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MORTEN HØGH

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by
Morten (Høgh) Hoegh

AALBORG UNIVERSITY
DENMARK

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PhD supervisor: Prof. Thomas Graven-Nielsen
Aalborg University

PhD committee: Associate Professor Christina Brock (chairman)
Aalborg University Hospital

Professor David Yarnitsky
Rambam Medical Center

Professor Stefan Lautenbacher
Otto-Friedrich-Universität Bamberg

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CV

Morten trained as a physical therapist (1996-1999) and after extensive post-graduate training and clinical experience he qualified as specialist physical therapist in musculoskeletal physical therapy (2005) and sports physical therapy (2006). He continued working full-time in clinical practice until 2010 where he attended the MSc Pain: Science & Society at King's College London (UK). After graduating he went back to clinical practice part-time while devoting more time to teaching graduates (medical doctors) and post-graduates (physiotherapists, occupational therapists and psychologists) in pain-related topics. In 2015 he applied for and was accepted as PhD-fellow at the *Center for Neuroplasticity and Pain* (CNAP), Aalborg University under a research grant funded by the Danish National Research Foundation (DNRF121).

Morten has been appointed to working groups within The Danish Medicine and Health Authorities, the Danish Council of Ethics and The European Pain Federation and has served as chairman of the Danish Society for Pain and Physiotherapy since 2006. He has authored or co-authored a number of peer-reviewed articles, a textbook on pain, several book chapters on pain and pain management, and he runs a blog on clinical aspects of pain. Morten insists on mentioning his family here as all of the above has been made possible through unlimited love and support from his wife, Lene, and their three kids; Signe, Andreas and Sarah.

ENGLISH SUMMARY

Around one in five adults suffer from chronic pain and the evidence from clinical and experimental studies suggest that it is associated with impaired pain inhibition, increased stress and less efficient executive functions. Pain modulation can be understood as a dynamic balance between facilitative and inhibitory pain mechanisms in the descending pathways. Common approaches to measuring the net-effect of descending pain modulation in humans are the *conditioned pain modulation* (CPM) paradigms. These paradigms study the effect of a painful conditioning stimulus on a test stimulus, compared to an unconditioned test stimulus; and can be categorised as a *bottom-up* (stimulus driven) mechanism. Conversely, pain can also be modulated via *top-down* (goal-oriented) modulatory mechanisms including expectation and attention. Social stress can be considered a hybrid between bottom-up (in relation to contextual allostasis) and top-down (influenced by perception) modulatory mechanisms. Bottom-up and top-down mechanisms are thought to share or have overlapping neurophysiological pathways.

This PhD project, comprising three studies, explored how repetition alone and in combination with stress or attention influences CPM in healthy men. In Study-I, the influence of repeated, painful stimuli on pain sensitivity and CPM was explored in two experiments: Repeated bouts with the same (*fixed*) conditioning stimulus intensity; and repeated bouts with *adapted* conditioning intensity. In both experiments a control session was applied, which included two unconditioned test-stimuli. In addition to exploring the temporal dynamics of pain sensitivity with and without conditioning, Study-I also provided rationale for the methods in Study-II and III.

Study-II combined a social stress model (Montreal Imaging Stress Task) and a comparable control-session with repeated pain measurements (with and without conditioning). In Study-III the Stroop task was used to

test the effects of attention on repeated painful stimuli (with and without conditioning). The overall aim of Study-II and III was to explore the effect of stress (Study-II) and attention (Study-III) on pain sensitivity and CPM, to explore the presumed interacting modulatory mechanisms.

Study-I showed that CPM-measurements could be repeated four times in 5-min bouts. Study-I also found the difference between the two test-stimuli in each bout (i.e. CPM effects and Control effects) were different; repeated test-stimuli (control session) led to cyclic increases in pain sensitivity with negative 'control effects' while positive CPM effects were found in the CPM-bouts. The study suggests that the temporal dynamic changes in painful stimuli involve non-linear effects and that the difference between control effects and CPM effects can provide a nuanced insight to the balance between descending facilitation and inhibition in healthy volunteers. In Study-II, CPM effects were found in all four sessions (before and after *stress* as well as before and after *control*). However, no significant changes in CPM effects from stress or control sessions could be found. In Study-III, it was found that application of Stroop to repeated test-stimuli, with or without conditioning, reduced pain sensitivity but not CPM effects. Study-III suggests that bottom-up and top-down modulatory mechanisms are independent of each other and that they may be seen as complementary rather than auxiliary mechanisms.

Individual differences in the response to conventional CPM paradigms provide indications for modality-specific differences. While the same modality was applied in all three studies an explorative analysis of the findings from all three studies suggest that 21% of the participants have a negative CPM effect during a pressure cuff CPM-paradigm. Furthermore, analysis indicates that responses to painful stimuli depend largely on how the individual reacts to the conditioning stimulus, rather than the test-stimulus.

This PhD-project indicates that CPM is a reliable and stable paradigm to study bottom-up pain modulation. In addition, it was shown that

repeated, unconditioned test-stimuli lead to negative, but cyclic, ‘control effects’ over time rather than to accumulated effects. Finally, this project finds that neither social stress, nor attention had any significant influence on CPM; and that attention can lead to analgesia independently of CPM.

DANSK RESUME

Ud af fem voksne vil én lide af kroniske smerter og det videnskabelige bevis, fra såvel kliniske som eksperimentelle studier, peger på, at der er en sammenhæng mellem kroniske smerter og smerteoverfølsomhed, øget stress og dårligere kognitive funktioner. Smertemodulation kan ses som en dynamisk balance mellem fremmende og hæmmende mekanismer i de signalvejene fra hjernen til rygmarven. Resultatet af de hæmmende og fremmende signaler (netto-effekten) måles ofte som med "Konditioneret smertemodulation" (eng. *Conditioned Pain Modulation*, CPM). Denne særlige model sammenligner et konditioneret (smertepåvirket) smertefuldt stimulus (*test-stimulus*) med et ukonditioneret test-stimulus, og kan kategoriseres som en *bottom-up* (provokeret) mekanisme. På den anden side kan smerte også påvirkes af *top-down* (motiverede) mekanismer, som bl.a. forventninger og koncentration. Kontekstuel stress (social stress) kan betragtes som en hybrid mellem bottom-up og top-down fordi det på den ene side er skabt af kontekstuelle provokationer og på den anden side er påvirket af vores tanker. Bottom-up og top-down mekanismer menes at benytte de samme eller i overlappende signalveje i nervesystemet.

Dette PhD-projekt, der består af tre studier, har undersøgt hvordan gentagelser alene og i kombination med stress eller koncentration påvirker CPM i raske mænd. Studie-I undersøgte hvordan smertesensitivitet og CPM blev påvirket af at blive gentaget med korte mellemrum i to forskellige eksperimenter: Gentagne runder med samme (*fixed*) intensitet af det konditionerende stimulus og gentagne runder med tilpasses (*adapted*) intensitet. Begge eksperimenter inkluderede desuden en kontrol-session med to test-stimuli uden konditionering. Studie-I skulle både give ny viden om hvordan gentagne runder af smertefulde stimuli påvirker raske mænd og bygge et metodisk rationale under de følgende studier.

Studie-II kombinerede en social stress model (Montreal Imaging Stress Task), og en sammenlignelig kontrol-session, med gentagne smertefulde

stimulationer med og uden konditionering. Studie-III undersøgte relationerne mellem koncentration og gentagne smertefulde stimulationer med og uden konditionering, ved hjælp af Stroop-task. Det overordnede mål for Studie-II og III var at undersøge hvordan stress (Studie-II) og koncentration (Studie-III) påvirkede smertesensitivitet og CPM, under formodning af, at dette kan give ny viden om evt. overlappende signalveje.

Studie-I viste at det er muligt at foretage fire på hinanden følgende CPM-målinger i omgange på 5 minutter. Studiet viste også, at forskellen mellem de to smertemålinger i hver omgang (hhv. CPM-effekterne og control-effekterne) gav forskellige resultater, hvor control-effekterne var negative og CPM-effekterne positive. Resultaterne peger på at smertefulde stimuli ikke udvikler sig lineært og at forskellen mellem control-effekter og CPM-effekter kan give et mere nuanceret indblik i balancen mellem de hæmmende og fremmende signaler i det centrale nervesystem hos raske forsøgspersoner. Resultaterne fra Studie-II viser, at der var positive CPM-effekter før og efter stress- samt før og efter kontrol-sessioner men at disse ikke er signifikant forskellige. I Studie-III viste resultaterne smerteoplevelsen i forbindelse med gentagne test-stimuli med og uden konditionering var reduceret i forbindelse med Stroop når man sammenligner med smerteoplevelsen uden Stroop, men der var ingen effekt af Stroop på CPM-effekterne. Resultaterne i Studie-III peger derfor på, at bottom-up og top-down modulation er uafhængige af hinanden, og at de skal ses som supplerende mere end som overlappende.

Resultater i litteraturen peger på, at typen af stimuli har betydning for hvordan individer reagerer på smertefulde stimulationer. I en undersøgende analyse af resultaterne fra alle tre studier, hvor den samme metode blev brugt på samtlige forsøgspersoner, viser resultaterne, at 21% af forsøgspersonerne reagerer med negative CPM-effekter (øget smertesensibilitet). Analysen peger desuden på, at der ikke er forskel på hvordan forsøgspersonerne reagerer på smertefulde stimuli generelt, men at en del af forskellen består i hvordan de reagerer på konditionerende stimuli.

Dette PhD-projekt indikerer at CPM er en reliabel og stabil model til at undersøge bottom-up smertemodulation. Her ud over viser resultaterne, at gentagne smertefulde stimulationer, uden konditionering, medfører ikke-lineære påvirkninger over tid. Projektet peger desuden på, at hverken social stress eller koncentration har signikant indflydelse på CPM og at koncentration i sig selv kan have en smertelindrende effekt.

ACKNOWLEDGEMENTS

No man is an island and this thesis would have never been possible without moral, technical, professional and personal support from more people than I can mention here. My first gratitude goes to all of you whose name did not make it into this section – you know who you are and I thank you.

A few people have been pivotal for this project. First and foremost, my family who have put up with me with a smile when I needed it the most. None of this would make any sense without you. As a 'late bloomer' in academia I'm very thankful to Thomas Graven-Nielsen for giving me a 'way in' to full-time academic work, and not least for agreeing to be my supervisor. I thank you for your arms-length support, for respectfully smiling with me over my mistakes, and for stepping in to help when I needed it.

I also want to express a special appreciation to the colleagues at Aalborg University who invited me into their social network and with whom I have shared ideas and frustrations (Kristian, Hjalte, Thorvaldur, Jeppe, Megan, Steffan, Enrico, Shellie and many others).

In addition to Thomas Graven-Nielsen, I have been fortunate to collaborate on research projects with Kristian Kjær Petersen, David Seminowicz, Jeppe Poulsen, Laura Petrini and Rogerio Hirata.

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PREFACE

The project leading to this PhD-thesis was initiated in May 2015. The data was collected at Aalborg University from October 2016 through March 2018. Analysis and submission of the articles comprising this PhD-thesis was finalized in January 2019. The entire project was funded by the Center for Neuroplasticity and Pain (CNAP) and supported by the Danish National Research Foundation (DNRF121). The third study in this thesis was conducted in collaboration with associate professor David A. Seminowicz of the Department of Neural and Pain Sciences, School of Dentistry, Center to Advance Chronic Pain Research, University of Maryland, Baltimore, United States.

The thesis summarises, compares and discusses the three studies in the light of the existing evidence. The first chapter introduces the topic of the project and chapter two provides a brief overview of pain modulation, as well as a contemporary understanding of the relationship of stress and attention on pain. The third and fourth chapters present the methods used to study pain sensitivity and descending pain modulation respectively, and explores the methods used to provoke them. Finally, in chapter six, conclusions are drawn and perspectives are proposed.

TABLE OF FIGURES

CHAPTER 1. INTRODUCTION

By definition, pain is an experience and thus only available to the person who experiences it[1]. In modern medicine, researchers and clinicians have extensive knowledge about the body at a systems level, at a molecular level and even at a genetic level. Yet, there still remains an explanatory gap[2] between what happens in the body and what a person perceives. This remains a scientific conundrum although treatment of pain has been everyday practice since the earliest of times by wise men, bonesetters and doctors who have been applying contemporary theories to their observations[3]. In their hallmark paper, Patrick D. Wall and Ronald Melzack, proposed for the first time in 1962 that molecular mechanisms in the spinal cord, not a psyche, were responsible for the modulation of nociceptive signals from the periphery and the experience of pain[4,5]. This monistic approach was later known as the Gate Control Theory[6]. Also, novel discoveries within the field of neuroscience, including neuroplasticity[7-12], endogenous inhibition of nociception and pain through painful stimuli[13,14], social stress[15,16], and cognition[17], have given an increased understanding of the relation between the body and painful experiences.

Chronic pain is a considerable burden on the individual, their families and the society[18,19]. The prevalence of chronic, non-malignant pain is estimated to be around 19% in Europe[20] and is associated with impaired pain inhibition[21-24], comorbidities such as stress[25-27], and less efficient executive functions[21-23,28]. Chronic widespread pain, which includes fibromyalgia syndrome, is estimated to affect 1 in 10 adults with twice the prevalence in women compared to men[29,30]. It has been suggested that heightened sensitivity to pain could be caused by dysfunctional pain inhibition[21].

When the perception of a painful stimulus (i.e. the pain sensitivity) is modulated by a heterotopic painful stimulus (conditioning stimulus), it is referred to as Conditioned Pain Modulation (CPM). CPM is believed to be

a proxy of the descending modulatory signals from subcortical regions of the CNS to the dorsal horn[31]. However, painful stimuli are not exclusive in utilising these neuronal pathways; similar activity is seen in situations involving cognitive load[32] and stress[33].

A recent meta-analysis found a reduced CPM effect in patients with widespread pain[34], compared with healthy volunteers and those with chronic low-back pain[35]. However, despite suggestions of impaired CPM in many clinical populations, it does not seem to be a good measure of clinical vulnerability[36]. Furthermore, only some patients suffering from painful syndromes[37] have been found to have impaired hypothalamic-pituitary responses, although it seems a fairly consistent finding in patients diagnosed with fibromyalgia[38-40]. This particular patient group have also been shown to have lower levels of cortisol, compared to healthy subjects and to patients with shoulder and neck pain[41,42]. A hallmark symptom of fibromyalgia is impairment of cognitive functions and studies find that attention is affected[43-45]. In summary, chronic pain – and widespread pain in particular – appears to be associated with dysfunctional CPM, dysfunctional stress-response and impaired cognitive functioning.

1.1. AIMS OF THE PHD THESIS

Neuroplasticity can be conceptualised as the ability of the nervous system to react to contextual changes through neuronal activity. At a clinical level, this is important because neuroplasticity is both regarded as a hallmark of persistent pain, and at the same time a possible pathway to treatment of persistent pain.

The aims of the studies leading up to this PhD thesis were to explore the plasticity of CPM over time, under stress and during cognitive load (Figure 1.1). See also Study aims, hypotheses and conclusions in Appendix B.

1.2. HYPOTHESES

Based on the body of evidence available at the time of planning the studies comprising this thesis, it was hypothesised that:

- Pain sensitivity with and without a parallel conditioning stimulus (CS) will habituate over four bouts within 20 minutes
- The pressure intensity of a CS will habituate over four bouts within 20 minutes
- Social stress will affect CPM more than a control condition, and
- Social stress will not reduce pain sensitivity
- Cognitive load can affect pain sensitivity and CPM.

1.3. OVERVIEW OF THE THESIS

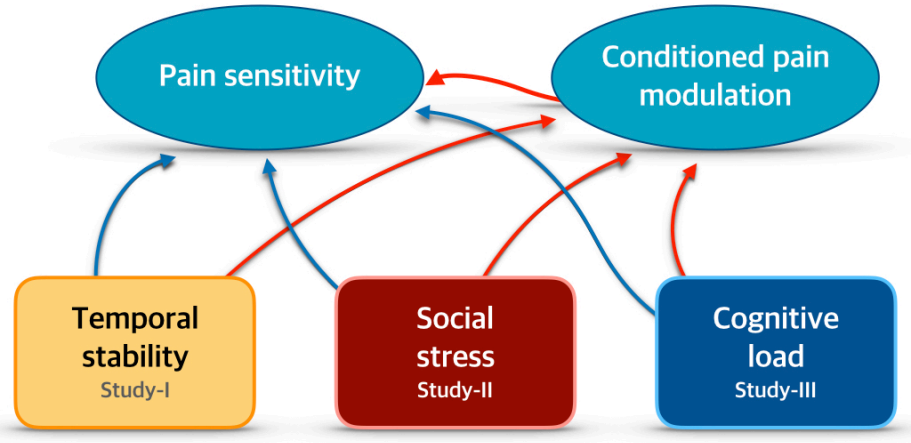


Figure 1.1 Overview of thesis

The thesis explored the stability of pain sensitivity and conditioned pain modulation (CPM) over time (Study-I), under stress (Study-II) and during cognitive loading (Study-III).

1.4. STUDIES INCLUDED IN THE THESIS

Study-I: Hoegh M, Petersen KK, Graven-Nielsen T. Effects of repeated conditioning pain modulation in healthy volunteers. *Eur J Pain*. June 2018. doi:10.1002/ejp.1279.

Study-II: Hoegh M, Poulsen JN, Petrini L, Graven-Nielsen T. The Effect of Stress on Repeated Painful Stimuli With And Without Painful Conditioning (under review)

Study-III: Hoegh M, Seminowicz DA, Graven-Nielsen T. The Effect of Attention on Pain Sensitivity (under review)

CHAPTER 2. A MECHANISM-BASED APPROACH TO PAIN MODULATION

While end-organ or line-labelling theories of pain[46] do not convey the complexity of pain experiences, it is still helpful to acknowledge possible contributions from the specialised nociceptive system [47,48]. A mechanism-based approach includes a comprehensive understanding of how nociceptive stimuli are conveyed to the brain and can be conceptualised as shown in Figure 2.1.

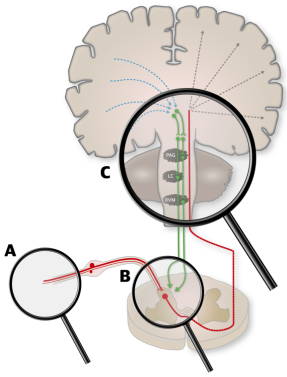


Figure 2.1 Pain modulation
Schematic overview over peripheral (A), central (B) and descending (C) modulation. Peripheral and central sensitization are the substrate of primary and secondary hyperalgesia. Descending modulation includes facilitatory and inhibitory mechanisms, which are commonly measured by their net-effect on pain sensitivity.

2.1. DESCENDING MODULATION OF NOCICEPTION

It has been established that noxious stimuli can facilitate descending signals, with facilitatory and inhibitory capacities, and thus provide a substrate for multifaceted, neuronal modulation from subcortical nuclei at the level of the spinal cord[49,50]. Much attention has been given to this mechanism with an emphasis on the (net) inhibitory responses[49] since it was described as *diffuse noxious inhibitory controls* (DNIC) by Le Bars and colleagues[14,51]. That nociceptive stimuli can lead to reduced pain sensitivity (i.e. that 'pain inhibits pain') has provided a framework for understanding how noxious stimuli can lead to a *bottom-up* pain inhibition. Other studies have found that cognition[50] and stress[52] can have analgesic effects on healthy subjects, thus providing evidence for *top-down* (i.e.

cognitive) pain inhibition. The underlying mechanisms for descending modulation of pain sensitivity have been studied extensively over the last 40 years[13,53] and several pathways have been suggested.

At the most basic level, dynamic, descending modulation predicts that descending signals affect pre- and post-synaptic nociceptive signalling and that the net-response will either be pro-nociceptive (painful) or anti-nociceptive (pain-reducing)[54]. In this framework, pain sensitivity in response to CPM and temporal summation of pain, can be seen on a spectrum with room for individual and contextual influences. It has also been suggested that saliency of any stimulus (sensory or not) is more closely related to the actual experience of pain than nociception itself[55], indicating that attention and repeated exposure to nociception may change pain intensity and/or sensitivity. At a mechanistic level, however, there seems to be consensus that descending modulation, whether *bottom-up* or *top-down*, is related to the descending pathways that connect the higher cortical neurons with the spinal neurons via central areas of the sub-cortical, supra-spinal nervous system[50].

PAG-RVM Pathway

The most well-described pathways involved in modulation of nociceptive transmission are the opiodergic-serotonergic-noradrenergic signalling pathways; from the periaqueductal grey area (PAG) in the midbrain via the rostroventromedial medulla (RVM) in the medulla oblongata to the spinal cord[50,56], see Figure 2.2. Data also suggests that acetylcholine plays a role in this pathway and that it may have a particular function in attention-related pain modulation via the amygdala[57].

Neurons in PAG (or RVM) can be activated by stimulus-driven, ascending nociceptive signals (bottom-up) and goal-driven, descending signals from the cortex or subcortical areas including the amygdala (top-down)[58,59]. In the RVM, two subsets of neurons (so-called *off-cells* and *on-cells*) project to the dorsolateral funiculus of the spinal cord where they have anti-nociceptive and pro-nociceptive effects on pre- and post-synaptic cells[58,60]. It has been suggested that the PAG-RVM pathway rely on

GABAergic interneurons that disinhibit otherwise tonically inhibited anti-nociceptive outputs at the spinal levels[52].

Locus Coeruleus

Located in the pons, the locus coeruleus (LC) is an area of the brain frequently associated with descending, noradrenergic anti-nociception in the dorsal horn[61]. In fact, the LC is key for the DNIC and very likely to also play a major role in CPM[62]. Furthermore, projections between the LC and the noradrenergic pathways in the cortex suggests an intimate relation between the LC and cognitive function, including attention[63].

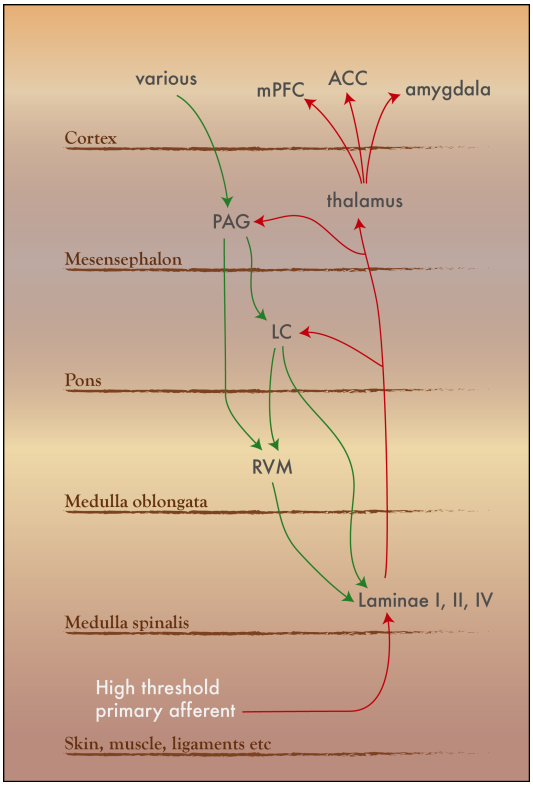


Figure 2.2 Schematic showing ascending and descending pathways

Detailed schematic of ascending and descending pathways involved in modulation of nociception. Abbreviations: Rostroventromedial medulla (RVM), Locus Coeruleus (LC), Periaqueductal Grey Area (PAG), medial Prefrontal Cortex (mPFC), Anterior Cingulate Cortex (ACC).

Cortical involvement in pain-inhibition

The associations between pain sensitivity and cortical activity are not fully understood although a relevant connection[64,65] has been established. A landmark study showed that while experimental pain did associate with stimulus intensity, neuronal activity in the cortex did not[55]. Currently, the *Default Mode Network theory* is the dominant thinking/hypothesis. It proposes that activity in the brain at rest has a time-independent, stimulus-dependent, brain-activation pattern[66], which can be studied. However, developments to this theory suggest that brain activities are dynamic and therefore bound to change with cognition (e.g. attention) as well stimuli (e.g. high threshold pressure)[67] which may end up confound many findings. Indeed, two phenotypes have been suggested; those who by default are more likely to attend to pain during pain, and those who by default are better at attending away during pain[68,69]. At a structural level there is evidence to suggest that attention away from pain, as well as stress, involves the frontal lobe (medial prefrontal cortex, mPFC), anterior cingulate cortex (ACC), amygdala and PAG[50,58,70-72].

Just as a conditioning, painful stimulus can activate descending pathways, it is believed that stress[73], attention[74], expectations[75] and exercise[76] can do the same.

2.2. STRESS AND PAIN

Stress is a normal response to changes in or around mammals when exposed to demands, which challenge homeostasis[77,78]. At a neurobiological level, stressful situations are closely associated with the hypothalamic-pituitary-adrenal (HPA) axis (Figure 2.3). This implies that triggers in the hypothalamus can signal to the adrenal medulla via the LC and other subcortical areas[79]. From the adrenal gland, monoamines and other signalling molecules are then secreted[80]. In parallel, but slightly slower, signals from the hypothalamus to the pituitary gland activate receptors on the cortex of the adrenal gland, via the bloodstream, which in turn releases corticosteroids (cortisol)[75]. The instant, neurochemical

signal to the adrenal medulla is responsible for the early responses in the nervous system (seconds to minutes), whereas the effect of corticosteroids will increase slowly thereafter[73,79].

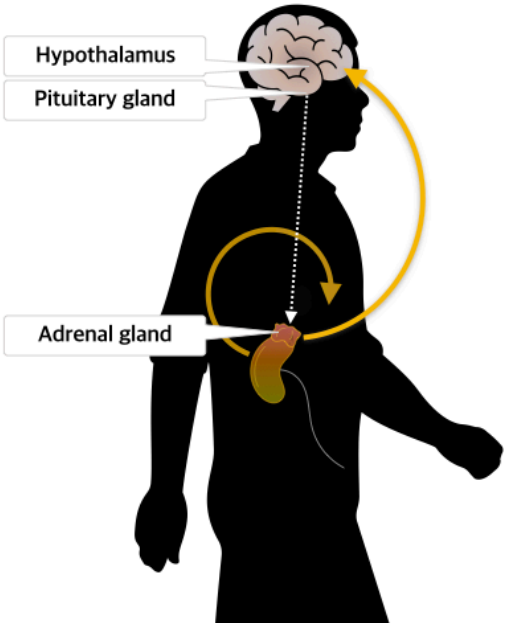


Figure 2.3 HPA-axis

The hypothalamic-pituitary-adrenal (HPA) axis. Signals from the hypothalamus are sent to the medulla of the adrenal gland as neurochemical signals via LC and via the pituitary gland as hormonal signals to the cortex of the adrenal gland. Circulating cortisol subsequently released from the adrenal gland will provide feedback to the hypothalamus.

Cortisol can be easily measured in saliva but the relationship between saliva-cortisol and stressors is non-linear, which makes interpretations difficult[81]. Less than 10% of the produced cortisol is unbound at any time and this residue can be measured in blood, saliva and urine[82]. Furthermore, cortisol levels in the body have a diurnal rhythm, which mean that levels are higher in the morning albeit still under influence of daylight, physical activity and stressful events[79,83]. Besides diurnal changes in cortisol production, there are a myriad of factors that can influence the level of freely available cortisol, including sex hormones and oral

contraceptives[81]. Furthermore, the cortisol measurements are only moderately associated with perceived stress and the two should be considered supplementary to each other rather than alternatives[81]. Beyond these limitations, salivary cortisol is safe and stress-free to sample; relatively uncomplicated to analyse; and has moderate-high intersession reliability[84].

Peak-levels of cortisol are difficult to predict but it has been found to be increased at various time points from 10 to 40 minutes after a stressful event or task[85,86]. In healthy volunteers the physiological stress-responses are regulated by negative feedback mechanisms, including inhibition of corticotrophin releasing hormones and adrenergic neurons[79].

It has been suggested that stress can lead to reduction in pain sensitivity, so-call stress-induced analgesia, via similar or overlapping mechanisms as CPM[52,72,87,88], including the PAG-RVM pathway[52] with a possible predisposition towards cannabinoid-dependent mechanisms[89,90]. However, it is currently unknown whether stress-induced analgesia also relies on activation of the HPA-axis[27]. Also, a study found that pharmacological suppression of cortisol in healthy twins could impact the effectiveness of descending modulation (i.e. CPM), which signifies that dysregulation of cortisol may be relevant in clinical populations[42,91]. In addition cortisol could have a direct influence on nociception via co-localisation of glucocorticoid receptors, substance P-receptors and CGRP-receptors[92]; and via regulation of cannabinoids[27] in the dorsal horn.

2.3. COGNITION AND PAIN

Cognition is a highly complex phenomena that includes the process of conscious decision making (i.e. ‘thinking about things’), and despite common agreement that the brain is involved in cognition, the mechanisms are far from understood[93]. Moreover, patients with chronic pain suffer from impairment of executive functions, such as attention[32,94,95]. Likewise, patients with chronic pain show less efficient pain-inhibition during CPM compared to healthy individuals[24,96]. The

suggestion has been made that there is an overlap between the mechanisms associated with CPM and selective attention[32,97-99] (see Chapter 2.1).

Given that attention is a subjective construct, studies can collect first-hand reports on perceived attention (e.g. 0 equal to 'no attention at all' up to 10 qualified as 'highest possible attention') or proxies such as reaction time. Experimental models for testing attention include the Dot-probe task (attentional bias), 3-Back task (working memory), variations of the Stroop task, and many more[100].

2.4. SUMMARY

The mechanism-based approach provides a neurobiological correlate to pain sensitivity that can be modulated in the periphery, at the spinal level and in the higher areas of the CNS. A well-established method to study descending modulation of pain sensitivity in humans is the CPM-paradigm but other methods exist. Evidence suggests that descending modulation stimulated by attention[101], nociception[102] and emotional stress[53] utilise the same brain regions, descending pathways and signalling molecules. Furthermore, the nervous system has the potential to be modulated (e.g. sensitisation or habituation) and thus displays an astonishingly high degree of adaptability and possibly individuality. The driving question in this thesis is therefore: how stable is the CPM-response when it is provoked repeatedly, or exposed to either social stressors or cognitive manipulations?

CHAPTER 3. PAIN SENSITIVITY

3.1. ASSESSING PAIN SENSITIVITY

Clinical manifestations of pain can often be spontaneous in nature, i.e. they appear in the absence of external stimuli. This is very common in inflammatory[103,104], neuropathic[105] and some widespread pain conditions[106,107]. Clinical examination of patients reporting pain commonly involves pain provocation tests[108] such as the application of manual pressure or stress on specific tissues. Experimental pain can be induced by well-established paradigms, e.g. injection of hypertonic saline[109], capsaicin[110] or nerve-growth-factor[111]. Experimental pain can also be evoked by more short lasting stimuli including pressure[112] and thermal stimuli[113]. Ideally, a dose-response relationship (Figure 3.1) between perceived pain and stimulus intensity is found in healthy subjects but certain factors, including individual (e.g. age[114,115] and gender[116,117]) as well as contextual (e.g. stress and attention), play an important role in pain perception.

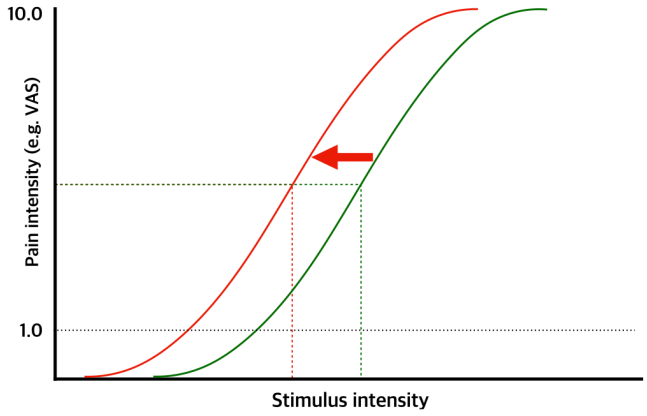


Figure 3.1 Stimulus-response graph

Schematic showing the ideal relationship between stimulus and pain intensity (in green) and how hyperalgesia represents a shift to the left (in red).

In clinical as well as experimental settings increased pain from a stimulus that normally causes pain is referred to as hyperalgesia. In order to assess pain sensitivity and its modulation, Study-I, II and III applied noxious test-stimuli, which were intended to be painful, to healthy volunteers. The following section will introduce the methods and discuss the results.

3.1. THE TEST STIMULUS

A test-stimulus (TS) can be any noxious stimulus but the most commonly used are noxious heat or pressure[118]; although cold water[17] and electrical stimuli[119] are frequently used too. The TS is intended to evoke pain, which can then be quantified[118] and recorded as *pressure-pain detection threshold* (PDT); *pressure-pain tolerance threshold* (PTT); sometimes as a stimulus intensity (e.g. kPa,°C, mA) equal to a pain-intensity (e.g. pain equal to 6 out of 10)[112]; or if the intensity of the test-stimulus is unmodifiable, *exposure time* (seconds) can be recorded as the output[120].

The different modalities (e.g. heat vs. pressure) do not produce identical results when it comes to pain sensitivity and CPM[121,122]. One reason may be that thermal stimuli affect nociceptors in the skin rather than the deeper tissues[123]; and mechanical stimuli, which are typically delivered via a probe[124] or a cuff[112], are considered appropriate for testing deeper tissues including the musculoskeletal system[125]. Reliability of heat as TS in CPM paradigms range from fair to excellent[118]. Intrasession reliability for pressure-pain threshold (administered via a probe) is excellent and both intra- and intersession reliability for pressure-pain detection threshold (measured by a pressure cuff on the calves) are good [112,126].

All studies included in this thesis used the computerised pressure cuff algometry system and an overview of the protocols is shown in Figure 3.2. A more detailed overview is found in Appendix B. The user-independent pressure algometer system was attached to the calves of the participants throughout each experiment and the software allowed for meticulous programming to ensure easy interaction with the software used to modulate stress (Study-II) and attention (Study-III).

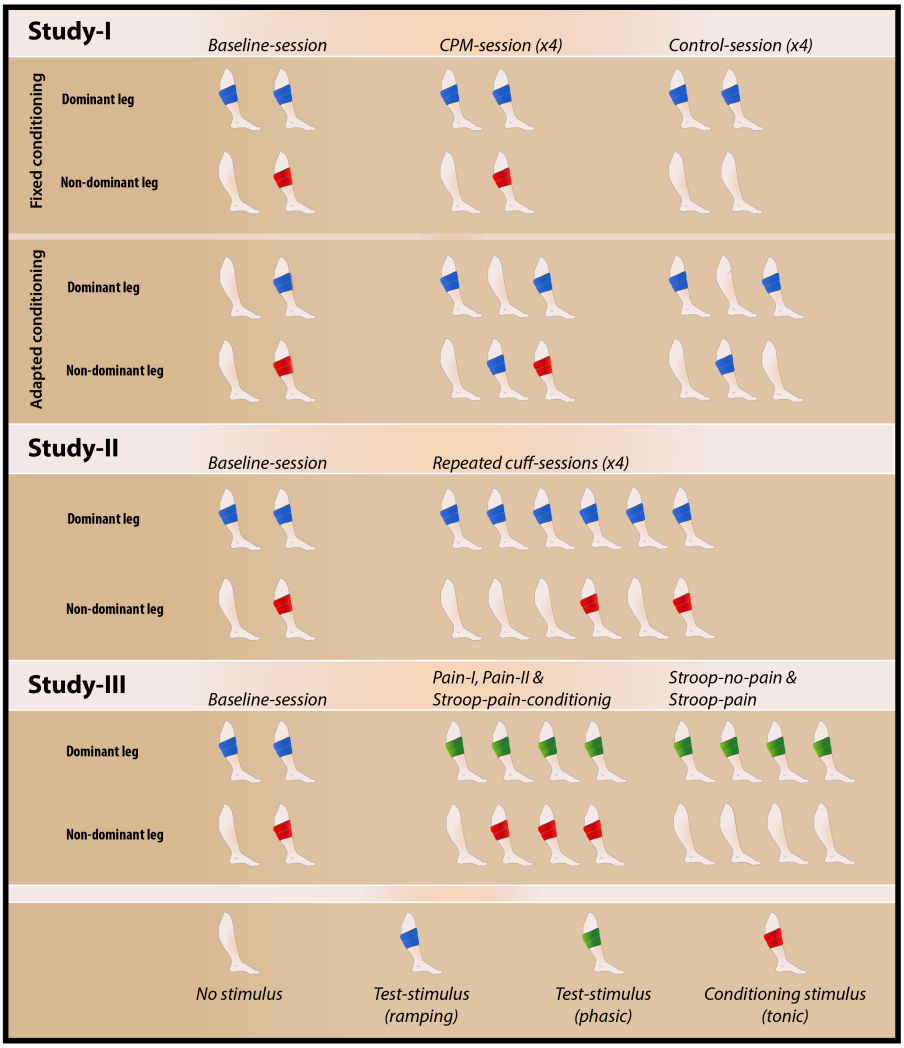


Figure 3.2 Pressure cuff protocols at a glance

Pressure cuff algometry protocols at a glance. Baseline test were similar for all experiments. Repeated TS in Study-I and II measured thresholds while Study-III recorded pain intensities on an electronic visual analogue scale (VAS, 10 cm), see Appendix B for further details.

3.1.1. PRESSURE CUFF ALGOMETER

The slowly increasing (1kPa/s) mechanical pressure, induced by computerized cuff algometry has been shown to be reliable[112,127,128]. A total of 90 baseline measurements of PDT and PTT were included in the three studies (see Figure 3.2). The pressure intensities equal to PDT or PTT, can be found in Table 3.1.

Table 3.1 Pressure-pain intensities at baseline

	Test stimulus (TS)		
	PDT	**PTT**	**N**
Study-I (fixed)	27.1 ± 3.0	63.6 ± 4.9	20
Study-I (adapted)	24.6 ± 2.9	64.4 ± 4.4	20
Study-II	26.4 ± 2.4	65.8 ± 4.3	25
Study-III	31.7 ± 2.3	68.7 ± 4.1	25
Mean	27.6 ± 1.3	65.8 ± 2.2	90

Pressure intensities in kPa (± SEM) at baseline

Participants were instructed that "*0 cm on VAS is equal to no pain"* and that "*10 is equal to the maximum tolerable pain*". PDT was defined as 1.0 and in most cases PTT was equal to 10.0 on VAS (maximal pain) although some participants in Study-I did not reach VAS 10.0 before the limits of the pressure cuff machine (100 kPa)[129]. A recent study found that supra-threshold pressure-pain ratings show good reliability (inter- and intrasession)[130]. In an analysis of the variance of test-stimuli from baseline PDT and PTT in all four experiments, no statistical differences were found between the mean (Figure 3.3). Analysis of all participants in the four experiments (n = 90) showed that mean PDT was 27.6 ± 1.3 kPa with a 95% Confidence Interval (CI) of 25.0 – 30.2 kPa, while mean PTT was 65.8 ± 2.2 kPa (95% CI 61.5 – 70.1 kPa).

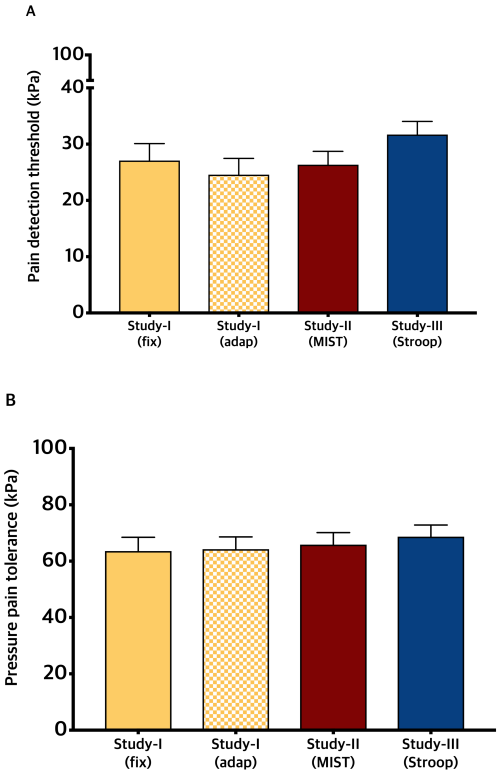


Figure 3.3 PDT and PTT at baseline

A: *Pressure (kPa) necessary to induce pain (PDT) presented as mean ± SEM for each of the four baseline measurements in Study-I, II and III. There is no statistical difference between the four means (ANOVA, P > 0.35)* ***B:*** *PTT in each of the four experiments presented as mean ± SEM.*

3.1.2. PHASIC PRESSURE TEST-STIMULUS

In addition to the more conventional *ramping* pressure test-stimulus it was necessary to implement a *phasic* pressure test-stimulus paradigm in Study-III. Rather than slowly increasing the pressure and measuring PDT and PTT, the phasic test-stimulus was set to 100% of PTT at baseline. Earlier studies have tested the influence of Stroop on pressure-pain sensitivity and used phasic pressure measured on a VAS-scale to fit the measurements to the Stroop paradigm[131,132]. Both studies found that Stroop was associated with pain inhibition, however no studies have

measured how long the effect of Stroop on pain sensitivity lasts . It was therefore decided to use a rapid, phasic TS (100 kPa/s for 5 seconds) rather than a ramping TS (1 kPa/s until PTT). Pilot studies showed that 5 seconds with the phasic TS was enough time for the participants to rate the intensity of the stimulus on an electronic VAS.

A phasic paradigm was used twice in other studies[133,134]. However, one study did not repeat the paradigm to assess reliability[133] and the other reported poor reliability of both tonic and phasic heat-TS[134]. Accordingly, two identical sessions with four phasic test-stimuli (three of which were conditioned) were compared as part of Study-III (Figure 3.4).

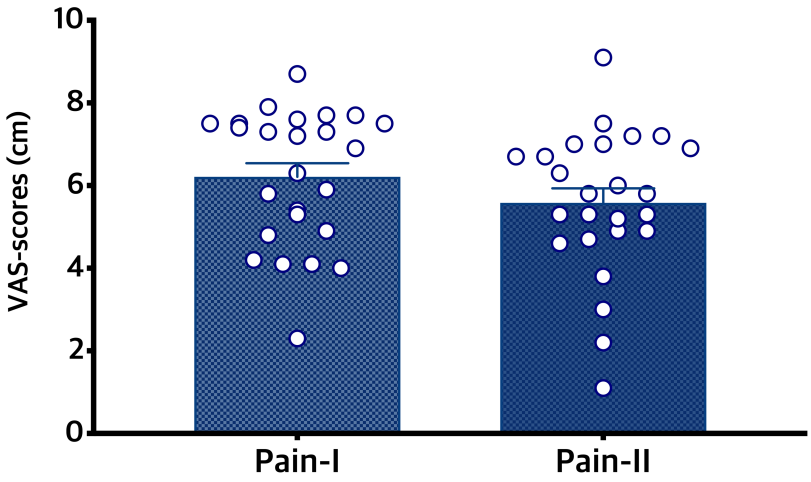


Figure 3.4 Phasic test-stimuli
VAS scores (cm) during the first test-stimulus in each of the two sessions without Stroop in Study-III.

The VAS scores (cm) of the first (unconditioned) TS were good (ICC = 0.8, 95% CI = 0.53 – 0.91). For all four TS as a group, reliability was excellent (ICC = 0.96, 95% CI = 0.92 – 0.98). The average VAS scores of the two sessions (Pain-I and Pain-II) before conditioning was 5.9 ± 0.3 cm. These findings support the majority of existing literature on reliability of a pressure cuff TS[135] although contrasts with the findings by Lie et al. (2017)[134]. Possible explanations for the discrepancy to Lie et al. (2017) can be both

gender and modality differences, as already mentioned, but could also relate to differences in the protocols: In Study-III participants did not move or remove the cuff between sessions, and sessions were identical as they were controlled by a computer (i.e. was user-independent). Also, the two sessions in Study-III were only separated by 5 minutes, whereas the sessions were separated by 30 mins in the study by Lie et al. (2017).

3.2. THE CONDITIONING STIMULUS

A conditioning stimulus (CS) is a painful stimulus applied to another part of the body during, in parallel to, or immediately after a test-stimulus. The CS can be any modality (thermal, chemical, electrical or mechanical[118,136,137]) and is thought to engage descending modulatory pathways (see Figure 2.2)[138]. The CS-modality may play a role in pain modulation[137,139], and one study found that electrical and heat TS, tested separately, in combination with pressure cuff conditioning were unable to generate CPM effects[126]. A recent study combined pressure-pain and heat pain, with hot water CS and found similar results[122]. Two perspectives arise from these findings: CS may be more effective for CPM represented by the same physical modality as TS (e.g. pressure TS and pressure CS) or when the same structures are influenced by TS and CS (e.g. skin)[126]. The other perspective is that perceptually incongruent stimuli (e.g. burning vs. pressure) may work less well in a CPM paradigm compared to perceptually congruent stimuli[122]. While the first two perspectives relate to the neuroanatomical arrangement, the latter implies that perception influences the effect of CS on TS. To optimise the effect of CS, the automated pressure cuff system was used for CS in all three studies since it incorporated the abovementioned factors, and because it provided the benefit of a user-independent CS, which was implemented into the same software as was used for the TS in all three studies.

In all four studies CS was applied to the calf muscle of the non-dominant leg while TS was applied to the dominant leg calf muscle. CS was defined as 70% of PTT on the non-dominant calf muscle and an analysis of variance found no differences between the pressure intensity of the CS

(kPa) between the four experiments (Figure 3.5 A), indicating a stable CS. The mean CS intensity (n = 90) was 42.8 ± 1.6 kPa (95% CI 39.6 – 46.0). Pain intensity of the CS was assessed on a numeric rating scale (NRS) from 0 (no pain) to 10 (worst imaginable pain). Mean pain-intensity ratings are shown in Figure 3.5 B.

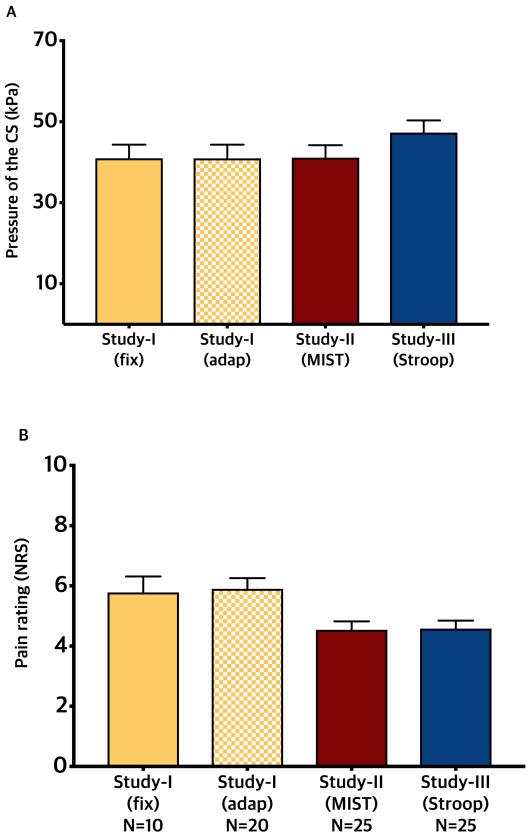


Figure 3.5 Intensity of conditioning stimuli at baseline

***A**: Intensity of the CS presented as mean ± SEM (kPa) for each of the four experiments showed no statistical differences (ANOVA: P > 0.91). **B:** Pain intensity of the CS (NRS, 0-10) was 5.8 in Study-I (fix, n = 10), 5.9 in Study-I (adap, n = 20), 4.5 in study-II (n = 25) and 4.6 in study-III (n = 25).*

3.2.1. HABITUATION OF CONDITIONING STIMULI AND CPM?

While the literature has focused primarily on pain facilitation over time (e.g. *temporal summation of pain*), habituation is known to play a role in pain sensitivity in healthy individuals as a result of tonic[140] and phasic cuff-pressure[111,141]. This was a focus in Study-I.

It seems that a noxious CS is necessary for CPM paradigms to have effect and studies suggest an association between conditioning intensity and CPM effects may be present[142,143,144]. Moreover, a study has suggested that higher intensities are necessary to maintain pressure-induced pain over time due to habituation to the stimulus[140]. Study-I explored the influence of repeated bouts of CS on CPM. The primary concern was whether changes in CS over time (pressure or pain intensity) could influence CPM. It is plausible that a moderately painful stimulus (CS was equal to 70% of PTT, see Methods at a glance) can induce either facilitative or habituative responses depending on the delivery of the painful stimuli[140,145]. It would seem that tonic stimuli appear to lose saliency[55] over time; while cuff-induced pressure or pin-prick repeated 10 times with less than 3s between stimuli, will typically lead to facilitation (increased pain)[24,141]. To test this, Study-I was split into two experiments on the same cohort (n = 20). The two experiments were separated by 1-4 weeks. In one study the same CS was used throughout the experiment (fixed according to baseline sensitivity) and in the other, CS was adapted changes in pain sensitivity on the non-dominant leg within each bout (adapted).

Based on the existing literature it was hypothesised that a fixed intensity of the CS (i.e. 70% of PPT at baseline) would habituate over time, while an adjustment of CS-intensity within each bout would void habituation over time (see Appendix A.1 and ***Figure 3.6***). No differences were found between baseline CS. As hypothesised, the pressure intensity of the *adapted* CS increased over the four bouts compared to baseline ($P \leq 0.05$) and to comparable time points in the *fixed* experiment ($P \leq 0.01$) as shown in ***Figure 3.6*** A. The NRS-rating (pain intensity) of the CS was, however, not different between the two experiments (***Figure 3.6*** B).

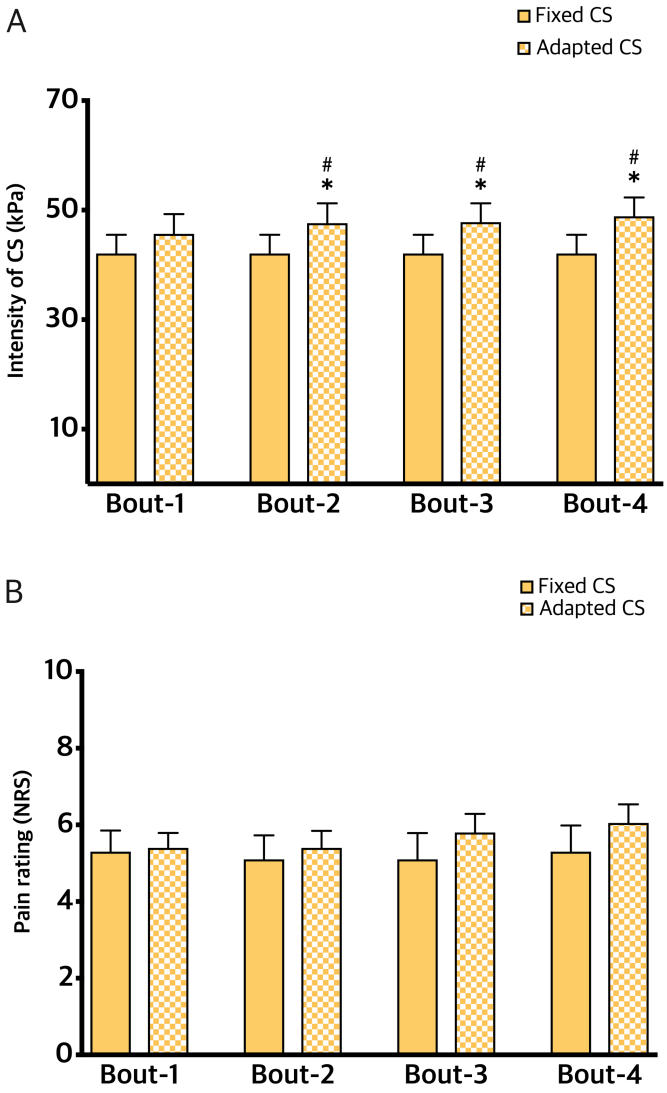


Figure 3.6 Pressure and pain during conditioning (Study-I)

A: *Pressure intensity of the CS increased in the adapted experiment compared to baseline (*, P ≤ 0.05) and to the fixed CS (#, P < 0.01).* ***B:*** *Pain intensity (NRS) of the CS did not change over time and were not different between experiments. All data are displayed as mean + SEM.*

3.3. EFFECTS OF REPEATED TEST-STIMULI WITHOUT CONDITIONING

Most studies that have examined repeated TS have tested intra-session reliability (see Appendix A.1 for overview). One study tested repeated TS following up to two mins conditioning with cold water[146] and concluded that dynamic changes in pain sensitivity increases the chances of detecting differences in CPM efficacy over time. In Study-I, repeated, unconditioned test-stimuli were used to study normal responses over time and as a control session for conditioned, repeated TS (see ***Figure 3.2***). A recent study, tested six repetitions of heat- and pressure-induced painful stimuli respectively, and found an increase in pain rating (mean vs baseline), for both modalities[122]. In contrast, repeated painful test-stimuli with electrical stimulation[147] and repeated pressure cuff[128], which both show signs of habituation over time.

To assess the difference between baseline and TS of the (unconditioned) control sessions in the two experiments in Study-I, a comparative analysis was conducted. The only difference between the two experiments was the additional TS on the non-dominant leg in the adapted experiment (used to calculate CS-intensity). Analysis shows that the mean of the repeated TS in the control session of the fixed experiment did not change compared to baseline ($P = 0.3$) and that there was a significant increase in PDT in the control-session of the adaptive experiment ($P = 0.047$); indicating that habituation only occurred over time when both legs were stimulated (see ***Figure 3.7***). There are two possible explanations for this: Firstly, the additional TS on the non-dominant leg might unintentionally act as a serial CS and secondly, the additional TS on the non-dominant leg has increased the nociceptive/sensory barrage to the CNS and given less time without stimuli. It is possible that the two explanations could be reflecting the same mechanisms (i.e. more stimuli into the CNS). To measure this, it would be necessary to compare the protocol for the adapted experiment to a similar protocol but with three stimuli on the same leg.

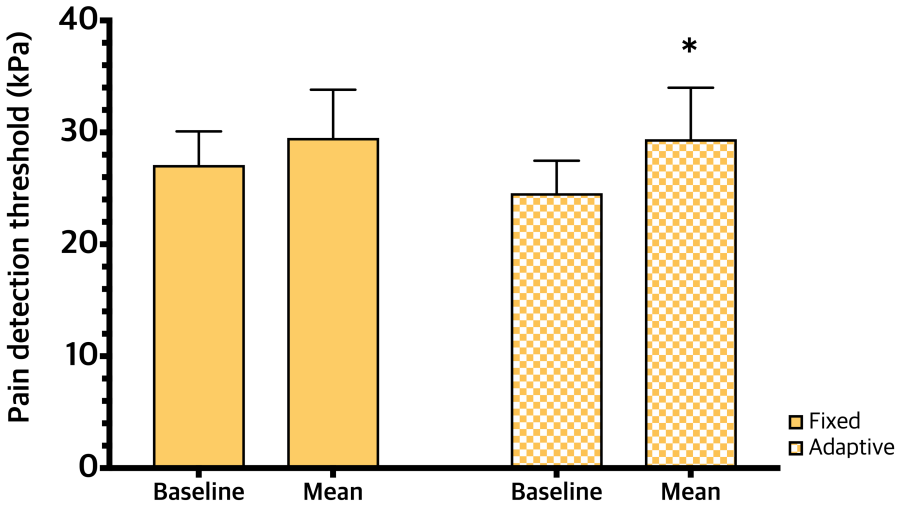


Figure 3.7 Fixed and adaptive PDT (Study-I)

Results from two paired T-test (baseline TS vs mean of all 8 TS in the control-sessions) from the fixed- and adapted-experiments in Study-I (N = 20). No statistical difference was found in the fixed experiment (yellow bars) but PDT increased over time in the adapted experiment (P = 0.047), which may indicate habituation.

The intention of the repeated TS in the control session of Study-I, was not to explore mean effects but to evaluate the effect of paired TS within each bout as a control for the repeated CPM session. Similar to the calculation of a CPM effect by subtracting threshold values during conditioning from threshold values without conditioning, it was suggested that a control effect could be calculated within each bout in a similar way (2^{nd} TS subtracted from 1^{st} TS). In this way a positive control effect would mean the same as a positive CPM effect, i.e. pain inhibition.

Unexpectedly, in the control sessions there was a decrease in PDT from the first to the second TS (i.e. a negative control effect) in Study-I (see Figure 3.8). Mean control effect over the four bouts was minus 0.3 ± 0.9 kPa for the fixed and minus 3.3 ± 1.3 kPa for the adapted experiment.

Due to the apparent cyclical change in PDT from the first to the second TS, the control effects are unlikely to be as a result of time-dependent habituation and so are more likely to relate to the repeated stimuli.

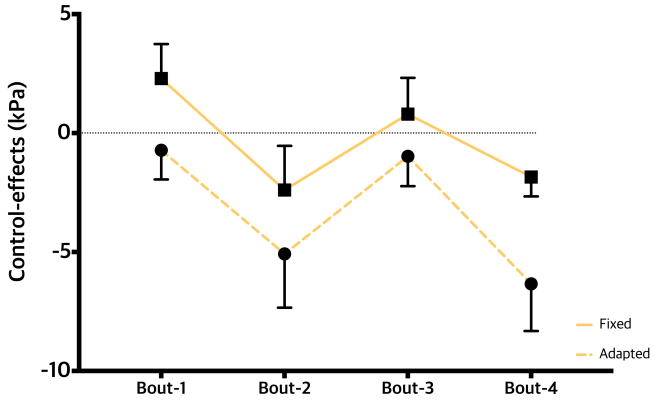


Figure 3.8 Control effects graph

Control effects in Study-I (N = 20) presented with in kPa with SEM. In the control session of the fixed CS experiment, two of the four control effects were positive, whereas all of the control effects were negative in the adapted experiment.

The mean control effect over four bouts was closest to zero (neutral) in the fixed experiment, which seems most rational for a control condition. The influence of time on TS is, however, not trivial and future studies could look into the influence of bilateral vs unilateral TS. If the additional TS on the non-dominant leg in the adaptive experiment acted as a serial CS and thus led to CPM effects rather than control effects, it is interesting that these effects were negative since most people, including those in Study-I, react to CPM with positive effects (i.e. pain inhibition).

3.4. CONDITIONED PAIN MODULATION

A range of factors are associated with the effectiveness of CPM in healthy humans and understanding individual differences may help clinicians differentiate patients with existing chronic pain conditions[21,35] and thereby personalise pain medicine[148].

3.4.1. INDIVIDUAL RESPONSES TO CPM

Larger CPM effects have been associated with 5-HTTLPR (an allele related to serotonin-transport), and CPM effects are generally larger in physically active people compared to less physically active[149]. Gender and hormonal status may also matter since higher CPM effects have been found in men compared to women[117,149]. In women, higher CPM effects are found during the ovulatory phase of the menstrual cycle[149]. CPM has been reported to be higher in younger adults compared to older[149], however no effect of age on CPM was found in a large cohort of healthy men and women in Denmark[140]. In addition, cognitive factors, including positive expectations and attention towards the CS are also associated with larger CPM effects[149]. Interestingly, there seems to be some overlap between CPM effects and placebo (or nocebo) effects likely due to expectations playing a role in both situations[150,151]. Despite this overlap, the analgesic effects from CPM paradigms and placebo/nocebo are not correlated but may potentiate or neutralise each other when applied simultaneously[50].

Study-I, II and III included only healthy men to reduce any variability related to hormonal influence. The median age was 26 years (mean 29.4 ± 1.2) and the age span was between 18-72 years of age.

3.4.2. CATASTROPHIC THINKING

Studies have suggested that catastrophic thinking can influence pain sensitivity[152-154] and a negative correlation with CPM has been reported[155]. The *pain catastrophizing scale* (PCS) is a questionnaire that is frequently used in clinical and experimental pain to measure the influence of catastrophic thinking and pain[156]. In clinical populations pre-treatment scores above 24 points have been associated with higher pain scores post-treatment[157]. There are no cut-off scores for high vs. low pain 'catastrophizers' in experimental pain settings but correlations between experimental pain sensitivity and the PCS have been found[158].

In Study-II and Study-III the PCS[152,159] was used to assess catastrophic thinking. The mean score (n = 50) was 9.6 ± 0.9 (of 52 possible points, Figure 3.9), which is slightly lower than a comparable cohort of 118 healthy Danish men (mean 10.3 ± 0.6)[152]. The results indicate that participants were not 'catastrophizing' about pain, and part of the reason why no correlations were found between catastrophic thinking and pain sensitivity or CPM in either of the studies, could relate to this.

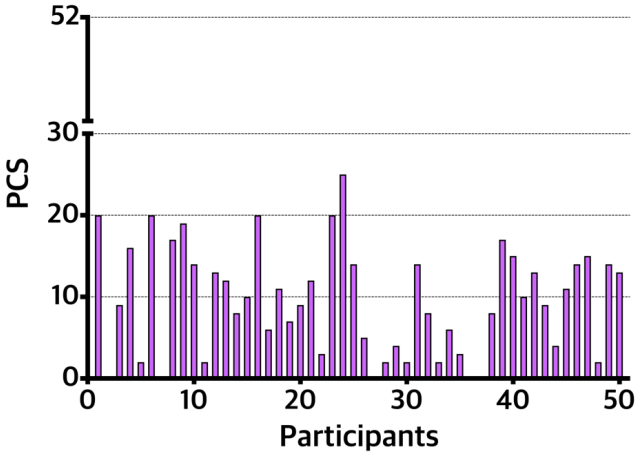


Figure 3.9 Pain Catastrophizing Scale
Individual pain catastrophizing scores (PCS) in Study-II and Study-III (n = 50).

3.4.3. ASSESSING CPM EFFECTS

The common denominator in CPM paradigms are two painful TS; one TS alone and one in combination with CS (parallel or serial) at a heterotopic site[118,160,161]. The TS and CS do not have to be of similar modalities and the paradigms in general show good-moderate reliability[118,135] although previous studies indicate that modality combinations may matter[118,126,162]. Pressure cuff algometry was used in all three studies in this thesis and the CPM effect was calculated by subtracting pain sensitivity from a TS during conditioning from a baseline TS[161], and was considered positive if pain ratings were reduced or if increased stimulus intensity was tolerated during CS[31]. The difference between PDT for the conditioned and unconditioned TS at baseline in Study-I, II and III are shown in Figure 3.10.

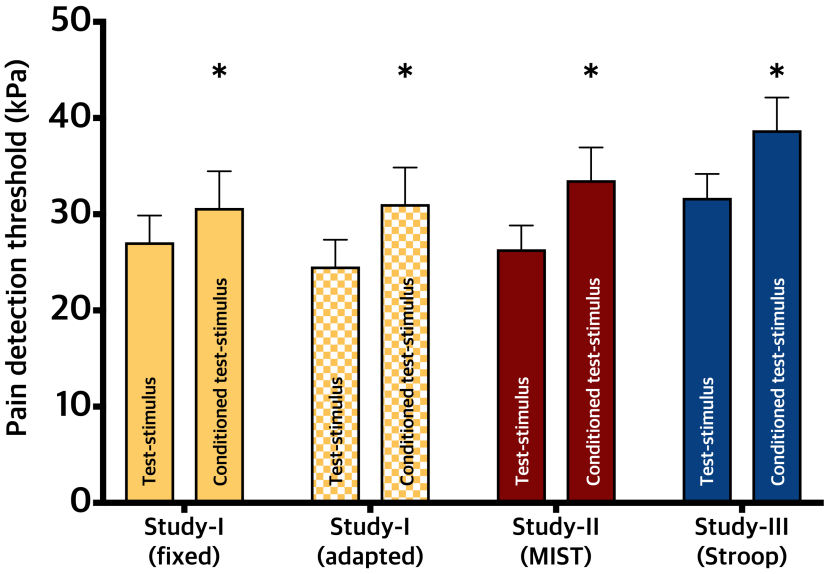


Figure 3.10 PDT with and without conditioning

PDT of a test-stimulus before and during CS in each of the four experiments. PDT was increased during conditioning compared to before conditioning (ANOVA: P < 0.0005), indicating a positive CPM effect at group-level (n = 90).

In Study-I PTT was analysed during the experiments but effects were found to generally be smaller than for PDT and subsequently Study-II and III only analysed PDT-based CPM effects (see Table 3.2). The difference in CPM effects based on PDT compared to PTT may relate to ceiling effects since the limitations of the computerised pressure cuff machine does not allow for pressure above 100 kPa, which in some participants was insufficient to reach PTT. Another possible explanation is that accumulated effects influence PTT since the CS lasts longer before reaching PTT compared to PDT.

The mean CPM effect, based on PDT in the four experiments (ANOVA: P = 0.25), was 6.2 kPa ± 0.9 (95% CI: 4.4 – 8.0) equal to 17.1% of PDT. As can be seen in Table 3.2 there are consistent differences between CPM effects based on PDT compared to PTT (ANOVA: P < 0.0005) with no difference between the experiments.

Table 3.2 CPM effects

CPM effects	PDT	PTT	TS-SEM	η_p^2
Study-I (fixed)	3.6 ± 2.0	1.6 ± 1.1	± 3.0	0.146
Study-I (adapted)	6.5 ± 1.6	2.6 ± 1.5	± 2.9	0.454
Study-II	7.2 ± 1.9	1.8 ± 1.8	± 2.3	0.358
Study-III	7.0 ± 1.7	0.1 ± 0.9	± 2.3	0.507
Mean ± SEM	**6.1 ± 0.9**	**1.4 ± 0.7**	± **1.3**	**0.339**

CPM effects (± SEM) at baseline in the three studies (I, II, III). Mean CPM effects calculated as PDT are higher than those from PTT (P < 0.0005). TS-SEM indicates the standard error of measurement in the unconditioned TS. Effect sizes (η_p^2) are calculated individually and as a mean. *The effect size (*η_p^2*) was large*[163] *in all studies but highest in Study-III.*

A recent reliability study[137] suggested that positive CPM effects should be higher than SEM of the unconditioned TS, which in the case of all four experiments was true. The mean effect size was 0.339, and according to Richardson (2011) η_p^2 above 0.138 can be considered 'large'. However, a review on CPM methodology found an approximated median CPM effect of 29% but noted that variability is huge between studies[164] indicating methodological and individual differences. A cohort study of 926 male participants from a random sample of the Danish population found a median CPM effect of 32.1% using a combination of the cold pressor test (as CS) and pressure algometry (as TS)[117]. If the increase in PDT during conditioning, compared to PDT before conditioning, is used to calculate the percentage increase in CPM on all baseline data in Study-I, II and III (N = 90), the pressure cuff paradigm in this project provided an increase of 17.1%, which is lower than previous findings. A likely explanation is the differences between modalities (heat, cold, pressure algometry) and deeper tissues (pressure cuff algometry). Despite these differences, recent studies using heat, probe-pressure and a combination of modalities suggest that CPM effects – albeit different in sizes – are stable over time[122,137], which fits very well with the results of all three studies in this thesis (see Table 3.2).

A comprehensive study on the pressure cuff for use in CPM paradigms shows that higher intensity of the CS can lead to higher CPM effects[112]. To test if this was true on the complete dataset from all four experiments at baseline, a regression analysis was made. The analysis found a significant correlation suggesting that higher CS-intensity can predict 20.6% of the variation in CPM effects (see Figure 3.11).

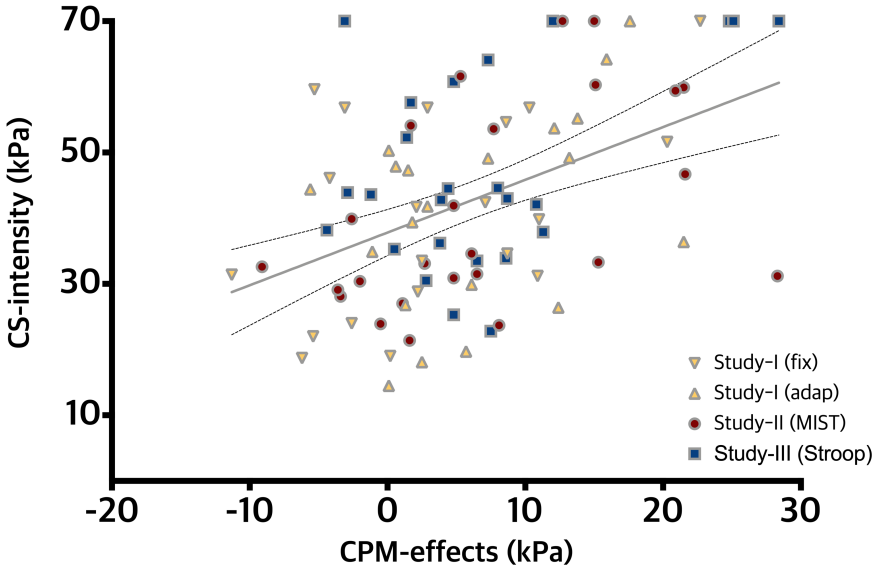


Figure 3.11 Intensity of CS correlates with CPM effect
There was a significant correlation between CS-intensity and CPM effects at baseline ($P < 0.0005$, $n = 90$) and higher CS-intensity predicted higher CPM effects ($R^2 = 0.206$, $Y = 0.803x + 37.8$).

3.4.4. NOVEL PARADIGMS FOR ASSESSING CPM (STUDY-III)

In addition to CPM paradigms based on threshold assessment, a range of variations have been used to explore different aspects of descending modulation[162,165-167]. In Study-III it was necessary to use a phasic TS in combination with a conventional, tonic conditioning. Reliability of the conditioned TS in this paradigm was excellent (average of three conditioned TS) with ICC of 0.92 and 95% CI = 0.82 – 0.97. Average VAS

score of the two sessions (Pain-I and Pain-II, see Figure 3.4) during conditioning was 6.3 ± 0.3. Overall the phasic paradigm produced a mean CPM effect of minus 0.4 ± 0.2 cm on VAS in the reliability study, indicating a facilitative response. Two other studies have used a similar paradigm and only one found a positive CPM effect, which is smaller than CPM effects from a conventional paradigm[133,134].

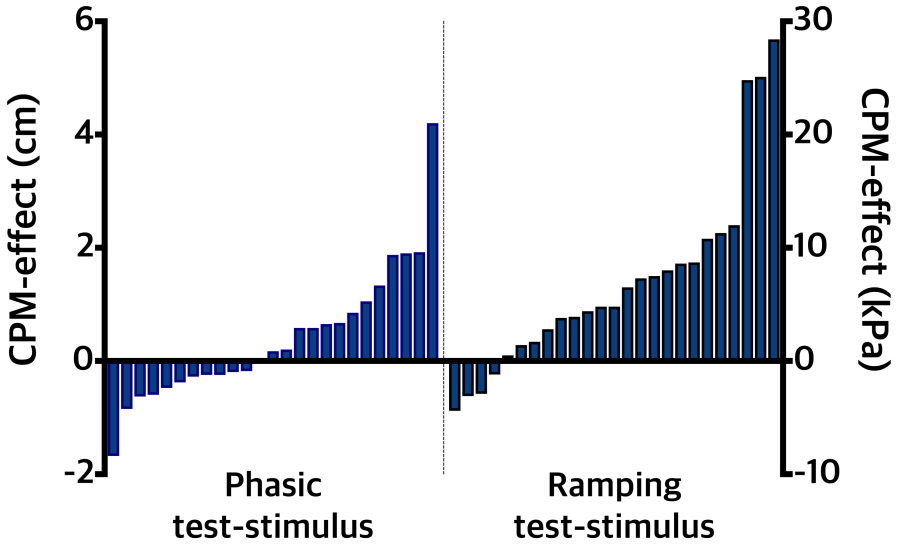


Figure 3.12 Ranked distribution of CPM effects

Ranked distribution of CPM effects in Study-III (n = 25). On the left side CPM effects in the phasic sessions (mean of both sessions) are shown as change in cm on VAS. On the right side CPM effects at baseline in the conventional CPM paradigm are shown (kPa).

In conventional CPM paradigms, an estimated 20% of healthy individuals will show a negative CPM response[21,117,168]. In the cohort used for Study-III, four subjects (16%) had a negative response during the conventional CPM-paradigm, while half of the participants had a negative CPM response in the new phasic paradigm (see Figure 3.12). These results align with the existing evidence[133,134] and suggest that the new phasic paradigm yields smaller effects. It is possible that the difference between paradigms reflect TS duration, which in the conventional paradigm lasts

up-to 100s compared to only 5s in the phasic paradigm. It could also relate to a difference in compression rates between the conventional (1 kPa/s) and phasic (100 kPa/s). Albeit speculative, the phasic paradigm could identify subtle individual differences related to the efficacy of the underlying mechanisms of CPM.

3.4.5. PRO- AND ANTI-NOCICEPTIVE PHENOTYPES?

It has been proposed that dynamic responses to nociceptive stimuli could place individuals on a spectrum between pro- and anti-nociceptive phenotypes[54]. This was explored in subgroup analysis[21,121] and a recent study suggests that the user-independent pressure cuff algometry method could be appropriate for studying differences between CPM responders and CPM non-responders[137]. The distribution of the CPM effects at baseline in all three studies is shown in Figure 3.13.

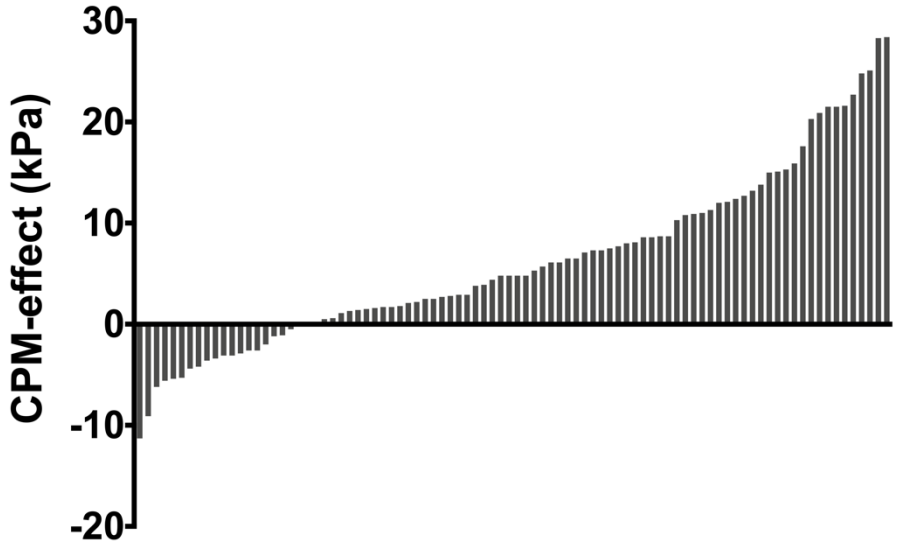


Figure 3.13 Ranked distribution of CPM effects at baseline
19 assessments showed negative CPM effects at baseline (n = 90) in kPa (Study-I, II and III).

In the four experiments, a total of 21% (19 assessments) had a negative response to the CS compared to before CS at baseline (Figure 3.13), and

could thus be classified on the pro-nociceptive spectrum and considered CPM non-responders.

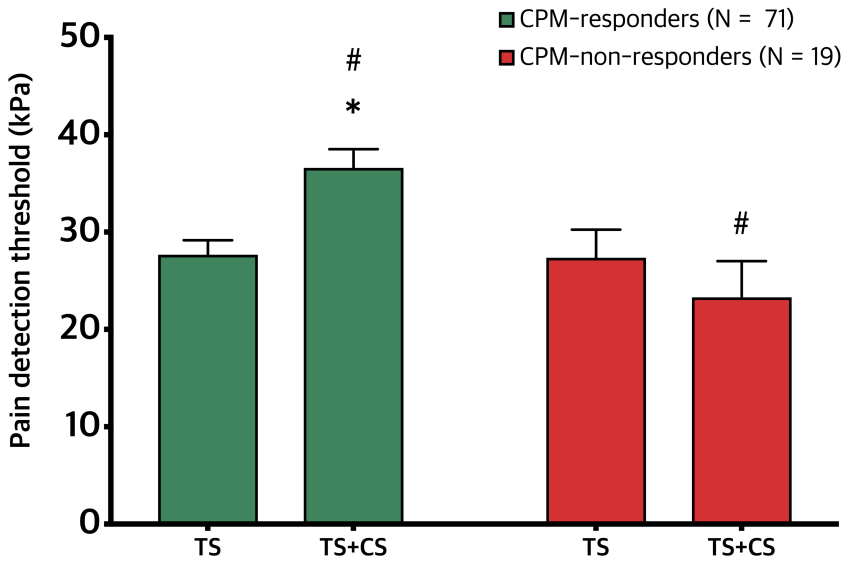


Figure 3.14 CPM-responders vs CPM non-responders at baseline

PDT was higher during conditioning for CPM-responders (in green: #, P < 0.0005) and compared to TS+CS for CPM non-responders (, P = 0.002), while PDT was lower for the CPM non-responders during conditioning compared to before (in red: #, P = 0.01) as would be expected. No differences were found between TS between the two subgroups (P = 0.9). Data from Study-I, II and III.*

An analysis of the full dataset (n = 90) finds no differences between responders and non-responders for the unconditioned TS (P = 0.9), indicating that subjects respond differently to the CS, not the TS (Figure 3.14). Potvin and Marchand (2016) used thermal pain threshold as TS and cold water as CS on healthy controls and patients with fibromyalgia. No data was provided regarding the difference in pain threshold for healthy controls but for the fibromyalgia participants, pain threshold was 38.7°C and 38.9°C for CPM non-responders and CPM-responders, respectively[21].

These data were not analysed or discussed by the authors but they could be interpreted to align with the results found across this study. It is unlikely that peripheral sensitisation would fully explain the mechanism underlying these findings as it could not account for bilateral effects. The difference in CPM effect at baseline could support the idea that application of a CS (i.e. bilateral, parallel stimulation) relates to central mechanisms, possibly via summation-like effects.

Together this indicates that the cohorts respond normally to conventional CPM (approximately 80:20 distribution between anti-:pro-nociceptive phenotypes) and that CPM non-responders are not 'immune' to CS, rather they fall into a pro-nociceptive phenotype.

In Study-III the phasic paradigm without Stroop also found no difference between TS for the two subgroups (P = 0.7). Furthermore, this paradigm seemed to enhance the pro-nociceptive effects of CS in the CPM-non-responder group (Figure 3.15; *, P = 0.01). The two studies that have used phasic TS did not provide data to explore subgroup differences but McPhee and Graven-Nielsen (2018)[133] showed no main effect of phasic pressure cuff CPM and reported no data on pain thresholds, while Lie et al. (2017)[134] report only that a tonic heat TS reached higher CPM effects than a phasic and that there was not a positive mean CPM effect in all individual bouts.

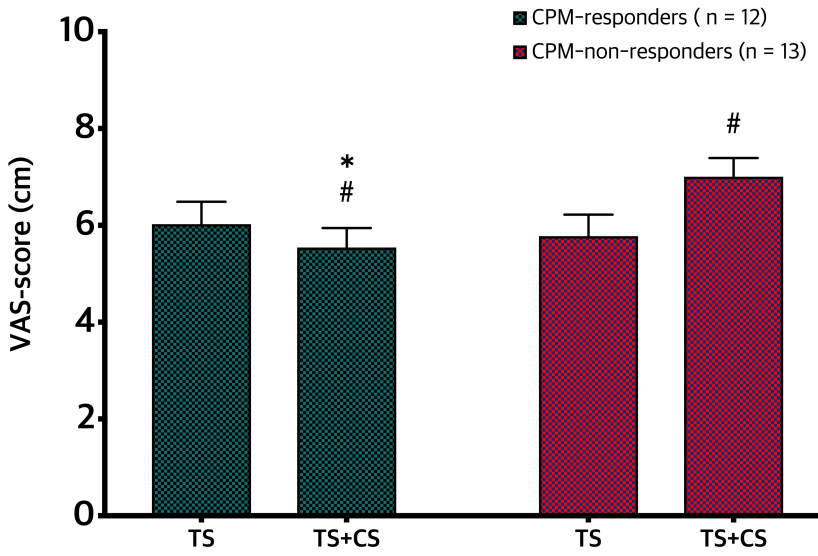


Figure 3.15 CPM-responders and CPM non-responders (phasic TS)

VAS scores (cm) for the average of the 1st phasic test-stimuli (TS) and the average of the 2nd-4th phasic test-stimuli (with tonic conditioning, TS+CS). Participants who responded with pain facilitation to conditioning (n = 13) were classified as CPM-non-responder phenotype. TS+CS was lower during for CPM-responders (, P = 0.01) compared to CPM non-responders. Both subgroups showed significant differences between TS and TS+CS, albeit in opposite directions (#, P < 0.05). No differences were found between TS between the two subgroups (P = 0.7). Data from Study-III.*

Based on the existing evidence a phasic TS in combination with a tonic CS seems to be less effective to test CPM in healthy volunteers. The phasic TS alone seems reliable and in combination with a tonic CS it may be a relevant paradigm to differentiate between individuals who 'facilitate' during bilateral stimuli (TS + CS) and those who 'inhibit' pain (i.e. CPM-responders). Future studies could explore this paradigm if the aim is to further subgroup healthy men.

3.5. SUMMARY

There are relevant modality-dependent differences for TS and it may be relevant to choose TS-modality based on tissue depth (skin vs. musculoskeletal tissue). The choice of CS-modality may also be important and indications from the evidence base suggest that it should match the physiological and perceptual qualities of the TS to optimise CPM effects.

Pressure cuff is a reliable paradigm, both when pressure is applied slowly (1 kPa/s) and instantly (100 kPa/s), and PDT as well as pressure intensities were similar across the three studies. Tonic pressure effect (e.g. CS) is likely to habituate over time although the consequences are unknown. The temporal dynamics of repeated TS are likely to be influenced by habituation and a range of unknown factors, which could be relevant to study in the future.

A positive CPM effect was found in all three studies at baseline and a combined analysis of all data from Study-I, II and III indicate that pressure cuff algometry provided stable and reliable CPM effects with an acceptable effect size for CPM effects calculated on the differences between PDT, with and without conditioning stimuli. Across all four experiments 21% of the participants had a negative CPM effect based on PDT, which is in alignment with the existing evidence.

A phasic TS appears to be best suited to study facilitation during repeated, painful stimuli or to subgroup facilitators from inhibitors. The model may hold potential for studying descending facilitation in healthy subjects but effects are likely lower compared to conventional CPM paradigms, which is methodologically important (e.g. for power calculations). Interestingly, results from this project show that CPM-responders and CPM non-responders react alike to TS without CS. It is unknown why healthy people may react differently to a parallel CS and it is suggested that differences between unilateral and bilateral stimulations may be relevant targets for future studies.

CHAPTER 4. TEMPORAL STABILITY OF CPM

Study-I

The effect of repeated, unconditioned TS on healthy men has been discussed in Chapter 3. A study in healthy volunteers and patients with migraine showed that repeated CPM-testing was able to detect subtle dysfunctions in those with migraine, which were not found in the first bout[169]. This could indicate that modulation of CPM over time is dynamic and possibly a more subtle way to study individual differences in descending modulation[129]. Despite this, no studies have previously focused on how CPM effects are influenced over repeated bouts in healthy volunteers.

4.1. REPEATED CPM EFFECTS

CPM is considered a reliable paradigm that tests the net-effects of descending facilitation and inhibition in humans (see Chapter 3.4). Evidence suggests, however, that CPM effects are not the only possible tests for descending modulation and "non-CPM paradigms" such as exercise[170] and placebo[171] have also been found to be analgesic. Interestingly, CPM effects appear to be transient in nature while exercise-induced analgesia last longer (15 mins or more)[96].

The pressure cuff paradigm has been shown to be able to repeat painful TS in the same location without signs of peripheral sensitisation[128]. Both heat and pressure TS, however, may habituate over time when applied in the same location[128,172,173] (see Chapter 3). This could potentially affect the efficacy of the CS and influence the CPM effect, since studies show that CS must be perceived as painful in order to give a CPM effect[144,160].

Even in the presence of a painful CS, different CPM effects can be found in the same individuals, depending on modality[174].

In Study-I, repeated CPM effects were calculated within each bout as described in Chapter 3.3. Individual CPM effects for each bout are shown in ***Figure 4.1***.

The mean CPM effect from all four bouts was 3.2 ± 0.7 kPa (fixed) and 2.8 ± 0.8 kPa (adapted) and CPM effects were higher than control effects ($P < 0.0005$) providing the first evidence for repeatability of CPM effects in 5-min bouts. One other study has reported the effect of repeated CPM-bouts in healthy subjects[169] albeit in a different paradigm and with heat as TS. Like Study-I, this study found positive CPM effects in each of the bouts, which strongly indicates that CPM effects are transient and not likely to accumulate when repeated in 5-minute bouts[129,169].

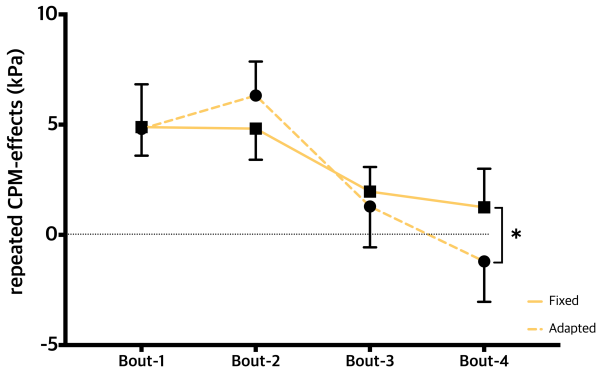


Figure 4.1 Repeated CPM effects

Repeated CPM effects in each of the four bouts in both experiments (Study-I). Analysis showed a main effect indicating that CPM effects in the fixed experiment were 4.4 ± 1.8 kPa higher than in the adapted experiment (, P = 0.02).*

There has not been much research exploring the role of CS in repeated CPM-bouts. Study-I found that a fixed CS generates higher CPM effects compared to an adapted CS (***Figure 4.1***). This is unexpected since previous studies have found a correlation between CS-intensity and CPM

effects[112]. However, it aligns with other studies, which indicate that perceived pain from the CS may be more important than the actual intensity, and that increased intensity in itself does not give higher CPM effects[175,176].

There was a difference in CS-intensity between the two experiments in Study-I, and as a consequence also longer duration of the CS in the adapted compared to the fixed experiment. Three studies find that duration of the CS has no effect on single-bout CPM[112,142,177], indicating that the difference between the results in the two experiments are not related to duration.

Another possibility is that the additional TS in the adaptive experiment could have influenced the CS as in serial conditioning. If this was the case, however, the pain ratings should decrease (as a sign of pain inhibition), which did not occur in Study-I.

Based on the current evidence base, it is uncertain why the two experiments in Study-I show different results but it seems that the fixed CS paradigm is more likely to provide the largest CPM effects[129].

4.2. A NET-CPM EFFECT?

Study-I explored dynamic changes in repeated TS with and without conditioning (i.e. CPM and control effects). An explorative analysis was conducted to understand the relationship between these two effects under the assumption that they share all properties but the CS (within each experiment). By subtracting the control effects from the CPM effects in each bout a 'netCPM effect' was found (Figure 4.2). A positive netCPM effect should represent an increase in CPM effects after the change in control effects is considered, and it is suggested that the netCPM effect can be interpreted as a proxy for the balance between habituation and sensitisation.

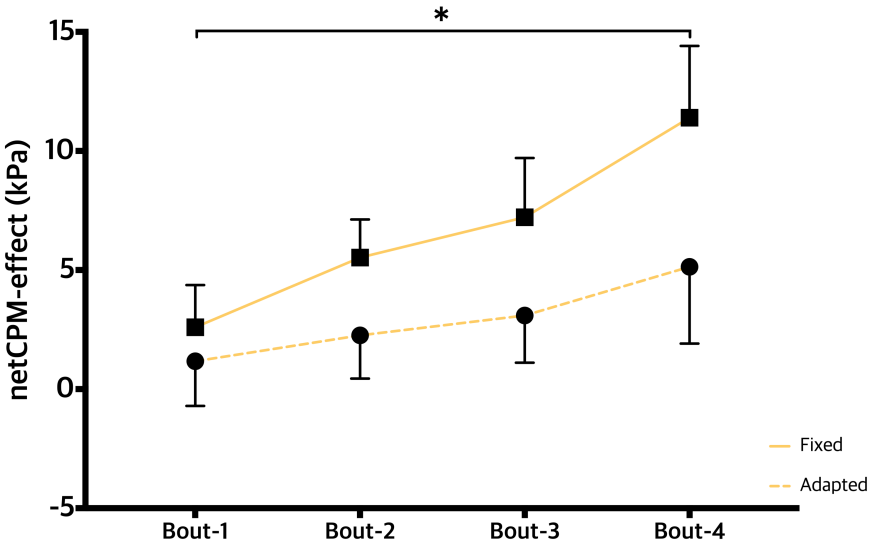


Figure 4.2 NetCPM effects

The netCPM effect in each bout for the fixed and adapted experiments. The fixed-conditioning experiment increased over time (, P = 0.03). No difference was found for the adapted experiment P = 0.5) and a trend was found for differences between the two netCPM effects (P = 0.07).*

As it turned out, the netCPM effect accumulated over time (P = 0.03) when the same (i.e. fixed) CS-intensity was used throughout the experiment; whereas no significant netCPM effects were found for PDT during the *adapted* conditioning experiment (Figure 4.2).

In conventional, single-bout CPM paradigms, the difference between a conditioned TS and an unconditioned TS is believed to represent the net balance between facilitative and inhibitory descending modulation[24]; yet it also represents one end of the dynamic pain spectrum[54] in most healthy individuals (anti-nociception). The netCPM uses the same logic but implements *time* as a factor without moving to the pro-nociceptive end of the spectrum. While the concept is unpretentious it is by no means assumed that netCPM represents the mere linear difference between conditioned and unconditioned TS over time. Rather, it may be a simplified method to encapsulate the net-response of CS over time, given that the

two TS are equally affected by time; and a way to exclude methodological confounders (e.g. type-I errors). In other words, netCPM could be a way to explore the dynamic interplay between TS with and without the influence of a CS.

As mentioned earlier, studies suggest that CPM effects are stable over time[122,137] and data from Study-I, II and III add to this that individual responses to CPM (i.e. inhibition or facilitation) relate less to the TS than to the CS. Knowledge regarding the influence of accumulated effects in control- and CPM effects are unknown and will likely involve transient adaptations to the stimuli and possibly be influenced by the intensity of the stimuli. In future studies, it would be interesting to understand more about how the 1st TS is influenced by the 2nd TS in the previous bout (serial conditioning), considering that two CS can eradicate the CPM effect[178] and the possibility that there could be ceiling effects for CPM.

4.3. SUMMARY

Study-I explored CPM effects over time and results indicate that the effects of repetitions are influenced by additional factors compared to single-measurements and further research is necessary to understand how best to use this paradigm. Based on Study-I, it seems that CPM- and control effects can be measured repeatedly over 20 minutes and that netCPM effects should increase over time in healthy men. At the same time, the results indicate a high degree of complexity related to temporal dynamics in nociceptive signalling during repeated painful stimuli. Consequently, it appears that repeated measures will not be ideal to study simple time-bound changes such as treatment-effects over a period of 20 minutes. Rather it is suggested that repeated stimuli can provide additional insight to the intricate balance between descending facilitation and descending inhibition in humans. Importantly, nothing in the results seems to indicate that repeated painful stimuli increase risks of type-I errors. Standard protocols record repeated measures but use the average for calculation of threshold[179] and CPM effects[117]. It could be argued that individual

measurements may give more information about the ability of the CNS to adapt to noxious stimuli.

Overall, it is likely that repeated assessment of pain sensitivity is influenced by multiple factors (e.g. habituation and non-linear effects) and future studies should pay attention to such factors and include a control-session to account for temporal dynamics of repeated measures. For repeated CPM-measurement, a fixed conditioning intensity based on individual PTT at baseline is recommended because of more stable responses in both CPM- and control-sessions.

CHAPTER 5. STRESS MODULATION OF PAIN SENSITIVITY AND CPM

Study-II

Stress, like pain, is an experience that has biological correlates and it could affect the balance between the descending facilitation and inhibition of nociception via overlapping pathways (see Chapter 2.1).

A range of stressors such as cold water, mirror drawing, anger recall, mock job-interviews and various mental tasks have been applied to facilitate acute stress in humans[180]. Responses to experimental stress can be measured via biomarkers or self-report. Measurement of self-perceived stress has been used in relation to social stressors[78,181] where it can be measured on a 11-point Likert scale (0 being no stress and 10 being maximum stress). Questionnaires, such as the widely used Perceived Stress Scale, have been used in cohort studies but are less ideal for acute, experimental stress because of its short-lived nature[182-184]. Proxies of the biological stress-responses can be measured in 'real-time' (e.g. heart rate variability[185]) and the slower cortisol pathway, which is the most commonly used biomarker of a physiological stress-response[58,72,84,88,186,187].

5.1. MONTREAL IMAGING STRESS TASK

At least three experimental paradigms have been established to induce cognitive stress based on contextual stressors such as negative feedback during mental arithmetic or mock job interviews[188-190]. Many studies have used social stress to explore the relationship between perceived stress, biological correlates (see Appendix A.2) and pain sensitivity in healthy humans[181,191-200]. The Trier Social Stress Task[188] is a well-established, resource demanding paradigm that implements more elements than most, including a mock-job-interview and an arithmetic task – both in front of a

panel of trained assistants who give negative feedback during the stress-session. The Montreal Imaging Stress Task[190] (MIST) was developed to be used in an fMRI scanner and with less resources than the Trier Social Stress Task[190]. It has been used to study the influence of perceived stress and stress-related cortisol changes on pain sensitivity and CPM experiments[181,201]. Since this was the aim for Study-II, the MIST was ideal. The MIST can be modified to fit individual protocols and has the option to include a control-task (MIST-control) in addition to the stress-task (MIST-stress), see Figure 5.2. Prior to the present work, no studies had compared the effects of MIST-stress on pain sensitivity or CPM to the effects of MIST-control on pain sensitivity or CPM.

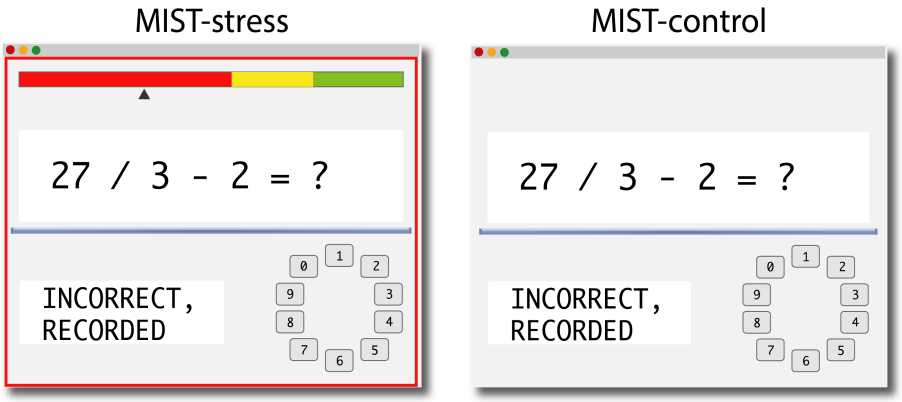


Figure 5.1 Montreal Imaging Stress Task
A schematic showing the interface of the MIST-software. The stress-task (left) included a performance indicator providing incorrect, negative feedback as well as other stressors.

Increased levels of salivary cortisol have been shown to correlate with the MIST-stress paradigm but not with MIST-control[190], and two pain-related studies have found increased cortisol during MIST-stress compared to baseline[181,191].

The sessions in MIST (stress, control) are built into a software application that contains a computerized algorithm and the ability to deliver an output based on performance. The software adjusts arithmetic tasks according to

settings (e.g. 'stress' or 'control') and to the individual so that time restraints and levels of difficulty are adjusted depending on responses from the participant. During the stress task, the ideal setting will allow the participant to answer correctly 40-50% of the time, while in the control task the ideal setting will allow 90% correct answers[190,191].

The stress paradigm differentiates from the control by application of negative feedback to the participant (performance indicator and a stressing, high tone of increasing intensity indicating the time left to calculate each task). The performance indicator is set to always suggest that the participant is performing under average. Finally, the researcher gives the participant negative feedback (verbally) in accordance with a manuscript. The feedback from the researcher may indicate that the results are too low and that the data might have to be discarded if performance requirements are not met.

5.2. PAIN SENSITIVITY AND STRESS

In Study-II, the effect of MIST on repeated TS with and without CS was explored in addition to the effect of stress and control on CPM. Studies have previously looked at the relationship between pain and stress with similar methods[181,191,192] and found associations between CPM and/or pain sensitivity and salivary cortisol and/or perceived stress. However, there is still a lot of uncertainty with regard to the interaction between pain sensitivity and perceived or 'biological' stress[191]. It was hypothesised that CPM would be further reduced by stress than a during the control session.

Previously, two studies have found an association between social stress and hyperalgesia[192,198] whereas the majority of the literature has found no change in pressure-pain sensitivity during acute, experimental stress[84,91,181,191,193-195,201] (see Appendix A.2). MIST-stress has been used twice to study changes in pain sensitivity during stress. In one of these, perceived stress was associated with heat-pain hyperalgesia[192] while the other study found no change in heat-pain thresholds[181].

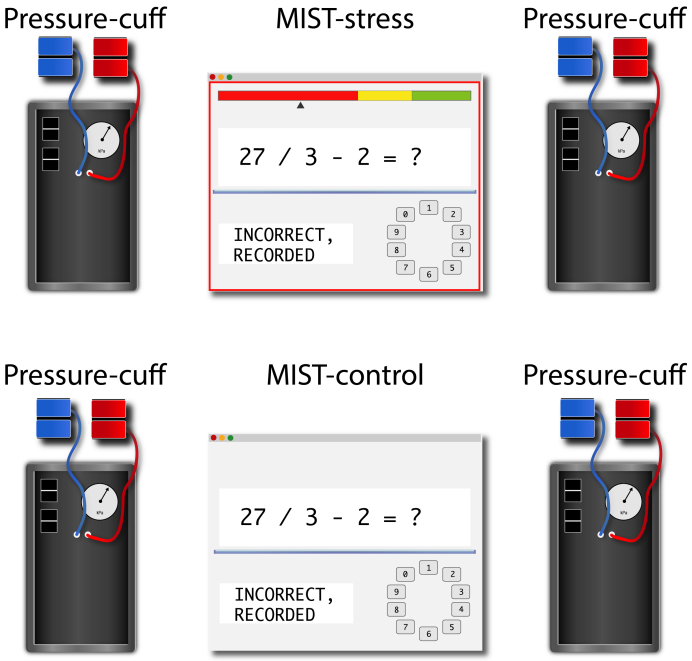


Figure 5.2 Stress (MIST) protocol

PDT was measured in repeated test-stimuli with and without conditioning before and after MIST-stress and MIST-control. MIST-sessions were separated by a 15-minute break.

In Study-II, TS with and without conditioning were compared before and after MIST-stress and MIST-control, respectively, and no significant changes were seen (Figure 5.3; $P > 0.36$). The results are in keeping with existing studies[181,194,196,197,199,200,202,203], suggesting that stress and pain sensitivity are unrelated and independent of modality (e.g. pressure, cold and heat). The robustness of pressure-pain thresholds is well established[198-200] and in the context of a competing stressor, acute pain could be considered more salient[204], at least theoretically, providing a conceptual understanding of why acute pain is not affected by social stress.

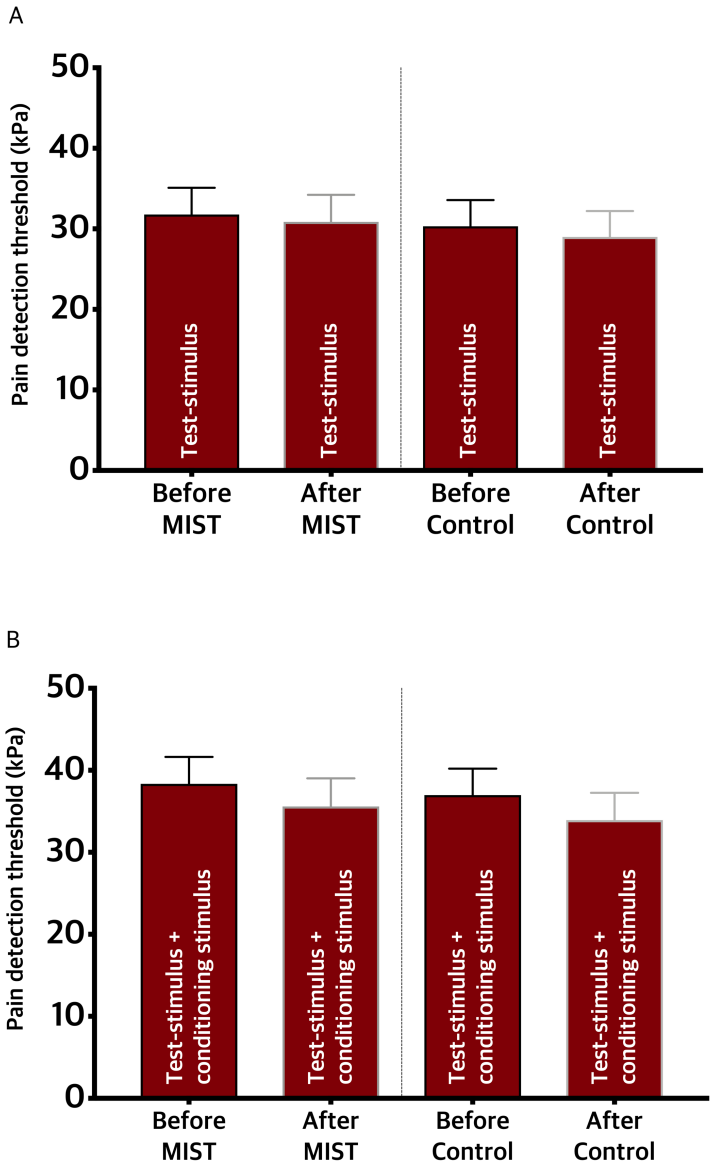


Figure 5.3 PDT before and after stress and control

*Average PDT for the unconditioned test-stimuli (**A**) and conditioned test-stimuli (**B**) did not change as a consequence of MIST compared to MIST-control (P > 0.36, n = 25). Data from Study-II.*

5.3. CPM EFFECTS AND STRESS

CPM effects can be reduced during social stress[181,191-193] with only a single study reporting that stress does not affect CPM[196]. Four studies. which found an effect of perceived stress on CPM, used heat-pain as stimuli. Nilsen et al. (2012), found an effect of stress on heat-evoked CPM and although data on pressure-pain was collected, this was not analysed because of indications of carry-over effects[193]. Cathcart et al. (2010), who did not find any effect of perceived stress on CPM, used pressure-pain for TS and occlusion for CS[196]. The existing findings may indicate some influence of modality but it is also relevant to note CPM effects during experimental stress were compared to baseline/recovery[181,191,192], listening to music[193] or reading newspapers[196], rather than to a comparable control-session.

In Study-II, CPM was measured twice before and twice after CPM-stress and CPM-control, respectively. More measurements were not possible due to the expected timeline of peak salivary cortisol (see below). PDT was increased during conditioning in all four sessions compared to PDT of the unconditioned TS, i.e. there was a significant CPM effect in all four CPM-sessions (Figure 5.4, $P < 0.011$). Contrary to the hypothesis, no significant differences were found between CPM before and after MIST-stress or between MIST-stress and MIST-control (Figure 5.5).

The results of Study-II show significant effects of conditioning; but no significant difference before and after social stress; or when compared to a control. While this supports the ability of the repeated TS, with and without CS, to show CPM effects; it contradict four previous studies on CPM and stress reporting a decrease in CPM effectiveness during stress[181,191-193].

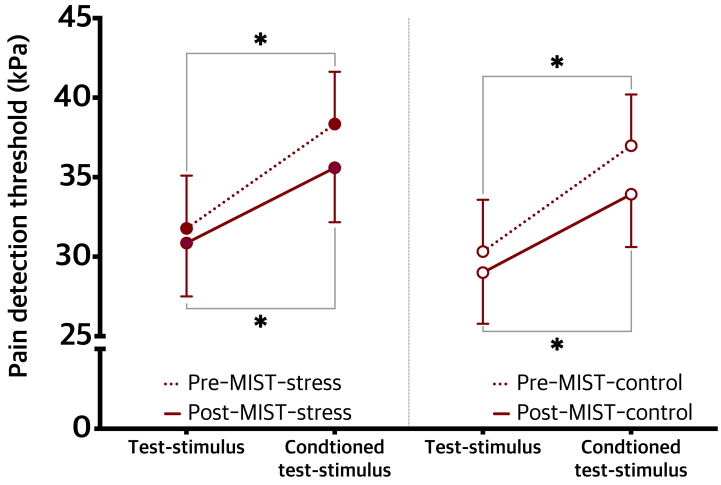


Figure 5.4 PDT before vs after stress and control

There was a significant increase in PDT during conditioning compared to before conditioning in all four sessions in Study-II (N = 25, P < 0.011). No significant differences were found between TS or conditioned TS before or after MIST-stress or MIST-control (P > 0.18), indicating that there was no effect of MIST-stress on pain sensitivity.

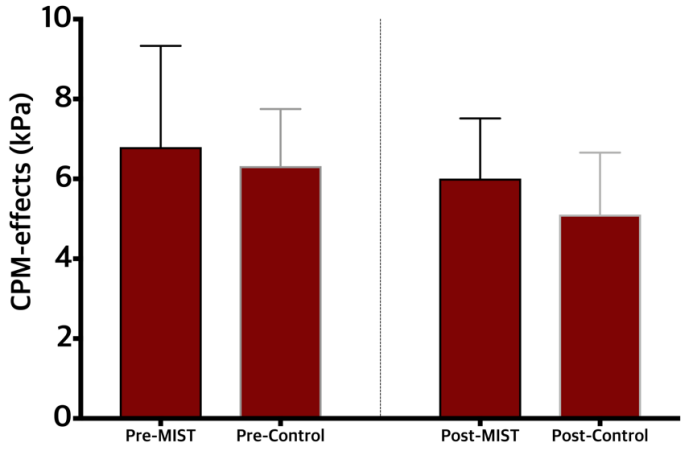


Figure 5.5 CPM effects before and after stress and control

CPM effects before MIST-stress and MIST-control (left side) and after MIST-stress and MIST-control (right side) were not significantly different (P > 0.36). Likewise, there were no differences between CPM effects before compared to after MIST or MIST-control (P > 0.68, n = 25).

The four studies that were able to show an effect of stress on CPM used heat as TS and heat or ischemia for CS, but as stated by Nilsen et al. (2012)[193] the effects were small-to-medium and results might be modality specific. Cathcart et al. (2010)[196], used pressure for TS and ischemia for CS and found no differences in CPM between the stress-condition and 'reading newspapers'. Despite discrete neuroanatomical differences in skin compared to musculoskeletal tissue (e.g. a subset of pressure-sensitive afferents in the lateral spinal nucleus, which only respond to deep-tissue stimulation[205]), no obvious explanation for any modality difference exists but future studies, using comparable control conditions, could explore this topic.

5.4. CPM, CORTISOL AND PERCEIVED STRESS

A correlation between perceived stress and CPM has previously been described[181,191,192], whereas the same studies did not find a correlation between cortisol and CPM. Others have found that morning cortisol may mask an effect from a stress task[189]. The majority of studies measure CPM less than five minutes post-stress[181,191-199,203] leaving very little time for cortisol to influence the nociceptive mechanisms, although a single study suggests that effects may be delayed since they found no change in pain sensitivity immediately after stress but pain reduction 15 minutes later[200].

A single study using social stress, found a negative correlation between cortisol and hypoalgesia[200] and no studies found any positive association between CPM and cortisol (see Appendix A.2). Four studies found changes in cortisol albeit with quite different definitions: Gaab et al. (2017)[203] found increased cortisol 10 minutes after Trier Social Stress Task compared to a matched control; Bement et al. (2010)[197] and Geva et al. (2018)[191] showed increased salivary cortisol compared to baseline measurements; and Geva (2014)[181] showed increased cortisol after MIST compared to both baseline and rest. The last study (Geva et al., 2016)[192] found a borderline main difference in salivary cortisol. The literature on

social stress and cortisol, therefore, does not provide data to support a correlation between CPM and cortisol, only weak evidence in relation to pain sensitivity and no clear evidence on cortisol response after mental arithmetic.

For convenience, saliva sampling was preferred over serum or urine sampling in Study-II. Cortisol was sampled at baseline and several times in Study-II (see Figure 5.6). To avoid unnecessary variance only healthy men were included and all sampling was done in the morning. Participants were instructed to avoid exercise, smoking, coffee and food three hours prior to baseline.

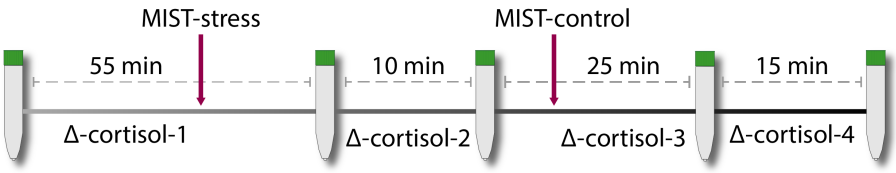


Figure 5.6 Sampling of salivary cortisol (Study-II)
Sampling of salivary cortisol in Salivette® was done five times during the study with respect to expected peak values (approx. 20 min after MIST-stress) and with sufficient time to expect cortisol levels to return to baseline (50 mins post MIST-stress[190]*).*

MIST-stress and MIST-control resulted in 45.3 ± 0.6 % and 90.1 ± 2.1 % correct answers, respectively, indicating a successful manipulation. Participants confirmed this in self-report. However, salivary cortisol measurements did not change over time (Figure 5.7).

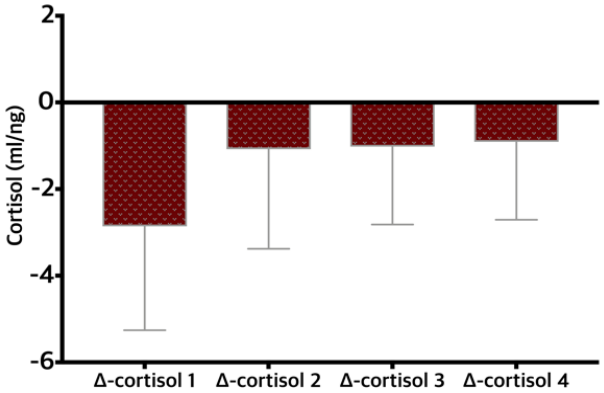


Figure 5.7 Changes in salivary cortisol

Study-II salivary cortisol was measured at baseline and four times during the study. Changes between measurements (Δ-cortisol) are shown. There was no difference between cortisol measurements (P = 0.85, n = 25).

No correlations were found between cortisol and PDT (P > 0.2) but a study suggested that individual variations in cortisol-recovery after stress may be a relevant factor[91]. An explorative regression analysis was made between Δ-cortisol 1 and the change in pain sensitivity before and after MIST-stress. The analysis showed small, but significant correlation between Δ-cortisol 1 and conditioned TS *(R^2 = 0.19, P = 0.03)*. No associations were found for PDT of the unconditioned TS. The result suggests that the pain sensitivity during CS is most reduced in participants with the highest change in Δ-cortisol 1. This is in alignment with Godfrey et al. (2016), who found that less efficient CPM (i.e. less change) after dexamethasone was associated with lower recovery in cortisol[91].

The correlation was only present for *conditioned* TS, and while speculative, it could be interesting to investigate if individual differences in CPM effects after MIST-stress are linked to the CS, as was proposed for repeated CPM effects (see Chapter 3.4.5). This interpretation could fit with the literature, which found no effect of stress on pain sensitivity[181,194-197,199,200,203]; but rather that CPM and cortisol share mechanisms[199,200], which are utilised under different situations. Furthermore, future studies

could explore whether repeated bouts of CPM can encapsulate changes over a period of time rather than at a specific time-point.

The results from Study-II on the relationship between cortisol and CPM echo the diversity of existing literature. As was the case for Geva et al. (2016), Study-II found borderline significant changes in cortisol. However, they tested participants later in the day (1 p.m.). Nonetheless, results suggest that the change in cortisol between baseline and the first post-stress measurement correlated with the change in conditioned, but not unconditioned, TS between pre- and post-stress pain measurements.

The results in Study-II also indicated that CPM effects were not influenced by morning cortisol since no differences were found for participants tested at 8.30am compared to those tested at 10.30am. This is in line with one other study, which showed that pressure-induced pain and CPM (heat-TS, cold-CS) did not vary during the day[206].

5.5. SUMMARY

Findings seem to suggest that social stress has little influence on pain sensitivity. The relationship between perceived stress and CPM is still unclear but results from Study-II highlight that methodological considerations in relation to control conditions are essential for the interpretation of existing and future studies. Furthermore, the relationship between cortisol and CPM is unclear but future studies should explore if cortisol recovery after stress can explain individual variability in CPM-responses during or after stress.

CHAPTER 6. COGNITIVE MODULATION OF PAIN SENSITIVITY AND CPM

STUDY-III

6.1. STROOP TASK

One of the most commonly applied paradigms in studies of the relationship between selective attention and pain sensitivity is the Stroop Task (see Appendix A.3 for overview). The Stroop Task was originally developed to test the capability of an individual to react against primary responses under time pressure[97]. Study-III used Numbers Stroop Task[68,207], which in essence requires a participant to stick to one rule (count and report the amount of words as quickly and correctly as possible) under conditions where there is congruency (e.g. the word '*two'* written twice) or incongruency (e.g. the word '*one'* written twice). The words were displayed on a computer screen (see Figure 6.1) and responses were made on a numeric keyboard with the keys for each of the four options highlighted. Outcome measures are reaction time (ms) and accuracy (as a percentage) of the responses[207]. Each participant was asked to finish three sessions with each four bouts of Stroop Numbers (1 min).

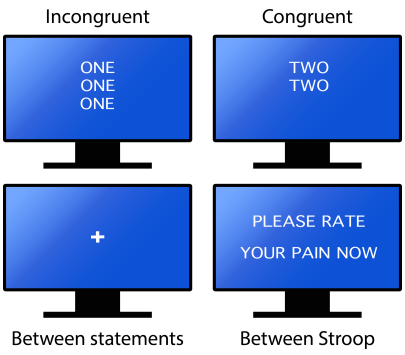


Figure 6.1 Stroop Task interface

The Stroop task was displayed on a computer screen. During the test congruent and incongruent statements were shown and between each statement a fixation cross *was shown. After each 1-min trial participants were informed to rate pain intensity of the test-stimulus.*

A majority of the studies on Stroop and pain sensitivity conclude that pain sensitivity is reduced in healthy volunteers after the Stroop task, even after confounders have been taken into account[32,131,132,208-210], however, a single study found that Stroop testing leads to hyperalgesia[99] and it has also been found that the effect of Stroop declines with age[211].

6.2. ATTENTION, DISTRACTION AND STROOP

Attention (i.e. cognitive load) is closely related to *distraction* (i.e. conscious focus *away* from something specific, e.g. a painful stimulus) and research has shown that distraction is analgesic[212]. Methodologically, the difference between attention to (e.g. Stroop Task) and distraction from a painful stimulus may only be the sequence of the stimuli: In order for the participant to focus on Stroop Task first and pain afterwards, the painful stimulus must be placed between the individual Stroop-sessions. If Stroop was intended to be a distraction task, the painful stimulus should have been produced in parallel to the Stroop-sessions.

The Stroop Numbers test was used in Study-III to explore the relationship between pain sensitivity, CPM and selective attention. The advantage with Stroop over e.g. 3-back or simpler method of mental arithmetic was that results could be quantified as reaction time (ms) and correct answers (as a percentage) simultaneously. The competitive element of being quick and correct appealed to the participants in the pilot study, who also found that Stroop was good at maintaining their focus on a task. Also, the paradigm could be designed to work in tandem with the pressure cuff algometry system, and had the advantage of being easily translated into both Danish and English.

In Study-III, three Stroop sessions were included of which one only served as a control condition. In the control session, TS was equal to 90% of PDT (i.e. not painful). There were no statistical differences between reaction time or accuracy between the three sessions. However, during congruent trials participants were significantly more accurate and had shorter

reaction time compared to incongruent trials (***Figure 6.2***), indicating successful assimilation of the paradigm[68].

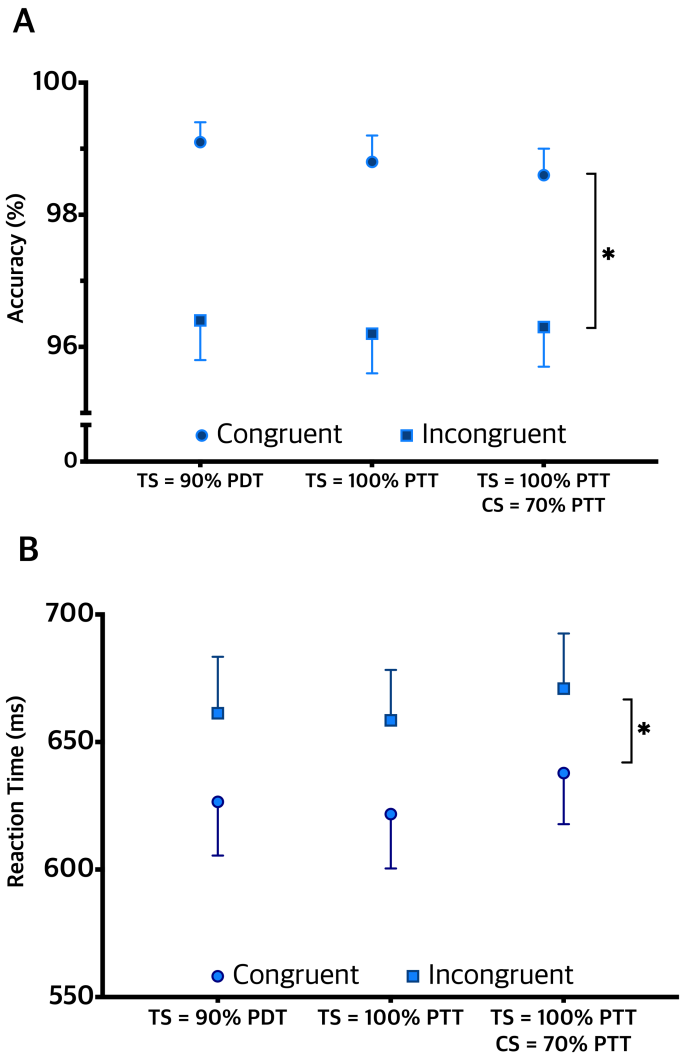


Figure 6.2 Stroop performance

Accuracy (A) and reaction time (B) were not different between the three Stroop-sessions in Study-III (P = 0.3) but congruent and incongruent trials were different from each other (P < 0.0005). Together these results suggest successful implementation of Stroop and no effect of pain on Stroop from TS and CS.

In the two sessions with painful stimuli, Stroop and TS were run in sequence. Attention (i.e. focus on Stroop Task) was deliberately not run in parallel to the painful stimuli. First of all because a study, which combined painful stimuli with Stroop did not find any effect on pain sensitivity[213] and secondly because this could be considered more a test of distraction, which was not the aim of this study.

6.3. STROOP AND PAIN SENSITIVITY

The majority of previous studies exploring the effect of Stroop on pain sensitivity show that Stroop correlates with reduced pain sensitivity, although a single study reported increased electrically induced pain sensitivity with increased cognitive load[99] (see also Appendix A.3). However, two other studies, which also used electrical TS, found Stroop to be associated with pain inhibition[210,214] indicating that modality is unlikely to explain the difference in pain sensitivity. The remaining studies have shown that Stroop has a pain inhibitory effect on cold-induced pain[32], capsaicin[209] and pressure-induced pain[131,132]. The difference in modalities used to study Stoop effects on pain sensitivity further supports that the effect is independent of modality.

As hypothesised, Study-III found that Stroop has an analgesic effect, i.e. TS VAS scores were lower during Stroop compared to without Stroop (***Figure 6.3***). This is in line with the existing literature[32,131,132,208-210,214].

Since no differences between congruent and incongruent tasks were found, it is suggested that selective attention rather than cognitive load is the most likely explanation. Two other studies found no difference between congruent and incongruent tasks[131,132]. The only study to report a difference between the two tasks[208] had participants rate pain intensity only once on completion of both tasks and thus is likely to be subject to recall bias[215].

In addition to attention, results may be influenced by placebo/nocebo effects including expectations[50]. Methodologically this was managed by randomisation between sessions and application of control conditions (without pain, without Stroop and without CS). Furthermore, results show that there are no differences between a conditioned and an unconditioned test-stimulus in combination with Stroop suggesting that expectations are unlikely to play a role[151,175]. Rather, it would seem that attention in itself has the capacity to inhibit pain, independent of expectation and conditioning[32,131,132,208-210].

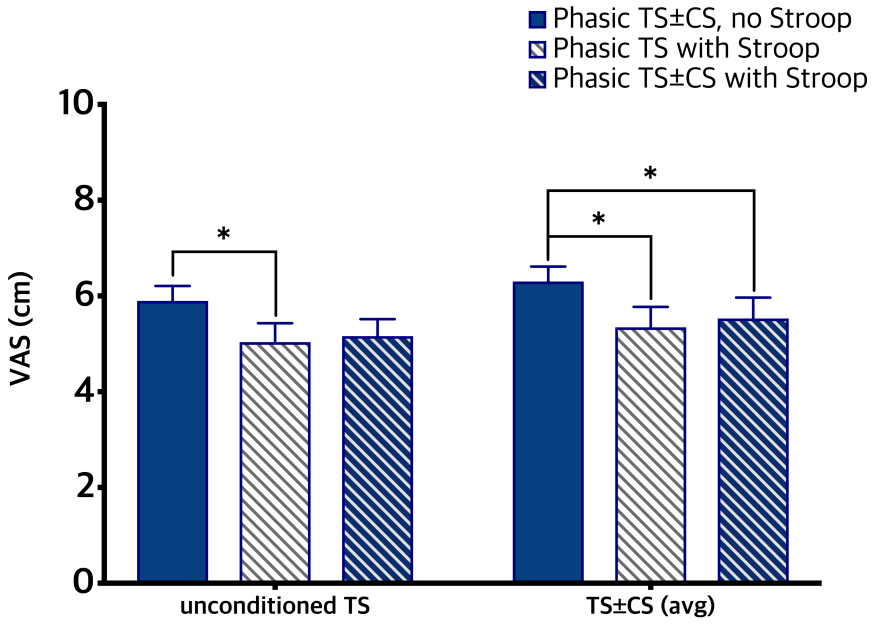


Figure 6.3 PDT before and during Stroop-task

The first TS in Study-III (n = 25) was unconditioned in all sessions (left). The following three TS were conditioned in the "Phasic TS±CS, no Stroop" session and the "Phasic TS±CS with Stroop" session. The mean of all three TS in each session is shown (right). VAS scores of the first (all unconditioned) TS were lower during "Phasic TS with Stroop" compared to the "Phasic TS±CS, no Stroop" (, P = 0.03). Both Stroop-sessions were lower than the "Phasic TS±CS, no Stroop" during the last three TS (P < 0.05) although the "Phasic TS±CS, with Stroop" was also conditioned.*

6.4. STROOP AND CPM

Previously, four studies have looked at the effect of Stroop on CPM[209,211,216,217], however, none of them controlled for baseline CPM-responses or compared the effect of Stroop on CPM to a control session. One of the studies found that Stroop reduced experimental pain more in healthy controls than in patients with functional dyspepsia, and that more abdominal pain correlated with reduced pain inhibition during Stroop in the patients[209]. The other three studies reported a correlation between CPM-efficacy and reaction time[211,216,217] albeit results were not significant in one study[211] and no CPM effects were found in the other two[216,217]. See Appendix A.3 for overview.

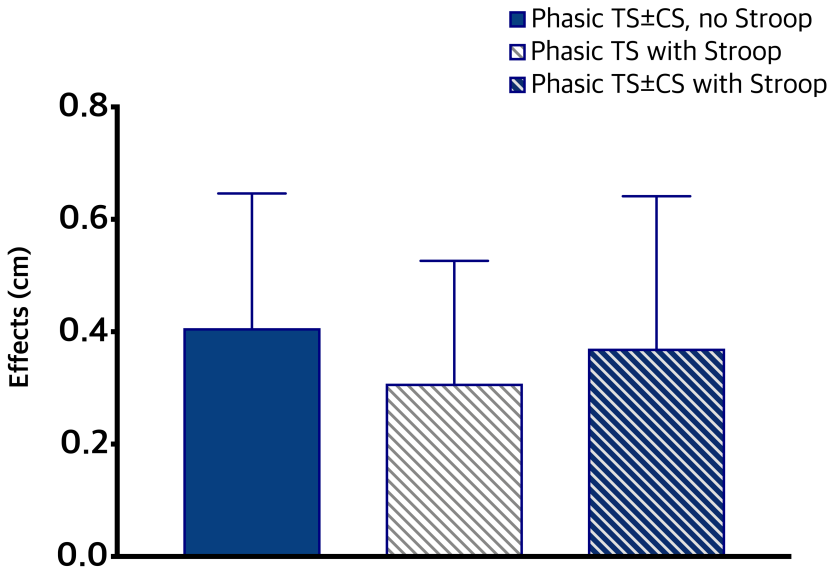


Figure 6.4 Effects before and during Stroop

Difference between the first and mean of the following three TS are shown for each of the three sessions in Study-III. The difference is equal to CPM effects in "Phasic TS±CS, no Stroop" and "Phasic TS±CS with Stroop" but no conditioning was applied in the "Phasic TS with Stroop" session. Analysis shows no difference between the three (P = 0.7) and no interaction with CPM-responders and CPM non-responders (P = 0.2) in Study-III.

Contrary to the hypothesis, no differences in CPM were found between the three sessions (P = 0.9), indicating that there was no effect of Stroop on CPM effects in Study-III (Figure 6.4).

In Study-III, differences between CPM-responders and CPM non-responders were analysed (see Chapter 4) and results show that Stroop, albeit hypoalgesic, could not change a facilitative response during CS into an inhibitory response (i.e. could not 'reverse a negative CPM'). Also, participants with an inhibitory response to CS did not show any significantly different responses during Stroop in combination with a CS (i.e. no change in participants with a positive CPM effect). These results were interpreted to suggest that Stroop and CPM did not have auxiliary effects in Study-III.

Overall, these results indicate that CPM and Stroop both have analgesic effects, but that they have different effects on healthy men. In support of this, a recent study concludes that attention and CPM both have analgesic effects but that they seem not to accumulate[147]. Three studies looked at correlations between CPM and Stroop and one found no correlation[214], which support the findings in Study-III, while the two others report positive correlations between CPM and Stroop[32,209].

Neither of the abovementioned findings provides a clear picture of the relationship between Stroop and CPM. Even in the case of overlapping mechanisms, studies using BOLD-signals to measure brain activity found that there are individual differences in responses to painful stimuli and Stroop with some participants being more prone to focus on the painful stimuli and others on Stroop[68] and that this may be reflected in the dynamic, rather than structural, functions of the brain[69,70,101]. Thus, it could be the case that stimulus-driven (*bottom-up*) and goal-oriented (*top-down*) modulation of pain[59,218] may involve the same pathways but with different dynamics, depending on individual differences.

6.5. SUMMARY

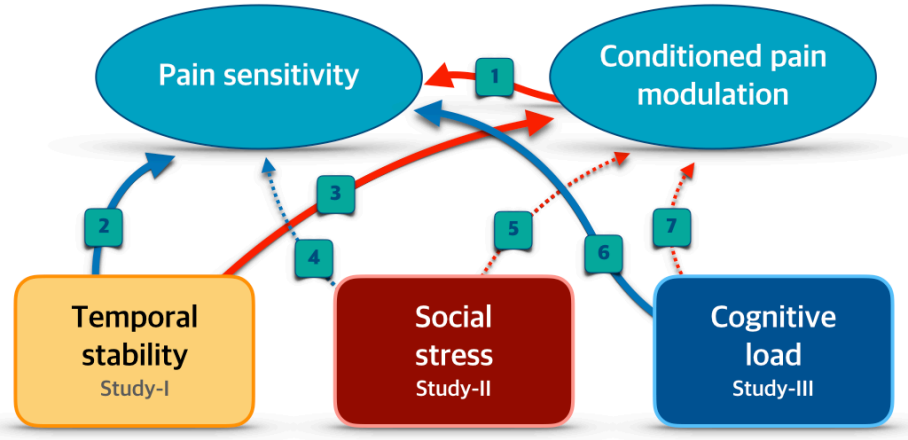
Selective attention has analgesic properties, which appear to utilise different mechanisms as those involved in CPM. This conclusion is based on the findings that the reduction in pain scores (VAS) during Stroop alone (*Phasic TS with Stroop*) was significantly larger than the first, unconditioned TS as well as the three conditioned TS in the *Phasic TS±CS, no Stroop* session (see Figure 6.3).

Study-III indicates that even participants who experienced increased pain intensity during the phasic paradigm (with and without CS) could benefit from attention-analgesia. Thus, Study-III concludes that painful stimuli and attention both have the capacity to reduce pain sensitivity.

CHAPTER 7. CONCLUSION

Pain modulation can occur after painful stimuli (*bottom-up*) or related to goal-oriented tasks such as stress and attention *(top-down*) and an overlap of the neural substrate behind both phenomena has been suggested. Thus, the aim of this thesis was to explore the dynamic stability of conditioned pain modulation (CPM) and to explore the suggested overlap between attention and stress with pain modulation. Results are summarised in Figure 7.1.

Figure 7.1 Findings from Study-I, II and III



Findings from Study-I, II and III at a glance. 1: The conventional CPM-paradigm was successful in all three studies in reducing pain sensitivity during conditioning. 2: Repeated test-stimuli without conditioning lead to an increase in pain sensitivity over time. 3: Repeated test-stimuli with conditioning decreased pain sensitivity over time when compared to a control-condition. The difference (i.e. netCPM effect) between control- and CPM effects increased over time. 4: Stress had no significant effect on pain sensitivity. 5: Stress had no significant effect on CPM. 6: Pain sensitivity was higher in sessions without Stroop compared to sessions with Stroop. 7: Pain sensitivity

had no effect on Stroop performance and Stroop Task did not affect CPM effects.

It was hypothesised that CPM effects and the effectiveness of a CS would adapt over time. While it was true that pain ratings (NRS) during CS were reduced over time, results suggest that CPM and CS-intensity can be maintained over time. Furthermore, it was found that the conventional CPM-paradigm showed stable responses in all three studies. In addition, a novel paradigm with phasic TS was tested and found reliable.

In the conventional CPM-paradigm, 21% of the datasets (n = 90) showed pain facilitation during conditioning (i.e. negative CPM effects). In the phasic CPM-paradigm, 52% of the participants (n = 25) had a negative CPM effect, possibly indicating that it could have potential for studying pain facilitation in future studies.

Over time, CPM effects increase more than the effects of repeated, control stimuli without conditioning. Furthermore, it is suggested that repeated TS with and without conditioning may be a subtle model to test for the balance between descending inhibitory and facilitatory mechanisms in clinical subgroups.

Explorative analysis on the full dataset from all three studies suggest that different responses to CPM (i.e. facilitatory or inhibitory net-effect) relates more to the CS than the TS. In support of these findings, a small, negative correlation between changes in cortisol and changes in conditioned, but not unconditioned, TS was found in Study-II. This could imply that stress and painful conditioning may have overlapping mechanisms, albeit with no detectable effect on pain sensitivity or CPM effect.

In Study-II, normal responses to conditioning were found (i.e. pain inhibition) and CPM effects were not influenced by a stress-control. Moreover, there was no effect of social stress on pain sensitivity, which supports the existing literature. However, Study-II also found no effect of social stress on CPM, which is in alignment with a single study and in disagreement with four reports of reduced CPM effects during stress. The

most likely reason for this discrepancy is modality-specific differences between heat and pressure induced pain thresholds. The findings in Study-II could indicate that an overlap between CPM- and stress-mechanisms is either too small to affect the stability of CPM during acute stress or too multifaceted to be detected in the applied model. Study-II concludes that future studies should include a control-session and that the modality should be carefully considered.

Study-III shows that attention has an analgesic effect on TS induced pain and that pain from the CS and/or test-stimuli did not influence Stroop-performance, which is in line with the hypothesis. There were no significant differences in the effectiveness of Stroop between CPM-responders and CPM non-responders, which could indicate that CPM and attention activate descending modulation in different, not overlapping, ways. In other words, while the conditioning stimulus may be essential to understand individual differences in CPM and stress-related influences on CPM, there seems to be no interaction between attention and the conditioning stimulus. In support of this theory, attention was insufficient to change pain facilitation in CPM non-responders into inhibition during repeated, painful stimuli, indicating an independent rather than auxiliary mechanism. It is thus concluded that attention and CPM are both capable of activating descending modulation, and they are complementary rather than supplementary to each other.

In conclusion, this project indicates that bottom-up pain modulation (CPM) is only discreetly influenced by stress-related changes. Furthermore, top-down modulation (attention) is likely to achieve pain modulatory effects independently of those utilised by bottom-up modulation. From a clinical perspective, this suggests that individual differences are likely to play a major role in how patients respond to painful stimuli during stress and cognitive challenges, and that CPM may not be a clinical marker of top-down pain modulatory capacities.

REFERENCES

1. Apkarian AV. Nociception, Pain, Consciousness, and Society: A Plea for Constrained Use of Pain-related Terminologies. *J Pain*. July 2018:1-3. doi:10.1016/j.jpain.2018.05.010.

2. Levine J. Materialism and qualia: The explanatory gap. *Pacific Philosophical Quarterly*. 1983.

3. Roselyne R. The history of pain. *Harvard University Press*. 1993.

4. Melzack R, Wall PD. On the nature of cutaneous sensory mechanisms. *Brain*. 1962;85:331-356.

5. Duncan G. Mind-body dualism and the biopsychosocial model of pain: what did Descartes really say? *J Med Philos*. 2000;25(4):485-513. doi:10.1076/0360-5310(200008)25:4;1-A;FT485.

6. Wall PD. Comments After-30 Years of the Gate Control Theory. *Pain Forum*. 1996;5(1):12-22. doi:10.1016/S1082-3174(96)80063-8.

7. Bliss TV, Lomo T. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J Physiol (Lond)*. 1973;232(2):331-356.

8. Lømo T. The discovery of long-term potentiation. *Philos Trans R Soc Lond, B, Biol Sci*. 2003;358(1432):617-620. doi:10.1098/rstb.2002.1226.

9. Wall PD, Woolf CJ. Muscle but not cutaneous C-afferent input produces prolonged increases in the excitability of the flexion reflex in the rat. *J Physiol (Lond)*. 1984;356:443-458.

10. Woolf CJ. Central sensitization: uncovering the relation between pain and plasticity. *Anesthesiology*. 2007;106(4):864-867. doi:10.1097/01.anes.0000264769.87038.55.

11. Lüscher C, Malenka RC. NMDA receptor-dependent long-term potentiation and long-term depression (LTP/LTD). *Cold Spring*

Harb Perspect Biol. 2012;4(6). doi:10.1101/cshperspect.a005710.

12. Dudek SM, Bear MF. Bidirectional long-term modification of synaptic effectiveness in the adult and immature hippocampus. *J Neurosci*. 1993;13(7):2910-2918.

13. Anderson SD, Basbaum AI, Fields HL. Response of medullary raphe neurons to peripheral stimulation and to systemic opiates. 1977;123(2):363-368.

14. Le Bars D, Villanueva L, Bouhassira D, Willer JC. Diffuse noxious inhibitory controls (DNIC) in animals and in man. *Patol Fiziol Eksp Ter*. 1992;(4):55-65.

15. Langford DJ, Crager SE, Shehzad Z, et al. Social modulation of pain as evidence for empathy in mice. *Science*. 2006;312(5782):1967-1970. doi:10.1126/science.1128322.

16. Colloca L, Benedetti F. Placebo analgesia induced by social observational learning. 2009;144(1-2):28-34. doi:10.1016/j.pain.2009.01.033.

17. McCaul KD, Haugtvedt C. Attention, distraction, and cold-pressor pain. *J Pers Soc Psychol*. 1982;43(1):154-162.

18. James SL, Abate D, Abate KH, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *The Lancet*. 2018;392(10159):1789-1858. doi:10.1016/S0140-6736(18)32279-7.

19. Murray CJL, Vos T, MD PRL, et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. 2012;380(9859):2197-2223. doi:10.1016/S0140-6736(12)61689-4.

20. Breivik H, Collett B, Ventafridda V, Cohen R, Gallacher D. Survey of chronic pain in Europe: prevalence, impact on daily life, and treatment. *Eur J Pain*. 2006;10(4):287-333. doi:10.1016/j.ejpain.2005.06.009.

21. Potvin S, Marchand S. Pain facilitation and pain inhibition during conditioned pain modulation in fibromyalgia and in healthy controls. *PAIN*. 2016;157(8):1704-1710. doi:10.1097/j.pain.0000000000000573.

22. Skou ST, Graven-Nielsen T, Rasmussen S, Simonsen OH, Laursen MB, Arendt-Nielsen L. Widespread sensitization in patients with chronic pain after revision total knee arthroplasty. *PAIN*. 2013;154(9):1588-1594. doi:10.1016/j.pain.2013.04.033.

23. Koelbaek Johansen M, Graven-Nielsen T, Schou Olesen A, Arendt-Nielsen L. Generalised muscular hyperalgesia in chronic whiplash syndrome. 1999;83(2):229-234.

24. Arendt-Nielsen L, Morlion B, Perrot S, et al. Assessment and manifestation of central sensitisation across different chronic pain conditions. *Eur J Pain*. 2018;22(2):216-241. doi:10.1002/ejp.1140.

25. Gore M, Sadosky A, Stacey BR, Tai K-S, Leslie D. The burden of chronic low back pain: clinical comorbidities, treatment patterns, and health care costs in usual care settings. *Spine*. 2012;37(11):E668-E677. doi:10.1097/BRS.0b013e318241e5de.

26. Nordstoga AL, Nilsen TIL, Vasseljen O, Unsgaard-Tøndel M, Mork PJ. The influence of multisite pain and psychological comorbidity on prognosis of chronic low back pain: longitudinal data from the Norwegian HUNT Study. *BMJ Open*. 2017;7(5):e015312. doi:10.1136/bmjopen-2016-015312.

27. McEwen BS, Kalia M. The role of corticosteroids and stress in chronic pain conditions. *Metab Clin Exp*. 2010;59 Suppl 1:S9-S15. doi:10.1016/j.metabol.2010.07.012.

28. Plesner KB, Vaegter HB. Symptoms of Fibromyalgia According to the 2016 Revised Fibromyalgia Criteria in Chronic Pain Patients Referred to Multidisciplinary Pain Rehabilitation: Influence on Clinical and Experimental Pain Sensitivity. *J Pain*. 2018;19(7):777-786. doi:10.1016/j.jpain.2018.02.009.

29. Mansfield KE, Sim J, Jordan JL, Jordan KP. A systematic review and meta-analysis of the prevalence of chronic widespread pain in the general population. 2016;157(1):55-64. doi:10.1097/j.pain.0000000000000314.

30. Andrews P, Steultjens M, Riskowski J. Chronic widespread pain prevalence in the general population: A systematic review. *Eur J Pain*. 2018;22(1):5-18. doi:10.1002/ejp.1090.

31. Yarnitsky D, Arendt-Nielsen L, Bouhassira D, et al. Recommendations on terminology and practice of psychophysical DNIC testing. *Eur J Pain*. 2010;14(4):339-339. doi:10.1016/j.ejpain.2010.02.004.

32. Oosterman JM, Dijkerman HC, Kessels RPC, Scherder EJA. A unique association between cognitive inhibition and pain sensitivity in healthy participants. *Eur J Pain*. 2010;14(10):1046-1050. doi:10.1016/j.ejpain.2010.04.004.

33. Oken BS, Chamine I, Wakeland W. A systems approach to stress, stressors and resilience in humans. *Behav Brain Res*. 2015;282:144-154. doi:10.1016/j.bbr.2014.12.047.

34. O'Brien AT, Deitos A, Pego YT, Fregni F, Carrillo-de-la-Peña MT. Defective Endogenous Pain Modulation in Fibromyalgia: A Meta-Analysis of Temporal Summation and Conditioned Pain Modulation Paradigms. *J Pain*. April 2018:1-18. doi:10.1016/j.jpain.2018.01.010.

35. Gerhardt A, Eich W, Treede R-D, Tesarz J. Conditioned pain modulation in patients with nonspecific chronic back pain with chronic local pain, chronic widespread pain, and fibromyalgia. *PAIN*. 2017;158(3):430-439. doi:10.1097/j.pain.0000000000000777.

36. Wan DWL, Arendt-Nielsen L, Wang K, Xue CC, Wang Y, Zheng Z. Pain Adaptability in Individuals With Chronic Musculoskeletal Pain Is Not Associated With Conditioned Pain Modulation. *J Pain*. 2018;19(8):897-909. doi:10.1016/j.jpain.2018.03.002.

37. Tak LM, Bakker SJL, Rosmalen JGM. Dysfunction of the hypothalamic-pituitary-adrenal axis and functional somatic symptoms: a longitudinal cohort study in the general population. *Psychoneuroendocrinology*. 2009;34(6):869-877. doi:10.1016/j.psyneuen.2008.12.017.

38. Adler GK, Kinsley BT, Hurwitz S, Mossey CJ, Goldenberg DL. Reduced hypothalamic-pituitary and sympathoadrenal

responses to hypoglycemia in women with fibromyalgia syndrome. *Am J Med*. 1999;106(5):534-543.

39. Torpy DJ, Papanicolaou DA, Lotsikas AJ, Wilder RL, Chrousos GP, Pillemer SR. Responses of the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis to interleukin-6: a pilot study in fibromyalgia. *Arthritis Rheum*. 2000;43(4):872-880. doi:10.1002/1529-0131(200004)43:4<872::AID-ANR19>3.0.CO;2-T.

40. Neeck G. Neuroendocrine and hormonal perturbations and relations to the serotonergic system in fibromyalgia patients. *Scand J Rheumatol Suppl*. 2000;113:8-12.

41. Riva R, Mork PJ, Westgaard RH, Lundberg U. Comparison of the cortisol awakening response in women with shoulder and neck pain and women with fibromyalgia. *Psychoneuroendocrinology*. July 2011. doi:10.1016/j.psyneuen.2011.06.014.

42. Riva R, Mork PJ, Westgaard RH, Rø M, Lundberg U. Fibromyalgia syndrome is associated with hypocortisolism. *Int J Behav Med*. 2010;17(3):223-233. doi:10.1007/s12529-010-9097-6.

43. Grace GM, Nielson WR, Hopkins M, Berg MA. Concentration and memory deficits in patients with fibromyalgia syndrome. *Journal of Clinical and Experimental Neuropsychology*. 1999;21(4):477-487. doi:10.1076/jcen.21.4.477.876.

44. Moriarty O, McGuire BE, Finn DP. The effect of pain on cognition function: A review of clinical and preclinical research. *Prog Neurobiol*. 2011;93(3):385-404. doi:10.1016/j.pneurobio.2011.01.002.

45. Park DC, Glass JM, Minear M, Crofford LJ. Cognitive function in fibromyalgia patients. *Arthritis Rheum*. 2001;44(9):2125-2133. doi:10.1002/1529-0131(200109)44:9<2125::AID-ART365>3.0.CO;2-1.

46. Moayedi M, Davis KD. Theories of pain: from specificity to gate control. *J Neurophysiol*. 2013;109(1):5-12. doi:10.1152/jn.00457.2012.

47. Moqrich A. Peripheral pain-sensing neurons: from molecular diversity to functional specialization. *Cell Reports*. 2014;6(2):245-246. doi:10.1016/j.celrep.2014.01.018.

48. Mason P. Placing pain on the sensory map: Classic papers by Ed Perl and colleagues. *J Neurophysiol*. 2007;97(3):1871-1873. doi:10.1152/jn.01327.2006.

49. Millan MJ. Descending control of pain. *Prog Neurobiol*. 2002;66(6):355-474.

50. Damien J, Colloca L, Bellei-Rodriguez C-É, Marchand S. Pain Modulation: From Conditioned Pain Modulation to Placebo and Nocebo Effects in Experimental and Clinical Pain. In: *Neurobiology of the Placebo Effect Part II*. Vol 139. International Review of Neurobiology. Elsevier; 2018:255-296. doi:10.1016/bs.irn.2018.07.024.

51. Le Bars D, Dickenson AH, Besson JM. Diffuse noxious inhibitory controls (DNIC). I. Effects on dorsal horn convergent neurones in the rat. *PAIN*. 1979;6(3):283-304.

52. Lau BK, Vaughan CW. Descending modulation of pain: the GABA disinhibition hypothesis of analgesia. *Curr Opin Neurobiol*. 2014;29:159-164. doi:10.1016/j.conb.2014.07.010.

53. François A, Low SA, Sypek EI, et al. A Brainstem-Spinal Cord Inhibitory Circuit for Mechanical Pain Modulation by GABA and Enkephalins. *Neuron*. 2017;93(4):822-839.e826. doi:10.1016/j.neuron.2017.01.008.

54. Yarnitsky D, Granot M, Granovsky Y. Pain modulation profile and pain therapy: Between pro- and antinociception. 2014;155(4):663-665. doi:10.1016/j.pain.2013.11.005.

55. Iannetti GD, Hughes NP, Lee MC, Mouraux A. Determinants of laser-evoked EEG responses: pain perception or stimulus saliency? *J Neurophysiol*. 2008;100(2):815-828. doi:10.1152/jn.00097.2008.

56. Fields HL. Is there a facilitating component to central pain modulation? *APS Journal*. 1992;1(2):71-78. doi:10.1016/1058-9139(92)90030-g.

57. Naser PV, Kuner R. Molecular, Cellular and Circuit Basis of Cholinergic Modulation of Pain. *Neuroscience*. 2018;387:135-148. doi:10.1016/j.neuroscience.2017.08.049.

58. Heinricher MM, Tavares I, Leith JL, Lumb BM. Descending control of nociception: Specificity, recruitment and plasticity. *Brain Res Rev*. 2009;60(1):214-225. doi:10.1016/j.brainresrev.2008.12.009.

59. Katsuki F, Constantinidis C. Bottom-up and top-down attention: different processes and overlapping neural systems. *Neuroscientist*. 2014;20(5):509-521. doi:10.1177/1073858413514136.

60. Fields HL, Heinricher MM. Anatomy and Physiology of a Nociceptive Modulatory System. *Philos Trans R Soc Lond, B, Biol Sci*. 1985;308(1136):361-374.

61. Tsuruoka M, Willis WD. Descending modulation from the region of the locus coeruleus on nociceptive sensitivity in a rat model of inflammatory hyperalgesia. *Brain Research*. 1996;743(1-2):86-92. doi:10.1016/S0006-8993(96)01025-6.

62. Bannister K, Dickenson AH. The plasticity of descending controls in pain: translational probing. *J Physiol*. 2017;595(13):4159-4166. doi:10.1113/JP274165.

63. Berridge CW, Waterhouse BD. The locus coeruleus–noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. *Brain Research Reviews*. 2003;42(1):33-84. doi:10.1016/S0165-0173(03)00143-7.

64. Reidler JS, Mendonca ME, Santana MB, et al. Effects of Motor Cortex Modulation and Descending Inhibitory Systems on Pain Thresholds in Healthy Subjects. 2012;13(5):450-458. doi:10.1016/j.jpain.2012.01.005.

65. Schabrun SM, Christensen SW, Mrachacz-Kersting N, Graven-Nielsen T. Motor Cortex Reorganization and Impaired Function in the Transition to Sustained Muscle Pain. *Cereb Cortex*. 2016;26(5):1878-1890. doi:10.1093/cercor/bhu319.

66. Baliki MN, Geha PY, Apkarian AV, Chialvo DR. Beyond feeling: chronic pain hurts the brain, disrupting the default-mode network

dynamics. *J Neurosci*. 2008;28(6):1398-1403. doi:10.1523/JNEUROSCI.4123-07.2008.

67. Kucyi A, Scheinman A, Defrin R. Distinguishing Feigned From Sincere Performance in Psychophysical Pain Testing. *J Pain*. 2015;16(10):1044-1053. doi:10.1016/j.jpain.2015.07.004.

68. Seminowicz DA, Mikulis DJ, Davis KD. Cognitive modulation of pain-related brain responses depends on behavioral strategy. *PAIN*. 2004;112(1):48-58. doi:10.1016/j.pain.2004.07.027.

69. Erpelding N, Davis KD. Neural underpinnings of behavioural strategies that prioritize either cognitive task performance or pain. *PAIN*. 2013;154(10):2060-2071. doi:10.1016/j.pain.2013.06.030.

70. Kucyi A, Salomons TV, Davis KD. Mind wandering away from pain dynamically engages antinociceptive and default mode brain networks. *Proc Natl Acad Sci USA*. 2013;110(46):18692-18697. doi:10.1073/pnas.1312902110.

71. Wiech K. Deconstructing the sensation of pain: The influence of cognitive processes on pain perception. *Science*. 2016;354(6312):584-587. doi:10.1126/science.aaf8934.

72. Ossipov MH, Dussor GO, Porreca F. Central modulation of pain. *J Clin Invest*. 2010;120(11):3779-3787. doi:10.1172/JCI43766.

73. Vachon-Presseau E. Effects of stress on the corticolimbic system: implications for chronic pain. *Prog Neuropsychopharmacol Biol Psychiatry*. 2018;87(Pt B):216-223. doi:10.1016/j.pnpbp.2017.10.014.

74. Tracey I, Ploghaus A, Gati JS, et al. Imaging attentional modulation of pain in the periaqueductal gray in humans. *J Neurosci*. 2002;22(7):2748-2752.

75. Goffaux P, de Souza JB, Potvin S, Marchand S. Pain relief through expectation supersedes descending inhibitory deficits in fibromyalgia patients. *PAIN*. 2009;145(1-2):18-23. doi:10.1016/j.pain.2009.02.008.

76. Sluka KA, Frey-Law L, Hoeger Bement M. Exercise-induced pain and analgesia? Underlying mechanisms and clinical

translation. *PAIN*. 2018;159:S91-S97. doi:10.1097/j.pain.0000000000001235.

77. Cohen S, Gianaros PJ, Manuck SB. A Stage Model of Stress and Disease. *Perspectives on Psychological Science*. 2016;11(4):456-463. doi:10.1177/1745691616646305.

78. Koolhaas JM, Bartolomucci A, Buwalda B, et al. Stress revisited: a critical evaluation of the stress concept. *Neurosci Biobehav Rev*. 2011;35(5):1291-1301. doi:10.1016/j.neubiorev.2011.02.003.

79. Tsigos C, Chrousos GP. Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. *J Psychosom Res*. 2002;53(4):865-871.

80. Chrousos GP. Stress and disorders of the stress system. *Nat Rev Endocrinol*. 2009;5(7):374-381. doi:10.1038/nrendo.2009.106.

81. Hellhammer DH, Wüst S, Kudielka BM. Salivary cortisol as a biomarker in stress research. *Psychoneuroendocrinology*. 2009;34(2):163-171. doi:10.1016/j.psyneuen.2008.10.026.

82. El-Farhan N, Rees DA, Evans C. Measuring cortisol in serum, urine and saliva - are our assays good enough? *Ann Clin Biochem*. 2017;54(3):308-322. doi:10.1177/0004563216687335.

83. Nees F, Löffler M, Usai K, Flor H. Hypothalamic-pituitary-adrenal axis feedback sensitivity in different states of back pain. *Psychoneuroendocrinology*. 2018;101:60-66. doi:10.1016/j.psyneuen.2018.10.026.

84. Sobas EM, Reinoso R, Cuadrado-Asensio R, Fernández I, Maldonado MJ, Pastor JC. Reliability of Potential Pain Biomarkers in the Saliva of Healthy Subjects: Inter-Individual Differences and Intersession Variability. Nater UM, ed. *PLoS ONE*. 2016;11(12):e0166976. doi:10.1371/journal.pone.0166976.

85. Dawans von B, Kirschbaum C, Heinrichs M. The Trier Social Stress Test for Groups (TSST-G): A new research tool for controlled simultaneous social stress exposure in a group

format. *Psychoneuroendocrinology*. 2011;36(4):514-522. doi:10.1016/j.psyneuen.2010.08.004.

86. Kalman BA, Grahn RE. Measuring salivary cortisol in the behavioral neuroscience laboratory. *J Undergrad Neurosci Educ*. 2004;2(2):A41-A49.

87. Chapman CR, Tuckett RP, Song CW. Pain and Stress in a Systems Perspective: Reciprocal Neural, Endocrine, and Immune Interactions. 2008;9(2):122-145. doi:10.1016/j.jpain.2007.09.006.

88. Segerstrom SC, Miller GE. Psychological stress and the human immune system: a meta-analytic study of 30 years of inquiry. *Psychol Bull*. 2004;130(4):601-630. doi:10.1037/0033-2909.130.4.601.

89. Hohmann AG, Suplita RL, Bolton NM, et al. An endocannabinoid mechanism for stress-induced analgesia. *Nature*. 2005;435(7045):1108-1112. doi:10.1038/nature03658.

90. Ford GK, Finn DP. Clinical correlates of stress-induced analgesia: Evidence from pharmacological studies. *PAIN*. 2008;140(1):3-7. doi:10.1016/j.pain.2008.09.023.

91. Godfrey KM, Herbert M, Strachan E, et al. Dexamethasone-suppressed Salivary Cortisol and Pain Sensitivity in Female Twins. *The Clinical journal of Pain*. June 2016:1-8. doi:10.1097/AJP.0000000000000398.

92. Pinto-Ribeiro F, Moreira V, Pêgo JM, Leão P, Almeida A, Sousa N. Antinociception induced by chronic glucocorticoid treatment is correlated to local modulation of spinal neurotransmitter content. *Mol Pain*. 2009;5:41. doi:10.1186/1744-8069-5-41.

93. Price CJ, Friston KJ. Functional ontologies for cognition: The systematic definition of structure and function. *Cogn Neuropsychol*. 2005;22(3-4):262-275. doi:10.1080/02643290442000095.

94. Mazza S, Frot M, Rey AE. A comprehensive literature review of chronic pain and memory. *Prog Neuropsychopharmacol Biol Psychiatry*. 2018;87(Pt B):183-192. doi:10.1016/j.pnpbp.2017.08.006.

95. Reyes Del Paso GA, Pulgar A, Duschek S, Garrido S. Cognitive impairment in fibromyalgia syndrome: The impact of cardiovascular regulation, pain, emotional disorders and medication. *Eur J Pain*. December 2011. doi:10.1002/j.1532-2149.2011.00032.x.

96. Vaegter HB, Handberg G, Graven-Nielsen T. Similarities between exercise-induced hypoalgesia and conditioned pain modulation in humans. *PAIN*. 2014;155(1):158-167. doi:10.1016/j.pain.2013.09.023.

97. Stroop JR. Studies of interference in serial verbal reactions. *J Exp Psychol Gen*. 1935;18(6):643-662. doi:10.1037/h0054651.

98. MacLeod CM. The Stroop task: The “gold standard” of attentional measures. *J Exp Psychol Gen*. 1992;121(1):12-14. doi:10.1037/0096-3445.121.1.12.

99. Silvestrini N, Rainville P. After-effects of cognitive control on pain. *Eur J Pain*. 2013;17(8):1225-1233. doi:10.1002/j.1532-2149.2013.00299.x.

100. Buhle J, Wager TD. Performance-dependent inhibition of pain by an executive working memory task. 2010;149(1):19-26. doi:10.1016/j.pain.2009.10.027.

101. Kucyi A, Davis KD. The dynamic pain connectome. *Trends Neurosci*. 2015;38(2):86-95. doi:10.1016/j.tins.2014.11.006.

102. Tobaldini G, Sardi NF, Guilhen VA, Fischer L. Pain Inhibits Pain: an Ascending-Descending Pain Modulation Pathway Linking Mesolimbic and Classical Descending Mechanisms. *Molecular Neurobiology*. June 2018:1-14. doi:10.1007/s12035-018-1116-7.

103. Haack M, Lee E, Cohen DA, Mullington JM. Activation of the prostaglandin system in response to sleep loss in healthy humans: potential mediator of increased spontaneous pain. 2009;145(1-2):136-141. doi:10.1016/j.pain.2009.05.029.

104. Djouhri L, Koutsikou S, Fang X, McMullan S, Lawson SN. Spontaneous pain, both neuropathic and inflammatory, is related to frequency of spontaneous firing in intact C-fiber nociceptors. *J Neurosci*. 2006;26(4):1281-1292. doi:10.1523/JNEUROSCI.3388-05.2006.

105. Haroutounian S, Nikolajsen L, Bendtsen TF, et al. Primary afferent input critical for maintaining spontaneous pain in peripheral neuropathy. 2014;155(7):1272-1279. doi:10.1016/j.pain.2014.03.022.

106. Kosek E, Ekholm J, Hansson P. Sensory dysfunction in fibromyalgia patients with implications for pathogenic mechanisms. *PAIN*. 1996;68(2-3):375-383.

107. Kosek E, Ekholm J, Hansson P. Modulation of pressure pain thresholds during and following isometric contraction in patients with fibromyalgia and in healthy controls. *PAIN*. 1996;64(3):415-423. doi:10.1016/s0304-3959(02)00036-2.

108. Palsson TS, Graven-Nielsen T. Experimental pelvic pain facilitates pain provocation tests and causes regional hyperalgesia. *PAIN*. 2012;153(11):2233-2240. doi:10.1016/j.pain.2012.07.013.

109. Gibson W, Arendt-Nielsen L, Graven-Nielsen T. Referred pain and hyperalgesia in human tendon and muscle belly tissue. *PAIN*. 2006;120(1-2):113-123. doi:10.1016/j.pain.2005.10.023.

110. Serra J, Campero M, Ochoa J. Flare and hyperalgesia after intradermal capsaicin injection in human skin. *J Neurophysiol*. 1998;80(6):2801-2810.

111. Nie H, Madeleine P, Arendt-Nielsen L, Graven-Nielsen T. Temporal summation of pressure pain during muscle hyperalgesia evoked by nerve growth factor and eccentric contractions. *Eur J Pain*. 2009;13(7):704-710. doi:10.1016/j.ejpain.2008.06.015.

112. Graven-Nielsen T, Izumi M, Petersen KK, Arendt-Nielsen L. User-independent assessment of conditioning pain modulation by cuff pressure algometry. *Eur J Pain*. 2017;21(3):552-561. doi:10.1002/ejp.958.

113. Sato H, Droney J, Ross J, et al. Gender, variation in opioid receptor genes and sensitivity to experimental pain. *Mol Pain*. 2013;9(1):20-20. doi:10.1186/1744-8069-9-20.

114. Tumi El H, Johnson MI, Dantas PBF, Maynard MJ, Tashani OA. Age-related changes in pain sensitivity in healthy humans: A

systematic review with meta-analysis. *Eur J Pain*. 2017;21(6):955-964. doi:10.1002/ejp.1011.

115. Lautenbacher S, Peters JH, Heesen M, Scheel J, Kunz M. Age changes in pain perception: A systematic-review and meta-analysis of age effects on pain and tolerance thresholds. *Neurosci Biobehav Rev*. 2017;75:104-113. doi:10.1016/j.neubiorev.2017.01.039.

116. Racine M, Tousignant-Laflamme Y, Kloda LA, Dion D, Dupuis G, Choinière M. A systematic literature review of 10 years of research on sex/gender and experimental pain perception - part 1: are there really differences between women and men? *PAIN*. 2012;153(3):602-618. doi:10.1016/j.pain.2011.11.025.

117. Skovbjerg S, Jørgensen T, Arendt-Nielsen L, Ebstrup JF, Carstensen T, Graven-Nielsen T. Conditioned Pain Modulation and Pressure Pain Sensitivity in the Adult Danish General Population: The DanFunD Study. *J Pain*. 2017;18(3):274-284. doi:10.1016/j.jpain.2016.10.022.

118. Kennedy DL, Kemp HI, Ridout D, Yarnitsky D, Rice ASC. Reliability of conditioned pain modulation: a systematic review. *PAIN*. 2016;157(11):2410-2419. doi:10.1097/j.pain.0000000000000689.

119. Teepker M, Kunz M, Peters M, KUNDERMANN B, Schepelmann K, Lautenbacher S. Endogenous pain inhibition during menstrual cycle in migraine. *Eur J Pain*. 2014;18(7):989-998. doi:10.1002/j.1532-2149.2013.00444.x.

120. Vaegter HB, Handberg G, Jørgensen MN, Kinly A, Graven-Nielsen T. Aerobic exercise and cold pressor test induce hypoalgesia in active and inactive men and women. *Pain Med*. 2015;16(5):923-933. doi:10.1111/pme.12641.

121. Vaegter HB, Graven-Nielsen T. Pain modulatory phenotypes differentiate subgroups with different clinical and experimental pain sensitivity. *PAIN*. 2016;157(7):1480-1488. doi:10.1097/j.pain.0000000000000543.

122. Horn-Hofmann C, Kunz M, Madden M, Schnabel E-L, Lautenbacher S. Interactive effects of conditioned pain modulation and temporal summation of pain-the role of stimulus

modality. *PAIN*. 2018;159(12):2641-2648. doi:10.1097/j.pain.0000000000001376.

123. Treede RD, Meyer RA, Raja SN, Campbell JN. Peripheral and central mechanisms of cutaneous hyperalgesia. *Prog Neurobiol*. 1992;38(4):397-421.

124. Waller R, Straker L, O'Sullivan P, Sterling M, Smith A. Reliability of pressure pain threshold testing in healthy pain free young adults. *Scandinavian Journal of Pain*. 2015;9:38-41. doi:10.1016/j.sjpain.2015.05.004.

125. Arendt-Nielsen L. Reliability of pressure pain threshold testing (PPT) in healthy pain free young adults. *Scandinavian Journal of Pain*. 2015;9:28-29. doi:10.1016/j.sjpain.2015.06.002.

126. Imai Y, Petersen KK, Mørch CD, Arendt-Nielsen L. Comparing test–retest reliability and magnitude of conditioned pain modulation using different combinations of test and conditioning stimuli. *Somatosens Mot Res*. September 2016:1-9. doi:10.1080/08990220.2016.1229178.

127. Graven-Nielsen T, Vaegter HB, Finocchietti S, Handberg G, Arendt-Nielsen L. Assessment of musculoskeletal pain sensitivity and temporal summation by cuff pressure algometry: a reliability study. *PAIN*. 2015;156(11):2193-2202. doi:10.1097/j.pain.0000000000000294.

128. Polianskis R, Graven-Nielsen T, Arendt-Nielsen L. Computer-controlled pneumatic pressure algometry—a new technique for quantitative sensory testing. *Eur J Pain*. 2001;5(3):267-277. doi:10.1053/eujp.2001.0245.

129. Hoegh M, Petersen KK, Graven-Nielsen T. Effects of repeated conditioning pain modulation in healthy volunteers. *Eur J Pain*. 2018;22(10):1833-1843. doi:10.1002/ejp.1279.

130. Dissanayaka TD, Farrell M, Zoghi M, Egan GF, Jaberzadeh S. Test-retest reliability of subjective supra-threshold scaling of multiple pressure-pain sensations among healthy individuals: a study using hydraulic pressure algometry. *Somatosens Mot Res*. 2018;25:1-9. doi:10.1080/08990220.2018.1505608.

131. Martinsen S, Flodin P, Berrebi J, et al. Fibromyalgia patients had normal distraction related pain inhibition but cognitive impairment reflected in caudate nucleus and hippocampus during the Stroop Color Word Test. *PLoS ONE*. 2014;9(9):e108637. doi:10.1371/journal.pone.0108637.

132. Martinsen S, Flodin P, Berrebi J, et al. The role of long-term physical exercise on performance and brain activation during the Stroop colour word task in fibromyalgia patients. *Clin Physiol Funct Imaging*. 2018;38(3):508-516. doi:10.1111/cpf.12449.

133. McPhee M, Graven-Nielsen T. Alterations in Temporal Summation of Pain and Conditioned Pain Modulation Across an Episode of Experimental Exercise-Induced Low Back Pain. *J Pain*. September 2018. doi:10.1016/j.jpain.2018.08.010.

134. Lie MU, Matre D, Hansson P, Stubhaug A, Zwart J-A, Nilsen KB. A tonic heat test stimulus yields a larger and more reliable conditioned pain modulation effect compared to a phasic heat test stimulus. *PAIN Reports*. 2017;2(6):e626. doi:10.1097/PR9.0000000000000626.

135. Petersen KK, Vaegter HB, Arendt-Nielsen L. An updated view on the reliability of different protocols for the assessment of conditioned pain modulation. *PAIN*. 2017;158(5):988. doi:10.1097/j.pain.0000000000000833.

136. Lewis GN, Rice DA, McNair PJ. Conditioned pain modulation in populations with chronic pain: a systematic review and meta-analysis. *J Pain*. 2012;13(10):936-944. doi:10.1016/j.jpain.2012.07.005.

137. Vaegter HB, Petersen KK, Mørch CD, Imai Y, Arendt-Nielsen L. Assessment of CPM reliability: quantification of the within-subject reliability of 10 different protocols. *Scandinavian Journal of Pain*. 2018;0(0):663.

138. Nir R-R, Yarnitsky D. Conditioned Pain Modulation. *Topics in Pain Management*. 2015;30(11):1-8. doi:10.1097/01.TPM.0000467011.25779.e8.

139. Nahman-Averbuch H, Yarnitsky D, Granovsky Y, Gerber E, Dagul P, Granot M. The role of stimulation parameters on the

conditioned pain modulation response. *Scandinavian Journal of Pain*. 2013;4(1):10-14. doi:10.1016/j.sjpain.2012.08.001.

140. Polianskis R, Graven-Nielsen T, Arendt-Nielsen L. Spatial and temporal aspects of deep tissue pain assessed by cuff algometry. *PAIN*. 2002;100(1-2):19-26.

141. Nie H, Arendt-Nielsen L, Andersen H, Graven-Nielsen T. Temporal summation of pain evoked by mechanical stimulation in deep and superficial tissue. *J Pain*. 2005;6(6):348-355. doi:10.1016/j.jpain.2005.01.352.

142. Razavi M, Hansson PT, Johansson B, Leffler A-S. The influence of intensity and duration of a painful conditioning stimulation on conditioned pain modulation in volunteers. *Eur J Pain*. 2014;18(6):853-861. doi:10.1002/j.1532-2149.2013.00435.x.

143. Lautenbacher S, Kunz M, Burkhardt S. The effects of DNIC-type inhibition on temporal summation compared to single pulse processing: does sex matter? *PAIN*. 2008;140(3):429-435. doi:10.1016/j.pain.2008.09.019.

144. Granot M, Weissman-Fogel I, Crispel Y, et al. Determinants of endogenous analgesia magnitude in a diffuse noxious inhibitory control (DNIC) paradigm: do conditioning stimulus painfulness, gender and personality variables matter? *PAIN*. 2008;136(1-2):142-149. doi:10.1016/j.pain.2007.06.029.

145. Weissman-Fogel I, Dror A, Defrin R. Temporal and spatial aspects of experimental tonic pain: Understanding pain adaptation and intensification. *Eur J Pain*. 2015;19(3):408-418. doi:10.1002/ejp.562.

146. Mlekusch S, Neziri AY, Limacher A, Jüni P, Arendt-Nielsen L, Curatolo M. Conditioned Pain Modulation in Patients With Acute and Chronic Low Back Pain. *The Clinical journal of Pain*. 2016;32(2):116-121. doi:10.1097/AJP.0000000000000238.

147. Ladouceur A, Rustamov N, Dubois J-D, et al. Inhibition of Pain and Pain-Related Brain Activity by Heterotopic Noxious Counter-Stimulation and Selective Attention in Chronic Non-Specific Low Back Pain. *Neuroscience*. 2018;387:201-213. doi:10.1016/j.neuroscience.2017.09.054.

148. Yarnitsky D, Granot M, Nahman-Averbuch H, Khamaisi M, Granovsky Y. Conditioned pain modulation predicts duloxetine efficacy in painful diabetic neuropathy. *PAIN*. 2012;153(6):1193-1198. doi:10.1016/j.pain.2012.02.021.

149. Hermans L, Van Oosterwijck J, Goubert D, et al. Inventory of Personal Factors Influencing Conditioned Pain Modulation in Healthy People: A Systematic Literature Review. *Pain Pract*. 2016;16(6):758-769. doi:10.1111/papr.12305.

150. Peerdeman KJ, van Laarhoven AIM, Keij SM, et al. Relieving patients' pain with expectation interventions: a meta-analysis. 2016;157(6):1179-1191. doi:10.1097/j.pain.0000000000000540.

151. Bjørkedal E, Flaten MA. Expectations of increased and decreased pain explain the effect of conditioned pain modulation in females. *JPR*. 2012;5:289-300. doi:10.2147/JPR.S33559.

152. Kjøgx H, Zachariae R, Pfeiffer-Jensen M, et al. Pain frequency moderates the relationship between pain catastrophizing and pain. *Front Psychol*. 2014;5. doi:10.3389/fpsyg.2014.01421/abstract.

153. Kjøgx H, Kasch H, Zachariae R, Svensson P, Jensen TS, Vase L. Experimental manipulations of pain catastrophizing influence pain levels in patients with chronic pain and healthy volunteers. 2016;157(6):1287-1296. doi:10.1097/j.pain.0000000000000519.

154. Niederstrasser NG, Meulders A, Meulders M, Slepian PM, Vlaeyen JWS, Sullivan MJL. Pain Catastrophizing and Fear of Pain Predict the Experience of Pain in Body Parts Not Targeted by a Delayed-Onset Muscle Soreness Procedure. *J Pain*. 2015;16(11):1065-1076. doi:10.1016/j.jpain.2015.07.008.

155. Weissman-Fogel I, Sprecher E, Pud D. Effects of catastrophizing on pain perception and pain modulation. *Exp Brain Res*. 2007;186(1):79-85. doi:10.1007/s00221-007-1206-7.

156. Leung L. Pain catastrophizing: an updated review. *Indian J Psychol Med*. 2012;34(3):204-217. doi:10.4103/0253-7176.106012.

157. Scott W, Wideman TH, Sullivan MJL. Clinically meaningful scores on pain catastrophizing before and after multidisciplinary

rehabilitation: a prospective study of individuals with subacute pain after whiplash injury. *The Clinical journal of Pain*. 2014;30(3):183-190. doi:10.1097/AJP.0b013e31828eee6c.

158. Kristiansen FL, Olesen AE, Brock C, et al. The role of pain catastrophizing in experimental pain perception. *Pain Pract*. 2014;14(3):E136-E145. doi:10.1111/papr.12150.

159. Sullivan MJL, Bishop SR, Pivik J. The Pain Catastrophizing Scale: Development and validation. *Psychological Assessment*. 1995;7(4):524-532. doi:10.1037/1040-3590.7.4.524.

160. Klyne DM, Schmid AB, Moseley GL, Sterling M, Hodges PW. Effect of types and anatomic arrangement of painful stimuli on conditioned pain modulation. *J Pain*. 2015;16(2):176-185. doi:10.1016/j.jpain.2014.11.005.

161. Yarnitsky D, Bouhassira D, Drewes AM, et al. Recommendations on practice of conditioned pain modulation (CPM) testing. *Eur J Pain*. 2015;19(6):805-806. doi:10.1002/ejp.605.

162. Granovsky Y, Miller Barmak A, Goldstein O, Sprecher E, Yarnitsky D. CPM Test-Retest Reliability: "Standard" vs “Single Test-Stimulus” Protocols. *Pain Med*. 2016;17(3):521-529. doi:10.1111/pme.12868.

163. Richardson JTE. Eta squared and partial eta squared as measures of effect size in educational research. *Educational Research Review*. 2011;6(2):135-147. doi:10.1016/j.edurev.2010.12.001.

164. Pud D, Granovsky Y, Yarnitsky D. The methodology of experimentally induced diffuse noxious inhibitory control (DNIC)-like effect in humans. *PAIN*. 2009;144(1-2):16-19. doi:10.1016/j.pain.2009.02.015.

165. Wilson H, Carvalho B, Granot M, Landau R. Temporal stability of conditioned pain modulation in healthy women over four menstrual cycles at the follicular and luteal phases. 2013;154(12):2633-2638. doi:10.1016/j.pain.2013.06.038.

166. Pud D, Sprecher E, Yarnitsky D. Homotopic and heterotopic effects of endogenous analgesia in healthy volunteers. *Neurosci Lett*. 2005;380(3):209-213. doi:10.1016/j.neulet.2005.01.037.

167. Lautenbacher S, Roscher S, Strian F. Inhibitory effects do not depend on the subjective experience of pain during heterotopic noxious conditioning stimulation (HNCS): a contribution to the psychophysics of pain inhibition. *Eur J Pain*. 2002;6(5):365-374. doi:10.1016/S1090-3801(02)00030-7.

168. Klyne DM, Moseley GL, Sterling M, Barbe MF, Hodges PW. Individual Variation in Pain Sensitivity and Conditioned Pain Modulation in Acute Low Back Pain: Effect of Stimulus Type, Sleep, and Psychological and Lifestyle Factors. *J Pain*. 2018;19(8). doi:10.1016/j.jpain.2018.02.017.

169. Nahman-Averbuch H, Granovsky Y, Coghill RC, Yarnitsky D, Sprecher E, Weissman-Fogel I. Waning of "Conditioned Pain Modulation": A Novel Expression of Subtle Pronociception in Migraine. *Headache: The Journal of Head and Face Pain*. 2013;53(7):1104-1115. doi:10.1111/head.12117.

170. Naugle KM, Fillingim RB, Riley JL. A meta-analytic review of the hypoalgesic effects of exercise. *J Pain*. 2012;13(12):1139-1150. doi:10.1016/j.jpain.2012.09.006.

171. Vase L, Riley JL, Price DD. A comparison of placebo effects in clinical analgesic trials versus studies of placebo analgesia. 2002;99(3):443-452.

172. Greffrath W, Baumgärtner U, Treede R-D. Peripheral and central components of habituation of heat pain perception and evoked potentials in humans. *PAIN*. 2007;132(3):301-311. doi:10.1016/j.pain.2007.04.026.

173. Treister R, Pud D, Ebstein R, Laiba E, Gershon E. Associations between polymorphisms in dopamine neurotransmitter pathway genes and pain response in healthy humans. 2009.

174. Aparecida da Silva V, Galhardoni R, Teixeira MJ, Ciampi De Andrade D. Not just a matter of pain intensity: Effects of three different conditioning stimuli on conditioned pain modulation effects. *Neurophysiologie Clinique / Clinical Neurophysiology*. 2018;48(5):287-293. doi:10.1016/j.neucli.2018.06.078.

175. Nir R-R, Yarnitsky D, Honigman L, Granot M. Cognitive manipulation targeted at decreasing the conditioning pain perception reduces the efficacy of conditioned pain modulation. *PAIN*. 2012;153(1):170-176. doi:10.1016/j.pain.2011.10.010.

176. Nir R-R, Granovsky Y, Yarnitsky D, Sprecher E, Granot M. A psychophysical study of endogenous analgesia: The role of the conditioning pain in the induction and magnitude of conditioned pain modulation. *Eur J Pain*. 2011;15(5):491-497. doi:10.1016/j.ejpain.2010.10.001.

177. Smith A, Pedler A. Conditioned pain modulation is affected by occlusion cuff conditioning stimulus intensity, but not duration. *Eur J Pain*. 2017;22(Pt 4):816-819. doi:10.1002/ejp.1093.

178. Arendt-Nielsen L, Sluka KA, Nie HL. Experimental muscle pain impairs descending inhibition. 2008;140(3):465-471. doi:10.1016/j.pain.2008.09.027.

179. Rolke R, Baron R, Maier C, et al. Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): standardized protocol and reference values. 2006;123(3):231-243. doi:10.1016/j.pain.2006.01.041.

180. Steptoe A, Hamer M, Chida Y. The effects of acute psychological stress on circulating inflammatory factors in humans: a review and meta-analysis. *Brain Behav Immun*. 2007;21(7):901-912. doi:10.1016/j.bbi.2007.03.011.

181. Geva N, Pruessner J, Defrin R. Acute psychosocial stress reduces pain modulation capabilities in healthy men. *PAIN*. 2014;155(11):2418-2425. doi:10.1016/j.pain.2014.09.023.

182. Andreou E, Alexopoulos EC, Lionis C, et al. Perceived Stress Scale: Reliability and Validity Study in Greece. *IJERPH*. 2011;8(8):3287-3298. doi:10.3390/ijerph8083287.

183. White RS, Jiang J, Hall CB, et al. Higher Perceived Stress Scale Scores Are Associated with Higher Pain Intensity and Pain Interference Levels in Older Adults. *J Am Geriatr Soc*. 2014;62(12):2350-2356. doi:10.1111/jgs.13135.

184. Østerås B, Sigmundsson H, Haga M. Perceived stress and musculoskeletal pain are prevalent and significantly associated

in adolescents: an epidemiological cross- sectional study. *BMC Public Health*. October 2015:1-10. doi:10.1186/s12889-015-2414-x.

185. Usui H, Nishida Y. The very low-frequency band of heart rate variability represents the slow recovery component after a mental stress task. *PLoS ONE*. 2017;12(8):e0182611. doi:10.1371/journal.pone.0182611.

186. Chapman CR, Tuckett RP, Song CW. Pain and stress in a systems perspective: reciprocal neural, endocrine, and immune interactions. *J Pain*. 2008;9(2):122-145. doi:10.1016/j.jpain.2007.09.006.

187. Dickerson SS, Kemeny ME. Acute Stressors and Cortisol Responses: A Theoretical Integration and Synthesis of Laboratory Research. *Psychol Bull*. 2004;130(3):355-391. doi:10.1037/0033-2909.130.3.355.

188. Kirschbaum C, Pirke KM, Hellhammer DH. The “Trier Social Stress Test”–a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology*. 1993.

189. Atchley R, Ellingson R, Klee D, Memmott T, Oken B. A cognitive stressor for event-related potential studies: the Portland arithmetic stress task. *Stress (Amsterdam, Netherlands)*. 2017;20(3):277-284. doi:10.1080/10253890.2017.1335300.

190. Dedovic K, Renwick R, Mahani NK, Engert V, Lupien SJ, Pruessner JC. The Montreal Imaging Stress Task: using functional imaging to investigate the effects of perceiving and processing psychosocial stress in the human brain. *J Psychiatry Neurosci*. 2005;30(5):319-325.

191. Geva N, Defrin R. Opposite Effects of Stress on Pain Modulation Depend on the Magnitude of Individual Stress Response. *J Pain*. 2018;19(4):360-371. doi:10.1016/j.jpain.2017.11.011.

192. Geva N, Pruessner J, Defrin R. Triathletes Lose Their Advantageous Pain Modulation under Acute Psychosocial Stress. *Med Sci Sports Exerc*. 2016;49(2):1-341. doi:10.1249/MSS.0000000000001110.

193. Nilsen KB, Christiansen SE, Holmen LB, Sand T. The effect of a mental stressor on conditioned pain modulation in healthy subjects. *Scandinavian Journal of Pain*. 2012;3(3):142-148. doi:10.1016/j.sjpain.2012.04.005.

194. Caceres C, Burns JW. Cardiovascular reactivity to psychological stress may enhance subsequent pain sensitivity. *PAIN*. 1997;69(3):237-244.

195. Cathcart S, Petkov J, Pritchard D. Effects of induced stress on experimental pain sensitivity in chronic tension-type headache sufferers. *Eur J Neurol*. 2008;15(6):552-558. doi:10.1111/j.1468-1331.2008.02124.x.

196. Cathcart S, Winefield AH, Lushington K, Rolan P. Noxious Inhibition of Temporal Summation is Impaired in Chronic Tension-Type Headache. *Headache: The Journal of Head and Face Pain*. 2010;50(3):403-412. doi:10.1111/j.1526-4610.2009.01545.x.

197. Bement MH, Weyer A, Keller M, Harkins AL, Hunter SK. Anxiety and stress can predict pain perception following a cognitive stress. *Physiol Behav*. 2010;101(1):87-92. doi:10.1016/j.physbeh.2010.04.021.

198. Crettaz B, Marziniak M, Willeke P, et al. Stress-Induced Allodynia – Evidence of Increased Pain Sensitivity in Healthy Humans and Patients with Chronic Pain after Experimentally Induced Psychosocial Stress. Paul F, ed. *PLoS ONE*. 2013;8(8):e69460. doi:10.1371/journal.pone.0069460.t003.

199. Reinhardt T, Kleindienst N, Treede R-D, Bohus M, Schmahl C. Individual modulation of pain sensitivity under stress. *Pain Med*. 2013;14(5):676-685. doi:10.1111/pme.12090.

200. Timmers I, Kaas AL, Quaedflieg CWEM, Biggs EE, Smeets T, de Jong JR. Fear of pain and cortisol reactivity predict the strength of stress-induced hypoalgesia. *Eur J Pain*. 2018;22(7):1291-1303. doi:10.1002/ejp.1217.

201. Bali A, Jaggi AS. Clinical experimental stress studies: methods and assessment. *Reviews in the Neurosciences*. 2015;26(5):555-579. doi:10.1515/revneuro-2015-0004.

202. Cathcart S, Winefield AH, Lushington K, Rolan P. Effect of mental stress on cold pain in chronic tension-type headache sufferers. *J Headache Pain*. 2009;10(5):367-373. doi:10.1007/s10194-009-0131-5.

203. Gaab J, Jiménez J, Voneschen L, et al. Psychosocial Stress-Induced Analgesia: An Examination of Effects on Heat Pain Threshold and Tolerance and of Neuroendocrine Mediation. *Neuropsychobiology*. 2017;74(2):87-95. doi:10.1159/000454986.

204. Legrain V, Iannetti GD, Plaghki L, Mouraux A. The pain matrix reloaded: a salience detection system for the body. *Prog Neurobiol*. 2011;93(1):111-124. doi:10.1016/j.pneurobio.2010.10.005.

205. Sikandar S, West SJ, McMahon SB, Bennett DL, Dickenson AH. Sensory processing of deep tissue nociception in the rat spinal cord and thalamic ventrobasal complex. *Physiol Rep*. 2017;5(14):e13323–13. doi:10.14814/phy2.13323.

206. Aviram J, Shochat T, Pud D. Pain Perception in Healthy Young Men Is Modified by Time-Of-Day and Is Modality Dependent. *Pain Medicine*. 2015;16(6):1137-1144. doi:10.1111/pme.12665.

207. Bush G, Whalen PJ, Rosen BR, Jenike MA, McInerney SC, Rauch SL. The counting Stroop: an interference task specialized for functional neuroimaging--validation study with functional MRI. *Hum Brain Mapp*. 1998;6(4):270-282.

208. Bantick SJ, Wise RG, Ploghaus A, Clare S, Smith SM, Tracey I. Imaging how attention modulates pain in humans using functional MRI. *Brain*. 2002;125(Pt 2):310-319.

209. Wilder-Smith CH, Li X, Shen L, Cao Y, Ho KY, Wong RK. Dysfunctional endogenous pain modulation in patients with functional dyspepsia. *Neurogastroenterol Motil*. 2013;26(4):489-498. doi:10.1111/nmo.12291.

210. Fechir M, Schlereth T, Kritzmann S, et al. Stress and thermoregulation: different sympathetic responses and different effects on experimental pain. *Eur J Pain*. 2009;13(9):935-941. doi:10.1016/j.ejpain.2008.11.002.

211. Marouf R, Caron S, Lussier M, Bherer L, Piché M, Rainville P. Reduced pain inhibition is associated with reduced cognitive inhibition in healthy aging. 2014;155(3):494-502. doi:10.1016/j.pain.2013.11.011.

212. Buhle JT, Stevens BL, Friedman JJ, Wager TD. Distraction and Placebo: Two Separate Routes to Pain Control. *Psychol Sci*. 2012;23(3):246-253. doi:10.1177/0956797611427919.

213. Ivanec D, Pavin T, Kotzmuth A. Possibilities of attentional control of pain: Influence of distractive Stroop task on pain threshold and pain tolerance. *Review of psychology*. 2007;13(2):87-94.

214. Marouf R, Caron S, Lussier M, Bherer L, Piché M, Rainville P. Reduced pain inhibition is associated with reduced cognitive inhibition in healthy aging. *PAIN*. 2014;155(3):494-502. doi:10.1016/j.pain.2013.11.011.

215. Serbic D, Pincus T. Diagnostic uncertainty and recall bias in chronic low back pain. *PAIN*. 2014;155(8):1540-1546. doi:10.1016/j.pain.2014.04.030.

216. Meeus M, Van Oosterwijck J, Ickmans K, et al. Interrelationships between pain processing, cortisol and cognitive performance in chronic whiplash-associated disorders. *Clin Rheumatol*. 2015;34(3):545-553. doi:10.1007/s10067-013-2446-5.

217. Coppieters I, Ickmans K, Cagnie B, et al. Cognitive Performance Is Related to Central Sensitization and Health-related Quality of Life in Patients with Chronic Whiplash-Associated Disorders and Fibromyalgia. *Pain Physician*. 2015;18(3):E389-E401.

218. Hauck M, Lorenz J, Domnick C, Gerloff C, Engel AK. Top-Down and Bottom-Up Modulation of Pain-Induced Oscillations. *Front Hum Neurosci*. 2015;9.

Appendix A. Overview of the literature

Appendix A.1 – A.3 summarises literature on repeated TS and CPM (A.1); social stress, pain sensitivity and CPM (A.2); and Stroop, pain sensitivity and CPM (A.3) in healthy volunteers. Studies that have also examined clinical populations have been summarised for the data related to healthy subjects only.

Literature searching used PubMed, keywords in Google Scholar and reference lists in studies with a specific relation to any of the three studies. Literature searches have been ongoing throughout the period (from 2016) but have not been structured beyond what is mentioned here.

The following keywords have been applied for the structured search on PubMed and Google Scholar:
CPM: Conditioned pain modulation, CPM, pain modulation, DNIC, diffuse noxious inhibitory controls, heterotopic noxious conditioning stimulation, endogenous analgesia, counter irritation
Stress: Social stress, acute experimental stress, Trier Social Stress Task, Montreal Imaging Stress Task, Saliva cortisol, perceived stress, stress induced hypoalgesia, stress induced hyperalgesia, stress analgesia, stress induced pain modulation
Stroop: Stroop, Stroop Task, Attention task, cognitive load, top-down inhibition of pain, cognitive analgesia, cognitive induced hyperalgesia.

Table A.1: Repeated pain sensitivity and CPM in healthy volunteers

Studies are summarised by paradigm and main findings and organised by study aim (repeated TS, repeated CPM, reliability) and next by TS (cuff, handheld pressure, heat, cold, electrical)

Reference	Participants	Design	TS and effect on pain sensitivity	CS and effect on CPM	Other
Repeated stimuli (TS or CS)					
Isselée H, De Laat A, Lesaffre E, Lysens R. Short-term reproducibility of pressure pain thresholds in masseter and temporalis muscles of symptom-free subjects. Eur J Oral Sci. 1997;105(6):583-587.	N = 22 (11 males) 27 yrs (21-35 yrs)	PPT testet in the morning and afternoon. Two sessions at each time point: 5 min between each session. Each session had two trials, separated by > 30s.	TS: PPT twice on two different muscles (bilaterally). Afternoon PPT < morning PPT 1st PPT > 2nd PPT = habituation	Not tested	
Treister R, Eisenberg E, Gershon E, Haddad M, Pud D. *Factors affecting - and relationships between-different modes of endogenous pain modulation in healthy volunteers.* Eur J Pain. 2010;14(6):608-614.	N = 191 (87 males) 24.5 yrs ± 0.25	Baseline TS + 4 identical TS (TS1-4) used to test for habituation. Same protocol – but with CS – used for CPM and control (water = 25˚C)	TS: Heat pain 3s (47˚C, 12s ISI). Habituation = control (non-painful CS)	CS: Cold water parallel to TS2 and TS3 (12˚C). CPM > control (25˚C) CPM > 'habituation' (only TS)	
Zheng Z, Wang K, Yao D, Xue CCL, Arendt-Nielsen L. Adaptability to pain is associated with potency of local pain inhibition, but not conditioned pain modulation: a healthy human study. PAIN. 2014;155(5):968-976.	N = 41 (20 males) 28.3 yrs ± 7.9 SD	Cross-over: Effect of hot and cold induced pain (CS) on PPT and pinprick.	TS: PPT and 512 mN HeatCS = +ve CPM on both ColdCS = PPT +ve CPM	CS-hot: 46˚C for 7 minutes CS-cold: 1-4˚C for 5 minutes Heat-pain increased from 0-3 min and remained stable. Cold-pain habituated slowly after 2-3 minutes	
Fujii K, Motohashi K, Umino M. *Heterotopic ischemic pain attenuates somatosensory evoked potentials induced by electrical tooth stimulation: diffuse noxious inhibitory controls in the trigeminal nerve territory.* Eur J Pain. 2006;10(6):485-504.	N = 19 (7 males) 29.7 yrs ± 4.5 SD	2 sessions: Noxious vs Control	TS: Electrical tooth stimulation, approximately 1.4 x pain detection threshold (VAS = 55.3). Pain evaluated at baseline, during cuff, 5-min after CS and 15 min after cuff.	CS: Tourniquet cuff; ischeamic, 10 mins CPM < Control (i.e. +ve) during CS and 5-min post CS	
Ernst M, Lee WH, Dworkin B, Zaretsky HH. *Pain perception decrement produced through repeated stimulation. PAIN.* 1986;26(2):221-231.	N = 11 (5 males) Age not reported	13 trials (1 trial = 3 mins). Time 0-60 = repetitive stimulation (RS), constant intensity. Time 60-120 free of stimulation (post-RS) ; Compare RS with post-RS.	TS: Electrical (pain detection threshold) decreased during RS. No sign of potentiation. Pain threshold remained at adapted level 60 min post-RS.	Not tested	The results were not affected by Naloxone (separate experiment, same study)

Reference	Participants	Design	TS and effect on pain sensitivity	CS and effect on CPM	Other
Repeated CPM / Comparing CS					
Smith A, Pedler A. *Conditioned pain modulation is affected by occlusion cuff conditioning stimulus intensity, but not duration*. Eur J Pain. 2017;22(Pt 4):816-819	S1: N = 27 (19 males) 24.9 yrs ± 4.5 SD S2: N = 25 (12 male) 22.5 yrs ± 2.7 SD No significant gender differences	S1: 1: PPT x 3 (Baseline 1) 2: Occlusion cuff NRS2 + PPT x 1 3: 5-min rest 4: PPT x 3 (post-CS 1) 5. Occlusion cuff NRS5 + PPT x 1 6: PPT x 3 (post-CS 2) S2: 1: PPT x 3 (Baseline 2) 2: 5-min rest 3: Occlusion cuff (3 min) +PPT at 1, 2 and 3 min 4: 5-min rest 5: PPT x 3 (post-CS)	S1 + 2: PPT (20s ISI)	S1: Occlusion cuff (NRS2 or NRS5) S2: Occlusion cuff (NRS2) Pain intensity from CS = NRS (0-10) **Results:** S1: No carry-over effects. CPM-effects increased with CS-intensity (NRS5 > NRS2). No CPM-effect with NRS2. S2: NRS2 did not induce CPM-effect. Pain-intensity from CS increased at 2 and 3-min compared to baseline. "…pain intensity, but not duration of the CS, was associated with increased heterotopic PPTs"	No significant interactions with physical acivity.
Klyne DM, Schmid AB, Moseley GL, Sterling M, Hodges PW. *Effect of types and anatomic arrangement of painful stimuli on conditioned pain modulation.* J Pain. 2015;16(2):176-185	N = 31 (14 males) 25 yrs ± 6 SD	Combination of R/L forearm and R/L lower back (homotopic, heterotopic and different anatomic regions) 4 blocks of (TS x 2), 15 min break, 2nd TS was conditioned. 1 block = 3 x PPT + 3 x Pain-45 (approximately 90s total). A sham bloc was included (control).	TS1 = PPT (mean of 3 trials < 10s ISI) TS2 = Heat (Pain-45, mean of 3 trials) Change in TS over time not analysed	CS = Heat pain threshold (mean of 5 trials), approx 90s. CS-intensity = NRS (0-100) at 0, 30 and 90s. CS below 35/100 = exclusion. PPT: Increased with heterotopic and different anatomic regions (= +ve CPM). Not homotopic or sham. Pain-45: +ve CPM in all combinations, including sham. CPM: Only PPT is valid for CPM since Pain-45 increase HPT independent of CS and during homotopic stimuli. Best CPM with same location on each side of the body.	
Honigman L, Yarnitsky D, Sprecher E, Weissman-Fogel I. *Psychophysical testing of spatial and temporal dimensions of endogenous analgesia: conditioned pain modulation and offset analgesia.* Exp Brain Res. 2013;228(4):493-501	N = 29 (15 males) 27.6 yrs ± 3.4 SD	2 CPM-assessments, parallel design	TS: 49 ℃ (heat, 30s), pain intensity rated 6 x (NPS).	CS: Hot water (46 ℃, 60s) pain intensity rated 2 x (NPS) 10s, 20s **Result**: *"no significant changes in pain ratings during the CPM versus 'constant' stimu- lation at any of the time points were demonstrated"*	Also tested offset analgesia, and *"main finding of our study is the additive effect of CPM and OA on pain inhibition in males, suggesting that the two paradigms represent at least partially different aspects of EA. On the other hand, the moderate association that was found between the CPM and OA magnitude suggests some commonality in their mechanisms"*
Mlekusch S, Neziri AY, Limacher A, Jüni P, Arendt-Nielsen L, Curatolo M. *Conditioned Pain Modulation in Patients With Acute and Chronic Low Back Pain.* The Clinical journal of Pain. 2016;32(2):116-121.	N = 30 (14 males) 37.4 yrs ± 10.9 SD	Pressure pain tolerance threshold (Somedic) before Cold Pressor Test (< 2 min) and after 0, 3, 5 and 10 minutes. Compare healthy to acute (N=40) and chronic LBP (N=34)	TS not reported (only baseline level)	Pain tolerance increased during CS (normal CPM) and TS remained higher than baseline, although declining, at 3, 5 and 10 min post CS.	CPM is less effective in LBP (albeit present) compared to healthy controls, i.e. 'shorter duration of CPM in LBP'. *"…repeating the test stimulus over time can increase the chance of detecting alterations in endogenous pain modulation."*

Nilsen KB, Olsen IC, Solem AN, Matre D. *A large conditioned pain modulation response is not related to a large blood pressure response: a study in healthy men*. Eur J Pain. 2014;18(9):1271-1279.	N = 25 (all males) 26.1 yrs ± 5.4 SD	Two bouts (randomised) with same (heat) TS for 120s but with different CS (also 120s). 30 mins between CPM-bouts, 5 mins between TS and TS+CS.	Heat pain; Pain-5 on NRS. Continuous pain rating. No difference in TS-pain between the two bouts or over time.	CS1 = Ischaemic cuff CS2 = Cold pressor test CPM-effects: CS2>CS1 (CS-pain intensity not different).	No association between CPM-effect and blood pressure.
Nahman-Averbuch H, Granovsky Y, Coghill RC, Yarnitsky D, Sprecher E, Weissman-Fogel I. Waning of *"Conditioned Pain Modulation": A Novel Expression of Subtle Pronociception in Migraine.* Headache: The Journal of Head and Face Pain. 2013;53(7):1104-1115.	N = 35 (0 males) 29.3 yrs ± 9.3 SD	Repeated assessment: TS alone x 4 TS + (TS+CS-parallel x 3) TS + (TS+CSserial x 3) 8-min wash-out time between sessions.	TS: Tonic heat (30s, 47.5℃)	CS: Cold water (60s, 10℃): No carry over-effects detected. No significant difference in CPM over time (healthy): *"This suggests a waning process of the CPM response for the migraineurs as opposed to control subjects."* Habituation to CS over time (parallel < serial)	Migraine pt: *"...waning of CPM .. a novel test protocol for revealing the mild pronociceptive state of migraineurs."* *"..waning of CPM efficiency as indicator for a more pronociceptive profile of the migraine"*
Treister R, Eisenberg E, Gershon E, Haddad M, Pud D. *Factors affecting - and relationships between-different modes of endogenous pain modulation in healthy volunteers.* Eur J Pain. 2010;14(6):608-614.	N = 191 (87 males) 24.5 yrs ± 0.25	Baseline TS + 4 identical TS (TS1-4) used to test for habituation. Same protocol – but with CS – used for CPM and control (water = 25℃)	TS: Heat pain 3s (47℃, 12s ISI). Habituation = control (non-painful CS)	CS: Cold water parallel to TS2 and TS3 (12℃). CPM > control (25℃) CPM > 'habituation' (only TS)	
Ladouceur A, Rustamov N, Dubois J-D, et al. *Inhibition of Pain and Pain-Related Brain Activity by Heterotopic Noxious Counter-Stimulation and Selective Attention in Chronic Non-Specific Low Back Pain*. Neuroscience. 2018;387:201-213.	N = 17 (10 males) 42.7 yrs ± 11.1 SD	3 sessions (randomized): 4 blocks of 60 electrical stimuli (ISI 6-15s); baseline, cool, hot, recovery. Session 1: Control Session 2: Painful CS Session 3: Cool 'CS'	TS: TENS Control: Pain habituated over time	Non-painful cool: 3-min 16-19℃ CS: Ice-pack, 3-min, -12℃ Cool: habituation, but boarderline 'CPM-effect' Painful CS: Pain inhibition, i.e. normal CPM-effect	Normal CPM-effect = attention-hypo-algesic effect: No additive effect of attention on CPM-effect.

Reference	Participants	Design	TS and effect on pain sensitivity	CS and effect on CPM	Other
Reliability (intra-session)					
Imai Y, Petersen KK, Mørch CD, Arendt-Nielsen L. *Comparing test–retest reliability and magnitude of conditioned pain modulation using different combinations of test and conditioning stimuli*. Somatosens Mot Res. September 2016:1-9.	N = 26 males 25.3 yrs ± 5.7 SD	Intra-session reliability: 1: TS x 4 + TSCS x 4 (CPT) 2: Pause 45 min 3: TS x 4 + TSCS x 4 (cuff)	Pain detection threshold: 1: Electric (1 sec x 200 Hz) x 5 2: Heat 3: PPT 4: Cuff PDT and PDT Inter-session reliability (cuff) TS noCS (0.75 and 0.84) TS noCS (0.82 and 0.87) TS +CS (0.75 and 0.82) TS +CS (0.83 and 0.94)	1: Cold pressor test (CPT), hand 2. Pressure-cuff, leg equal to VAS7 (PPT + CPT) and (cuff-PDT + CPT) = most reliable CPM	Within-session ICC: 1: Highest ICC (0.96) = electrical and PPT Modality: No significant effect of electrical or heat (TS) with Cuff (CS)
Graven-Nielsen T, Masahi I, Petersen KK, Petersen K, Arendt-Nielsen L. *User-independent assessment of conditioning pain modulation by cuff pressure algometry*. August 2016:1-37.	N = 20 (10 males) 30 yrs ± 5 SD	Intra-session reliability: 1: TS (cuff x 3, PPT x 3) 2: 5 min break 3: TSCS (cuff x 2, PPT x 2) 8 variations of arm-leg compressions including leg-leg (as Study-I)	Cuff: TS-variations 1: Cuff (ramp PDT, PVAS6, PTT) 2: PPT Not analysed (only 1 baseline-TS in each session)	Cuff: CS-variations 1: 10; 30; 60 kPa (60 sec) 2: 30; 60 kPa (10 sec) 3: VAS=7 (60 sec) Ad1: 60 kPa highest CPM-effect Ad2: 60s higher CPM than 10s Median CPM-increase 13% - 22%	TS: 3% (PVAS6) and 1% (PTT) reached 100 kPa. For TSCS: 4% (PVAS6) and 11% (PTT) reached 100 kPa. Leg-leg combination (protocol 7) was consistently high on ICC and low 95% CI.
Lewis GN, Heales L, Rice DA, Rome K, McNair PJ. *Reliability of the conditioned pain modulation paradigm to assess endogenous inhibitory pain pathways*. Pain Res Manag. 2012;17(2):98-102.	N = 20 (7 males) 25 yrs ± 8 SD	Intra-session reliability: 4 bouts, 15 mins wash-out. CS-pain assessed at 100s (NRS), TS during CS assessed at knee at 45s and 90s.	TS: Pressure (motor-driven, 20 N/s), participants pressed 'stop' = NRS50 (average of two TS with 30-45s ISI). *"The intrasession ICC value indicated an excellent level of reliability while the intersession value was good"*	CS1 (twice): Ischaemic + handgrip exercises; 120s CS2 (twice): Cold water; 120s, 12℃. *"no difference in the change in pressure-pain threshold during the ischemic arm test compared with the cold pressor test"* The intrasession ischemic: excellent level of reliability | cold pressor: excellent.	
Isselée H, De Laat A, Lesaffre E, Lysens R. Short-term reproducibility of pressure pain thresholds in masseter and temporalis muscles of symptom-free subjects. Eur J Oral Sci. 1997;105(6):583-587.	N = 22 (11 males) 27 yrs (21-35 yrs)	PPT testet in the morning and afternoon. Two sessions at each time point: 5 min between each session. Each session had two trials, separated by > 30s.	TS: PPT twice on two different muscles (bilaterally). Afternoon PPT < morning PPT 1st PPT > 2nd PPT = habituation	Not tested	
Granovsky Y, Miller Barmak A, Goldstein O, Sprecher E, Yarnitsky D. *CPM Test–Retest Reliability: "Standard" vs 'Single Test-Stimulus' Protocols*. Pain Med. August 2015:pme12868	S1: N = 35 (15 males) 26.1 yrs ± 2.5 SD S2: N = 30 (15 males) 25.9 yrs ± 2.6 SD	Inter-session reliability: S1 and S2 separated by 3-7 days S1: Parallel S2 A: Serial, B: Parallel	S1: Heat pain (30s) S2: Heat pain (45s). In B the TS was delivered in parallel and only once (not three times as in A and S1). This may be a more reliable measure but can also be more distracting. No separate analysis.	S1: Cold pressor test (60 s) Pain intensity rating at 10; 20 and 30s on 0-100 NPS **Results**: CS increased HPT at all three time points, and habituation from 10 to 20 s and 10 to 30 s. S2: Heat pain (A: 65s / B: 25s) **Results**: CS increased HPT at all 20 and 30s in serial (A) and at 20s for parallel (B). No significant habituation.	Increased CS may increase habituation and mask CPM-effects

Table A.2: Social stress, pain sensitivity and CPM in healthy volunteers

Studies are summarised by paradigm and main findings and organised by stress paradigm (MIST, TSST, MAST, MMST, mental arithmetic)

Ref	Participants	Paradigm (stress and pain)	TS and effect on pain sensitivity	CS and effect on CPM	Perceived stress	Cortisol	Other
Geva N, Pruessner J, Defrin R. Acute psychosocial stress reduces pain modulation capabilities in healthy men. 2014;155(11):2418-2425	N = 29 (males) 33 yrs ± 10 SD	The Montreal Imaging Stress Task (MIST)	Heat (threshold and tolerance) **No significant difference** in sensitivity HP-threshold and -tolerance)	CS = heat, VAS5 (25s, 15s after TS) TS = heat, VAS5 (10s) **"weaker CPM during stress"**. The greater the impact of stress, the greater was the reduction in CPM (r = - 0.36)	0-10 self-report, increased during stress	Saliva (01.00 pm, all), cortisol **increased during stress**.	" … comparing the pain measurements before MIST vs after MIST (stress). " Obs no MIST-control but recovery
Geva N, Pruessner J, Defrin R. Triathletes Lose Their Advantageous Pain Modulation under Acute Psychosocial Stress. Med Sci Sports Exerc. 2016;49(2):1-341	N = 25 (male triathletes or ironmen) 35.9 yrs ± 10 SD	The Montreal Imaging Stress Task (MIST)	Heat (threshold and tolerance) Increase pain sensitivity (HP-threshold) during stress (**hyperalgesia**) but no change in HP-tolerance.	CS + TS = heat (VAS5) **CPM reduced during stress** "the greater the (perceived) stress response of the individuals, the smaller was the reduction in CPM"	0-10 self-report, increased during stress r = -0.48 to PDT and r = - 0.39 to CPM	Saliva (01.00 pm, all), increased during stress (**"borderline effect"**)	Anxiety, HRV, GSR. TSP Athletes = non-athletes (stress-related drop in CPM), but athletes had higher CPM-effects. No control (see above)
Geva N, Defrin R. Opposite Effects of Stress on Pain Modulation Depend on the Magnitude of Individual Stress Response. J Pain. 2018;19(4):360-371	N = 31 (males) 34 yrs ± 11 SD	The Montreal Imaging Stress Task (MIST)	Not measured (only as 'CPM' or 'Pain adaptation')	CS + TS = heat (VAS5-6) **CPM reduced during stress**	0-10 self-report, increased from rest (not BL) to stress r = - 0.45 to CPM	Saliva (01.00 pm, all), **increased from rest (not BL) to stress**	Anxiety, HRV, GSR, stimulus-response-function, pain adaptation No control (see above)
Gaab J, Jiménez J, Voneschen L, et al. Psychosocial Stress-Induced Analgesia: An Examination of Effects on Heat Pain Threshold and Tolerance and of Neuroendocrine Mediation. Neuropsychobiology. 2017;74(2):87-95	N = 29 (males) 24.6 yrs (18-40 yrs)	Trier Social Stress Test (TSST)	Heat (threshold and tolerance) Increase HP-tolerance (hypoalgesia), **no significant effect on HP-threshold** "Marginally but significant stress induced analgesia"	Not tested	Not tested	Saliva, **increased during stress** "Neither objective heat pain threshold or tolerance nor subjective pain intensity or valence correlated with cortisol level, sAA activity, or state anxiety."	Randomised (Control/Stress) with one week inbetween Control: waiting in a quiet room
Crettaz B, Marziniak M, Willeke P, et al. Stress-Induced Allodynia – Evidence of Increased Pain Sensitivity in Healthy Humans and Patients with Chronic Pain after Experimentally Induced Psychosocial Stress. PLoS ONE. 2013;8(8)	N = 10 (women). 27.7 yrs ± 5.58 SD	Trier Social Stress Test (TSST)	Thermal pain thresholds (CPT, HPT), mechanical pain detection thresholds to pinprick stimuli (MPT), blunt pressure pain thresholds (PPT) HPT was reduced from 44.2 to 42.2, p = 0.057 (**hyperalgesia**) in healthy	Not tested	None reported	Not tested	N = 13 (women, FMS), 50 yrs ± 10.6 SD No control session (but recovery)

Timmers I, Kaas AL, Quaedflieg CWEM, Biggs EE, Smeets T, de Jong JR. Fear of pain and cortisol reactivity predict the strength of stress-induced hypoalgesia. Eur J Pain. 2018;22(7):1291-1303	N = 19, 7 males 23.5 yrs ± 0.7 SEM *"Lower trait anxiety and being male was associated with more stress-induced hypoalgesia."*	Maastricht Acute Stress Task (MAST)	Heat-pain threshold and tolerance **No significant effect** in healthy male controls	Not measured	stress-group > control group	Increase cortisol = increase PDT (i.e. hypoalgesia) "the effect on pain thresholds was only present in cortisol responders (i.e. participants showing a cortisol response)"	N = 20, 8 men in Stress-group. Compare to control-group (healthy) The main result is **increased HPT 15 mins after** MAST in stress group, but not control group.
Reinhardt T, Kleindienst N, Treede R-D, Bohus M, Schmahl C. Individual modulation of pain sensitivity under stress. Pain Med. 2013;14(5):676-685	N = 80 (women) 25.4 yrs ± 0.5 SEM	Mannheim Multicomponent Stress Test (MMST)	Thermal (Medoc); HP-threshold, CP-threshold, repetitive heat, repetitive cold ANOVA showed **no significant effect of stress** However, pain sensitivity was not significantly changed by stress in the majority of subjects.	Not tested	Increased stress-ratings (0-10) during MMST	Not tested	No control condition "different factors might influence the individual shift of pain sensitivity under stress"
Caceres C, Burns JW. Cardiovascular reactivity to psychological stress may enhance subsequent pain sensitivity. PAIN. 1997;69(3):237-244.	N= 52 (26 males) 23.9 yrs ± 4.9 SD No gender difference reported	Randomised to 'mental arithmetics' (MA) + negative feedback or CPT	Cold-pressor-test (pain threshold = seconds until first report of 'painful sensation') **No difference in pain threshold** when MA-first (stress)	Not tested	Not tested	Not tested No time-of-day reported.	Randomised, cross-over study (but no control session) "These interactions coupled with the non-significant zero-order correlations between [arterial pressure] reactivity and pain threshold and tolerance suggest that stress-induced reactivity and pain sensitivity may not simply covary"
Cathcart S, Petkov J, Pritchard D. Effects of induced stress on experimental pain sensitivity in chronic tension-type headache sufferers. Eur J Neurol. 2008;15(6):552-558. doi:10.1111/j.1468-1331.2008.02124.x.	N = 15 (8 males), 31.8 yrs ± 10.1 SD No gender difference reported	(15 min) solve difficult mental arithmetic problems + negative feedback	Cold-pain threshold (ice cubes, threshold = seconds from application to first pain) Pressure-pain threshold (PPT) CPT and PPT: **Time (pre-2-post stress) effects** but no group difference. And "split-file Pearson's correlation indicated significant relationships between stress and pain thresholds for the **headache-group only**"	Not tested	0-10 self-report Increased pre-to-post (no group differences)	Not tested	Chronic tension-type headace N = 16, 34 yrs ± 11.3 SD No control-session but comparison to patients They claim that "a mildly stressful mental task increased sensitivity to subsequent pain in healthy controls"
Cathcart S, Winefield AH, Lushington K, Rolan P. Noxious Inhibition of Temporal Summation is Impaired in Chronic Tension-Type Headache. Headache: The Journal of Head and Face Pain. 2010;50(3):403-412	N = 25 (9 males) No significant interactions with of gender	(60 min) solving anagrams and arithmetic problems + negative feedback	Temporal summation, TS (Wager, 10 pulses) to finger and trapezius **No effect of induced stress**	Occlusion cuff on the opposing (left) arm to the experimental (TS) pain stimulation **No effect of induced stress**	Not tested	Not tested Test from 9 am to 5 pm	Chronic tension-type headace (N = 23, 8 male) no relationship between TS and anxiety or depression in the healthy controls Control: Neutral condition (only patients), no control for HC

Bement MH, Weyer A, Keller M, Harkins AL, Hunter SK. Anxiety and stress can predict pain perception following a cognitive stress. Physiol Behav. 2010;101(1):87-92	N = 25 (12 males), 20.2 yrs ± 3.7 SD No significant interactions with of gender	(10 min) Continuous subtraction by 13 starting from a four digit number + negative feedback + metronome	Pressure to the finger **No significant differences** in pain threshold or pain rating	Not tested	0-10 self-report **Increased during test compared to baseline** Higher baseline stress = more likely higher pain	Saliva sample (salivette) **Increased during stress**. Measured 20 min after stress No correlation to pain threshold or -ratin.	Sub-grouping; pain increase vs decrease vs no change Control: Mental math vs quiet rest
Nilsen KB, Christiansen SE, Holmen LB, Sand T. The effect of a mental stressor on conditioned pain modulation in healthy subjects. Scandinavian Journal of Pain. 2012;3(3):142-148	N = 20 (10 males) 24.2 yrs ± 2.1 SD No significant interactions with of gender	(5 minutes) Subtract 7 from 1000, time-pressure and negative feedback	PPT, heat pain threshold (HPT) and tolerance (SHPL) TS was assess only at baseline, not assessed in all sessions (only TS+CS and 'recovery TS')	CS = tourniquet induced pain, TS = HPT/SHPL and PPT **The stressful task reduced the CPM effect** (HPT>SHPL). PPT was not analyzed.	0-10 self-report Increased during stress, but not correlated to CPM-effects	Not tested	Blinded, cross-over design = no effect of order Possibly modality specific findings Control: Math vs reading a childrens book

Table A.3: Stroop Task, pain sensitivity and CPM in healthy volunteers

Studies are summarised by paradigm, modality and main findings and organised by TS-modality (thermal; chemical; mechanical; electrical)

Ref	Participants	Stroop paradigm	TS paradigm	CS paradigm	Main findings	Other
Bantick SJ, Wise RG, Ploghaus A, Clare S, Smith SM, Tracey I. Imaging how attention modulates pain in humans using functional MRI. *Brain*. 2002;125:310-319	N = 8 (2 males) 30 yrs ± 9 SD	Counting Stroop	**Heat** 50-53.5 ℃ for 5s	None	**Pain lower** during interference (incongruent) than neutral condition.	Neutral + incongruent (not congruent/incongruent) No learning effects of repeated Stroop Pain stimuli during Stroop: Pain sensitivity was recorded retrospectively (at the end of the study).
Aniskin DB, Fink E, Prosser J, et al. The Effect of Pain on Stroop Performance in Patients With Opiate Dependence in Sustained Remission. Journal of Addiction Medicine. 2011;5(1):50-56	N = 24 (13 males) 30.4 yrs ± 10.9 SD (21 – 55 yrs)	Colour Stroop	**Heat** pain threshold	None	Stroop: Comparison > former addicts Stroop+Pain: Comparison = former addicts i.e. **pain does nothing (significant) to Stroop** in 'healthy' comparisons	Women>Men @stroop Former opioid addicts (n=23) vs comparison
Oosterman JM, Dijkerman HC, Roy P.C. Kessels, Scherder EJA. A unique association between cognitive inhibition and pain sensitivity in healthy participants. Eur J Pain. 2010;14(10):1046-1050.	N = 31 (13 males) 51.1 yrs ± 21.5 (SD) (21 – 86 yrs)	Stroop Colour Word Test	**Cold pressor test** (pain and unpleasantness)	None	Pearson: 1. Better Stroop reaction time = longer immersion in CPT 2. p=0.066 better **Stroop = less pain** and unpleasantness	"relationship between cognitive inhibition and pain sensitivity appears to be unique. ..: "possible confounding effects of age, sex, education, depressive symptoms, fear of pain, and pain catastrophizing, were all considered, but did not mediate the relationship between pain sensitivity and cognitive inhibition"
Wilder-Smith CH, Li X, Shen L, Cao Y, Ho KY, Wong RK. Dysfunctional endogenous pain modulation in patients with functional dyspepsia. Neurogastroenterol Motil. 2013;26(4):489-498	N = 37 (15 males) 39.1 yrs	Colour Stroop	Ingested **capsaicin** (or nocebo) Electrical (hand) VAS 30-54	Heat (VAS 30-54)	Gastric pain (capsaicin) **reduced by Stroop** in HC Pain-inhibition from hand-foot-CPM correlated +ve with pain inhibition from Stroop (HC: *r* = 0.59)	Functional dyspepsia patients (n=31) Gastric pain reduction from Stroop: HC > FD-ptt No differences in Gastric-CPM but only HC had a significant CPM-effect on foot/hand paradigm. Higher clinical pain (FD) correlated with less pain reduction during Stroop.
Martinsen S, Flodin P, Berrebi J, et al. Fibromyalgia Patients Had Normal Distraction Related Pain Inhibition but Cognitive Impairment Reflected in Caudate Nucleus and Hippocampus during the Stroop Color Word Test. Garcia AV, ed. PLoS ONE. 2014;9(10):e108637-e108639	N = 31 (females) 46.3 (20-63 yrs)	Colour Stroop	**PPT** before, during, after Stroop	None	1: Pain was **reduced during Stroop** (FMS=HC), independently of congruent or incongruent (i.e. "cognitive load did not influence the pain sensitivity)	FMS (n=29), 49.8 (25-64 yrs) Also looked at HR and BP during Stroop 2nd study: fMRI cerebral activation pattern HC vs FMS Intro: Incongruent Stoop = reduce heat and activate 'pain reducing brain areas' (Bantick 2002, Valet 200x) Intro: HC long RT = dACC > dlPFC (Floden 2010) Discussion: FMS vs CPM and EIH in the context of distraction: FMS = pain facilitators (pro-nociceptive) from CS/exercise

Coppieters I, Ickmans K, Cagnie B, et al. Cognitive Performance Is Related to Central Sensitization and Health-related Quality of Life in Patients with Chronic Whiplash-Associated Disorders and Fibromyalgia. *Pain Physician*. 2015;18(3):E389-E401.	N = 22 (8 males) 38.0 ± 13.9 (SD)	Colour Stroop	**PPT** (Wager) TS (10 pulses)	Inflatable cuff (VAS 3)	Healthy control: Longer RT = **lower CPM** efficacy Longer RT = lower TS in FMS-ptt	WAD (n=16), FMS (n=21) No control condition. No of conditioned vs unconditioned TS (i.e. CPM-effect). Did not find any differences in CPM btw groups (but found other differences incl TS) = CPM may be flawed (not computerized)
Meeus M, Van Oosterwijck J, Ickmans K, et al. Interrelationships between pain processing, cortisol and cognitive performance in chronic whiplash-associated disorders. *Clin Rheumatol*. 2013;34(3):545-553	N = 16 (6 males) 40.9 yrs ± 13.4 (SD)	Colour Stroop	**PPT** (Wager) finger and trapezius TS (10xPPT)	Occlusion cuff on the left arm	Better CPM correlated with shorter RT in Stroop Higher baseline cortisol correlated with longer RT on Stroop (HC only)	WAD (n=15) Cortisol dropped after the test Small sample and no (t-test?) of conditioned vs unconditioned TS (i.e. CPM-effect)
Martinsen S, Flodin P, Berrebi J, et al. The role of long-term physical exercise on performance and brain activation during the Stroop colour word task in fibromyalgia patients. Clin Physiol Funct Imaging. 2018;38(3):508-516.	N = 20 (women) 47.2 yrs, 20 – 63 yrs)	Stroop Colour	**PPT**, somedic (4-ceps)	None	PPT higher (**pain inhibition**) during Stroop (HC and FMS) Congruens/incongruens did not show any difference in pain-inhibition-by-Stroop	FMS (n=31) 15 wk exercise program (PT supervised) led to *increased speed of Stroop RT* in HC, but not to any change in pain reduction during Stroop or PTT in either group.
Marouf R, Caron S, Lussier M, Bherer L, Piché M, Rainville P. Reduced pain inhibition is associated with reduced cognitive inhibition in healthy aging. PAIN. 2014;155(3):494-502	Young (N = 21, 18-46 yrs) vs Old (N = 23, 56-75 yrs)	Colour Stroop	"Shock pain" (**TENS**), sural nerve	Cold pain (ice pack on forearm) – VAS **CPM-effect: Not significant in older** (but was in young) = CPM higher in young (…compared to no-CPM!)	Correlation between CPM and Stroop was not significant (trend: Good Stroop = Good CPM and viceversa) Stroop: **Pain inhibition decreased with age** (*r* = -0.42) = older persons are less good at inhibiting pain during Stroop	No difference in CS-VAS between groups
Fechir M, Schlereth T, Kritzmann S, et al. Stress and thermoregulation: Different sympathetic responses and different effects on experimental pain. *Eur J Pain*. 2009;13(9):935-941	N = 15 (9 male) 24 yrs (22 - 29 yrs)	Colour Stroop	**Electrical** (lower leg), VAS every minute	None	**Stroop = pain inhibition** (compared to BL pain)	Aim: SNS Also incl *mental arithmetric task*
Seminowicz DA, Mikulis DJ, Davis KD. Cognitive modulation of pain-related brain responses depends on behavioral strategy. *PAIN*. 2004;112(1):48-58	N = 16 (8 males) 26.4 yrs (19 - 34 yrs)	Counting Stroop	**TENS** (median nerve, left): pain, tingeling, no stimulation	None	1: Stroop did change activation in S1, S2 and anterior insula cortex (maybe also pain) 2: Pain did not interfere with brain-regions activated by Stroop (i.e. **no effect of pain on stroop** compared to the other sessions)	subjects freely chose one of two strategies to cope with the pain and perform the attention-demanding task. It seems that the A group focused more on the task during the painful stimulation, which presumably caused a reduction in pain-related activity. In the P group, on the other hand, pain presumably interfered with task performance by diverting attention away from the task.
Silvestrini N, Rainville P. After-effects of cognitive control on pain. *European Journal of Pain*. 2013;17(8):1225-1233	N = 24 (9 males) 23 yrs (18 – 34)	Numerical Stroop	**TENS** (30-ms, 10x1-ms pulses over sural nerve), VAS-pain and unpleasantness	None	**Higher pain** (and NFR) after Stroop (compared to a neutral condition), small effect size (nP2=0.16) = *the more 'demanding' the task, the more pain they experience*	1: Perception of depletion (not physically exhaustion) of ressources 2: More challenging tasks are more motivating or taking more willpower

Appendix B. Overview of the studies

Study aims, hypotheses and conclusions

	Study 1	Study 2	Study 3
Aim	• measure **effects of repeated test-stimuli**, with and without parallel conditioning • analyse differences between a **fixed conditioning and an adapted conditioning** paradigm	• explore the effect of a **stressful mental task** on pressure pain sensitivity and CPM • compare these effects to a comparable control-condition	• assess the difference between conditioned pain and unconditioned pain with and without **Stroop task** in subjects demonstrating low and high degree of CPM
Hypothesis	• the CPM-effect will be impaired by habituation of the condition stimulus when repeated four times in 20 min • the unconditioned test-stimuli will habituate when applied repeatedly	• pressure-induced CPM was reduced more by stress than a comparable control condition	• reaction time and the percentage of correct answers was unaffected by pain • lower reaction time (increased cognitive load) was associated with reduced pain sensitivity and effectiveness of the conditioning stimulus (i.e. CPM-effect).
Conclusion	• conditioned test-stimuli significantly increase pain thresholds in four consecutive 'CPM' bouts • the increase in pain thresholds (during CS) is higher than repeated, unconditioned test-stimuli • **CPM-effects are unchanged over time, which contrasts to Control-effects that decrease over time** • repeated unconditioned test-stimuli may be influenced by focal facilitatory effects, which apparently are reduced when using the conditioning protocol and activation of descending modulatory mechanisms	• no significant differences in pain sensitivity and CPM could be measured after a brief episode of experimental stress compared to before or after a control-stress condition. • could be related to modality (thermal vs mechanical stimuli) • effectiveness of the conditioning stimulus was related to cortisol levels during experimental stress • cortisol has minor influence on the effectiveness of the descending modulatory system under stress • **cortisol and descending modulation may rely on overlapping mechanisms**	• CPM-responders maintain pain inhibition during Stroop task and show facilitation during Stroop without conditioning • attention does not play a major role CPM-responders • In the group of CPM-non-responders, attention was more effective as pain-inhibitor in Stroop conditions compared to CPM-responders, • **differences in pain-modulation abilities** between these subgroups
Clinical perspective	• CPM-sessions and control-sessions together could be a novel tool to **explore the relationship between the facilitatory and inhibitory systems**	• There may be clinically relevant overlaps between **stress and pain sensitivity but should be considered individual problems** in terms of management since the overlap is unlikely to explain the co-existence.	• Participants with **insufficient CPM may benefit from cognitive strategies** for pain-relief

Methods at a glance

Measure	Procedure	Study-I	Study-II	Study-III
Participants	Healthy men (18-80 years)	N = 20	N = 25	N = 25
Test stimulus (dominant leg)	Tonic, ramping 1 kPa/s until PTT duration 0-100 s	**X**	**X**	**X**
	Phasic 100 kPa/s equal to PTT at baseline duration 5 s			**X**
	Phasic 100 kPa/s equal to 90% of PDT (at baseline) duration 5 s			**X**
Conditioning stimulus (non-dominant leg)	Tonic 100 kPa/s equal to 70% of PTT at baseline (fixed) 7.5 cm torniquets	duration < 104 s	duration < 100 s	duration = 210 s
	Tonic 100 kPa/s equal to 70% of PTT (adapted), 7.5 cm torniquets	duration < 104 s		
Number of stimuli per session/bout	Test-stimuli	8 stimuli	6 stimuli	4 stimuli
	Conditioning stimuli	4 stimuli	2 stimuli	1 stimulus
Condition pain modulation	Baseline 'standard' CPM (pressure-cuff)	**X**	**X**	**X**
	Conventionel CPM	**X**	**X**	
	Phasic CPM			**X**
	Number of CPM-measurements total	**4**	**2**	**3**
Repeated test-stimuli (control session)	Pre- and post-measurement comparison		**X**	
	Unconditioned control-session	**X**	**(X)**	**X**
Randomisation	Randomised order of experimental sessions	**X**		**X**
Pain sensitivity measures	Electronic *visual analogue scale*, VAS (test-stimuli)	**X**	**X**	**X**
	Numeric rating scale, NRS (conditioning stimuli)	**X**	**X**	**X**
	Pressure pain detection threshold (1.0 on VAS) quantified in kPa	**X**	**X**	**X**
	Pressure pain tolerance threshold (10.0 on VAS or 100 kPa)	**X**		
Stress	Perceived stress (NRS)		**X**	**X**
	Saliva cortisol (ng/ml)		**X**	
Attention	Perceived focus (NRS)			**X**
Catastrophizing	Pain Catastrophizing Scale		**X**	**X**

Study-I | Effects of repeated conditioning pain modulation in healthy volunteers

EJP European Journal of Pain

Original Article

Effects of repeated conditioning pain modulation in healthy volunteers

M. Hoegh, K.K. Petersen, T. Graven-Nielsen

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Abstract

Background

Conditioned pain modulation (CPM) may be impaired in chronic pain patients compared with healthy subjects. The CPM-effect is the difference between pain sensitivity assessments (test-stimuli) with and without a painful conditioning stimulus. CPM has been extensively explored but effects of repeated CPM-effects and differences between repeated CPM assessments and comparable control conditions are less studied.

Methods

In 20 healthy men, four 5-min bouts with a test-stimulus in the beginning and midway were applied by cuff-algometry to the dominant leg. The 2nd test-stimulus in each bout was conditioned in parallel by a painful cuff pressure on the contralateral leg. A control-session was performed without conditioning. The conditioning intensity was 70% of the pressure-pain tolerance threshold (PTT) assessed at baseline. Pain detection threshold (PDT) was extracted from test-stimuli. CPM/Control-effects were calculated as second minus first test-stimulus, and netCPM-effects were calculated as the difference between CPM-effects and Control-effects.

Results

Pain detection threshold increased in all four bouts ($p < 0.02$) compared to the unconditioned test-stimulus and compared to the 2nd test-stimulus in bout1, bout3 and bout4 of the control-session ($p < 0.04$). In the control-session, the 1st test-stimulus PDT increased from bout1 to bout2, bout3 and bout4 ($p < 0.03$). The netCPM-effect increased progressively over the four bouts ($p = 0.03$).

Conclusion

Conditioned pain modulation-effects were maintained over four consecutive bouts and in the control-session repeated pain thresholds assessments habituated more than in the CPM-session leading to an increase in netCPM-effect over the four bouts.

Significance

Conditioning pain modulation can be assessed in 5-min intervals by cuff algometry with a fixed conditioning stimulus. Without applying conditioning stimuli the pain sensitivity of test-stimuli habituated. As a consequence, it can be speculated that the conditioning stimulus may negate the temporal habituation effects during repeated sessions, whereas this may not be the case for unconditioned stimuli. Applying both conditioned and unconditioned repeated test-stimuli may be a way to assess different parts of the pain modulatory system, and a model for measuring a netCPM-effect, which could indicate a balance between habituation and sensitization, is proposed.

Study-II | The Effect of Stress on Repeated Painful Stimuli With And Without Painful Conditioning

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Pain Medicine

The Effect of Stress on Repeated Painful Stimuli With And Without Painful Conditioning

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Complete List of Authors:	Hoegh, Morten; Aalborg Universitet Det Sundhedsvidenskabelige Fakultet, Center for Neuroplasticity and Pain (CNAP), SMI® Poulsen, Jeppe; Aalborg Universitet Det Sundhedsvidenskabelige Fakultet, Center for Neuroplasticity and Pain (CNAP), SMI® Petrini, Laura; Aalborg Universitet Det Sundhedsvidenskabelige Fakultet, Center for Neuroplasticity and Pain (CNAP), SMI® Graven-Nielsen, Thomas; Aalborg University, Laboratory for Experimental Pain Research, Centre for Sensory-Motor Interaction, Department of Health Science and Technology
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Study-III | The Effect of Attention on Pain Sensitivity

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The Effect of Attention on Pain Sensitivity

--Manuscript Draft--

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Corresponding Author:	Thomas Graven-Nielsen, Professor, DMSc, Ph.D Center for Neuroplasticity and Pain (CNAP) Aalborg, DENMARK
First Author:	Morten Hoegh, MSc
Order of Authors:	Morten Hoegh, MSc
	David A. Seminowicz, Ph.D.
	Thomas Graven-Nielsen, Ph.D., DMSc
Abstract:	Background The efficacy of descending pain control is assessed as the difference in pain sensitivity during a heterotopic, painful conditioning, compared to before (conditioning pain modulation, CPM). Attention-related changes in pain sensitivity may involve similar mechanisms as CPM and can be assessed with the Stroop-task, in which participants report the number of words on a screen, either congruent or incongruent with the value of the words. Methods Healthy men (n=25) underwent a cuff-algometry CPM-assessment where the pressure-pain detection (PDT) and tolerance thresholds (PTT) were recorded on one leg with and without conditioning on the contralateral leg. Two identical sessions of four test-stimuli equal to PTT (5s, 1-min interval, scored on a visual analogue scale, VAS) with a painful conditioning from the second to the last test-stimulus were performed. Subsequently, test-stimuli were applied between four Stroop-sessions with painful conditioning (Stroop-pain-conditioning) or without (Stroop-pain). Results The VAS scores in the first two sessions showed excellent reliability (ICC=0.92). VAS scores were lower in sessions with Stroop compared to sessions without Stroop (P=0.05). Participants were sub-grouped into CPM-responders and CPM-non-responders according to CPM-effects in the first two sessions. CPM-non-responders (n=13) showed facilitation to repeated noxious stimuli in all sessions with no effect of conditioning or Stroop (P=0.02). Conclusion Attention can inhibit experimental pain but is not able to change a negative CPM-effect into a positive, suggesting that attention and CPM utilize different mechanisms.

SUMMARY

Dette PhD-projekt, der består af tre studier, har undersøgt, hvordan gentagelser alene og i kombination med stress eller koncentration påvirker Conditioned Pain Modulation (CPM) i raske mænd.
Studie-I undersøgte, hvordan smertesensitivitet og CPM blev påvirket af at blive gentaget med korte mellemrum og havde til formål at give ny viden om, hvordan gentagne runder af smertefulde stimuli påvirker raske mænd. Studie-II undersøgte hvordan akut, social stress påvirker smertefulde stimulationer med og uden konditionering. Formålet var at belyse om stress har en direkte relation til CPM. Studie-III undersøgte, hvordan koncentration både med og uden gentagne, smertefulde stimulationer kan påvirke eksperimentelle smerter. Formålet var at belyse om koncentration og CPM kan supplere hinanden.
Resultaterne indikerer, at CPM er en reliabel og stabil model til at undersøge bottom-up smertemodulation. Her ud over viser resultaterne, at gentagne smertefulde stimulationer, uden konditionering, medfører ikke-lineære påvirkninger over tid. Projektet peger desuden på, at hverken social stress eller koncentration har signifikant indflydelse på CPM og at koncentration i sig selv kan have en smertelindrende effekt.

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