to the dorsal root and after that to supraspinal areas. Wind-up could also be a factor that plays a role. It has been shown that 2 minutes of tonic heat stimulation results in temporal summation. Thereby it is demonstrated that multireceptive neurons are activated by wind-up of subthreshold stimuli.[23][56][**?**]

Overall, the majority of the studies mentions that it is important that a stimuli is painful to induce CPM. However, some studies point that the CPM could also be triggered by a strong but non-painful stimulus when it is applied for a longer time, probably by inducing wind-up. It seems that there is no relation between the subjective pain ratings and the magnitude of the CPM effects. That is why it appears that the activation of CPM has a more physiologic existence. A certain threshold of stimuli coming from peripheral nociceptors is required to activate CPM, independent of the subjective perception of the intensity of the stimulus.[53]

3.4 Psychological effects on the CPM

There seems to be alternative psychological mechanisms for pain modulation which results in CPM effects, like distraction, expectations, pain catastrophizing and perceptual adaptation levels. Some studies have further analysed this hypothesis and concluded that attentional processes are only minimally related to CPM. In sum, these studies have demonstrated that distraction leads to a minimal chance in CPM, especially when test and conditioning stimuli are performed in the same sensory modality. Although it is influencing CPM to a minimal extent, in general, attentional processes appear to have no effect on CPM.

Besides distraction, expectations of analgesia are thought to influence the prefrontal cortex and midbrain structures. Midbrains structures are involved in the CPM system, which leads to the suggestion that expectations of analgesia also have influence on the CPM mechanisms. It is found that expectations of pain-enhancing effects are able to block descending inhibitory processes and that is why there is the suggestion that brain structures can modulate CPM effects by expectations.

Pain catastrophizing is the exaggerated interpretation of a stimulus that is painful. There are a small number of studies that examined the associations between catastrophizing and CPM. That is why it remains unclear. Although the effect on pain sensitivity is well studied and it is shown that there is a positive correlation between pain catastrophizing and the perceived pain intensities.

When a painful experience is judged or compared to another painful experience, it is called the perceptual adaptation level perspective. For example, it has turned out that patients who had severe injuries have a higher pain threshold and tolerance value compared to patients who had only light injuries. This indicates that a reference framework affects the pain perception. The CPM effect could be partly explained by the perceptual adaptation levels.[53]

3.5 Duration of CPM

There are a lot of studies that investigated the time till CPM has worked out. However, the results are different between these studies. Most of the studies mention that the CPM is worked out after 5 minutes, but others also mention the CPM works for longer than 5 minutes. This difference in time is declared by the use of different kind of conditioned stimuli and an other interpretation of the term "the end of the conditioned stimulus". Some investigators interpret it as the moment when the conditioned stimulus is removed, while others interpret it like the moment when the conditioned pain is equal to zero. Conditioned stimuli may comprise thermal, electrical, chemical, mechanical or ischemic stimuli.[53]

Multiple studies have investigated the duration of the CPM effects after the conditioning stimulus was applied. They generally conclude that the CPM effects are worked out within about 5 minutes after application of the conditioning stimulus and the maximal pain inhibition is reached during application. However some studies report that inhibition effects are detected up to 5 to 8 minutes. Only a few studies reported pain-suppressive effects for more than 10 minutes and even one study detected pain inhibition effects one hour after the application of the conditioning stimulus.[53]

The study of Tsao et al.[40](2012) focuses amongst other things also on the duration of the CPM effect. The participants were children from 8 till 17 years old. They were exposed to a test stimuli consisting of different levels of pressure. This test stimuli were given at 4 different times. One before CPT, one during CPT, one 15 sec after CPT and the last one 50 sec after CPT. It was found out that during CPT and 15 sec after CPT the pain intensity was significant different than the measurement before CPT. However, 50 seconds after CPT there was not a significant difference anymore. This was not different for the multiple ages of the children. Though, it can not be established that this also applies for adults. As measured in the study of Lewis et al.(2012)[57], the duration of the CPM was 10 minutes. This varies a lot with the 15 seconds measured in this study.[40]

3.6 Possible effects of chronic pain on CPM

It is difficult to assess the CPM activation in patients with chronic pain. Patients with chronic pain have spontaneous ongoing pain, which could act as a conditioning stimuli and thereby will modulate other painful stimuli, because CPM is already activated. When ongoing pain is acting in the same way as a conditioning stimulus, it triggers CPM and will result in a higher pain threshold and lower pain ratings

compared to healthy persons without ongoing pain. Peters et al.(1992)[58] did not find a relation between clinical pain and the activation of CPM. It was found that patients with chronic low back pain and acute postoperative pain have a slightly higher (but not significantly) subjective pain threshold compared to healthy persons.

Also the RIII reflex threshold is observed in Clinical Pain Syndromes patients and control subjects. There was not a significantly difference in threshold and also the rating of clinical pain did not differ from each other. Similar results were found in the study of Boureau et al.(1991)[59] where no difference in RIII reflex thresholds were observed as well.[53]

A declaration could be that the intensity of the ongoing pain of the patients is not enough to induce inhibition by CPM. However, this declaration is not very plausible, because it is demonstrated that also non-painful stimuli be able to induce the CPM effect. Furthermore, a study of Bouhassira et al.(2003)[22] has shown that severe ongoing neuropathic pain did not result in a reduction of the nociceptive RIII reflex of the sural nerve or in a reduction of the subjective noted pain that the patients experienced after the test stimulus. A possible explanation is that clinical pain together with an applied conditioning stimulus could act like two contemporary applied conditioning stimuli, which potentially lead to a reduction of the CPM effects. This is suspected because recently it is found out that two contemporary conditioning stimuli result in a reduced CPM effect when compared with only one conditioning stimulus[53]. In addition, it could be that CPM is not able to be activated for a long time. Then CPM will only be activated at the beginning of the pain, but when it continues for a longer period of time, CPM will slowly exhaust. To clarify the effects of ongoing pain on CPM effects, more research is necessary.[22]

In the previous study, it is demonstrated that patients with a lower effectivity of CPM in a pain free state before surgery, are having a higher risk to develop chronic pain after surgical thoracotomy. However, not all chronic pain patients have less effective CPM effects, so it is not likely that deficient CPM effect are a prerequisite for the development of chronic pain after surgery. Although it is an essential risk factor.[53]

4 Method

4.1 WMO study

This prospective, observational research is a WMO study because it is a medical scientific research that requires participants to perform a specific action. Besides, they are required to behave in a way determined by the researchers. Therefore a METC subsidiary is presented and approved.

4.2 Participants

Potential participants were healthy men and women between the ages of 25 and 45. It has been tried to find a group of twenty volunteers between these ages. Hereby the in- and exclusion criteria were taken into consideration, which are listed in the appendix A.1. Necessarily there had to be made a deviation from the age restriction of the participants. Finally participants between the ages of 17 and 54 were accepted. Therefore the group of potential participants was higher so that enough people could be found to participate in the study. A part of the participants were employees or interns from the Sint Antonius Hospital in Nieuwegein, The Netherlands. The other participants were family, friends or acquaintances of the researchers. The participants were approached to participate in the study through advertising texts. Only basic information about the study was given to the participants so that their behaviour would not be affected. They all had to read and sign an informed consent before participating.

Informed consent

The participants were given explanation about the study and the required way of behaviour. By giving informed consent, the participant acknowledged he/she will participate voluntary and is aware of the fact that he/she can quit at every moment. Also he/she confirmed to had enough time to ask questions about the study. Furthermore the participants also gave permission to use the relevant collected data in the study anonymously and preserve the collected data for 15 years after the study.

4.3 Procedure

The study consisted of one session, during about an hour and a half. For five participants the study consisted of two sessions, each taking about one and a half hour on two separate days. Between these days there had to be a time of at leasts three days. These sessions are the same, with the exception that the first session includes a moment of explanation. The researchers explained well which pain the participant could expect. After this the NociTRACK electrodes were applied on the right arm and a training session followed. This training session consisted of a number of test stimuli so the participant could get familiar with the equipment and the stimulus it provides. Then the actual measurements started, the protocol (included in the appendix A.2) exists of the elements Cold Pressor Test and test stimuli.

Cold Pressor Test (CPT)

There are four CPTs which differ from each other. The four types were as follows:

1. The participant was instructed to place his right foot in cold water (0-2°C) until the pain becomes unbearable (NRS=10), minding a maximum of three minutes.
2. Likewise, but now the temperature is 33-34°C, since this tub should not hurt at all (NRS=0). Three minutes is standard now.
3. The foot is placed in cold water (0-2°C) until NRS=4.
4. The foot is placed in cold water (0-2°C) until NRS=6.

   The temperature of the water is measured before every session with use of CPT with a TC305K Digital Handheld Thermometer. This thermometer consists of a probe which can be held into the water.

During the Cold Pressor Test the participant is asked to keep his/her foot moving to create the effect of circulating water. The advantage of circulating water is the fact that the same water temperature at every place in the tub will be realised. When the participant gets his/her foot out of the water he/she is allowed to put his/her foot on a towel placed next to the ice water tub and dry it.

Test stimulus (EPDT)

With the term test stimulus is meant the action with the NociTRACK for detecting the Electric Pain Detection Threshold. Its electrodes (Red Dot 3M Cirkel Electrode) were placed at dermatome C6, on the right lower arm near to the elbow joint. Always the same two, round shaped electrodes were used. These were placed adjacent to each other so during test and re-test the same distance between them is realised. The participant self-administers the increasing current until the sensation becomes unpleasant. The participant hold the NociTRACK in the left hand so that the right hand can remain in rest position. The participant presses the red button of the NociTRACK whereby the electric stimuli start. The stimuli stop when the participant let the button go. The participant is asked to try finding the same unpleasant moment during the measurements when the electric stimuli is given.

Protocol

As is visually explained in the protocol in the appendix A.2, the test was always started with the CPT where a NRS of 10 had to be realised. The other three CPTs are performed in a randomized order. Before and after each CPT three test stimuli are given and the outcome is noted. Between the CPTs, a break of at least 20 minutes must be taken to minimize the effect of one CPT on the following. After the first CPT, the change in time of CPM is also tested so for a period of 20 minutes each 5 minutes and directly after CPT there are given three test stimuli. This period replaces the 20 minutes break that were taken for the other CPTs.

4.4 Randomisation and blinding

As said before, after the first CPT, three more follow (CPT 2, 3 en 4). The order of these tests was computer-randomised by the generator at http://www.randomization.com. Blinding of the researchers and participants was not possible, because one had to give the instruction and the other had to understand it and act according to it.

4.5 Datacollection and -processing

First, the participant filled out three pain questionnaires. The follow questionnaires were used: Brief Pain Inventory, Pain Anxiety Symptoms Scale(PASS) and Beck Depression Inventory. On the basis of these questionnaires it could be decided to exclude a participant afterwards. During the measurements the researchers filled out the form Meetdata ICE study that can be found in appendix A.3. Hereby, for every CPT test the time spend in the water bath was measured. For the first CPT each 15 seconds the participant gave a NRS score. Before and after each CPT the current strength at which the EPDT is reached was noted (always three tests), for the first CPT this includes the repeated stimulation each five minutes after immersion. During re-test these times and current strengths were collected again.

For determination of the EPDT always three test stimuli were given. From these three current strengths the average was calculated. These values for EPDT provide data for the three research questions of this study. The EPDTs before and directly after the CPT are referred to as respectively pre and post values. These are compared for each NRS value separately and displayed as relative increase, which is calculated by the difference between the post and pre EPDT value divided by the pre EPDT value. The relative increase is a rate for the CPM effect and that is why the calculated outcome is equal to the CPM effect. The EPDTs measured in the 20 minutes following the first CPT are plotted in time. Furthermore, the EPDTs measured in test and re-test are compared to each other. For all of the measurements the CPM effect is the outcome.

To reach an answer to the subquestions, figures of the CPM for the NRS values and for the CPM in time were created in SPSS (IBM SPSS Statistics 20) with the function; graph, error bar. Before creating these figures, the values for all the participants are compared in graphs to provide insight in how the within subject variability relates to between subjects variability. There were also additional figures created to validate the research itself. These figures include the EPDT values for the NRS values (created in Matlab 2011), test and retest differences for CPM (created in Excel) and the pre and post EPDT values categorised for order of administration (created in SPSS).

4.6 Statistics

The descriptive statistics is done in SPSS. The mean values and standard deviation of age and BMI of the participants were calculated. The figures for the correlation between test and retest were created in SPSS as well. A two way repeated measures ANOVA has been chosen to test if there was a significant difference between the EPDT score of the pre-test /post-test and the four NRS values. The four NRS values and the pre-test/ post-test were both within subject variables. The alpha that is used for the two way repeated measure ANOVA had the following value: 0,05. To test whether the CPM effect was significant for the NRS values , a one way repeated measures ANOVA has been chosen. The NRS values in this test were within subject variables. To test whether the pre EPDT values differ from the post EPDT values for the different conditions (NRS values), a paired sample t-test was chosen. For this test an alpha of 0.05 was used. To test if there was a significant difference in the CPM effect for time (0min 5min 10min 15min and 20min), a one way repeated measures ANOVA has been chosen. Time was a within subject variable. For this test an alpha of 0.05 was used. Finally to test the correlation between the test and retest for the NRS values and time an ICC was calculated.

5 Results

Process of research

In this study a group of 22 participants took the test. This group consisted of eleven women and eleven men. Two of the 22 participants were excluded because they did not reach their maximum EPDT within the limits of the NociTRACK. Also one participant was excluded because he did not reach NRS 10 during the first CPT. Another participant was also excluded because her answers to the pain questionnaires were too deviating. The remaining group consisted of nine women and nine men (age: mean=27.67 sd=11.65, BMI: mean=22.46, sd=2.13).

In the study protocol it was stated that the first EPDT value of the repeated measurements would be taken 1 minute after the CPT. During the data collection it appeared that a test stimulus can take longer than one third of a minute. This means that after the three test stimuli for zero minutes were done, already a minute was passed. So the moment to measure the EPDT at 1 minute was removed and the second moment was at five minutes.

During the measurements there were a few things that could affect the outcomes. Some participant saw the strength of the pulses in amperage during the different measurements after they released the red button of the NociTRACK. Another disturbing factor, like conversations that took place during the measurements, could distract the participants and influence the amount of attention they paid to the research. Some patients also experienced muscle contraction as a result of the electrical stimulation. These patients chose the moment of muscle contractions as the start of the unpleasant feeling.

5.1 Conditions

NRS values

All of the EPDT values of the participants are registered in appendix A.4. To be able to further understand these values a two way repeated measures ANOVA test was used. This tests the within subject variables EPDT level (before and after) and condition (one of the four CPTs). For this test, a significance level of 95% is chosen. The values of the significance in table 1 show a significant difference between both pre and post measurements as well as between the conditions. The table also shows a non significant difference for the interaction effect.

Table 1: Two way repeated measures ANOVA

Effect	F-value	Significance
PrePost	F(1,17)=15.297	0.007
Condtitions	F(3,15)=4.507	0.019
PrePost*Conditions	F(3,15)=0.666	0.586

Since a significant difference is shown for the conditions, a closer look is taken to determine which conditions differ from each other. The results are shown in table 2. All EPDT values, regardless of pre or post measurement are compared between the conditions.

Table 2: Pairwise comparison of EPDT for condition

Condition		Significance
NRS 10	NRS 6	0.012
NRS 10	NRS 4	0.046
NRS 10	NRS 0	0.043
NRS 6	NRS 4	1.000
NRS 6	NRS 0	1.000
NRS 4	NRS 0	1.000

Table 3: Paired sample t-test for pre and post values

Pre-Post	Significance
NRS 10	0.041
NRS 6	0.036
NRS 4	0.044
NRS 0	0.591

In figure 11 the average pre and post EPDTs values for all participants are plotted per NRS value. The asterixes indicate wich differences are significant according to tables 2 and 3.

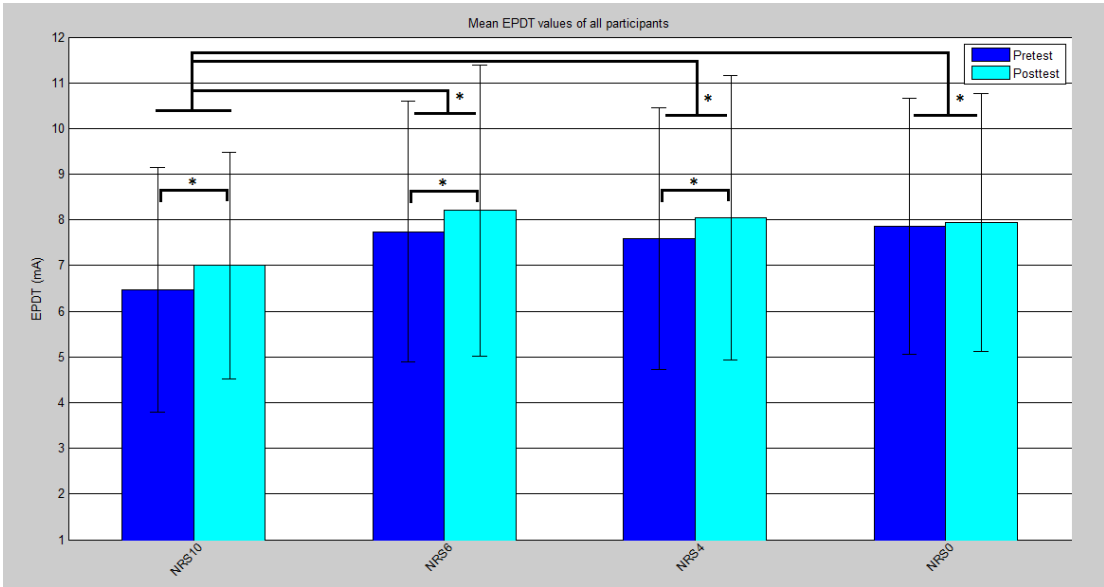

Figure 11: The average EPDT value of all participants divided in pre and post values per condition.
*Differs with a significance of P<0.05

In figure 11 all EPDT values are provided with an 95% confidence interval. From the mean values within these intervals can be seen that for all conditions the EPDT values after conditioning stimulus are elevated. The difference between pre and post EPDT shows to be significant for all conditions but NRS 0. Both pre and post values are the lowest for NRS 10 in comparison to the rest. In statistical analyses came forward that all EPDT values of NRS 10 differ significantly from NRS 6, 4 and 0.

Order of conditions

Besides from NRS 10, all conditions were administered in randomised order. Therefore the results are also separable for the relative moment of measurement. This is used for figure 12 where the different moments are presented as order on the x-axis.

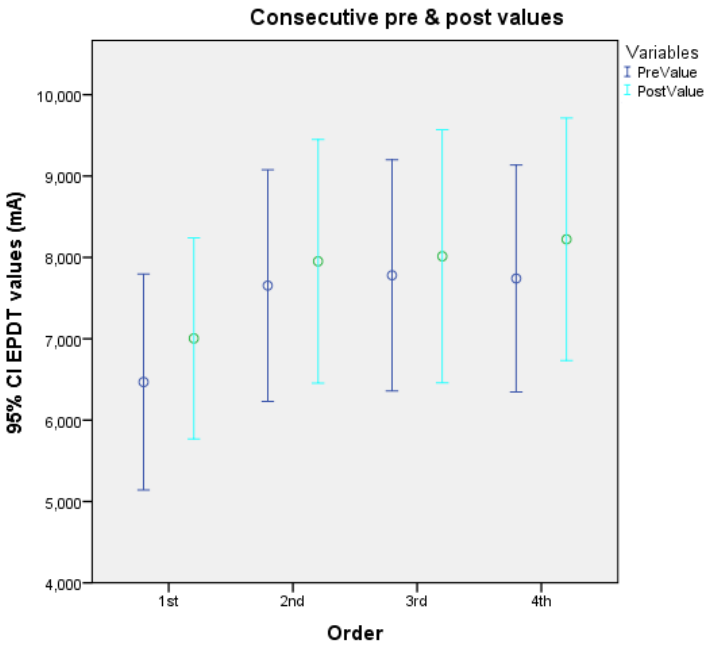

Figure 12: Pre and post EPDT values for all participants and all conditions in chronological order.

For figure 12 the measured EPDT values of all participants were categorised for chronological order in stead of condition. Since the CPT to reach NRS 10 was always performed first, the point above '1st order' only consists of these measurements. The other CPTs consist of NRS scores 6, 4 and 0 and were randomised so the other orders are mixed values for these NRS scores. The figure shows that the EPDT values are lowest at first order and the other EPDT values are at the same level. The difference between the orders is statistically underlined with a p value of 0.037.

To answer the question whether subjective experience of pain is necessary in order to activate the inhibiting function of the CPM system, the CPM effects are compared between the different CPTs. Every CPT has a different NRS level to reach; NRS 10, 6, 4 and 0. First, the relative difference between the EPDT after a CPT and before this CPT is calculated for the four different CPTs which is seen in appendix A.4. Next, these relative differences in EPDTs are graphed per CPM and thus per pain level (see figure 13).

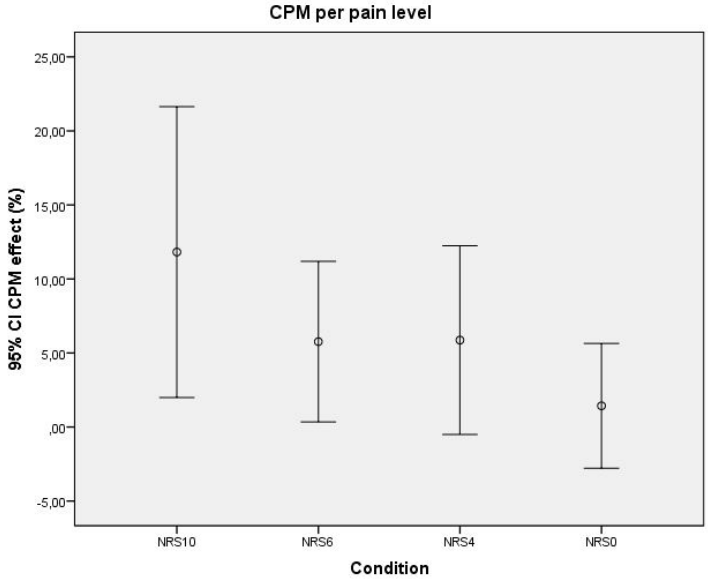

Figure 13: EPDT before detected NRS compared to EPDT after detected NRS for all participants

As seen from figure 13 it seems that the difference in EPDT is the largest for NRS 10 and decreases slightly for lower NRS values. Thus, the difference in EPDT seems to be highest for NRS 10. A statistical analysis follows to turn out whether the observed relative difference is significant. A one way repeated measures ANOVA test is used. The within variable is the condition.

Table 4: One way repeated measures ANOVA

Effect	F-value	Significance
Condtitions	F(3,15)=1.088	0.384

Table 5: Pairways comparison of CPM for all conditions

Condition		Significance
NRS 0	NRS 10	0.444
NRS 0	NRS 6	1.000
NRS 0	NRS 4	1.000
NRS 10	NRS 6	0.912
NRS 10	NRS 4	1.000
NRS 6	NRS 4	1.000

A significance level of 95% is chosen, this means there will be a significant difference for the within subject variable, when the significance value is lower than 0.05. The value of the significance in table 4 is thus compared to 0.05. This shows there is a non significant difference between the conditions. The value of the significance in table 5 is also compared to 0,05. This table shows there is no significant difference between any of the conditions.

5.2 Time

The mean average values of the post EPDT of all participants for time, are shown in figure 14. When paying attention to the mean values, it can be seen that the moments do not differ too much. It seems however that there is a slight increase in EPDT value in time.

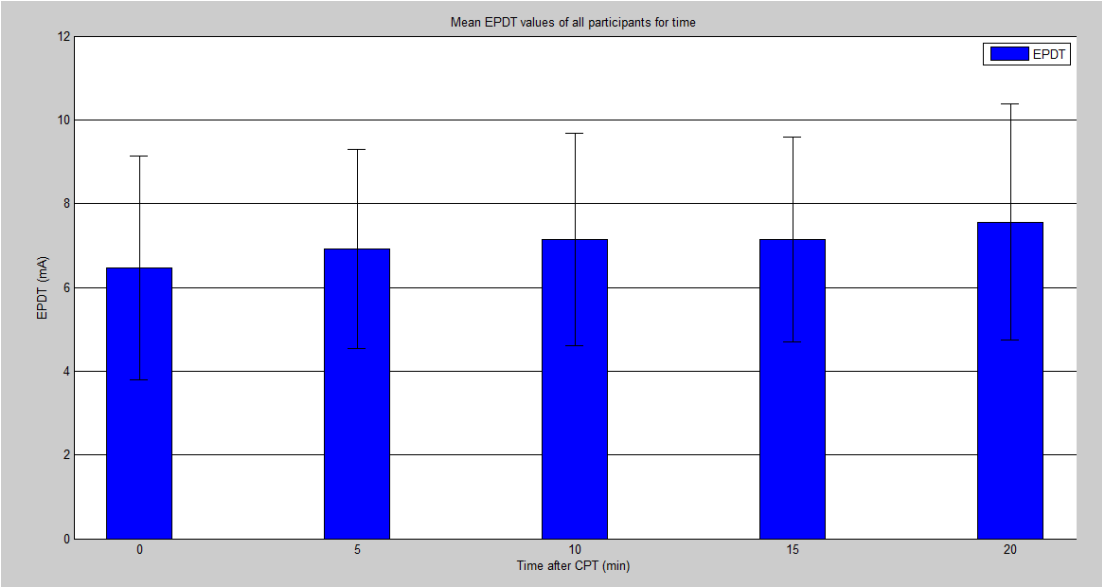

Figure 14: The average EPDT values of all participants for time

To answer how the inhibiting effect of the CPM behaves in time after removal of the conditioning stimulus, every 5 minutes the EPDT is registered after immersing till NRS 10. The values of the EPDTs are compared to the EPDT before immersing and the relative difference is calculated. This relative difference is plotted against the time in figure 15.

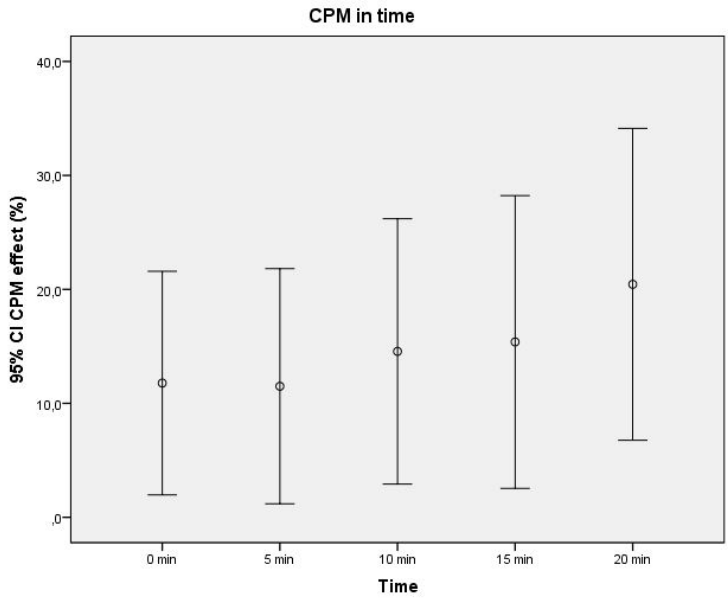

Figure 15: EPDT before detected NRS compared to EPDT after detected NRS

Seen from the graph it looks like the difference in EPDT increases exponentially in time. However, it is not conforming for all points. For example, when the measurement of 5 minutes is compared to the measurement of 0 minutes, the value is slightly decreased. In case of an exponentially growth it would have been increased. Also the value at 10 minutes should have been lower to portray an exponentially growth. Although these mentioned points at 5 minutes and 10 minutes deviate, the deviation is limited compared

to the 95% confidence interval for the relative difference in EPDTs, which is visible in figure 15. All these data is analysed with an one way repeated measures ANOVA, with all moments of measurement as within factors. The test showed these moments do not differ with a significance of 0.358. This is considerably larger than te 0.05 stated as limit for a statistical significant change of EPDT.

5.3 Reproducibility

The outcomes of the comparison between the test and re-test of the five participants are shown in appendix A.6. Appendix A.6 is showing the comparison between the test and re-test for NRS 10, 6, 4 and 0. In figure 16 is shown that three of the five participants had a larger CPM effect at the re-test and two of the five participants had a smaller CPM effect at the re-test. Figure 17 showed a different effect for NRS 6. Two participants had a larger CPM effect and two participants showed a smaller CPM effect. It is striking that one participant shows the same CPM effect at both test and re-test. For NRS 4 is shown in figure 18 the CMP effect of two participants decreases and that for the other three it increases. This seems to be the same effect as NRS 10. However each participant shows a different alteration when we compare NRS 4 and NRS 10. Figure 19 shows the comparison between test and re-test for NRS 0 where one participant is showing the same CPM effect at both tests. Furthermore two participants show an increase and two participants show a decrease in the CPM effect. Overall it can be seen that only participant 5 shows reproducibility for the re-test at the four conditions with a deviation at NRS 10.

Appendix A.6 shows the comparison between the test en re-test in the time. The outcomes of the measurements of the test and re-test at 0, 5, 10, 15 and 20 minutes after CPT 1 are compared. In figure 20 is shown that three of the five participants had a larger CPM effect at the re-test directly after CPT 1. On the other hand there were two participants who showed a decrease of CPM effect at the re-test directly after CPT 1. Figure 21 is showing the comparison between test and re-test 5 minutes after CPT 1. It is striking that only one of the five participants shows a decrease were the other four showed an increase of the CPM effect. The comparison of test and re-test for 10 minutes after CPT 1 is showed in figure 22. Here there are two participants who show a decrease and three who show an increase. Figure 23 is showing similarities with figure 21. The participants show the same effect 15 minutes after CPT 1 as 5 minutes after CPT 1. There is only a difference between the test and re-test of the participants at starting and ending points. Figure 24 shows similarities with figure 22. The participants show the same effect 20 minutes after CPT 1. The differences between the test and re-test of the participants are about equal. Overall there can be seen that participant 1 continuously shows a decrease when we compare the test and re-test to each other. Most participants show a decrease of the CPM effect when we compare the test and the re-test at the five different times after CPT 1.

6 Discussion

As mentioned in the results section there were a few things that could have affected the outcomes. Some participant saw the strength of the pulses in amperage during the different measurements after they released the red button of the nociTRACK. However, this value was not imaginable for the participants, so they had no clue what to do with it. Maybe some of them wanted to reach the same amperage, because they thought this was the purpose of the measurements. Even when they wanted to do this, it was hard to achieve because during application of the stimulus, the nociTRACK is not showing the value of the increasing current. So it would still have been a gamble for the participant at which amperage value he/she released the red button and this will therefore minimally affect the amperage of one participant.

Concerning the the distraction of participants, some studies mention that distraction leads to a decrease in the experience of pain of a test stimulus after a conditioning stimulus is applied.[60][61]. So there is a possibility that this distraction causes lower pain scores and thus a higher pain detection threshold. However, not all studies argue that distraction leads to a modulation of the experience of pain. In sum, the investigating studies demonstrated that distraction causes minimal changes in CPM and in general attentional processes are relatively insensitive to the effect of the CPM.[53] So it is thought that distraction has a minimal to none effect to the results of the pain detection threshold.

Furthermore, the use of muscle contraction as unpleasant feeling is not the most ideal strategy since this is a result of motoric stimulation by the electric current. In the experience of pain, the sensoric fibers of the neuronal network ought to be stimulated. This was discussed with the involved participants and they were asked to focus on the related unpleasant feeling. Since the values after discussing with the participants did not differ more than before the discussion took place, no bias resulting from this effect is expected.

A training session with the NociTRACK was executed to make patients acquainted with the feeling of increasing current. This neutralises the influence of different expectations. However, a moment where expectation is of influence but training does not help, is when the participant is searching for a recognisable point of EPDT. Since in the explanation of the study something about pain was written, and the test stimulus was the first feeling they got to experience, the participants were expecting pain. With some participants it was hard to overrule this idea however it was often repeated and described that "unpleasant" was the feeling that should be aimed for.

Some of the participants did not reach NRS 10 when immersing the foot in ice water for three minutes. In this study it is needed to conclude whether a maximal painful stimuli is required for activating the CPM effect or that also less painful stimuli are sufficient (NRS 4 and NRS 6). The ones who did not reach NRS 10 are not all excluded. It is decided that the level of NRS 9 is also high enough to be compared with NRS 4 and 6. However, one of the participants reached a maximal level of NRS 7. This level is not very deviating from NRS 6, that is why this participant is excluded. So this factor does not influence the results.

Conditions

The two way repeated ANOVA test (table 1) showed there was a non significant difference for the interaction effect of the pre-post and the condition variable, but there was a significant difference for both variables independently. To further explore the meaning of these significant values two additional tests are used. A pairwise comparison of conditions (table **??**) shows only NRS 10 is different from the other values. Figure 11 shows this result visually. This phenomenon can not be caused by the CPT itself because this does not influence the pre value. Therefore it is ascribed to a methodological factor. In figure 12 it can be seen that the first administered condition has the lowest related EPDT values for both before and after the CPT. This causes the suspection that NRS 10 differs from the other conditions because participants experience this at first but this can not be said for sure. Since the first condition is always NRS 10, the figure still shows EPDT values for NRS 10 are lower than for the other conditions. However, what can be said from figure 12 is that from the second condition on, the order is irrelevant. When separated for order, the combined results for NRS 6, 4 and 0 are the same. Another remarkable result from table 2 is that NRS 6 and 4 are also the same in EPDT values. One of the possible reasons why there is no difference between two conditions might have to do with the inadequacy of the NRS scale. Although participants

had already experienced NRS 10, they found it difficult to recognise a linear scale in their pain perception. Therefore it appeared to be difficult to separate NRS 6 and 4 from each other. Therefore the amounts of conditioning pain might have been closer to each other than intended with the two conditions. To avoid this from happening it might be advisable to use only three conditions, for example NRS 10, 5 and 0. Perhaps with this design it can be declared why both NRS 6 and NRS 4 show no significant difference with NRS 0.

The significance of pre and post values is also elaborated, in table 3. This table shows each condition has a significant difference between before and after the CPT, except in case of NRS 0. This suggests NRS 0 induces no CPM effect but since CPM effect is defined as relative increase in stead of difference, this should be tested separately. These relative increases are shown in figure 13. An one way repeated measures ANOVA points out the CPM effect does not differ significantly between the conditions. Because figure 11 indicates that during NRS 0 the EPDT did not change, the CPM effect for this condition is compared to the other three (table 5). From this table can be concluded that the CPM effect for NRS 0 does not differ from the other conditions.

This means the CPM effect occurs for each condition, and the effect is similar for each condition. This is not in line with the hypothesis since it would mean that a warm water tub is just as effective in pain inhibition as a most painful ice cold water tub. Possible factors that might have influenced this relation in line with this unlikely result are discussed below.

The temperature of the NRS=0 tub was set on 33 degrees Celsius. This was chosen because it is the average temperature of feet and therefore temperature should have had no influence. But every patient said the water was pleasantly warm. This is because all of the participants had already endured at least one cold water tub and therefore their foot temperature was decreased to below 33 degrees. So the temperature of the tub and foot was not the same and therefore might have had an effect on the CPM effect. To avoid this possible effect in further research the temperature of the foot should be measured and the water temperature adjusted to it.

Time

The results showed a seemingly increase in the post EPDT values in time. This could be a reflection of the true process of the CPM effect but this would not be in line with literature and the hypothesis. Most previous studies show that the CPM effect stops between 5 and 10 minutes, but there are studies that show that the CPM effect can even last one hour[**?**]. A factor that might play a role in this observation is habituation to the test stimulus. This idea came in mind because the pre-stimulation before the last randomised CPT was in every participant much higher than the pre-stimulation before CPT 1. This could influence the measuring of the CPM effect in time. The use of a repeated measures ANOVA pointed out that the change of EPDT in time is not significant. Therefore there is no change in time that can be pointed out.

Figure 12 gives more information regarding this point of discussion. This graph showed the pre value of the first CPT is significantly lower than of the later CPTs. To determine what causes this effect, figure 13 comes in handy. Since this figure shows the CPM effect for all the CPTs is almost the same, the sudden increase in EPDT value must be caused between the post value for the first CPT and the pre value for the second one. The only action performed between these moments is the five minutes repetition of test stimuli. So probably in this period habituation to the test stimuli advances.

In further research it is recommended to extensively prolong the training phase to make sure the process of habituation does not influence the research. Furthermore, it is important to measure longer than the 20 minutes after the first conditioned stimulus, because the CPM is still visible after these 20 minutes. Earlier research shows that one hour after the first conditioned stimulus there is still a CPM effect.

Reproducibility

During this study Red Dot 3M Cirkel electrodes were used to transfer the stimuli. The round shape of the electrodes made it difficult to place them precisely next to each other on the arm of the participants.

There is a chance that the space between the two stimuli is not the same for every session. This could give the participants different strengths of electrical stimuli during re-test compared to the first test.

To minimize the influence of multiple researchers, the test was practised together and instructions to be given were standardised as much as possible. Still, every researcher will respond differently to participants to explain the study. Furthermore, they will have a different way to clarify their instructions. This could affect the behaviour of the participants which could eventually affect the results of the measurements. The situation where it is most important to avoid this effect, test and re-test of one person were performed by the same researcher. In this way differences between researchers are not an obstacle for the comparison between the test and the re-test.

In the end 5 participants underwent a re-test. The results were displayed in charts to see if there was a link between the test an the re-test. Following the results only participant 1 showed reproducibility between test and re-test at different conditions. There was no participant who showed the same effect between test and re-test in time. Also participant 1 showed no link between the test and re-test in time. Because no clear point emerges from the charts, the ICC was calculated with the outcomes of these measurements for the four conditions and the five times. Although this showed some values, we can not draw a conclusion about the reproducibility. The reason for this is the fact that a group of five participants is to small in order to give reliable results. Further research is needed with at least 15 participants.

7 Conclusion

The study showed that there was no significant difference in CPM between the NRS values 10, 6, 4 and 0. So it can not be proven that subjective experience of pain is needed to induce the CPM effect. However this does not mean that a non or less painful stimulus will induce the CPM effect.

The inhibiting effect of CPM is not significant different within the 20 minutes after the first conditioning stimulus. The post values of all times after the first conditioning stimulus between 20 minutes are higher then the pre values before the conditioning stimulus. This means that the CPM effect stays the same over 20 minutes.

After this study it can not be concluded if the CPM effect is quantitatively reproducible by administering the same effect, because the study population is too small.

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A Appendix

A.1 Inclusion- and exclusion criteria

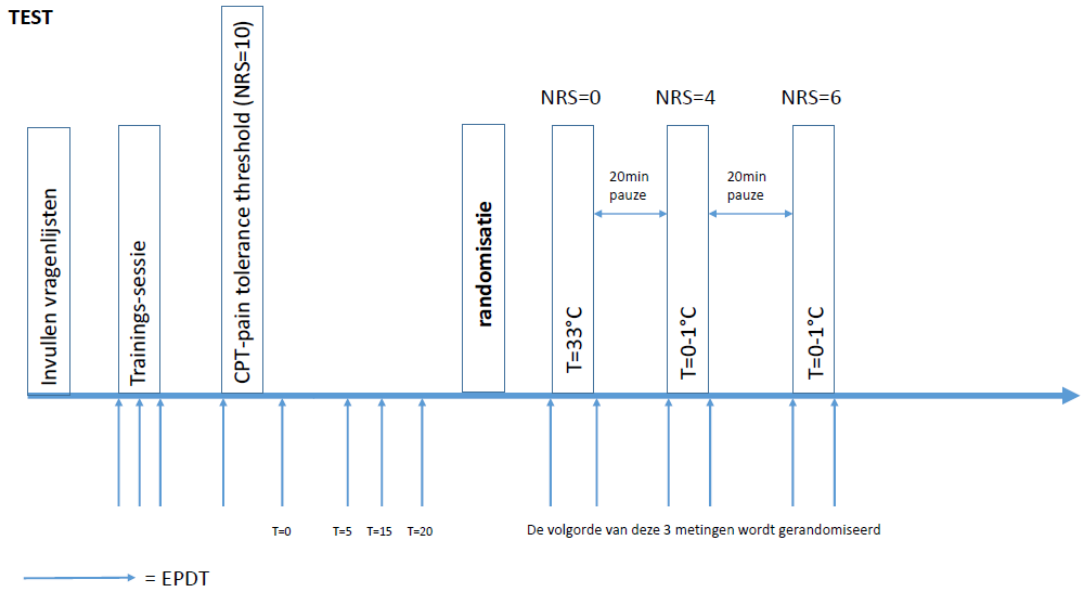
Inclusion criteria

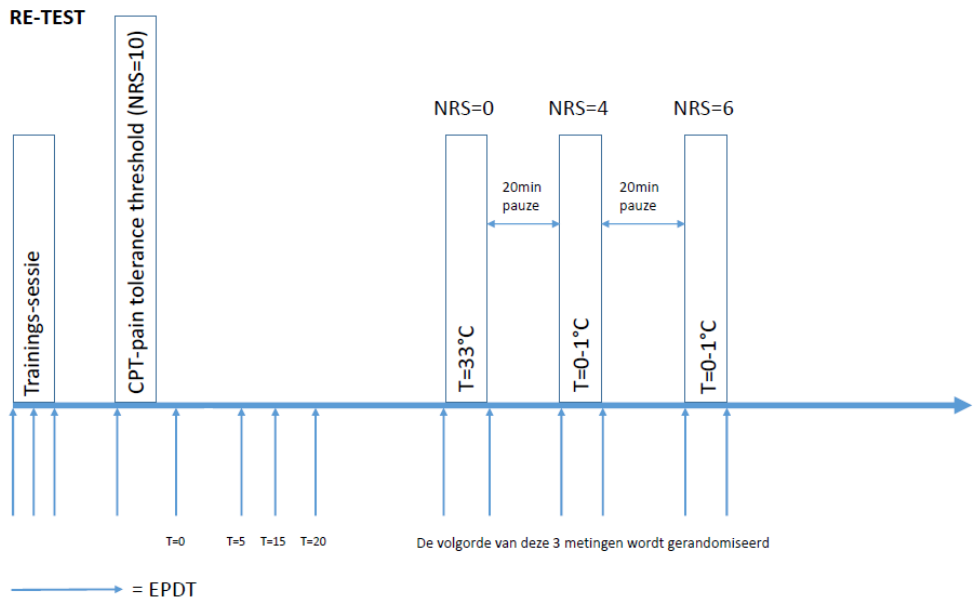
- Healthy subjects
- Ability to obtain informed consent
- Subject speaks Dutch

Exclusion criteria

- Use of analgetics
- History of psychiatric or neurological disease
- Chronic pain disorders
- Diabetes Mellitus
- Systemic illness
- (History of) substance abuse (drugs, alcohol)
- Kidney disease
- Disorders revealed during brief neurological examination
- Dermal lesions at the site of stimulation (i.e. psoriasis, ulcera, infection)
- Subject currently has an active implantable device including ICD/pacemaker
- Transmeridian travel within 1 month before the study

A.2 Protocol


TEST
Invullen vragenlijsten
Trainings-sessie
CPT-pain tolerance threshold (NRS=10)
randomisatie
NRS=0
NRS=4
NRS=6
T=33°C
20min pauze
T=0-1°C
20min pauze
T=0-1°C
T=0
T=5
T=15
T=20
De volgorde van deze 3 metingen wordt gerandomiseerd
= EPDT


RE-TEST
Trainings-sessie
CPT-pain tolerance threshold (NRS=10)
NRS=0
NRS=4
NRS=6
T=33°C
20min pauze
T=0-1°C
20min pauze
T=0-1°C
T=0
T=5
T=15
T=20
De volgorde van deze 3 metingen wordt gerandomiseerd
= EPDT

A.3 Form 'meetdata Ice study'

Meetdata ICE study

Proefpersoonnr: Onderzoeksdatum: sessie: 1 / 2

Geboortedatum	
Lengte (cm)	
Gewicht (kg)	
Geslacht (m/v)	

Meetdata

Meting 1:

eQST	EPDT												
Arm rechts (*pre*)													
IJswatertest	1-2 graden (max *3 minuten*)												
Tijd (sec)	0	15	30	45	60	75	90	105	120	135	150	165	180
NRS													
NRS (eind)													
CPT (sec)													
eQST	EPDT (t=0)												
Arm rechts (*post*)													

eQST					
T = 0	T = 1	T = 5	T = 10	T = 15	T = 20

Randomisatie volgende meetsessies (VAS=0; VAS=4; VAS=6)

Meting 2:

eQST	EPDT		
Arm rechts (*pre*)			
IJswatertest	33 graden (tot NRS = 0)		
Tijd (*sec*)			
eQST	EPDT		
Arm rechts (*post*)			

Meting 3:

eQST	EPDT		
Arm rechts (*pre*)			
IJswatertest	1-2 graden (tot NRS = 4)		
Tijd (*sec*)			
eQST	EPDT		
Arm rechts (*post*)			

Meting 4:

eQST	EPDT		
Arm rechts (*pre*)			
IJswatertest	1-2 graden (tot NRS = 6)		
Tijd (*sec*)			
eQST	EPDT		
Arm rechts (*post*)			

A.4 Collected data

Detailed values

Proefpersoon	Sessie	NRS10 Pre 1	NRS10 Pre 2	NRS10 Pre 3	NRS10 Post 1	NRS10 Post 2	NRS10 Post 3	NRS6 Pre 1	NRS6 Pre 2	NRS6 Pre 3	NRS6 Post 1	NRS6 Post 2
1	test	2,492	2,688	3,556	3,682	4,242	4,256	6,048	5,796	6,132	6,142	6,650
2	test	4,606	4,648	4,2	5,194	5,094	4,788	4,718	4,970	4,970	4,984	5,166
3	test	3,596	3,567	3,463	5,461	5,284	5,150	3,315	3,315	3,271	3,241	3,419
4	test	11,070	10,197	11,070	11,633	10,493	10,789	12,269	12,920	12,136	12,417	12,136
5	test	5,334	5,278	4,956	5,068	4,886	5,026	5,756	6,496	6,258	5,922	5,978
6	test	5,642	5,936	6,426	5,138	7,112	7,308	7,896	9,086	10,080	7,420	8,834
7	test	5,250	5,194	5,152	5,278	5,222	5,236	5,278	5,446	5,866	5,726	5,614
8	test	6,146	6,118	6,076	5,698	6,286	6,538	5,138	5,544	5,852	5,936	5,866
9	test	8,834	9,478	9,142	9,338	10,304	10,850	8,946	9,422	9,422	10,122	10,206
10	test	4,424	4,326	4,284	3,850	4,536	4,480	5,292	5,082	5,544	4,480	4,676
11	test	11,41	10,276	12,6	14,000	11,662	10,794	11,606	11,088	11,662	10,500	12,152
12	test	9,184	9,114	10,528	8,778	8,834	8,638	8,498	8,554	8,946	9,114	9,464
13	test	7,63	7,952	6,846	9,054	8,792	8,918	12,558	12,782	11,886	12,782	12,754
14	test	4,76	4,718	4,9	4,284	3,92	3,97	5,838	6,636	6,230	5,180	5,880
15	test	6,72	6,916	5,88	9,520	10,43	11,536	14,000	14,000	14,000	14,000	13,608
16	test	11,102	11,956	12,712	10,500	10,178	10,556	10,304	11,438	11,872	12,054	11,690
17	test	6,034	5,838	5,866	6,230	6,426	6,566	7,196	6,678	7,546	8,092	7,686
18	test	4,424	6,02	4,424	7,896	7,868	7,28	8,260	7,504	9,086	11,802	10,514
19	test	3,038	3,01	3,15	3,486	3,528	3,598	3,192	3,276	3,248	3,682	3,836
20	test	7,35	7,616	7,294	9,016	9,016	8,988	8,904	8,918	9,212	9,338	9,590
21	test	7,448	6,748	7,672	8,096	8,016	8,208	7,600	7,520	7,776	8,464	8,624
22	test	7,9032	8,4212	8,5692	8,806	9,1464	9,1908	9,946	10,449	11,796	12,314	12,817
1	retest	6,678	7,000	8,106	6,188	7,546	7,602	10,444	10,640	11,690	9,632	10,990
2	retest	5,88	7	7,24	7,5776	7,6072	7,7552	11,21	9,234	9,5756	9,694	10,4192
7	retest	5,46	5,432	5,376	7,658	7,742	8,022	5,642	5,978	6,062	6,916	7,112
9	retest	9,8272	9,8124	10,6116	10,0492	10,656	9,9752	9,9012	9,842	10,1084	11,47	11,396
20	retest	5,6	5,726	5,782	6,63+F4:F3	7,658	7,504	8,512	8,806	8,806	8,82	9,044
	mean	6,56	6,64	6,76	7,27	7,33	7,39	7,84	8,04	8,31	8,35	8,51
	std	2,58	2,50	2,90	2,83	2,50	2,59	3,02	3,09	3,07	3,29	3,20
	CV	0,39	0,38	0,43	0,39	0,34	0,35	0,39	0,38	0,37	0,39	0,38

NRS6 Post 3	NRS4 Pre 1	NRS4 Pre 2	NRS4 Pre 3	NRS4 Post 1	NRS4 Post 2	NRS4 Post 3	NRS0 Pre 1	NRS0 Pre 2	NRS0 Pre 3	NRS0 Post 1	NRS0 Post 2	NRS0 Post 3	NRS10 5min 1
6,342	6,020	6,314	6,622	6,468	7,028	7,070	7,098	7,672	7,350	7,882	8,288	8,582	4,452
5,530	5,866	5,782	5,572	5,376	5,992	6,174	5,264	5,866	6,188	4,816	5,488	5,824	4,368
3,419	2,560	2,768	2,723	2,353	2,575	2,753	3,300	3,256	3,271	3,108	3,226	3,138	4,928
11,529	11,189	11,500	12,476	12,980	12,373	11,796	11,085	10,538	11,130	12,151	12,550	12,210	11,736
6,202	5,712	5,908	5,516	6,272	5,880	6,188	5,586	5,796	5,908	6,958	7,028	7,448	5,334
8,680	9,646	9,660	10,024	10,010	9,814	9,436	9,366	9,408	9,632	9,996	10,570	9,842	8,050
6,006	5,166	5,376	5,502	5,222	5,656	5,544	5,224	5,572	5,488	5,110	5,432	5,586	5,236
5,852	5,180	5,474	5,640	5,348	5,488	5,446	5,068	4,984	5,054	5,096	4,984	4,998	6,538
10,850	7,588	9,100	9,184	9,030	8,848	9,044	8,610	8,862	8,582	8,960	8,890	8,708	8,722
4,480	4,480	5,642	4,956	4,522	4,830	4,480	4,172	4,186	4,634	4,466	4,354	4,284	4,452
11,550	12,306	13,300	12,530	14,000	14,000	14,000	12,922	11,830	10,542	9,310	11,116	11,214	11,648
9,366	8,694	9,114	8,344	8,778	8,498	8,428	8,848	8,120	8,582	8,484	7,462	8,358	8,722
13,566	11,816	12,796	12,572	12,012	12,460	12,810	9,338	10,738	10,668	10,514	10,262	9,828	9,016
6,482	5,250	5,810	5,698	6,510	4,984	5,166	5,838	6,636	6,230	6,460	6,692	6,482	4,270
14,000	14,000	13,972	14,000	14,000	13,930	14,000	10,360	10,332	12,614	13,762	13,225	14,000	11,340
12,362	11,564	11,988	11,438	11,732	11,634	12,740	11,704	12,054	11,970	11,746	11,858	11,830	10,234
7,686	6,286	6,496	6,804	6,790	6,734	6,930	6,090	6,860	6,944	7,056	6,986	6,986	5,768
10,794	5,782	6,762	5,950	9,240	8,988	9,254	9,604	9,142	8,610	7,658	8,414	9,366	6,244
3,920	3,486	3,528	3,542	3,668	3,850	3,752	3,108	3,220	3,248	3,234	3,500	3,304	3,234
9,632	9,240	9,478	9,604	9,478	9,982	9,898	11,712	12,544	12,016	11,472	12,272	12,128	8,540
8,416	8,432	8,144	8,016	8,320	8,160	8,768	8,368	10,144	8,608	7,920	8,384	8,176	8,096
12,787	10,967	9,502	9,087	12,506	12,654	12,240	9,442	10,419	10,079	7,770	9,827	10,256	9,590
10,890	10,038	11,284	12,376	11,200	12,166	12,726	10,752	11,284	12,810	11,438	11,942	11,788	7,714
10,1824	11,0704	11,1148	11,4404	11,1444	11,1296	10,5228	9,798	8,9984	9,472	10,7004	9,9308	11,1888	8,5692
7,168	6,118	5,586	5,152	6,902	5,838	6,944	7,826	7,42	7,644	6,23	7,154	6,426	7,742
11,3812	8,4212	8,0216	7,548	9,102	9,028	10,1676	8,6136	7,8292	8,7172	7,696	7,6664	7,5036	11,0852
9,548	9,8272	10,4044	10,3748	10,952	10,952	10,9816	10,0492	10,138	10,6116	10,3008	10,286	10,36	7,224
8,61	7,78	8,11	7,99	8,39	8,38	8,45	7,82	8,10	8,06	7,91	8,22	8,30	7,30
3,24	3,18	3,17	3,18	3,43	3,38	3,39	2,89	2,87	2,83	2,93	2,99	3,02	2,64
0,38	0,41	0,39	0,40	0,41	0,40	0,40	0,37	0,35	0,35	0,37	0,36	0,36	0,36

NRS10 5min 2	NRS10 5min 3	NRS10 10min 1	NRS10 10min 2	NRS10 10min 3	NRS10 15min 1	NRS10 15min 2	NRS10 15min 3	NRS10 20min 1	NRS10 20min 2	NRS10 20min 3
4,452	4,452	5,264	5,264	5,264	5,208	5,208	5,208	6,048	6,048	6,048
4,952	4,564	4,984	5,180	5,068	4,368	4,774	4,704	4,522	4,704	4,592
4,692	4,203	3,286	3,567	3,345	3,182	3,093	3,448	3,478	3,286	3,360
11,700	11,426	12,121	12,328	13,009	11,381	11,381	11,381	12,417	11,899	11,722
5,418	5,390	5,502	4,982	5,582	5,488	5,432	4,970	5,712	5,908	5,516
8,064	8,134	8,904	8,526	7,938	8,008	9,268	9,156	7,896	9,086	10,080
6,230	5,334	5,334	5,614	5,642	5,418	5,474	5,586	5,224	5,572	5,488
6,174	6,188	6,104	5,726	5,642	4,480	5,348	5,362	5,138	5,544	5,852
8,918	8,932	8,932	10,024	10,234	8,470	8,428	8,274	8,610	8,862	8,582
4,508	4,788	4,620	5,124	5,180	4,634	5,096	6,174	5,292	5,082	5,544
12,572	11,872	11,718	12,544	11,872	12,054	10,822	13,328	12,922	11,830	10,542
8,980	8,652	7,952	8,190	8,638	8,398	8,512	8,428	8,694	9,114	8,344
9,002	9,604	9,296	8,428	9,282	10,234	11,018	10,640	11,228	11,158	11,074
4,396	4,466	5,810	5,936	6,048	5,460	5,768	5,754	5,754	5,544	5,334
8,940	8,568	13,048	11,564	11,606	14,000	9,156	11,130	10,360	10,332	12,614
11,676	0,000	12,488	11,886	0,000	12,194	12,278	0,000	11,704	12,054	11,970
5,838	6,594	6,062	6,132	6,762	5,978	6,706	6,622	6,286	6,496	6,804
6,972	6,944	5,180	6,594	6,874	6,888	7,070	7,294	5,782	6,762	5,950
3,360	3,528	3,066	3,122	3,318	3,290	3,500	3,486	3,486	3,528	3,542
9,184	9,394	8,750	8,974	9,352	9,394	9,226	9,184	9,030	9,002	9,184
8,016	8,208	7,822	7,808	8,368	8,032	8,016	8,000	8,368	10,144	8,608
10,108	9,812	10,641	10,256	10,641	10,715	9,354	10,182	9,946	10,449	11,796
7,784	8,652	7,826	8,162	9,100	8,358	9,716	9,688	13,216	9,129	10,340
9,028	9,324	10,4192	10,478	10,5672	12,5356	10,952	12,4468	11,21	9,234	9,5756
7,826	7,854	8,722	8,106	8,022	8,316	7,14	6,888	7,826	7,42	7,644
11,4108	12,0916	9,1464	9,4276	9,398	10,36	11,174	10,9668	8,6136	7,8292	8,7172
7,84	7,854	8,036	7,882	8,106	8,218	8,302	8,554	8,512	8,806	8,806
7,46	6,87	7,59	7,63	7,26	7,60	7,50	7,20	7,63	7,84	7,84
2,67	2,88	3,02	2,86	3,18	3,14	2,64	3,14	2,88	2,82	2,93
0,36	0,42	0,40	0,37	0,44	0,41	0,35	0,44	0,38	0,36	0,37

Mean values

Proefpersoon	Sessie	NRS10Pre	NRS10Post	NRS6Pre	NRS6Post	NRS4Pre	NRS4Post	NRS0Pre	NRS0Post
1	test	2,91	4,06	5,99	6,38	6,32	6,86	7,37	8,25
2	test	4,48	5,03	4,89	5,23	5,74	5,85	5,77	5,38
3	test	3,54	5,30	3,30	3,36	2,68	2,56	3,28	3,16
4	test	10,78	10,97	12,44	12,03	11,72	12,38	10,92	12,30
5	test	5,19	4,99	6,17	6,03	5,71	6,11	5,76	7,14
6	test	6,00	6,52	9,02	8,31	9,78	9,75	9,47	10,14
7	test	5,20	5,25	5,53	5,78	5,35	5,47	5,43	5,38
8	test	6,11	6,17	5,51	5,88	5,43	5,43	5,04	5,03
9	test	9,15	10,16	9,26	10,39	8,62	8,97	8,68	8,85
10	test	4,34	4,29	5,31	4,55	5,03	4,61	4,33	4,37
11	test	11,43	12,15	11,45	11,40	12,71	14,00	11,76	10,55
12	test	9,61	8,75	8,67	9,31	8,72	8,57	8,52	8,10
13	test	7,48	8,92	12,41	13,03	12,39	12,43	10,25	10,20
14	test	4,79	4,06	6,23	5,85	5,59	5,55	6,23	6,54
15	test	6,51	10,50	14,00	13,87	13,99	13,98	11,10	13,66
16	test	11,92	10,41	11,20	12,04	11,66	12,04	11,91	11,81
17	test	5,91	6,41	7,14	7,82	6,53	6,82	6,63	7,01
18	test	4,96	7,68	8,28	11,04	6,16	9,16	9,12	8,48
19	test	3,07	3,54	3,24	3,81	3,52	3,76	3,19	3,35
20	test	7,42	9,01	9,01	9,52	9,44	9,79	12,09	11,96
21	test	7,29	8,11	7,63	8,50	8,20	8,42	9,04	8,16
22	test	8,30	9,05	10,73	12,64	9,85	12,47	9,98	9,28
1	retest	7,26	7,11	10,92	10,50	11,23	12,03	11,62	11,72
2	retest	6,71	7,65	10,01	10,10	11,21	10,93	9,42	10,61
7	retest	5,42	7,81	5,89	7,07	5,62	6,56	7,63	6,60
9	retest	10,08	10,23	9,95	11,42	8,00	9,43	8,39	7,62
20	retest	5,70	7,58	8,71	9,14	10,20	10,96	10,27	10,32
	mean	6,65	7,33	8,06	8,49	7,96	8,41	7,99	8,14
	std	2,63	2,60	3,04	3,23	3,16	3,39	2,83	2,96
	cv	0,40	0,35	0,38	0,38	0,40	0,40	0,35	0,36

NR$105min	NR$1010min	NR$1015min	NR$1020min
4,45	5,26	5,21	6,05
4,63	5,08	4,62	4,61
4,61	3,40	3,24	3,37
11,62	12,49	11,38	12,01
5,38	5,36	5,30	5,71
8,08	8,46	8,81	9,02
5,60	5,53	5,49	5,43
6,30	5,82	5,06	5,51
8,86	9,73	8,39	8,68
4,58	4,97	5,30	5,31
12,03	12,04	12,07	11,76
8,78	8,26	8,45	8,72
9,21	9,00	10,63	11,15
4,38	5,93	5,66	5,54
9,62	12,07	11,43	11,10
7,30	8,12	8,16	11,91
6,07	6,32	6,44	6,53
6,72	6,22	7,08	6,16
3,37	3,17	3,43	3,52
9,04	9,03	9,27	9,07
8,11	8,00	8,02	9,04
9,84	10,51	10,08	10,73
8,05	8,36	9,25	10,90
8,97	10,49	11,98	10,01
7,81	8,28	7,45	7,63
11,53	9,32	10,83	8,39
7,64	8,01	8,36	8,71
7,21	7,49	7,43	7,77
2,47	2,73	2,65	2,84
0,34	0,36	0,36	0,37

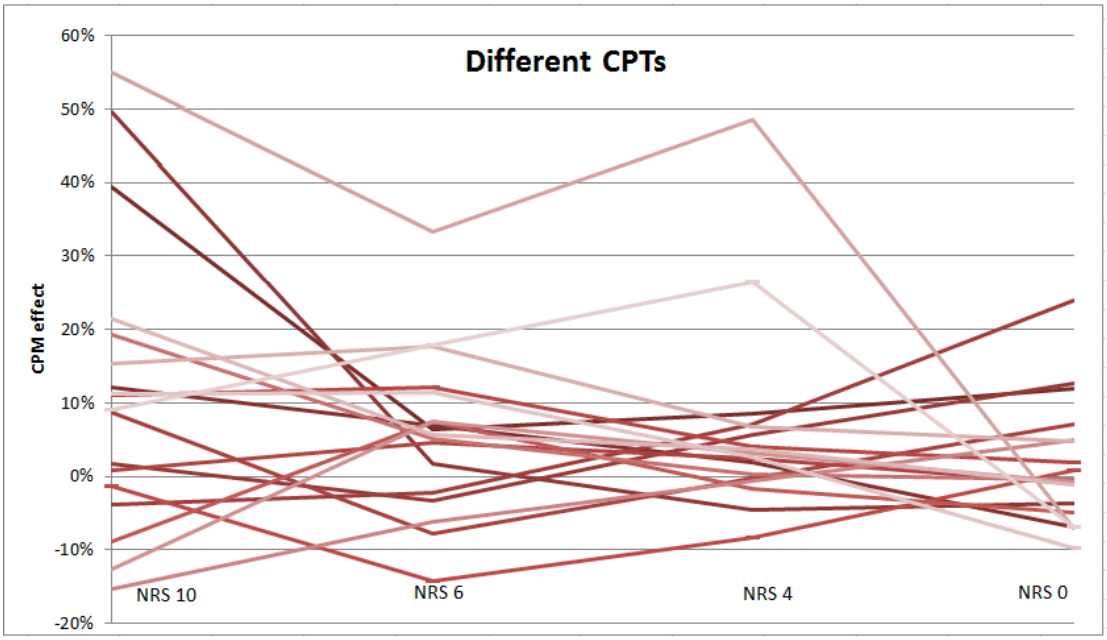
Relative difference values

Proefpersoon	Sessie	NR$10	NR$6	NR$4	NR$0	NR$105min	NR$1010min	NR$1015min
1	test	39%	6%	8%	12%	53%	81%	79%
2	test	12%	7%	2%	-7%	3%	13%	3%
3	test	50%	2%	-5%	-4%	30%	-4%	-8%
4	test	2%	-3%	6%	13%	8%	16%	6%
5	test	-4%	-2%	7%	24%	4%	3%	2%
6	test	9%	-8%	0%	7%	35%	41%	47%
7	test	1%	5%	2%	-1%	8%	6%	6%
8	test	1%	7%	0%	0%	3%	-5%	-17%
9	test	11%	12%	4%	2%	-3%	6%	-8%
10	test	-1%	-14%	-8%	1%	5%	15%	22%
11	test	6%	0%	10%	-10%	5%	5%	6%
12	test	-9%	7%	-2%	-5%	-9%	-14%	-12%
13	test	19%	5%	0%	0%	23%	20%	42%
14	test	-15%	-6%	-1%	5%	-9%	24%	18%
15	test	61%	-1%	0%	23%	48%	86%	76%
16	test	-13%	7%	3%	-1%	-39%	-32%	-32%
17	test	8%	10%	4%	6%	3%	7%	9%
18	test	55%	33%	49%	-7%	36%	25%	43%
19	test	15%	18%	7%	5%	10%	3%	12%
20	test	21%	6%	4%	-1%	22%	22%	25%
21	test	11%	11%	3%	-10%	11%	10%	10%
22	test	9%	18%	27%	-7%	19%	27%	22%
1	retest	-2%	-4%	7%	1%	11%	15%	27%
2	retest	14%	1%	-2%	13%	34%	56%	79%
7	retest	44%	20%	17%	-13%	44%	53%	37%
9	retest	1%	15%	18%	-9%	14%	-8%	7%
20	retest	33%	5%	7%	0%	34%	40%	47%
	mean	13%	5%	5%	2%	12%	16%	16%
	std	21%	10%	12%	9%	20%	27%	28%
	cv	1,59	1,86	2,15	4,68	1,69	1,64	1,76

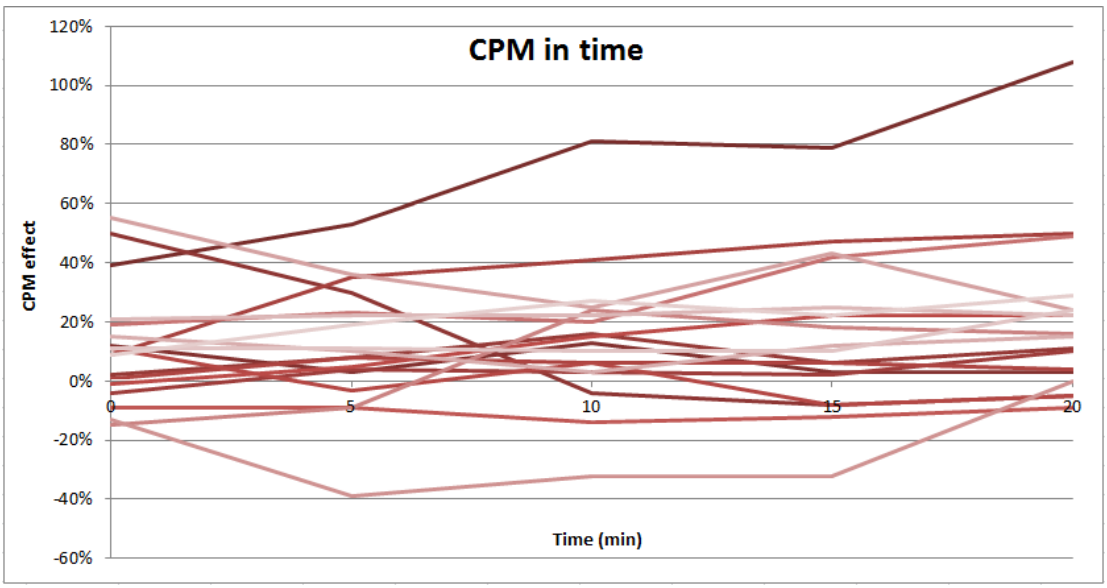
NR$1020min
108%
3%
-5%
11%
10%
50%
4%
-10%
-5%
22%
3%
-9%
49%
16%
71%
0%
10%
24%
15%
22%
24%
29%
50%
49%
41%
-17%
53%
20%
28%
1,40

A.5 Charts of collected data

Different CPTs



CPM in time



A.6 Test and re-test

Different CPTs

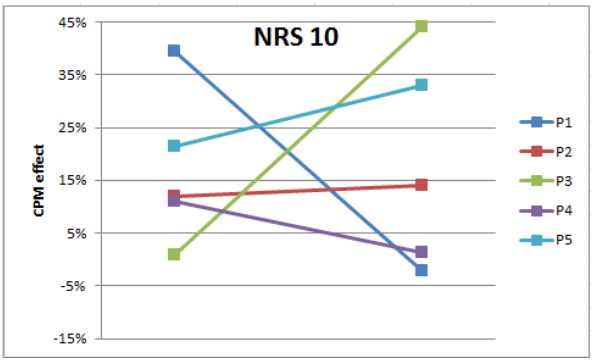


Figure 16: Combined test and re-test results of NRS 10 for the five participants.

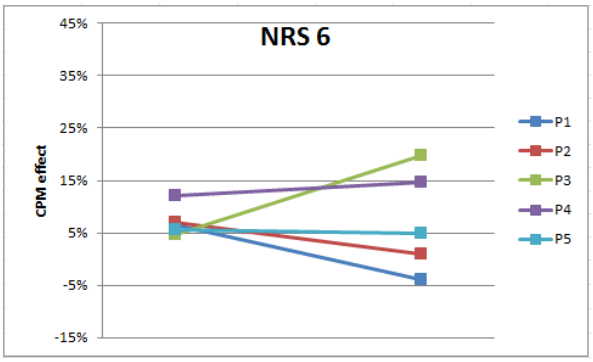


Figure 17: Combined test and re-test results of NRS 6 for the five participants.

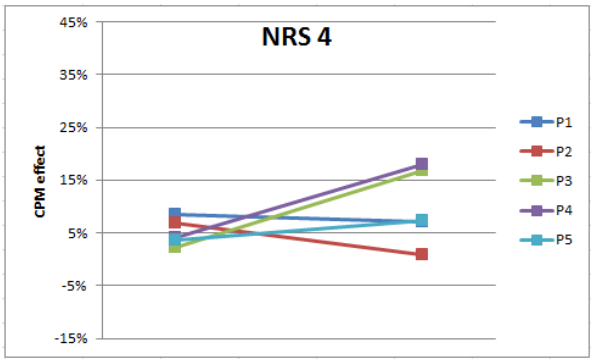


Figure 18: Combined test and re-test results of NRS 4 for the five participants

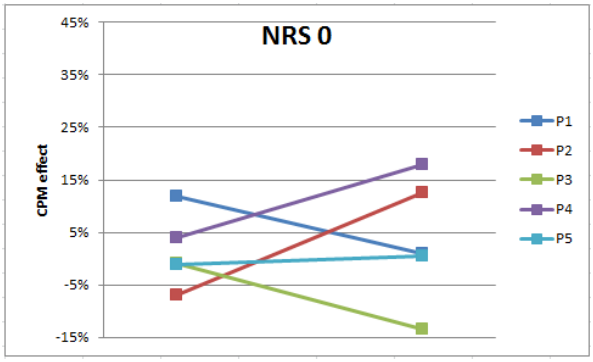


Figure 19: Combined test and re-test results of NRS 0 for the five participants

CPM in time

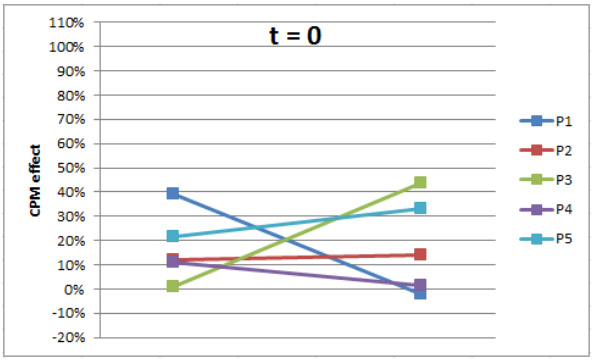


Figure 20: Combined test and re-test results after 0 minutes for the five participants. CPM

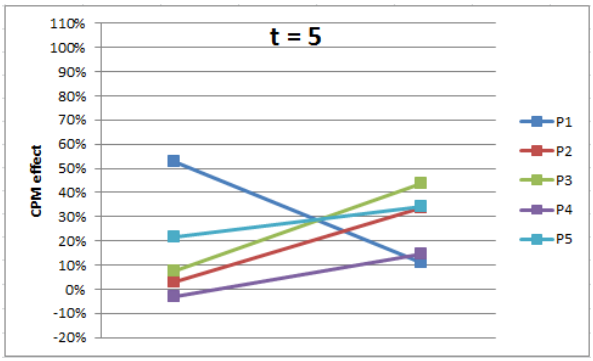


Figure 21: Combined test and re-test results after 5 minutes for the five participants.

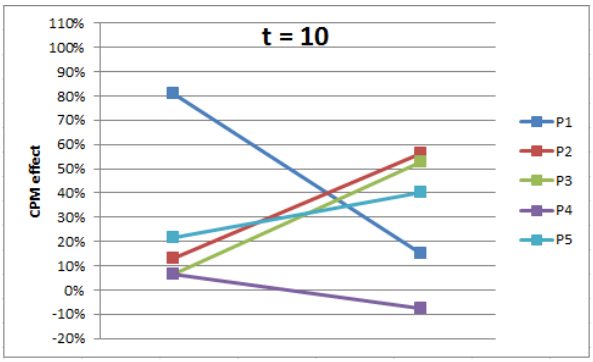


Figure 22: Combined test and re-test results after 10 minutes for the five participants.

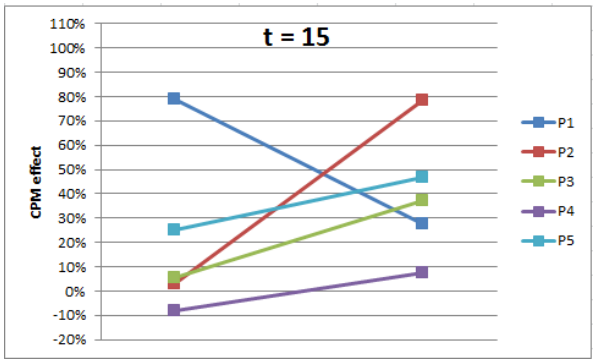


Figure 23: Combined test and re-test results after 15 minutes for the five participants.

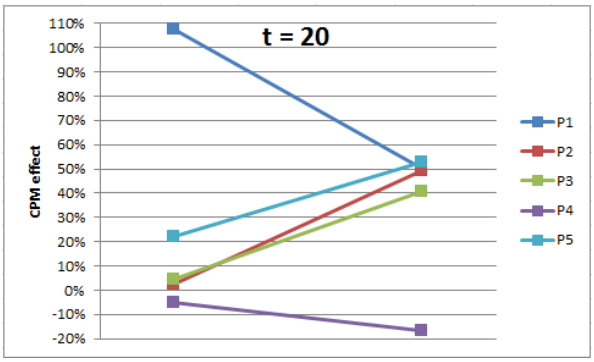


Figure 24: Combined test and re-test results after 20 minutes for the five participants.