# Mechanisms of pain facilitation and inhibition: autonomic outflow and conditioned pain modulation

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## List of Abbreviations

CPM: Conditioned pain modulation

CPT: Cold pressor test

MSNA: Muscle sympathetic nerve activity

BRS: Baroreflex sensitivity

HR: Heart rate

BP: Blood pressure

ACC: Anterior cingulate cortex

SG: Substantia gelatinosa

PAG: Periaqueductal gray

DNIC: Diffuse noxious inhibitory control

DCN: Descending control of nociception

RVM: Rostral ventromedial medulla

NRM: Nucleus raphe magnus

5-HT: Serotonin

NE: Norepinephrine

fMRI: Functional magnetic resonance imaging

ANS: Autonomic Nervous System

SNS: Sympathetic Nervous System

PNS: Parasympathetic Nervous System

MAP: Mean Arterial Pressure

NTS: Nucleus Tractus Solitarius

RVLM: Rostral ventrolateral medulla

CVLM: Caudal ventrolateral medulla

CRPS: Complex regional pain syndrome

BF: MSNA burst frequency

ECG: Electrocardiogram

BA: MSNA burst amplitude

HC: Healthy controls

FM: Fibromyalgia patients

HPTh: Heat Pain Threshold

VAS: Visual analog scale

NRS: Numerical rating scale

PCS: Pain Catastrophizing Scale

SBP: Systolic Blood Pressure

DBP: Diastolic Blood Pressure

ACh: Acetylcholine

AChEI: Acetylcholinesterase inhibitor

**Abstract**

Chronic pain is often comorbid with cardiovascular diseases, and this leads to increased rates of cardiovascular mortality in patients with chronic pain. Brain regions associated with autonomic control of the cardiovascular system overlap substantially with those related to nociceptive processing, and dysfunction in the nociceptive and autonomic systems are likely involved in the propagation of chronic pain. Conditioned pain modulation (CPM) protocols are used to measure the "capacity" of descending central nervous system pain inhibition mechanisms. Descending stress signals via the sympathetic or parasympathetic systems can also inhibit pain. The interaction of the nociceptive and autonomic nervous systems in healthy individuals is still incompletely understood, and their dysfunction in chronic pain patients even less so. Therefore, the purpose of this dissertation was to better understand the interactions between the nociceptive and autonomic systems, with the larger goal of better overall treatment of chronic pain and its comorbid health problems. *Study 1* examined the relationship between pain ratings and autonomic indices during a tonic cold pressor test (CPT) in male and female participants. We observed sex differences in responses to the CPT: males displayed a strong relationship between tonic pain ratings and heart rate, whereas females displayed strong and offsetting relationships between tonic pain and muscle sympathetic nerve activity (MSNA). Because heart rate is regulated in part by the parasympathetic system, and MSNA is a purely sympathetic variable, these findings suggested sex differences in autonomic regulation mechanisms during pain, the mechanisms of which were further explored in *Study 3. Study 2* followed up on a previous mouse finding in our lab, in which the intensity of the test stimulus during a CPM protocol affected the direction of CPM, leading to hyperalgesia, or "anti-CPM" when using lower intensity test stimuli. We were able to replicate this finding in a human cohort of 60 healthy participants. We also recruited 39 participants with

fibromyalgia to see if this phenomenon was present in individuals with the reduced efficacy of descending pain inhibition associate with chronic pain. We did not see this phenomenon in individuals with fibromyalgia, indicating that hyperalgesic "anti-CPM" is a naturally occurring part of endogenous pain modulation and may contribute to the variability we see in CPM study outcomes. Finally, *Study 3* combined methodologies of *Study 1* and *Study 2* to examine the relationship between the autonomic system and conditioned pain modulation. Participants underwent physiological data collection while experiencing a CPM protocol. We calculated both cardiovagal (i.e., parasympathetic) and sympathetic baroreflex sensitivity (BRS) for participants during rest and correlated their BRS with CPM. We found that greater sensitivity of the parasympathetic withdrawal system was significantly negatively associated with CPM-related reductions in pain, indicating that prolonged parasympathetic activity may promote CPM efficacy. Taken together these findings further elucidate the interactions between nociceptive processing and autonomic function and provide possible implications for pain patient treatment outcomes.

**Résumé**

La douleur chronique est souvent associée à des maladies cardiovasculaires, ce qui entraîne une augmentation des taux de mortalité cardiovasculaire chez les patients souffrant de douleur chronique. Les régions cérébrales impliquées dans le contrôle autonome du système cardiovasculaire se superposent à celles liées au traitement nociceptif, et un dysfonctionnement des systèmes nociceptif et autonome est probablement impliqué dans la propagation de la douleur chronique. Les protocoles de modulation de la douleur conditionnée (MDC) sont utilisés pour mesurer la « capacité » des mécanismes d'inhibition de la douleur descendante du système nerveux central. Les signaux de stress descendants via le systèmes sympathiques ou parasympathiques peuvent également inhiber la douleur. L'interaction des systèmes nerveux nociceptif et autonome chez des individus en bonne santé reste méconnue, et leur dysfonctionnement chez les patients souffrant de douleur chronique l'est encore moins. Par conséquent, le but de cette thèse était de mieux comprendre les interactions entre les systèmes nociceptif et autonome, avec l'objectif plus large d'un meilleur traitement global de la douleur chronique et de ses problèmes de santé comorbides. *L'étude 1* a examiné la relation entre les évaluations de la douleur et les indices autonomes lors d'un test au froid longtemps chez des participants masculins et féminins. Nous avons observé des différences entre les sexes dans les réponses au test au froid : les hommes ont montré une forte relation entre les évaluations de la douleur longtemps et la fréquence cardiaque, tandis que les femmes ont montré des relations fortes et compensatoires entre la douleur longtemps et l'activité nerveuse sympathique musculaire (ANSM). Étant donné que la fréquence cardiaque est régulée en partie par le système parasympathique et que l'ANSM est une variable purement sympathique, ces résultats suggèrent des différences entre les sexes dans les mécanismes de régulation autonome pendant la douleur. Ces mécanismes ont été explorés plus en détail dans

*l'étude 3*. *L'étude 2* a fait suite à une précédente découverte sur la souris dans notre laboratoire, dans laquelle l'intensité du stimulus de test pendant un protocole de la MDC affectait la direction de la MDC, conduisant à une hyperalgésie, ou « anti-MDC » lors de l'utilisation de stimuli de test de plus faible intensité. Nous avons pu reproduire cette observation dans une cohorte humaine de 60 participants en bonne santé. Nous avons également recruté 39 participants atteints de fibromyalgie pour voir si ce phénomène était présent chez les personnes présentant une efficacité réduite de l'inhibition descendante de la douleur associée à la douleur chronique. Nous n'avons pas observé ce phénomène chez les personnes atteintes de fibromyalgie, ce qui suggère que l'« anti-MDC » hyperalgésique fait partie de la modulation endogène de la douleur et pourrait contribuer à la variabilité que nous observons dans les résultats des études de la MDC. Enfin, *l'étude 3* a combiné les méthodologies de *l'étude 1* et de *l'étude 2* pour examiner la relation entre le système autonome et la MDC. Les participants ont subi une collecte de données physiologiques pendant qu'ils suivaient un protocole de la MDC. Nous avons calculé la sensibilité du baroréflexe (SBR) cardiovagal (c'est-à-dire parasympathique) et sympathique des participants au repos et avons corrélé leur SBR avec la MDC. Nous avons constaté qu'une plus grande sensibilité du système de retrait parasympathique était significativement associée positivement aux réductions de la douleur liées à la MDC, ce qui indique qu'une activité parasympathique prolongée peut favoriser l'efficacité du CPM. Pris ensemble, offrent une meilleure compréhension des interactions entre le traitement nociceptif et la fonction autonome et fournissent des implications possibles pour les stratégies de traitement des patients souffrant de douleur chronique.

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## Contributions of Authors

The following is a list of co-authors and their contributions to the manuscripts in this dissertation:

Sex differences in the relationship between pain and autonomic outflow during a cold pressor test

Yasmine Coovadia- Study creation, data collection and analysis, manuscript preparation

Brittany K. Schwende- Data collection and analysis

Danielle E. Berbrier- Data collection

Will Huckins- Data collection

Jinan Saboune- Data collection

Derek A. Skolnik- Data analysis

Emily K. Van Berkel- Data analysis

The direction and magnitude of conditioned pain modulation is dependent on test- stimulus intensity in healthy participants but not in those with fibromyalgia

Isabel Aboud- Participant recruitment, data collection and entry

Mathilde Ferland- Participant recruitment, data collection and entry

Natasha Stonebanks Cuillerier- Participant recruitment, data collection and entry

Simon Carrier- Data collection and entry

Elodie Nickner- Data collection

Marc O. Martel- Study creation, collaboration, material provision

Various:

Charlotte W. Usselman- Co-supervision, material provision, and manuscript preparation

Jeffrey S. Mogil- Supervision, data analysis, and manuscript preparation

Laila A. Chaudhry- Study creation, ethics application, participant recruitment, study logistics, data collection, data analysis, data presentation, and manuscript preparation

## Contributions to Original Knowledge

*Study 1* explored autonomic responses, including muscle sympathetic nerve activity (MSNA), to a tonic cold pressor test. It is one of only a few studies to investigate the relationship between prolonged pain and MSNA, and the first to assess pain at multiple timepoints during the stimulus. Finally, this is the only prolonged pain and MSNA study to recruit equal numbers of men and women and conduct a sex-based analysis.

*Study 2* examined test-stimulus intensity effects on conditioned pain modulation (CPM) outcomes in individuals with fibromyalgia and healthy controls. This is the first study to specifically examine and compare CPM outcomes in response to various test stimulus intensities. We believe our finding of hyperalgesic CPM in response to low-intensity test stimuli will help to explain variability of outcomes in the CPM field.

*Study 3* assesses different aspects of autonomic function and their relationship with CPM. The sequence method is used to assess cardiovagal baroreflex sensitivity (BRS), of which up and down sequences can provide us values of parasympathetic activation and withdrawal, respectively. Sympathetic BRS is an assessment of probability of bursts of MSNA in response to diastolic blood pressure changes. Though there are a small number of studies assessing cardiovagal BRS and CPM, this is the first to dissociate activation and withdrawal and relate them with pain. The MSNA and pain literature is already quite small, therefore this is one of the first studies to look at MSNA and CPM, sympathetic BRS and pain, and the only study assessing the relationship between sympathetic BRS and conditioned pain modulation.

## Preface

**Chapter 1** is a general introduction and comprehensive review of literature necessary to contextualize data and discussion in subsequent chapters**.**

**Chapter 2** or *Study 1* is an empirical data chapter which examined sex differences in the relationship between pain and autonomic indices, including sympathetic nerve activity, in response to a tonic cold pressor test and has been submitted for publication to Biology of Sex Differences: *Sex differences in the relationship between pain and autonomic outflow during a cold pressor test* by Chaudhry, L. A.; Coovadia, Y.; Schwende, B K.; Berbrier, D. E.; Huckins, W.; Saboune, J.; Skolnik, D. A.; Van Berkel, E. K.; Mogil, J.S.; Usselman, C.W.

**Chapter 3** or *Study 2* is a manuscript examining the role of test stimulus intensity in the variability of conditioned pain modulation outcomes in individuals with fibromyalgia and healthy controls. This manuscript has been submitted for publication to PAIN: *The direction and magnitude of conditioned pain modulation is dependent on test- stimulus intensity in healthy participants but not in those with fibromyalgia* by Chaudhry, L. A.; Aboud, I.; Ferland, M.; Stonebanks-Cuillerier, N.; Carrier, S.; Nickner, E.; Martel, M. O.; Mogil, J. S.

**Chapter 4** or *Study 3* is a manuscript focused on autonomic function and how it relates to conditioned pain modulation, using cardiovagal and sympathetic baroreflex sensitivity assessments: *Pain and autonomic function: the relationship between conditioned pain modulation and cardiovagal baroreflex sensitivity* by Chaudhry, L. A.; Mogil, J.S.; Usselman, C.W. The abstract for this manuscript has been submitted to the Canadian Journal of Pain, but has been updated for this dissertation.

**Chapter 5** serves as a comprehensive discussion of all manuscripts in this thesis.

# Chapter 1: Introduction

Pain is defined as "an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage" [202]. Pain typically signals the presence of a threat to the body, but this alarm signal can become faulty, as with the case of chronic pain. Chronic pain is the leading source of disability worldwide and presents a massive clinical, societal, and economic challenge [67; 130; 222]. The human body has evolved mechanisms that amplify and reduce pain perception. A decrease in the efficacy of pain inhibitory mechanisms has been observed in individuals with chronic pain [54; 104; 118; 122]. One of the phenomena produced by pain inhibitory mechanisms is conditioned pain modulation (CPM). The degree to which CPM is elicited using a counterirritation paradigm (i.e., comparing the intensity of pain caused by a test stimulus before and after another conditioning stimulus applied to another part of the body) [273], is considered to measure the capacity of these endogenous pain inhibition mechanisms. It has been observed that a conditioning stimulus of greater intensity generally results in greater pain inhibition [123; 240]. Contrarily, there is a lack of evidence exploring the relationship between the intensity of the test stimulus and CPM outcomes.

Pain can lead to significant cardiovascular/autonomic responses [37; 233], such as increased heart rate (HR), blood pressure (BP), and respiratory rate [3; 107; 241]. Some animal studies have demonstrated a relationship between pain modulatory mechanisms and autonomic activity [203; 275], which are supported by human studies demonstrating an inverse relationship between resting BP and pain sensitivity in humans (i.e., higher resting BP leads to lower pain sensitivity), as well as increases in stress-induced BP increases leading to decreases in pain sensitivity [4; 28; 66; 77]. Despite this evidence, very few researchers have explored the

relationship between autonomic activity and pain, and even less have explored the relationship between autonomic activity and CPM. Further exploring how autonomic influences as well as protocol parameters affect pain and CPM can allow us to better how their dysfunction contributes to chronic pain. Therefore, the objectives of this thesis are as follows.

*Study 1* aims to examine the relationship between pure sympathetic outflow and pain during a tonic cold pressor test, with the greater goal of understanding the complex relationship between pain and stress, with a focus on sex differences. *Study 2* aims to understand the effect of test-stimulus intensity on CPM outcomes, with the hypothesis that lower-intensity stimuli lead to hyperalgesic outcomes. We believe that this may explain a large amount of variability in participant outcomes in the CPM literature. Finally, *Study 3* combines methodologies from the previous two studies to explore the relationship between CPM and autonomic activity, using sympathetic and cardiovagal baroreflex sensitivity to assess, respectively, sympathetic and parasympathetic activation and withdrawal.

In this section, I will summarize relevant literature on pain, pain modulation, autonomic function and how it relates to pain, and finally how autonomic dysfunction is related to chronic pain. I will first discuss the neurophysiological mechanisms of pain to explain how a painful stimulus is transmitted from the periphery to the brain to be interpreted as a painful sensation.

### *1.1 Nociceptors and Ascending Nociceptive Pathways*

When the body detects a painful stimulus, this is referred to as nociception. Nociceptors are a class of primary afferents that detect noxious stimuli in the periphery and convey the signal to the central nervous system; they primarily consist of Aβ, Aδ and C-fibers. Aβ fibres are highly myelinated and large in diameter. They are typically responsible for transmitting non-nociceptive mechanosensory information, however, they can sometimes play a role in mechanical pain

transmission [57]. Aδ fibers are myelinated with medium-large diameter, producing high conduction velocities and the initial, localized “fast pain” immediately felt after contact with a noxious stimulus [18; 72; 134]. C fibers are unmyelinated, producing slow conduction velocities and the more diffuse, delayed “slow pain” which is typically described as more unpleasant.

In addition to these anatomical differences, different fiber types also typically terminate in different laminae in the spinal cord (see Fig 1.1). C fibers preferentially connect to second-order neurons in Rexed’s laminae I and II, with the delineating factor being whether they are peptidergic (lamina I) or non-peptidergic (lamina II) [106]. Aδ fibers also terminate in lamina I and II along with the C fibers; however, they also project to lamina V [10], synapsing with second-order interneurons. From here, second-order projection neurons—classified into nociceptive-specific neurons and wide dynamic range neurons—convey nociceptive signals to the contralateral ventral horn, then relay nociceptive messages upward along the anterolateral column. Nociceptive-specific neurons respond only to noxious mechanical or thermal stimuli from Aδ or C fibers, whereas wide dynamic range neurons respond in a graded manner to noxious and non-noxious stimuli (Aβ) [17]. Nociceptive afferent signals ascend through the spinothalamic, spinoparabrachial, and spinoreticular tracts, while non-nociceptive afferents from Aβ fibers travel through the dorsal column medium lemniscus [151]. Afferents from the spinothalamic pathway project to the

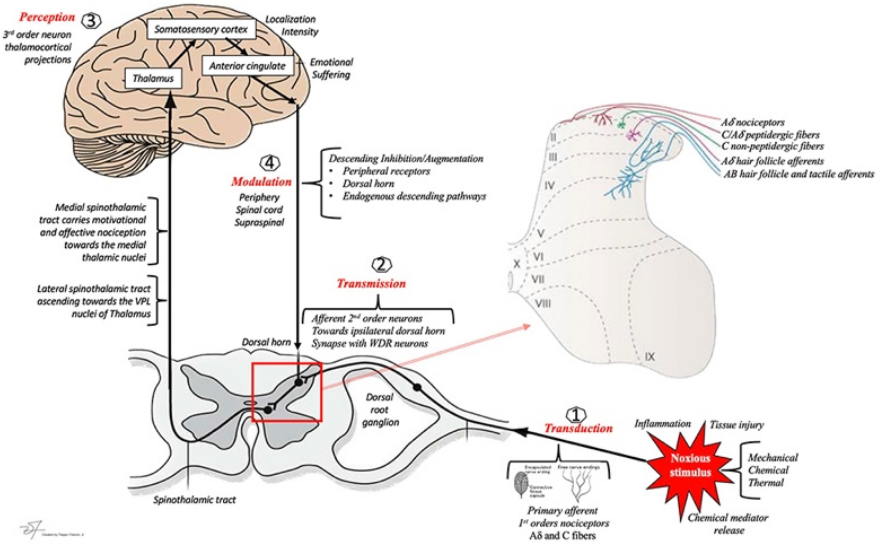


Fig 1. 1. Ascending nociceptive pathway (Karcz et al., 2024)

thalamus, then primarily to the primary and secondary somatosensory cortex (S1 and S2). S1 and S2 are primarily responsible for the discriminative and sensory components of pain (i.e., the assessment of pain intensity, quality, and location) [53; 201; 207]. Afferents from the spinoparabrachial and spinoreticular tracts project to different regions of the brainstem, limbic structures, insula, and the anterior cingulate cortex (ACC). These tracts are associated with the motivational-affective component of pain (i.e., how emotionally unpleasant the stimulus is). Once this nociceptive information has reached relevant cortical areas, its sensory and emotional components can be consciously perceived.

### *1.2 Modulation of Pain*

Ascending pain afferents may carry noxious information to the brain, but descending mechanisms can alter how that signal is perceived. Melzack and Wall's Gate Control Theory was the first to predict the existence of descending modulation [162], and numerous studies subsequently confirmed the presence of endogenous excitatory and inhibitory modulation mechanisms, involving various neurotransmitters, receptors, and neuromodulators [164]. Pain can be modulated at various levels of the central nervous system: the spinal cord, the brainstem, or the cerebrum.

#### 1.2.1 Spinal Mechanisms.

According to Gate Control Theory, proposed by Ronald Melzack and Pat Wall in 1965, pain perception is modulated by a "gate" that can be excited/opened (increased pain) or inhibited/closed (decreased

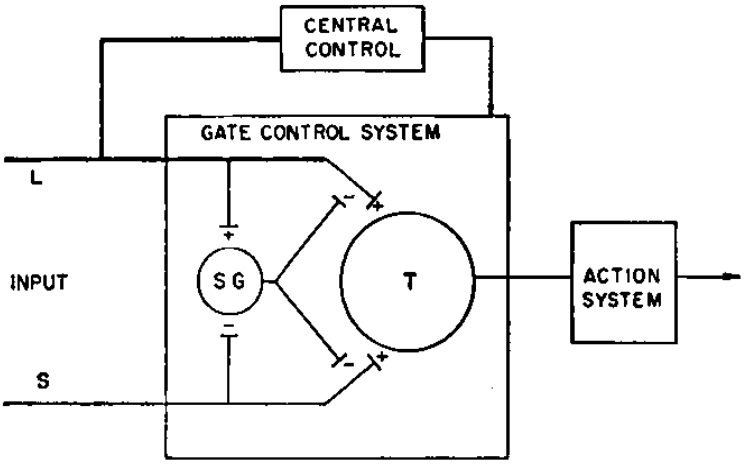


Fig 1. 2 Melzack & Wall's Gate Control Theory

pain). This theory accommodated for propagation of pain by small (Aδ and C) fibers which project to second-order transmission neurons in the spinal cord. These small fibers also inhibit inhibitory interneurons in the substantia gelatinosa (SG; laminae I and II) in the spinal cord, which facilitates pain signals. Contrarily, the non-noxious activation of large (Aβ) fibers activate the inhibitory SG interneurons, leading to inhibition of the pain signal. That "Central Control" from higher levels of the neuraxis can also open or close the gate was the first prediction of endogenous pain modulatory mechanisms, which would descend and exert their effects in the spinal cord.

**1.2.2 Brainstem mechanisms and descending pain modulation.** Shortly after the publication of Gate Control Theory, the periaqueductal gray (PAG) was discovered to be an area associated with analgesic activity [209]. Since then, many brainstem structures have been shown to play a role in inhibiting pain via descending projections to the spinal cord [74; 164; 272]. These pathways modify afferent nociception at the spinal level via release of various neurotransmitters, neuropeptides, and/or amino acids (e.g., NE, 5-HT, dopamine, vasopressin/oxytocin, glutamate) [164]. One of the most well-known forms of descending modulation is diffuse noxious inhibitory control (DNIC)—now referred to as descending control of nociception (DCN) [9]—in animal models and conditioned pain modulation (CPM) in humans.

DCN refers to the physiological process of intense pain reducing or inhibiting pain felt elsewhere in the body. DNIC was first discovered in Le Bars and colleagues in 1979 and was attributed to a spino-bulbar-spinal loop activated by Aδ and C fibers in the spinoreticular pathway, leading to diffuse, or body-wide, pain inhibition [128]. As stimulation of different brainstem structures such as the PAG, the rostroventral medial medulla (RVM), the nucleus raphe magnus (NRM) can cause diffuse analgesia [74; 272], it has been proposed that these structures may be involved in DCN, despite lesions of the same regions incompletely inhibiting DCN [22; 23; 25].

The subnucleus reticularis dorsalis receives input from the PAG, NRM, hypothalamus, and amygdala, and causes a significant decrease in DCN when lesioned [23; 25], so therefore is most likely responsible for DCN-related pain inhibition. Descending projections involved in DCN may inhibit wide dynamic range neurons [24; 125], but also release serotonin (5-HT), endogenous opioids, and norepinephrine (NE). Indeed, depletion of 5-HT, administration of naloxone, and administration of phenylephrine significantly reduce the analgesic efficacy of DCN by targeting these neurotransmitters, respectively [42; 126; 127; 145].

***1.2.2.1 Conditioned Pain Modulation.*** CPM is the consensus term for DCN in humans [273]. Despite the assumption that these two phenomena overlap significantly, the methods used to study CPM do not allow researchers to determine whether a spinobulbar-spinal loop or particular descending mechanisms involved in DCN are specifically activated in CPM. Therefore, CPM is a global measure of pain inhibition in humans [273], perhaps reflecting the activation of several descending inhibitory and facilitatory mechanisms. CPM is measured experimentally by comparing the intensity of a painful "test" stimulus before and after (or during) an also painful "conditioning" stimulus on another part of the body. There is great diversity in the methods used to activate and quantify CPM, and currently there is no standardization of the technique for testing CPM, presenting a barrier to pooled analyses.

Variability in test and conditioning stimuli parameters—including type, intensity, body area, and duration—contribute to discrepancies in the measured CPM effect across studies. CPM is generally triggered by a sufficiently intense and prolonged conditioning stimuli (e.g., cold pressor test [CPT], ischemic tourniquet test, noxious heat, intramuscular hypertonic saline) of which CPT is the most commonly used. Factors such as the intensity of the conditioning stimulus, demographic factors, and psychological traits (e.g., pain catastrophizing, anxiety) have been

shown to influence the magnitude of CPM [7; 35; 60; 69; 73; 75; 80; 183; 194; 195; 199; 200; 204; 229; 252].

While some studies suggest a positive correlation between conditioning stimulus intensity and CPM strength, other research has found no such association [7; 69; 80; 176; 183; 199; 200; 204]. Generally speaking, when the conditioning stimulus is sufficiently intense, applied over a large area, and for a long duration, CPM effects can last up to 10 minutes [262]. When the conditioning stimulus is applied simultaneously with the test stimulus, pain inhibition is more rapid, but this analgesia may be contributed to by a distraction effect from the conditioning stimulus [274]. This potential bias raises concerns regarding the accuracy of CPM measurement, particularly when the conditioning stimulus is substantially more intense than the test stimulus.

Demographic factors, including sex, age, and ethnicity [60; 73], also contribute to variability in CPM responses. Studies show that men typically exhibit more efficient CPM than women [32; 86; 193; 211], though this is not universally observed. The menstrual cycle also seems to have an (inconsistent) effect on CPM [210; 264]. Additionally, psychological factors like pain catastrophizing, anxiety, depression, and mental state can influence the efficacy of CPM [75; 80; 229]. For example, higher levels of pain catastrophizing are linked to less efficient CPM [43; 175], highlighting the role of cognitive and emotional factors in pain modulation.

Studies using nociceptive reflex muscle contractions to measure CPM responses have shown that CPM activation decreases nociceptive reflexes in healthy individuals, but not in those with spinal cord injury, underscoring the involvement of supraspinal structures [129]. Additionally, CPM is still observed in individuals with thalamic lesions, suggesting that the spinoreticular pathway, rather than the spinothalamic pathway, plays a significant role in CPM [52]. Pharmacologically, naloxone reduces the effectiveness of CPM [105; 263], and long-term

use of opioids has also been shown to reduce the effectiveness of CPM [154], possibly explaining the hyperalgesia seen in some chronic pain patients after prolonged opioid use. In contrast, GABA agonists, such as lorazepam, do not appear to impact CPM efficacy, though this may be dependent on dose [120]. Finally, functional magnetic resonance imaging (fMRI) studies have highlighted differences in brain structure activation between individuals with effective CPM and those with chronic pain, where CPM is markedly diminished. Such differences were observed in brain areas including the anterior cingulate cortex, thalamus, and hippocampus, suggesting that CPM dysfunction in chronic pain conditions may also involve emotional and autonomic responses [11; 261].

**1.2.3 Other Supraspinal Mechanisms.** Various cerebral cortical areas modulate pain perception via direct and indirect means. For example, the ACC projects to the PAG possibly influencing pain modulation [116]. Electrical stimulation of the motor cortex reduces pain and may potentiate the effect of CPM [205; 215]. Placebo, nocebo, hypnosis, exercise, and relaxation are all methods that have shown efficacy in altering pain perception [152], by acting on various psychological factors (e.g., stress, anxiety, emotional distress) and physiological factors (e.g., muscle tension, endogenous opioid release, BP) [4; 64 ; 78]. Physiological responses caused by the autonomic nervous system (ANS) such as increases in BP and HR generally accompany pain, so therefore it is reasonable that decreases in these responses are associated with pain modulation [12; 37]. A better understanding of the autonomic factors involved in pain modulation may allow for better understanding and treatment of pain.

### *1.3 The Autonomic Nervous System*

The human nervous system is separated into subdivisions, one of which, the ANS, controls involuntary functions used to maintain homeostasis such as cardiovascular function, respiration,

digestive activity, body temperature [153]. The ANS is composed of afferent sensory and efferent motor portions. The afferent part transmits information from both outside and inside the body, such as changes in temperature, pH, osmolarity, and concentration of gases ($O_2$, $CO_2$, etc.) in the body. The efferent part of the ANS always consists of two neurons in a series: preganglionic in the spinal cord, and postganglionic in the periphery. The efferent ANS is further divided into two subsystems: the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS). Most ANS target organs are innervated by both the SNS and the PNS, and although they can work independently, they generally work in complement (e.g., Resting HR increases via PNS withdrawal, but increases via SNS activation above resting threshold) to maintain homeostasis and respond to changes in the environment.

**1.3.1 The Parasympathetic Nervous System.** The PNS is primarily responsible for "rest and digest" functions [160; 255], or promoting activities that occur when the body is at rest, conserving energy, and facilitating processes essential for digestion, metabolism, and recovery [6; 160]. The majority of PNS preganglionic neurons originate in the medulla oblongata and pass through the vagus nerve to innervate the heart, lungs, liver, gallbladder, stomach, pancreas, kidneys, and proximal intestines [153]. Preganglionic neurons of sacral medullary origin synapse with terminal ganglia located in the walls of the distal large intestine, bladder, and genitals. The primary neurotransmitter released by the PNS is acetylcholine, which is released by both pre- and post-ganglionic neuron and binds to muscarinic G-protein coupled receptors at target organs [220]. Depending on the target organ, the effect of PNS can be activating (glands, gastrointestinal system) or inhibitory (heart). Specifically pertaining to BP, the PNS innervates the heart to alter cardiac parameters involved in acute BP regulation.

**1.3.2 The Sympathetic Nervous System.** The SNS is responsible for preparing the body

to respond to periods of stress, or “fight or flight” in emergency situations [100; 103]. The sympathetic arm of the ANS is heavily involved in BP regulation in both the short- and long-term [6; 99; 100; 103; 160; 255]. The main manifestations of SNS activation are increased HR, BP, and sweating. Sympathetic preganglionic neurons emerge from the thoracolumbar region (T1–L2) of the spinal cord to a ganglion of the sympathetic trunk [153]. Postganglionic SNS nerve terminals release neurotransmitters, including NE, epinephrine, acetylcholine, adenosine triphosphate, and neuropeptide Y, into the synaptic cleft [26; 27]. NE is the most abundant neurotransmitter released [160], and target organ activation by the SNS is regulated primarily through the release of NE. Although most target organs of the ANS are innervated by both the SNS and the PNS, sweat glands, erector pili (goosebumps), the adrenal medulla, and most importantly, arteries and veins, are innervated solely by the SNS. Therefore, their activity is only regulated by increases and decreases in sympathetic activity [153].

**1.3.3 Autonomic Control of Blood Pressure.** Mean arterial pressure (MAP) is a common measure of BP and a crucial physiological parameter that serves as an essential indicator for organ perfusion and oxygen delivery [101; 102]. It reflects the average arterial pressure and can be calculated as MAP = Diastolic BP + 1/3(Systolic BP - Diastolic BP), encapsulating both systolic and diastolic BP during a cardiac cycle. Additionally, MAP can be calculated using Ohm’s Law: MAP = Cardiac Output × Total Peripheral Resistance, all of three of which are variables involved in baroreflex control.

The baroreflex serves as a critical mechanism for the acute regulation of BP. It functions through a negative feedback loop (see Fig. 1.3), the sensitivity of which can be assessed through various methods, including vasoactive drugs and spontaneous BP fluctuations, which provide insights into autonomic control of the cardiovascular system [121; 191]. The baroreflex is

initiated by arterial baroreceptors [40; 48]; these mechanoreceptors, located in the carotid sinus and aortic arch, respond to changes in arterial pressure by altering their discharge patterns, which subsequently modulate sympathetic and parasympathetic outflows via the nucleus tractus solitarius (NTS) [112; 132]. The NTS processes afferent signals from baroreceptors, leading to decreased sympathetic outflow, which promotes vasodilation, and increased parasympathetic outflow, reducing HR [50; 137; 141].

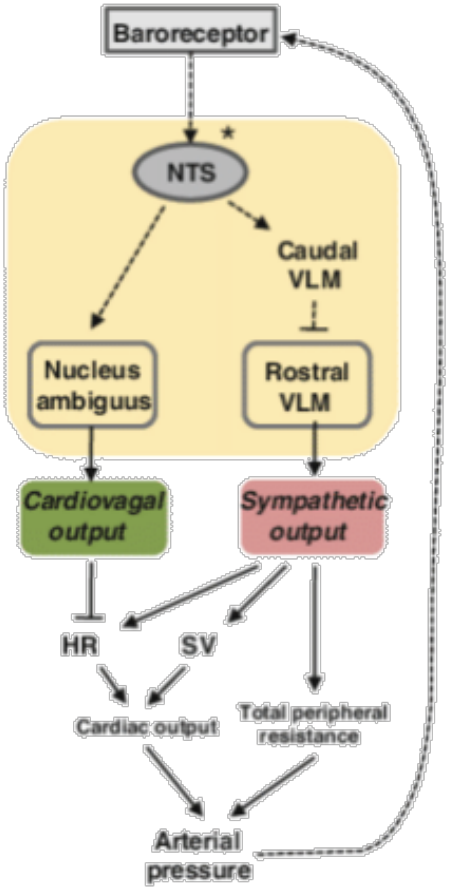


Fig 1. 3 Baroreflex loop (Hong et al., 2013)

Baroreceptor unloading, which occurs when BP decreases, results in decreased neural input to the NTS, leading to diminished parasympathetic activity and increased sympathetic outflow via the rostral ventrolateral medulla (RVLM) activation [137; 141]. Activation of the RVLM triggers a burst of sympathetic outflow, referred to as muscle sympathetic nerve activity (MSNA) [40; 256].

**1.3.4 Studying the human autonomic nervous systems: methods and measurements.** The ANS can be studied at rest or during reactivity. Though not unheard of, direct measurement of neuronal activity (i.e., microneurography) is technically difficult, and therefore rare. Consequently, most physiological measures serve as proxies of autonomic activity (e.g., BP, respiration, HR), and measures are quantified via changes over time (i.e., baroreflex sensitivity). The simultaneous innervation of several organs/glands by the SNS and PNS complicates the study of certain physiological measures. For example, an increase in HR could be attributable to

sympathetic activation, parasympathetic withdrawal, or both responses concomitantly. In contrast, some organs such as sweat glands and vascular smooth muscles are innervated only by the SNS, and measures such as BP and sweating and can be used as indirect measures of sympathetic activity [87]. MSNA serves as a direct measure of isolated sympathetic efferent activity acting on vasoconstriction and dilation, and recently, microneurographic recordings from the vagus nerve have been obtained, and serve as a measure of isolated parasympathetic efferent activity [184].

***1.3.4.1 Microneurography and MSNA.*** The microneurographic technique was developed in 1968 by Hagbarth and Vallbo [82]. Microneurography is a neurophysiological method in which a conductive tungsten needle microelectrode is inserted into a superficial peripheral nerve of an awake human subject to record and visualize the traffic of nerve impulses. This technique has been successfully employed to study motor control, touch, temperature, pain, and sympathetic activity [247]. When the electrode is positioned close to efferent muscle sympathetic nerve fibers, it allows for direct and real-time recordings of MSNA. This technique is considered the gold standard for studying the autonomic control of BP at the level of the peripheral vasculature. It provides direct recordings of the neural input that leads to vascular responses (i.e., vasoconstriction and dilation). The peroneal nerve, often used for sympathetic microneurography, is particularly advantageous as it is a large, easily accessible nerve containing many efferent sympathetic neurons [238; 260]. The microneurographic setup involves two tungsten electrodes: one reference electrode placed 1–3 cm from the recording site, and the second, the recording electrode, inserted transcutaneously into the peripheral nerve. The raw MSNA signal is then amplified, filtered, rectified, and integrated to reveal the characteristic bursts of activity, which are analyzed for firing frequency and burst size. This technique requires high precision, so few laboratories around the world utilize it due to the complexity involved in obtaining an adequate MSNA signal.

MSNA itself is a measure of the activity of sympathetic neurons innervating the smooth muscle around blood vessels. It plays a crucial role in regulating vascular tone by controlling vasoconstriction and vasodilation, with its regulation largely governed by the rostral ventrolateral medulla (RVLM). The RVLM is influenced by several brain regions, including the insula and prefrontal cortex [137; 139-141]. Under resting conditions, the RVLM is inhibited by the caudal ventrolateral medulla (CVLM), while the NTS integrates afferent inputs and projects excitatory signals to the CVLM [50; 51]. During periods of increased MSNA, the activity of NTS and CVLM decreases, while RVLM activity increases [138]. MSNA is heavily controlled by homeostatic baroreflex mechanisms, maintains cardiac rhythmicity, and displays an inverse relationship with BP variations. These mechanisms help regulate BP by adjusting sympathetic output. The quantification of MSNA includes measures of burst frequency (bursts/minute), burst incidence (bursts per 100 heartbeats), and burst amplitude, the latter of which is influenced by the number and size of neurons within the electrode's recording range [179; 231]. As burst amplitude can be affected by electrode placement, the method of normalization is often employed to control for interindividual differences [234], expressing burst activity relative to the maximal burst observed during baseline for each participant. These precise measurements allow researchers to investigate the complex integration of afferent inputs and central mechanisms that influence sympathetic output, contributing to our understanding of autonomic control over BP and vascular function [58; 88; 114; 146-149; 234].

***1.3.4.2 Baroreflex sensitivity (BRS).*** Because the baroreflex, like the autonomic system, has two branches (i.e., sympathetic and parasympathetic/cardiovagal), BRS can be assessed in both of them independently. The sensitivity of the cardiovagal baroreflex can be assessed by determining the acceleration/deceleration of HR driven by a given decrease/increase in BP,

respectively. There are different ways to assess the sensitivity of the baroreflex such as the use of vasoactive phenylephrine, external suction of the carotid arteries, and the Valsalva maneuver [121]. These techniques will cause fluctuations in BP, but spontaneous changes in BP can also be measured at rest. One of these methods, the sequence method of determining cardiovagal BRS, is as follows [186]: three or more consecutive beats are identified where increases/decreases in systolic BP are followed by lengthening/shortening of the interval between HR R-waves, or RR interval. The threshold values for including beat-to-beat systolic BP and RR interval changes in a sequence are typically set at 1 mmHg and 6 ms, respectively. A regression line relating one sequence of changes in systolic BP with changes in the RR interval is created and used to calculate one slope for BRS. The slopes of all sequences are then averaged to obtain a final value of cardiovagal BRS [259]. Sensitivity of the sympathetic baroreflex can be determined through alignment of BP waves with bursts of MSNA [10; 39]. Values of diastolic BP values corresponding with time-aligned MSNA bursts are extracted. The ratio of instances of diastolic BP corresponding with bursts to overall diastolic BP are stratified by increments of 2 mmHg, then expressed as a percentage, and finally graphed against DBP. The slope of this line is then used to determine sympathetic BRS. These techniques can be used during a long period of rest or used alongside different stimuli (e.g., nociceptive stimulation).

***1.3.4.3 The Cold Pressor Response.*** The CPT is performed by immersing a limb (usually the hand or forearm) into a container of cold water for a defined length of time. It is a sympathoexcitatory stimulus frequently used within the cardiovascular field [87; 249; 257; 265], and one of the most commonly used tools worldwide for producing experimental pain [104; 173; 198; 240]. The CPT is usually conducted using water temperatures of 0–7 °C [166], for a duration between 30 seconds and 6 minutes [47; 49; 63; 109; 250]. The cold pressor response refers to the

physiological responses associated with a painful cold stimulus: it evokes paradoxical local vasodilation in response to cold, diffuse vasoconstriction of blood vessels, and subjective perception of acute cold and ischemic pain in most people. These perceptions derive from activation of thermoreceptors and nociceptors, the signals of which travel to the spinal cord and brainstem, where they interface with the NTS and the rostral ventral regions of the medulla. Via these projections to the NTS and RVLM, downstream sympathetic outflow is increased.

### *1.4 Pain and the autonomic system*

Pain can be perceived as a stressor or threat to homeostasis, and in its presence, the ANS coordinates a range of physiological responses aimed at immediate survival. These responses typically include increases in HR [241], BP [188; 206], and sweating [135], largely driven by activation of the SNS and parasympathetic inhibition [95]. In extreme cases, such as with severe pain or fear, parasympathetic activity may also dramatically increase, leading to vasovagal syncope, potentially leading to collapse/loss of consciousness [248], and thereby greater blood flow to the brain. These autonomic reactions are believed to support short-term survival by providing the organism with a chance to escape, neutralize the source of pain, and/or support critical organ function. Beyond survival, these autonomic responses also play a crucial role in pain modulation [11]. The ANS and nociceptive activity are interconnected at multiple levels—from the periphery to the spinal cord, and up to the cortical regions of the brain.

At the peripheral level, NE released by sympathetic neurons can sensitize nociceptors, which can occur after nerve trauma or partial nerve injury (e.g., complex regional pain syndrome [CRPS] type I). This phenomenon, previously termed "sympathetically maintained pain," can lead to central sensitization, hyperalgesia, and allodynia [163]. Although the release of NE does not elicit pain under normal conditions, immune system responses in chronic pain (e.g., interleukins,

bradykinin) can further contribute to nociceptor sensitization [96]. Sympathetic nerve blocks, involving agents such as phenol or local anesthetics, are sometimes used to reduce pain in certain chronic conditions, although the exact mechanism remains unclear and may involve peripheral and/or central effects [36; 271].

In contrast, vagal afferents are thought to exert a tonic inhibitory influence on pain at the level of the spinal cord's dorsal horn [203]. Vagus nerve stimulation has been shown to reduce pain in animal models [20; 236] and to alleviate experimental pain in humans [115]. Conversely, vagotomy, or cutting the vagus nerve, can increase pain sensitivity in both cutaneous [113] and visceral tissues [90], underscoring the vagus nerve's critical role in pain inhibition. Moreover, the PAG is involved in both pain regulation and autonomic control, playing a crucial role in modulating these responses. Stimulation of the PAG can either increase sympathetic activity (via the dorsolateral PAG) or induce opioidergic analgesia (via the ventral PAG), with both pathways influencing pain perception and BP (Behbehani, 1995; Green et al., 2005). These findings suggest a complex interplay between the ANS and pain processing, with autonomic responses acting as modulators of pain intensity.

Sympathetic modulation of pain is complex, with acute pain generally inducing a stress response that temporarily suppresses pain (i.e., stress-induced analgesia) [34] [124]. The relationship between BP and pain sensitivity has been extensively studied. Hypertensive animals, for example, exhibit reduced pain sensitivity [144; 276], and similar inverse relationships between BP and pain sensitivity have been observed in humans across different populations, including hypertensive, normotensive, and hypotensive individuals [28; 59; 76]. This connection is mediated through the baroreceptor reflex, which influences pain sensitivity by activating pain-inhibitory pathways [203]. Chronic pain disrupts this normal relationship; in contrast to healthy individuals,

chronic pain is linked to prolonged SNS activation, which may contribute to the development and persistence of pain [11]. Chronic pain conditions like fibromyalgia are often associated with autonomic dysregulation, including reduced HR variability, altered BP responses [172], and even a reversal of healthy BP/pain sensitivity relationships [29; 30]. Dysregulation of pain modulation mechanisms such as CPM may contribute to these altered cardiovascular-pain dynamics.

The altered relationship between BP and pain sensitivity in chronic pain conditions may be linked to a failure of descending pain inhibitory systems. It is hypothesized that chronic pain arises not from increased pain transmission *per se*, but rather from the failure of these descending pain modulatory mechanisms, leading to a dominance of pain facilitatory systems [31; 143]. This dysregulation might be particularly evident in fibromyalgia, where both opioid and NE systems appear to play a role in modulating pain. Chronic pain sufferers, including those with fibromyalgia, often show diminished endogenous opioid function, which may reduce the efficacy of opioid-mediated pain relief [29; 31]. Additionally, decreased $\alpha_2$-adrenergic receptor activity in the dorsal horn has been observed in chronic pain models, further suggesting deficits in NE pathways that could exacerbate chronic pain perception [133; 232].

### *1.5 Chronic pain*

**1.5.1 Fibromyalgia.** Fibromyalgia is a chronic pain disorder characterized by widespread pain, allodynia, hyperalgesia, joint and muscle stiffness, and commonly, sleep disturbances, fatigue, difficulty concentrating, and gastrointestinal issues. Fibromyalgia affects around 2–3% of the population, with a much higher prevalence in women [268]. It was formally recognized in 1990 with diagnostic criteria from the American College of Rheumatology, which include diffuse pain lasting at least three months across multiple areas of the body. It is also frequently comorbid with psychiatric conditions like depression and anxiety [269]. More recent criteria also include non-

musculoskeletal symptoms, gastrointestinal issues, dizziness, and dry eyes, some of which may be linked to autonomic dysfunction [267].

Fibromyalgia's pathophysiology is still unclear. Conflicting evidence support small fibre neuropathy [62; 182; 244] or central sensitization [45] as the main causes of fibromyalgia, but the cause most-often pointed to is dysfunction in endogenous pain control mechanisms [104; 226]. Additionally, lower levels of neurotransmitters like serotonin, norepinephrine, and dopamine in the cerebrospinal fluid may impair pain inhibition [219]. Finally, autonomic dysfunction is believed to contribute to the heightened pain sensitivity, fatigue, and sleep disturbances seen in fibromyalgia patients [2; 157]. While there is no cure, treatment focuses on managing pain and improving sleep and quality of life [1]. Fibromyalgia can be more effectively treated with multiple simultaneous therapies [221], combining education, pharmacological approaches such as antidepressants (e.g., SNRIs, tricyclics), gabapentinoids, cannabinoids, opioids, and NSAIDs [84; 223; 224; 243], as well as non-pharmacological approaches like psychotherapy, physical exercise, and relaxation techniques [83].

***1.5.1.1 Fibromyalgia, the autonomic system, and pain modulation.*** The ANS, in many individuals with fibromyalgia, has been shown to function abnormally. Studies of HR variability have revealed that patients with fibromyalgia exhibit higher SNS activity and lower PNS activity at rest compared to healthy individuals of similar age [46; 70; 158; 230]. At rest, individuals with fibromyalgia have increased HR and diminished BRS, but no significant differences in resting BP when compared to controls [46; 55; 56; 70]. During autonomic reactivity, greater differences are observed. Orthostatic tests (e.g., tilt test) have revealed that fibromyalgia patients exhibit abnormal orthostatic hypotension and reduced SNS activity [21; 70; 158]. Additionally, cardiovascular responses to stress (e.g., HR and BP) are blunted in fibromyalgia patients [178]. Finally,

parasympathetic reactivity, measured through slow, deep breathing has also been shown to be lower in fibromyalgia patients compared to healthy controls [185; 245].

Few studies have examined the relationship between autonomic activity and pain modulation, and even fewer in fibromyalgia. In healthy participants, Chalaye et al. [37] observed that CPM magnitude was related to BP reactivity during the CPT. In a fibromyalgic sample, one study observed an inverse correlation between resting BP, baroreflex sensitivity, and clinical pain severity [56], suggesting that dysfunction in baroreflex-mediated pain inhibition may contribute to hyperalgesia. In another study, fibromyalgia patients who experienced a significant drop in BP during the tilt test also reported an increase in pain [21]. Finally, Chalaye et al. [39] observed that blunted BP reactivity during CPT was related to less efficacious CPM in patients with fibromyalgia.

Parasympathetic activity is also altered in fibromyalgia patients. Slow deep breathing, which enhances parasympathetic activity and reduces pain sensitivity in healthy individuals, has been shown to be ineffective in reducing pain sensitivity in fibromyalgia patients [278]. Additionally, reduced baroreflex sensitivity in fibromyalgia patients has been correlated with increased pain during the CPT. This suggests that impaired autonomic reactivity may be linked to both acute and clinical pain in fibromyalgia.

Pain modulation mechanisms also appear dysfunctional in fibromyalgia. Studies have found that fibromyalgia patients show greater temporal summation of pain when exposed to thermal stimuli compared to healthy controls [197; 227; 228], although there is conflicting evidence for this finding [196]. CPM is also diminished in fibromyalgia patients [54; 105; 118; 122; 180; 187]. Finally, fMRI studies showed that fibromyalgia patients exhibit decreased activity in the ACC and brainstem during painful stimulation, areas involved in pain inhibition as well as

the autonomic response to pain in healthy people [98; 142].

**1.5.2 Sex and gender considerations.** Sex differences in pain have been examined for decades [13; 246], and have been reviewed at length [65; 150; 167-170; 216; 225]. Sex and gender have historically been used interchangeably, despite having differences [19]. Sex is primarily associated with "physical and physiological features including chromosomes, gene expression, hormone levels and function, reproductive/sexual anatomy [and] is usually categorized as female or male" [44], although a small number of intersex individuals exist as well. In contrast, gender is associated with "psychological and sociocultural factors, such as beliefs, expectations, and stereotypes, and how men and women behave and interact with one another. Binary categories are commonly used (e.g., man/woman and boy/girl), although gender is not constrained to this and encompasses broader aspects" [111]. Even with these definitions, sex and gender are not completely independent; biological sex-related mechanisms can be shaped and influenced by psychosocial gender variables, and vice-versa. The term "sex/gender" has started to be used, as it highlights how difficult it is to disentangle these two interacting factors [92; 171]. Most of the research conducted to date primarily consists of a binary categorization of men and women, therefore, in this dissertation we will primarily address human sex/gender variables as a binary, using men/males and women/females interchangeably.

Chronic pain is a disease in which women are greatly overrepresented as patients [13; 94; 246], and for many pain disorders (fibromyalgia, migraine, temporomandibular disorder, etc.) female sex/gender has long been known to be the largest risk factor. Women have been shown to be more sensitive to and less tolerant of painful stimuli [167; 212], regardless of modality or measure. There are also known sex differences in the factors modulating pain. For example, in chronic low back pain patients, pain intensity was related to pain anxiety in men and fear of injury

in women [119]. Konietzny et al. [117] found that women express higher levels of depression related to back pain, and in terms of pain catastrophizing, rumination was found to be the mediating component of sex differences in CPT pain in young adults [161]. There are also recognized sex differences in brain activation during exposure to pain. Men are more likely to use prefrontal cortex-mediated threat-control circuits, whereas women relied on emotion-processing centers to cope with pain unpleasantness [79].

Previous reports have found that gender role expectation of pain accounts for significant variability in pain experience [213; 266], in that the more strongly one believes in gender disparities in pain experience, the more one will conform to that role. A related finding suggested that male pain study participants were more likely to identify with masculine gender roles and expressed higher levels of aggressiveness and competitiveness [159], potentially skewing the sex differences observed. It is apparent that gender plays a role in the perception of others' pain. Walsh et al. [253] demonstrated that female actors were rated as expressing more pain than male actors, even when the intensity of their expression was matched, but when men and women were asked to judge the pain of patients with back pain on video, women's pain was more often judged to be less intense, more exaggerated, and more psychologically based than men's pain [165]. Similarly, female chronic pain patients reported more dismissal of symptoms by physicians and males reported more hostility and avoidance [93]. These perceptual differences also alter coping strategies wherein men report more fear of movement and low activity, whereas women report greater reliance on social support [217] and feel that they are more empathetic to pain in others [8].

When viewed as a spectrum, gender can affect a number of pain outcomes. A review by Boerner et al. [19] outlines and defines various aspects of gender and describes how they interact

with pain. Gender identity, or "an individual's internal experience of one's own gender; the label one applies to oneself and one's self-concept" [85], likely contributes to the extent to which individuals internalize and engage in gendered pain behaviours; identifying more strongly with a particular gender group social norm has been associated with gendered pain behaviours [192]. Gender expression is defined as "the external or behavioural expression of one's gender, which may or may not be in line with one's internal perception or experience of their gender" [131], and expression of greater masculinity has been found to be associated with lower pain sensitivity [251]. Gender role orientation, is "the extent to which an individual demonstrates characteristics, attitudes, attributes, or behaviours considered to be typically associated with a specific gender within a specific cultural context" [190]. Numerous studies have described the impact of masculinity and feminine gender roles on the pain experience, finding that increased masculine gender roles are associated with decreased pain sensitivity [5] and both femininity and masculinity have been associated with certain health risk factors [110; 189]. Gender ideology is defined as "beliefs, attitudes, and expectations that are organized in line with expectations for specific genders, often internalized based on societal influences and pressures" [237]. Both men and women expect the typical man to be less willing to report pain, have higher pain endurance, and lower pain intensity than the typical woman [213; 254], and endorsement of such beliefs have been associated with pain outcomes such as temporal summation [214]. Gender bias is considered "differential treatment on the basis of an individual's gender, often informed by gendered cognitions" [218]. Systemic gender bias in clinical pain treatment has been documented at length, especially in patient-provider relationships [see 14; 15; 65; 89].

Unfortunately, there has not been substantial research to date examining the impact of gender identity and expression—when seen as a continuum—on pain, especially in gender-

nonconforming populations. This is due to the multiple difficulties that researchers have when seeking to quantify, examine, and understand gender. Though masculinity and femininity play important roles in understanding the pain experience [5; 110], gender goes beyond these two binaries, and we are only beginning to scratch the surface of understanding the full influence of the gender spectrum on pain [71; 208].

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# Chapter 2

## Sex differences in the relationship between pain and autonomic outflow during a cold pressor test

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**Abstract**

Chronic pain is partly maintained by the sympathetic nervous system, whose activity is best measured by muscle sympathetic nerve activity (MSNA). MSNA responses to acute pain have been thoroughly investigated, whereas MSNA responses to longer-lasting pain are poorly understood. Therefore, this study examined the relationship between pain ratings and peroneal MSNA during a tonic cold pressor test (CPT) in male and female participants. We obtained MSNA measures during a 6 min CPT in 18 young adult (20–33 years) men and women. Verbal pain ratings (0–10) and autonomic outcomes (heart rate [HR], mean arterial pressure [MAP], and MSNA) were assessed simultaneously at multiple time points across the CPT. Pain, HR, and MAP increased in the initial 30 s in both sexes. Females increased their MSNA burst frequency (BF) to a greater extent than males. Across the full CPT we observed a positive relationship between pain and HR in males, a positive relationship between pain and MSNA BF in females, and a negative relationship between pain and MSNA burst amplitude in females. Overall, males displayed a strong relationship between tonic pain and HR, an index of parasympathetic activity, whereas females displayed strong and offsetting relationships between tonic pain and purely sympathetic MSNA variables. These observations suggest sex differences in autonomic mechanisms during long-lasting pain, which may have relevance to ongoing efforts to modulate pain via manipulations of the autonomic nervous system.

## 2.1. Introduction

Chronic pain is often comorbid with other conditions, particularly cardiovascular diseases [7; 48], leading to increased rates of cardiovascular mortality in pain patients [2; 22; 40]. Patients who develop chronic postsurgical pain have almost twice the prevalence of hypertension than patients who do not [10], and chronic pain patients exhibit reduced heart rate variability [32; 43; 45; 50; 63], a marker for cardiovascular disease risk [21]. Brain regions associated with autonomic control of the cardiovascular system overlap substantially with those related to nociceptive processing [55], which may account for the link between chronic pain conditions and dysregulation in the autonomic—and, in particular, sympathetic—control of the cardiovascular system [2; 9; 60].

Sympathetic nervous system activation can be inferred from non-invasive cardiovascular parameters (e.g., BP) but the gold-standard method for quantifying sympathetic outflow in humans is microneurography [41; 42]. Microneurography is a neurophysiological technique in which a conductive microelectrode is inserted into a peripheral nerve of an awake human participant in order to record and visualize the traffic of nerve impulses [26]. Muscle sympathetic nerve activity (MSNA) can be measured via the microneurographic targeting and recording of efferent postganglionic sympathetic nerve fibers innervating vascular smooth muscle. MSNA is commonly quantified in cardiovascular research due to its role in homeostatic blood pressure regulation (e.g., baroreflex) mechanisms [56]. However, microneurographic recordings of MSNA have also been applied within the pain research field to directly quantify sympathetic responses to noxious stimuli and better understand relationships between sympathetic outflow and pain [13; 23; 37; 38; 51; 58].

In general, MSNA increases when a participant is exposed to a noxious stimulus. This relationship has been demonstrated using various and diverse pain assays, including: soap solution in the eye [51], nailbed mechanical pressure [51], mechanical skin pressure [58], and the cold

pressor test (CPT) in which a participant's hand is immersed in a cold water bath typically for 0.5–3 minutes [20; 38]. What remains poorly understood are MSNA responses to sustained (i.e., tonic or chronic) pain. Individuals with cluster headaches demonstrate higher levels of basal MSNA [52]. Moreover, Fazalbhoy and colleagues [23; 24] observed that a model of tonic visceral pain (i.e., bolus intramuscular injection of hypertonic saline, associated with pain lasting ~60 minutes) resulted in heterogeneous MSNA responses, with some participants progressively increasing and others progressively decreasing MSNA over the duration of the stimulus. Neither study included enough female participants to assess whether sex contributed to this variability, nor were sex disaggregated data reported in the existing CPT studies [20; 38]. Thus, the effects of tonic pain on MSNA remain to be firmly established in both sexes.

As experimental models of tonic pain have greater clinical relevance for chronic pain than do acute pain models [16; 57; 66], the primary aim of this study was to explore interactions between time, pain, and indices of autonomic activity, including MSNA, across a tonic pain stimulus (i.e., a 6 min-long CPT). Given the large body of evidence supporting sex and/or gender differences in pain perception [4; 35; 49] and blood pressure regulation [1], our secondary aim was to examine the effect of sex/gender on changes in pain and MSNA.

## 2.2 Materials and Methods

### *2.2.1. Participants*

We tested young (18–35 years), healthy men (n=9) and women (n=12) free from cardiovascular, respiratory, endocrinological, and chronic pain disorders. Three female participants were removed due to low-quality MSNA data, leaving 9 female participants. Although potential effects of sex and gender on our outcomes are likely intertwined [5; 35], this study was

not designed to assess the effects of sex and gender separately. Rather, we sought to analyze the effect of sex by dividing outcomes based on sex assigned at birth (male/female), and therefore the term 'sex difference' is used throughout. However, we note that our intake questionnaires did assess gender identity, and that all participants self-identified as cisgender.

All participants provided written, informed consent prior to participation. This study was conducted in accordance with the Declaration of Helsinki and was approved by the Faculty of Medicine Institutional Review Board at McGill University (A00-B14-20B).

### *2.2.2. Experimental Design*

Women were tested during the early follicular phase of the menstrual cycle (days 1-5; day being the first day of menses; n=2) or the low-hormone placebo phase of hormonal contraceptive use (n=7). All participants were tested at the same time of day (08:00 ± 1 h) to minimize any effects of circadian variations on resting MSNA. During a separate visit to the lab prior to testing, participants were familiarized with all non-invasive aspects of testing (see below), including an abbreviated CPT. Participants were coached to remain physically relaxed and to not hold their breath throughout the CPT in order to minimize movement- and respiration-induced alterations in the MSNA signal during testing [27; 39].

On the test day, all participants arrived at the laboratory having fasted a minimum of 3 h, and having abstained from caffeine, strenuous exercise, alcohol, and analgesics for 12 h. On arrival, participants were instructed to void their bladders. Participants were then positioned supine on a padded table for instrumentation. After ~15 min of stable supine rest, manual sphygmomanometry was used to obtain three manual blood pressure (BP) values that were used to calibrate finger photoplethysmography values. Microneurography was then conducted to obtain the MSNA signal. Following attainment of an adequate MSNA site, 10 min of quiet rest were

recorded to quantify baseline values of all outcomes. A 6-min CPT was then performed, consisting of placement of the participant's hand up to their wrist in ice water (~4 °C). This is an extended version of a well-established CPT protocol which elicits both pain and sympathetic reactivity in humans [e.g., 31; 53; 54; 61; 64; 65; 67]; our pilot work indicated that 6-min was the upper limit that could be tolerated by most participants. Participants reported pain ratings verbally on a numerical rating scale (0–10) at rest and at four predetermined time points over the course of the CPT (time points: 30 s, 2 min, 4 min, and 5.5 min).

### *2.2.3. Instrumentation*

Heart rate (HR) was measured using a standard 3-lead electrocardiogram (ECG). BP was measured on a beat-by-beat basis using finger photoplethysmography (Finometer MIDI, Finapres, Amsterdam, Netherlands), which involved the placement of a small cuff around the participant's third or fourth finger on the hand contralateral to the CPT. HR and continuous BP signals were sampled continuously at a frequency of 1.0 kHz and saved for offline analysis (PowerLab and LabChart v8, ADInstruments). Microneurography was used to record multiunit postganglionic MSNA from the common peroneal nerve (NeuroAmp EX, ADInstruments) [26]. Briefly, an insulated tungsten recording electrode (35 mm in length, 200 μm in diameter, 2 ± 0.4 MΩ impedance) was inserted transcutaneously into the peroneal nerve, and a reference electrode was inserted subcutaneously 1–3 cm away from the recording site. An adequate MSNA signal consisted of pulse-synchronous bursts of activity that increased in firing frequency during voluntary apnea and remained unchanged during arousal to a loud noise [19]. The raw sympathetic signal was amplified 100× by a head-stage and the total amplification was 20,000×. The signal was then band-pass filtered (700–2000 Hz), full wave rectified, and integrated (time constant 0.1 s). Sympathetic activity was recorded at 10.0 kHz.

### *2.2.4. Data Analyses*

ECG and calibrated BP waveforms were analyzed to determine HR and mean arterial blood pressure (MAP), respectively. Bursts of MSNA were detected using a semi-automated peak detection algorithm (LabChart V8) based on a 3:1 signal-to-noise ratio and confirmed by a trained microneurographer following a shift of the MSNA signal to account for the neural conduction latency within each subject, aligning each sympathetic burst with the cardiac cycle that initiated it [26]. Bursts of sympathetic activity were quantified as burst frequency (BF; number of bursts/min) and burst amplitude (BA; percentage of peak baseline voltage). Both are standard measures used to quantify MSNA: MSNA BF informs about sympathetic neuronal firing rates, and MSNA BA about axon size of recruited sympathetic neurons and resulting neurotransmitter release [59]. Finally, they can be multiplied together to calculate total MSNA, which functions as a measure of the combined effects of neural firing rates and neurotransmitter release on a blood vessel.

Statistical analyses were performed, and figures created using GraphPad Prism v.9 (La Jolla, CA). Shapiro Wilk tests for normality were conducted for all variables at each time point. Grubbs' test was used to identify outliers within each variable and analysis. All data were expressed as means ± standard error (SEM), and □ was set at <0.05 to establish statistical significance.

Baseline (BL) values of all outcomes were extracted as the average of 10 min of quiet rest prior to the CPT. To assess absolute levels of pain, HR, MAP, and MSNA (BF, BA, and total MSNA), we extracted the average of the 60-s period centered around each pain rating time point (i.e., 0 to 60 s, 90 to 150 s, 210 to 270 s, 300 to 360 s). Two-way repeated measures analyses of variance (ANOVAs; sex x time) were performed. Where main effects were significant, a post-hoc Sidak multiple comparisons test was used determine how variables changed throughout the duration of

the CPT within each sex (Fig. 1).

To assess cumulative effects of changes over time in pain, HR, MAP, and MSNA, we calibrated our variable values relative to baseline, and then calculated the area-under-the curve (AUC) of these baseline-relative values for pain, HR, MAP, and MSNA. For MSNA BA, where means decreased from baseline, absolute values of the area-over-the-curve was used instead. Unpaired Student's t-tests were used to compare AUC between the sexes (Fig. 2).

To assess whether overall changes in HR, MAP, and MSNA were associated with overall changes in pain and the strength of their relationships, we calculated linear regressions and Pearson correlations of AUCs of full CPT (baseline to 5.5-min) HR, MAP, and MSNA responses. We then conducted unpaired F-tests of slopes to assess if these relationships differed between the sexes (Fig. 3).

### 2.3. Results

All participants reported no pain (0) at baseline. When expressed relative to baseline, all variables were normally distributed as determined by Shapiro-Wilk tests. Using Grubbs' test for outliers, one male participant's data was removed from all MSNA BA analyses.

#### *2.3.1. Absolute levels across time course*

Repeated-measures ANOVA revealed a main effect of time for pain ($F_{4,64} = 113.1$, $p<0.001$; Fig. 1A), HR ($F_{4,64} = 24.9$, $p<0.001$; Fig. 1B), MAP ($F_{4,64} = 46.0$, $p<0.001$; Fig. 1C), and MSNA BF ($F_{4,64} = 10.4$, $p<0.001$; Fig. 1D). There was no main effect of time observed for MSNA BA ($p=0.23$; Fig. 1E). There were no main effects of sex, nor any time-by-sex interactions, on any of these variables ($0.16< p<0.94$).

Pain ($p<0.001$; Fig. 1A), HR ($p<0.001$; Fig. 1B), MAP ($p<0.001$; Fig. 1C) and MSNA BF

($p<0.001$; Fig. 1D) all increased significantly from baseline to 30-s. Both HR ($p=0.003$) and MAP ($p=0.001$) increased further from the 30-s to the 2-min time point.

### *2.3.2. Areas under the curve*

Although there were no significant time-by-sex interactions observed at any particular time point in absolute measures of pain, HR, MAP, or MSNA, we wanted to understand how the sexes may have differed in their overall CPT responses expressed relative to baseline over the entire 5.5 min time period. We thus calculated areas under the curve for each variable. There was no significant sex difference in pain ($t16 = 1.0$, $p=0.33$; Fig. 2A), HR ($t16 = 0.48$, $p=0.63$; Fig. 2B) or MAP AUC ($t16 = 0.59$, $p=0.56$; Fig. 2C). With respect to measures of MSNA, we observed a significant sex difference in MSNA BF AUC ($t16 = 2.4$, $p=0.03$; Fig. 2D) but not MSNA BA ($t15 = 0.64$, $p=0.53$; Fig. 2E). That is, over the full testing period, female participants displayed 2.2-fold higher changes in MSNA BF than male participants. We also calculated total MSNA (BF x BA), which was not significantly different between male and female participants (see Supplementary Fig. 1).

### *2.3.3. Correlations of autonomic indices with pain*

Males displayed a significant positive relationship between pain and HR ($r=0.69$, $p=0.04$; Fig. 3A) whereas females showed a positive but non-significant relationship between pain and HR ($r=0.41$, $p=0.27$; Fig. 3A). However, regression slopes were not significantly different between sexes ($p=0.59$). Pain and MAP were not related in males or females ($r= -0.11$ and $r= -0.49$ respectively; Fig. 3B). Females showed a significant positive relationship between pain and MSNA BF ($r=0.67$, $p=0.048$; Fig. 3C), whereas males did not ($r=0.26$, $p=0.49$; Fig. 3C). The slopes of the male and female regression lines only approached significance ($p=0.06$). Finally, females displayed a significant negative relationship between pain and MSNA BA ($r= 0.82$, $p=0.007$; Fig.

3D), whereas males showed a positive but non-significant relationship (r=0.57, p=0.14; Fig. 3D). Regression slopes for MSNA BA were significantly different by sex (F1,13 = 15.6, p=0.002).

## 2.4. Discussion

Despite the well-established positive linear relationship between acute pain and MSNA, our findings indicate that a tonic painful stimulus maintains a complex relationship with MSNA variables—as well as other autonomic indices like BP and HR—over time. Despite the difficulties in obtaining high-quality MSNA signals over a 6 min CPT, we were able to obtain such data in enough participants to examine sex differences during tonic pain for the first time and observed that autonomic responses to pain change in a sex dependent manner.

### *2.4.1. Pain and the autonomic nervous system*

As expected, the noxious stimulus significantly increased pain levels as well as HR, BP, and MSNA BF (see Fig. 1). The brain regions associated with control of the autonomic system are known to overlap substantially with those related to nociception [55], and thus it is not surprising that CPT-induced activation of this system led to parallel initial increases in both pain perception and autonomic variables. Although variability was noted both between participants and over time within participants, after the initial increase, mean responses for most variables stayed fairly constant over the entire time course of the CPT. The similar time courses of pain scores and the autonomic variables over time are likely due to the homeostatic relationship between pain and stress, in which acute pain can produce acute stress [44], and acute stress can inhibit pain (via stress-induced analgesia; [14]). This relationship may be mediated by a baroreceptor feedback loop, such that: 1) pain increases sympathetic arousal via a somatosensory reflex, thereby increasing BP; 2) increased BP stimulates baroreceptors, which trigger descending pain inhibition

[9]; and, 3) pain inhibition returns arousal levels to a state of homeostasis [25; 68]. Thus, this homeostatic loop likely involves the autonomic as well as the sensory nervous systems, with the nucleus tractus solitarius (NTS) serving as the interface between them. The NTS receives significant afferent input from the nociceptive spinal cord laminae and the vagus nerve, which regulates the baroreflex [9; 55]. The two efferent arms of the baroreflex, of course, are the parasympathetic and sympathetic nervous systems.

### *2.4.2. Effects of sex on the contribution of the parasympathetic system to pain*

In the first 30 s, pain, HR, and BP significantly increased in both sexes (see Fig. 1). Additionally, males displayed a significant positive relationship between HR and pain throughout the CPT (see Fig. 3A). HR and BP are both regulated by the parasympathetic system [28]. Given the inverse relationship between parasympathetic outflow and HR, we suggest that pain, at least in males, is influenced by parasympathetic withdrawal. In fact, a meta-analysis by Tracy and colleagues [62] showed that heart rate variability and parasympathetic outflow were disrupted in mixed-sex chronic pain patients, indicating that parasympathetic inhibition may be involved in the mediation of chronic pain as well.

Although females showed a moderate positive (but non-significant) correlation between HR and pain, they displayed much stronger relationships between pain and MSNA—a purely sympathetic variable (see below). These relationships suggest that physiological mechanisms involved in regulating pain initiation and maintenance differ between the sexes.

### *2.4.3. Effects of sex on the contribution of the sympathetic system to pain*

Across the CPT as a whole, females displayed greater MSNA BF to the CPT than did males (see Fig. 2D). It is fairly well established that young males have higher MSNA BF at rest than young females [33], which we observed as well (male: 8.2 ± 2.1; female: 5.3 ± 1.2), but sex

differences response to stressors including the CPT remain an area of ongoing investigation. Existing studies investigating the MSNA response to CPT have reported no differences between sexes [17; 29; 34; 47]. It is important to note, however, that the duration of the CPT in these studies were shorter than in the present study (i.e., 2-min [34; 47] and 3-min [17; 29]). It is possible that more prolonged cold pain may reveal sex differences in adaptation of cold afferents [69] or thermoregulatory mechanisms [12; 15] that could lead to the sex differences in cumulative MSNA BF observed here. It would be worthwhile to repeat this experiment using a different pain modality (e.g., hypertonic saline) to see whether the sex differences in MSNA BF relate to cold pain specifically or pain more generally.

In addition to changes in MSNA BF in females, we observed a significant positive relationship between pain and MSNA BF, as well as a significant negative relationship between pain and MSNA BA in females but not males (Fig. 3C, 3D). Taken together, we can conclude that females have strong sympathetic reactions in response to, or in association with, tonic pain. Opposing relationships between BF and BA may be explained as follows. BF and BA may act in mutual counterbalance as a homeostatic mechanism to maintain total MSNA levels (see Supplementary Fig. 1), and thereby BP, which was mostly stable throughout the CPT in females (see Fig. 1C). MSNA BF is a measure of neuronal firing rates and may be increased in females in order to dampen pain perception via the initiation of stress-induced analgesia. We speculate that MSNA BA, as the other variable making up total MSNA, is correspondingly reduced in order to ensure total MSNA outflow and resulting BP remain steady. Alternatively, because females' BA was negatively associated with pain, it is possible that the CPT leads to neural recruitment of smaller sympathetic neurons (i.e., decreased recruitment of larger axons) such that each MSNA burst releases less neurotransmitter. Indeed, neuronal size, and thus BA, can be regulated

independently from BF [18; 59], and smaller-diameter neurons likely release less neurotransmitter [36]. As such, these smaller neurons may in turn increase their BF to compensate, leading to the positive relationship with pain in females. If these smaller sympathetic neurons continue to be persistently activated alongside pain, the resultant increase in BF could sensitize spinal nociceptive pathways, overpower a depleted pain-inhibitory system, and lead to hyperalgesia [30]. Reversal of the normal negative pain-stress homeostatic feedback loop into a positive one has been observed in orofacial pain patients [6] and chronic low back pain sufferers [11] and can ultimately contribute to the development of chronic dysfunctional pain [9; 46].

#### *2.4.4. Strengths and limitations*

To our knowledge, this is the first study to examine relationships between repeated measurements of both tonic pain and MSNA in both sexes. We were able to recruit and obtain high quality MSNA data in 18 participants, a large sample size for microneurography studies. Despite this, there was a large amount of interindividual variability within the sample. Furthermore, additional data collection in the form of more frequent pain assessments would have allowed for more granularity in time-course analyses.

#### *2.4.5. Conclusions*

In summary, we find a dynamic relationship between pain and autonomic indices, a relationship that appears to vary between the sexes. These data suggest that pain in males may be regulated by mechanisms primarily associated with the parasympathetic system, whereas pain in females may be more closely tied to sympathetic mechanisms. Given that chronic pain has been associated with chronic activation of the sympathetic nervous system [3; 8], we suggest that treatment strategies involving manipulations of the autonomic nervous system should pay special attention to sex.

## 2,5. Acknowledgments

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## Figures

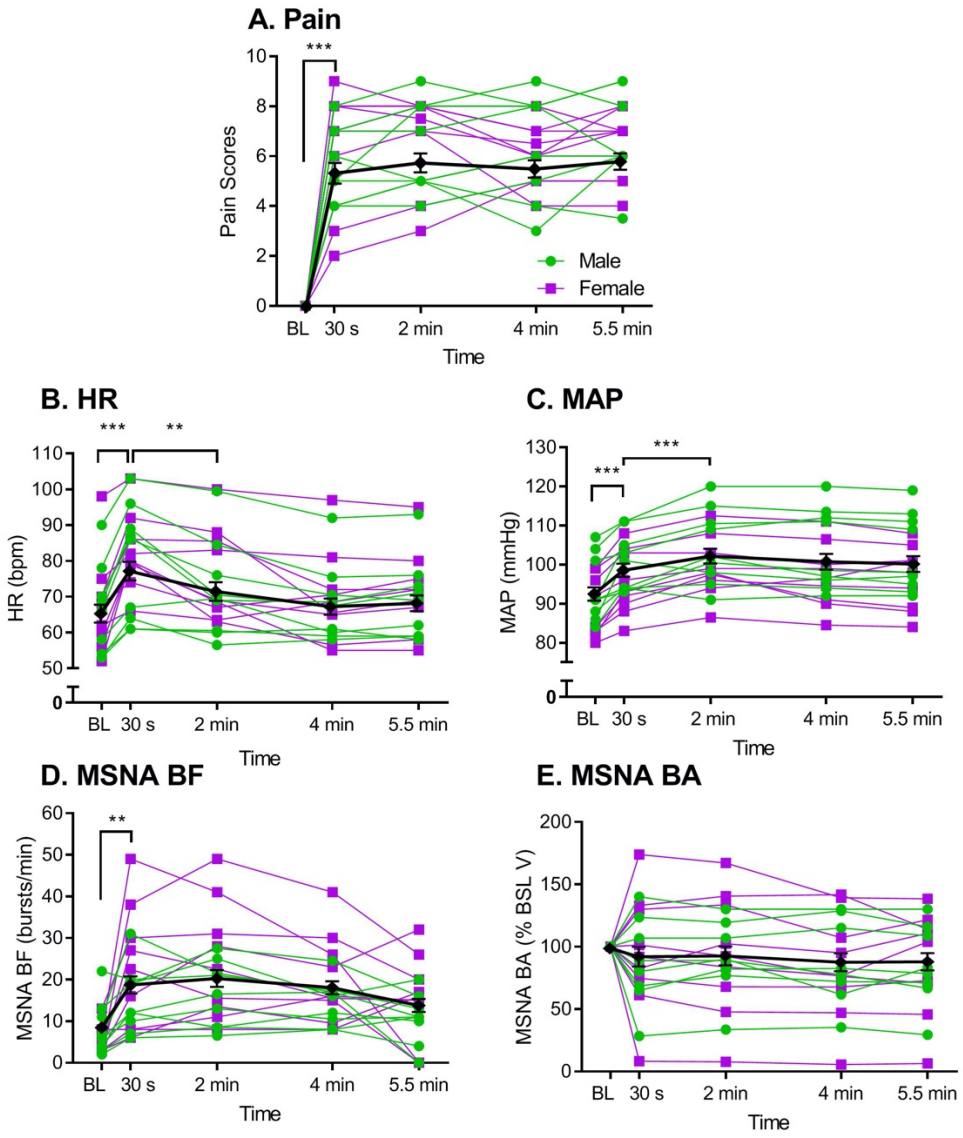


**Fig. 2.1.** Time course of pain (A) and autonomic variables (B, heart rate [HR]; C, mean arterial pressure [MAP]; D, muscle sympathetic nerve activity burst frequency [MSNA BF]; and E, muscle sympathetic nerve activity burst amplitude [MSNA BA]) before (i.e., at baseline [BL}) and at various time points during a CPT. Black symbols indicate mean ± SEM; purple symbols represent female participants ($n$=9) and green symbols represent male participants ($n$=9; $n$=8 for MSNA BA). **$p$<0.01, ***$p$<0.001 as indicated.

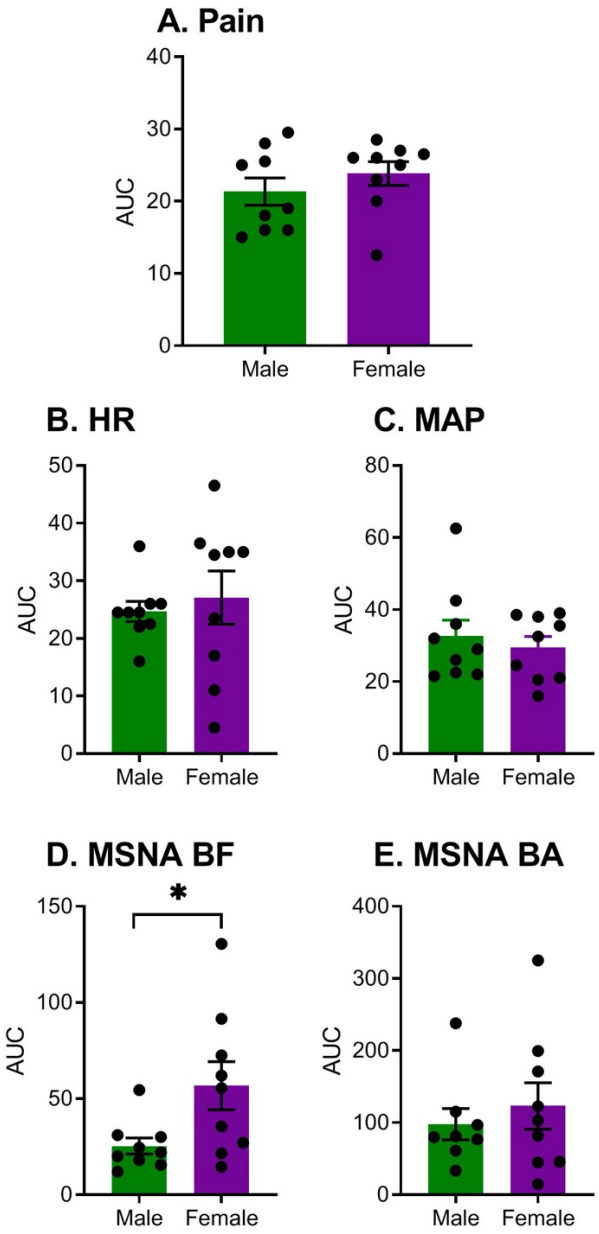


**Fig. 2.2.** Areas-under-the-curve (AUC) of pain (A) and autonomic responses (B, heart rate [HR]; C, mean arterial pressure [MAP]; D, muscle sympathetic nerve activity burst frequency [MSNA BF]; and E, muscle sympathetic nerve activity burst amplitude [MSNA BA]) to the CPT expressed as relative changes from baseline and stratified by sex. Bars represent mean ± SEM. *$p$<0.05 as indicated.

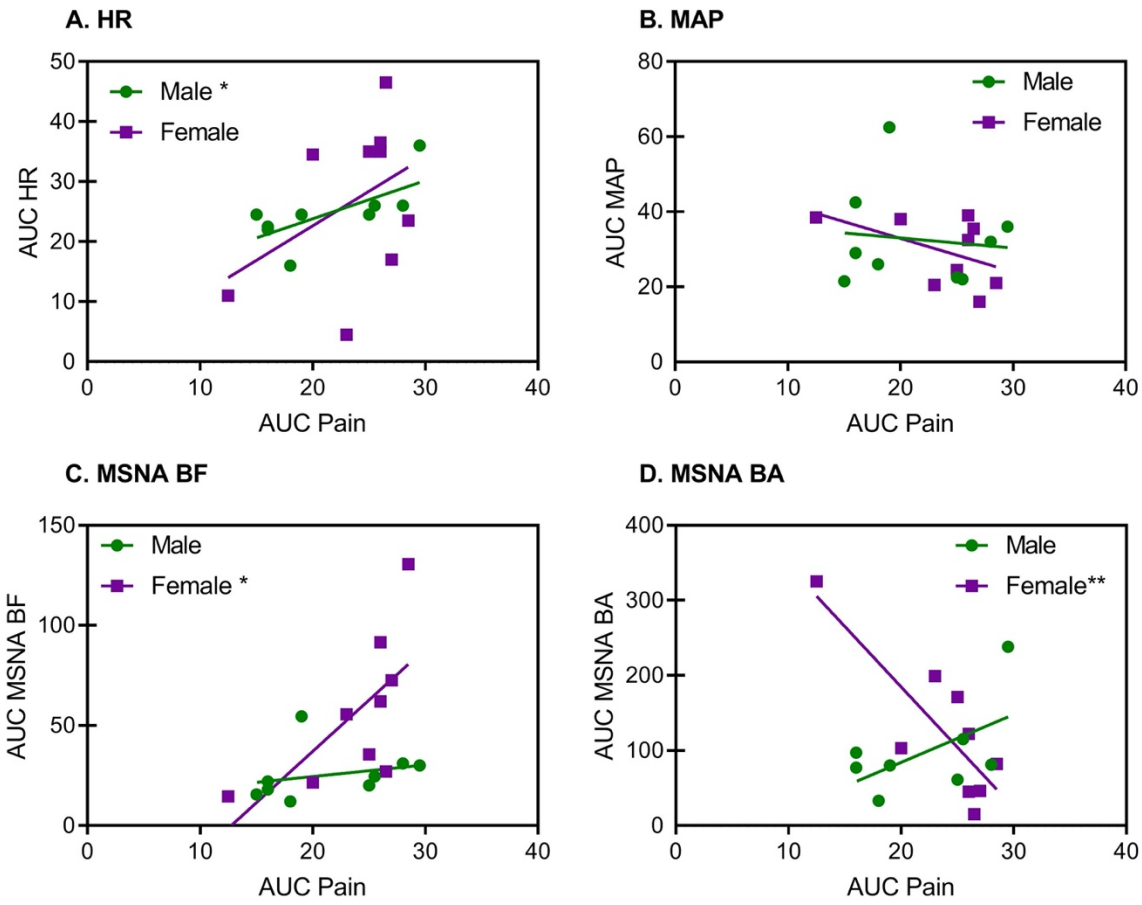


**Fig. 2.3.** Sex-dependent relationships between pain (AUC) and autonomic variables (A, heart rate [HR]; B, mean arterial pressure [MAP]; C, muscle sympathetic nerve activity burst frequency [MSNA BF]; and D, muscle sympathetic nerve activity burst amplitude [MSNA BA]). Lines represent linear regressions. *$p$<0.05, **$p$<0.01 within-sex.

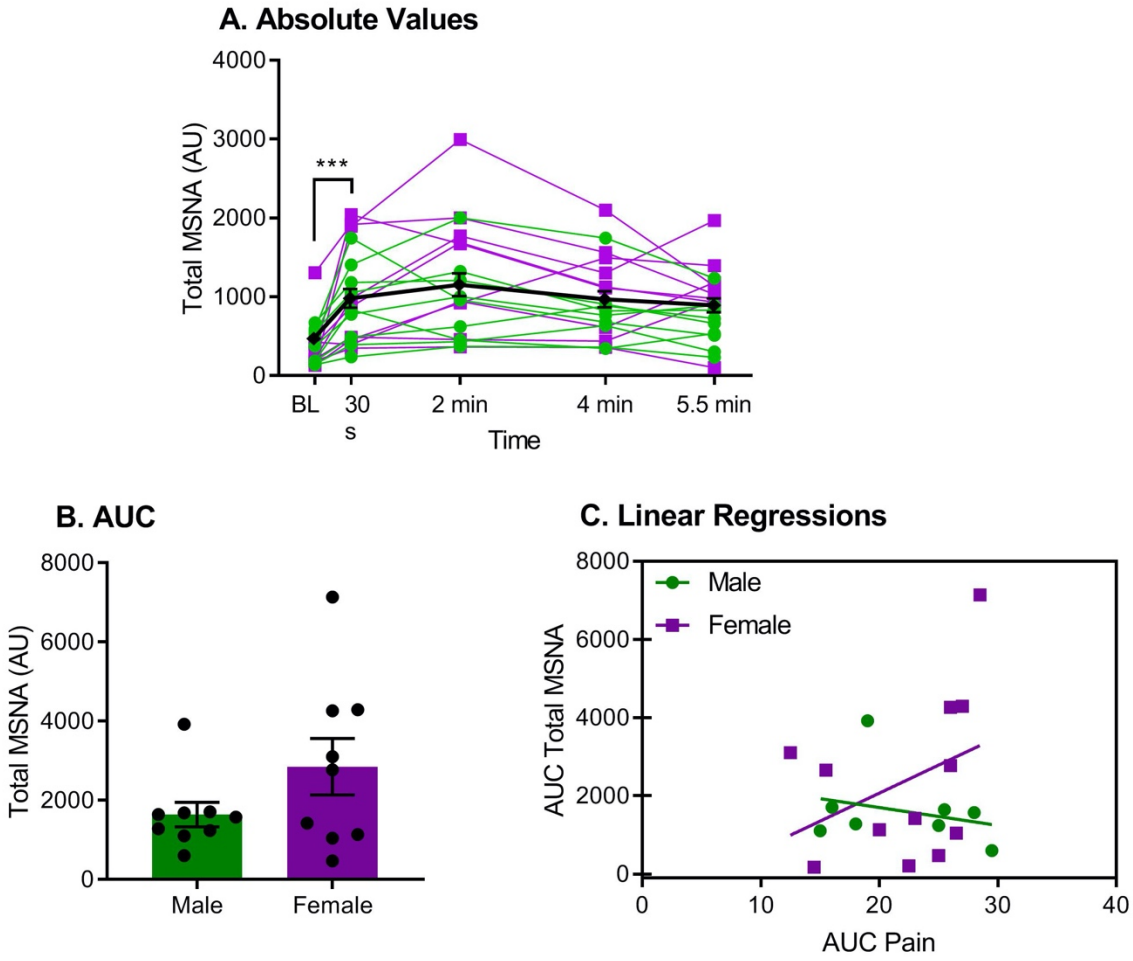


**Fig. 2.4.** Total MSNA values for previous time-course (A), area-under-the-curve (B), and regression analyses (C). Lines represent linear regressions. ***$p$<0.001

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## 2.7. Bridging text

As autonomic function has some relationship to the initiation and propagation of pain, it also has a relationship to the inhibition of pain [11]. To better understand how chronic pain can be inhibited via autonomic mechanisms, we must better understand pain inhibition mechanisms. One of these mechanisms, CPM, is still incompletely understood. The methods by which CPM is elicited still feature considerable variability across the field, which contributes to large variability in CPM outcomes, and presents a significant barrier to pooled analyses. Standardized protocols have been recommended, but these guidelines do not provide recommendations with respect to intensity of either the conditioning stimulus or the test stimulus during the CPM protocol. Much work has been done examining the effect of conditioning stimulus intensity on CPM efficacy (e.g., analgesic or hyperalgesic outcomes) [7; 69; 80; 183; 200; 204], but there are currently no studies specifically examining and comparing the effect of test stimulus intensities on CPM outcomes in humans.

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# Chapter 3

## The direction and magnitude of conditioned pain modulation is dependent on test- stimulus intensity in healthy participants but not in those with fibromyalgia

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**Abstract**

Conditioned pain modulation (CPM) is a psychophysical phenomenon considered to be a biomarker of endogenous descending pain modulatory mechanisms. Previous rodent data from our lab demonstrated that test stimulus intensity affects CPM's direction, with higher-intensity stimuli leading to hypoalgesia (i.e., CPM) and lower-intensity stimuli leading to hyperalgesia (i.e., "anti-CPM"). The primary aim of this study was to see if we could replicate these findings in humans. Because deficits in CPM suggest low capacity to inhibit pain—a risk factor for chronic pain—the secondary aim of this study was to see how this "anti-CPM" phenomenon presented itself in chronic pain patients with fibromyalgia, with a hypothesis that CPM and anti-CPM effects would either be attenuated or replaced entirely with hyperalgesia. Healthy (n=60) and fibromyalgic (n=39) participants underwent an individual heat pain threshold assessment, followed by a single CPM trial, at –1, +1, or +3 °C below/above their threshold. The CPM trial consisted of two baseline sub/suprathreshold heat pain stimulations (the test stimulus), a 30-s cold pressor test (4 °C) as a conditioning stimulus, and a final heat pain stimulation at the same temperature, with pain ratings provided throughout. Healthy controls displayed statistically significant analgesia +3 °C, no change at +1 °C, and hyperalgesia at -1 °C. Further analyses revealed that subjective intensity of the test stimulus determined the direction and magnitude of CPM. We observed no significant evidence for either analgesic CPM or anti-CPM in fibromyalgia patients, suggesting that the mechanism(s) subserving both phenomena are dysfunctional in them.

### 3.1. Introduction

Chronic pain presents with extremely high prevalence, societal economic burden, and unparalleled disability and morbidity [26]. In addition to experiencing amplification of pain signals via peripheral and/or central sensitization, evidence suggests that chronic pain patients also have limited endogenous pain inhibition. The status of these mechanisms can be measured using paradigms designed to elicit conditioned pain modulation (CPM) [34].

CPM is a psychophysical phenomenon first demonstrated in rats in 1979 by Le Bars and colleagues [41] and labelled diffuse noxious inhibitory controls (DNIC). CPM is considered to be a biomarker of human endogenous pain inhibitory mechanisms [18], and employs a "pain inhibits pain" (counter-irritation) paradigm whereby two painful stimuli (i.e., the test stimulus and the conditioning stimulus) are applied to different regions of the body [34]. CPM efficacy or capacity—the amount of test stimulus pain reduction caused by conditioned stimulus exposure—has been shown to be a predictive factor for pain treatment outcomes [82] and acute and postoperative pain levels [80], with low CPM capacity predicting the development of chronic pain [17; 43; 45]. However, although CPM is usually considered to be an analgesic phenomenon, both the magnitude and direction of the CPM phenomenon in healthy volunteers are, in fact, highly variable and related to various participant demographics [10; 17; 21; 22; 27; 63; 65; 66; 72; 76] as well as various parameters of the conditioned stimulus, such as modality (e.g., mechanical pressure, cold pressor test [CPT], heat, cuff ischemia), intensity [4; 20; 27; 56; 67; 70], method of stimulation (sequential or simultaneous) [81], and body area [27; 52; 66].

Despite the large literature on how variability in features of the conditioned stimulus can affect CPM, very little, if any, information exists on the effects of the test stimulus [69]. Based on our studies in rodents, we hypothesize that variations in the intensity of the test stimulus can help to

explain the robust interindividual variability across CPM studies. Previously, we observed that after an intraperitoneal injection of acetic acid, mice displayed increased sensitivity to noxious thermal heat on the plantar hind paw [38]. This apparent thermal hyperalgesia was contrary to the hypoalgesia that is expected of a counterirritation phenomenon. Given the surprising direction of this effect, we performed a series of parametric studies using multiple conditioning stimuli (acetic acid and orofacial formalin), test stimuli (hind paw and forepaw withdrawal, tail-withdrawal, hot plate, and von Frey tests) and species/genotypes (CD-1, DBA/2, and C57BL/6 mice, and Sprague-Dawley rats) [74]. We discovered a test stimulus intensity-dependent effect in which higher-intensity stimuli reliably led to hypoalgesia (i.e., CPM), and lower-intensity stimuli reliably led to hyperalgesia (i.e., "anti CPM").

Given the importance of CPM as an assessment tool for chronic pain risk, the aims of this study were to: 1) determine if, as in rodents, test stimulus intensity modulates the size and direction of responses to CPM paradigms in humans, and 2) examine how this "anti CPM" phenomenon presents itself in chronic pain patients with fibromyalgia.

## 3.2. Methods

Healthy participants (HCs) were recruited from the McGill undergraduate population (n=60). Individuals with fibromyalgia (FMs) were recruited from the Alan Edwards Pain Management unit as well as the general population (n=39). FM participants were asked for a rating of their clinical pain on a 0–100 scale. Participant demographics are shown in Table 1. All experiments were approved by a local Research Ethics Board at McGill University.

### *3.2.1. Heat Pain Threshold Assessments*

Heat pain was used as the test stimulus, chosen because of its reliability and ubiquity in

CPM studies, and to better equate baseline sensitivity between HC and FM groups, who would be expected to differ greatly if mechanical pain was used instead. To determine each participant's heat pain threshold, heat stimuli were delivered to the volar forearm using a 3 cm x 3 cm contact thermode (Medoc Advanced Medical Systems TSA II, Israel). Participants received four successive temperature ramps, separated by 30 s, and were asked to indicate at what temperature the stimulus first became painful. The average was then taken of these four temperatures, to establish each participant's individual heat pain threshold.

### *3.2.2. CPM Protocol*

Temperatures used in the CPM trial were calculated based on each participant's individual heat pain threshold and occurred shorly after the pain thresholds were established. Participants were assigned (by quasi-random assignment) to groups in which they received heat stimuli -1 °C, +1 °C, or +3 °C below or above their individual threshold established as described above. Deviation from random assignment occurred for 4 participants (n=2 HC; n=2 FM) who were assigned to the -1 °C group, due to threshold temperatures that were so high that increased test stimulus temperatures would cross the 50 °C safety cut-off. Inclusion and exclusion of these data yielded similar results.

Participants were asked to place their arm on the thermode, and two baseline 7-s heat stimulations (2-s ramp and 5-s plateau, 30-s interval) were administered. Participants were asked to rate their level of pain on an electronic (Microsoft Surface tablet) 0–100 visual analogue scale (VAS) after each of these stimulations. Within 30 s of the second VAS pain rating, the participant began a 30-s CPT, immersing the right hand in a circulating cold-water bath maintained at 4 °C (the conditioned stimulus). Participants were asked to verbally rate the intensity of the CPT pain on a 0–10 numeric rating scale (NRS) after 20 s of immersion. Immediately following the CPT, a

single 7-s heat pain stimulation (at the same temperature) was administered to the volar forearm, and participants were asked to rate their level of pain a final time on a 0–100 VAS. CPM was defined as the difference between the rating immediately preceding (pre-CPT) and the rating immediately following the CPT (post-CPT).

#### *3.2.3. Questionnaires*

Participants were administered a battery of questionnaires to assess demographics, health history (including chronic pain and medications), mental state (PHQ-4) [37], pain catastrophizing (Pain Catastrophizing Scale) [73], and (for those with fibromyalgia), gender (GENESIS-PRAXY) [59; 60; 62].

#### *3.2.4. Data Analysis*

Statistical analyses were performed in SPSS (v. 29), and figures created using GraphPad Prism (v. 10). Groups were compared using ANOVA, Student's t test, or in one case, Welch's t test followed by post hoc analyses, as appropriate. Correlations were assessed using Pearson's r statistics. Normality of groups was assessed using Shapiro-Wilk tests. In all cases an α criterion of 0.05 was adopted.

### 3.3. Results

Due to the very small number of male participants (n=6), data could not be meaningfully disaggregated by sex. In all cases, conclusions presented below are equally valid in female-only cohorts.

#### *3.3.1. Heat Pain Thresholds*

Heat pain thresholds of all participants are shown in Figure 1. Heat pain thresholds ranged from 37.6–48.7 °C; there was no significant difference ($t98 = 0.2$, $p=0.42$) between mean

thresholds of HCs (mean: 43.7 °C; SD: 2.9 °C) and FMs (mean: 43.6 °C; SD: 2.7 °C). In the HC participants ($F_{2,58} = 6.6$, $p=0.003$), those assigned to the -1 °C group had significantly higher thresholds than the +1 °C group (Tukey $p=0.002$) but not the +3 °C group (Tukey $p=0.11$). This difference was due entirely to the fact that HC participants with particularly high thresholds were transferred to the -1 °C group as a strategy to avoid ceiling effects (see Methods). There were no threshold differences between groups in the FM participants ($F_{2,36} = 0.6$, $p=0.53$). Note that the actual heat pain stimuli provided to all participants in the CPM trial were calibrated based on their individual thresholds determined here.

### *3.3.2. CPT and Pre- vs. Post-CPT Heat Pain Ratings*

Ratings of CPT pain 20 s into the 30-s cold water immersion ranged from 3–10 on an 11-point NRS, with FMs giving almost significantly higher ratings than HCs ($t_{97} = 2.0$, $p=0.052$) (see Supplementary Fig. 1A). CPT pain ratings correlated significantly with heat pain thresholds in HC participants ($r = 0.37$, $p=0.004$; Supplementary Fig. 1B) but not FM participants ($r = 0.11$, $p=0.52$; Supplementary Fig. 1C). However, CPT pain ratings correlated significantly with ratings of clinical pain in participants with FM ($r = 0.44$, $p=0.009$; Supplementary Fig. 1D).

Heat pain ratings before and after the application of the cold pressor conditioning stimulus are shown in Figure 2. One-way ANOVAs on baseline (pre-CPT) data revealed the expected relationship between stimulus temperature and pain ratings in both HC ($F_{2,58} = 31.8$, $p<0.001$) and FM ($F_{2,36} = 4.0$, $p=0.03$) participants. HC and FM baseline pain ratings differed significantly in the -1 °C groups ($t_{31} = 6.7$, $p<0.001$), +1 °C groups ($t_{30} = 7.4$, $p<0.001$), and +3 °C groups ($t_{33} = 3.0$, $p<0.002$), with FM participants giving higher pain ratings in each case. For each participant type/stimulus temperature group comparison a repeated measures ANOVA was performed. Significant CPM (i.e., analgesia) was observed in the HC/+3 °C group ($F_{1,21} = 4.6$, $p=0.04$),

whereas significant anti-CPM (i.e., hyperalgesia) was observed in the HC/-1 °C group ($F_{1,19} = 4.7$, $p=0.04$). All other repeated measures ANOVAs yielded non-significant values ($0.14<p<0.88$).

### *3.3.3. Difference Scores*

Pre- and post-CPT data were transformed into difference scores for each participant (Fig. 3). A two-way ANOVA (HC vs. FM, stimulus temperature group) on all data revealed a significant interaction ($F_{2,94}=3.7$, $p=0.03$). Subsequent one-way ANOVAs performed on HC and FM data separately revealed a statistically significant effect of stimulus temperature in HCs ($F_{2,58}=4.4$, $p=0.02$) but not FMs ($F_{2,36}=3.1$, $p=0.06$). As a final way of considering statistical significance, we compared each difference score to 0 using one sample t-tests. Difference scores in the HC/+3 °C group were found to be significantly below zero ($t_{21}=2.0$, $p=0.03$), indicative of analgesia. Difference scores in the HC/-1 °C group were found to be significantly above zero ($t_{19}=1.9$, $p=0.04$), indicative of hyperalgesia. All other comparisons to zero were non significant ($0.12<p<0.88$).

### *3.3.4. CPM and Pain Threshold*

CPM was predicted by trial (test stimulus) temperature, and trial temperatures in each participant were determined by individual heat pain thresholds, and thus we sought to assess the relationship between pain thresholds and CPM responses. Figure 4 illustrates the correlation between heat pain thresholds of each participant and their CPM difference scores. As can be seen, a significant positive relationship was obtained in HC participants, such that those with lower pain thresholds (i.e., higher pain sensitivity) were more likely to display CPM analgesia (i.e., negative difference scores) and those with higher pain thresholds were more likely to display CPM hyperalgesia, regardless of their test stimulus intensity group allocation (Fig. 4A). There was no evidence for such a relationship in FM participants (Fig. 4B).

A statistically significant relationship between CPT ratings and subsequent CPM was also observed in HC (r=0.32, p=0.03) but not FM (r=0.02, p=0.89) participants (see Supplementary Fig. 1E–F). Unlike with the test stimulus, HC participants' rating of the conditioning stimulus predicted the magnitude of CPM, but not its direction. Clinical pain ratings in FMs did not predict CPM outcomes (r=0.08, p=0.69).

### *3.3.5. Median Split Analyses*

To further investigate the impact of test stimulus pain sensitivity on CPM direction and magnitude, data were divided by group and trial temperature, then further subdivided into "low" and "high" subgroups based on a median split of baseline pain ratings. Results of this analysis are shown in Figure 5. Overall, analgesic CPM was higher in high subgroups than low subgroups ($t_{97}=3.6$, $p<0.001$). Furthermore, three pairs of subgroups demonstrated significant differences. In the FM/ 1 °C group the high subgroup displayed significant analgesia compared to the low subgroup ($t_{11}=2.2$, $p=0.02$); this difference, however, was largely driven by two extreme data points. In the HC/+3 °C group, the subgroups diverged ($t_{20}=2.7$, $p=0.007$) such that only the high subgroup showed statistically significant analgesia ($t_{10}=3.5$, $p=0.003$). Furthermore, in the HC/-1 °C group, the subgroups diverged ($t_{17}=2.5$, $p=0.01$) such that only the low subgroup showed statistically significant hyperalgesia ($t_{9}=3.6$, $p=0.003$).

### *3.3.6. Demographic Covariates and Correlations*

Age did not significantly affect either pain thresholds or CPM difference scores in either HC or FM participants. Because there were large differences in the ethnic makeup of the FM and HC participants (see Table 1), we examined whether ethnicity affected pain thresholds or CPM but found no significant effects. Smoking also was found to have no significant effects. A trend

(p=0.14) towards increased analgesic CPM in HC (but not FM) participants who reported exercising regularly was observed.

A limited analysis by self-reported gender identity was attempted, with n=5 FM participants identifying as non-binary, and no FM participants identifying as men. Non-binary individuals displayed hyperalgesic CPM compared to women (Welch's t9=2.8, p=0.02; see Supplementary Fig. 2A). Furthermore, we investigated the influence of gender, as quantified by GENESIS-PRAXY scores ("masculine" = 0, "feminine" =100; mean: 47.5, range: 29.9–66.2), of FM participants on self-reported chronic pain intensity, heat pain thresholds, and CPM (see Supplementary Fig. 2B–D). More feminine gender strongly trended towards predicting higher clinical pain intensity (r=0.32, p=0.06). No relationships were observed between gender and heat pain threshold (r=0.20, p=0.22) or CPM difference scores (0.16<p<0.90).

#### *3.3.7. Psychometric Covariates and Correlations*

Correlations between pain thresholds, CPM difference scores, and psychological variables such as those in the PHQ4 (nervousness, worry, anhedonia, depression) or the Pain Catastrophizing Scale (PCS) revealed no significant findings with the following exception. Despite the fact that FM participants had higher total PCS scores than HCs (23.4/39 vs. 18.1/39, respectively; t98 = 2.4, p=0.009), a significant positive correlation was observed between total PCS score and CPM hyperalgesia in HCs (r=0.30, p=0.02) but not FMs (r=0.02, p=0.90) participants (see Supplementary Fig. 3).

### 3.4. Discussion

Through application of a CPM protocol using three different heat pain threshold-calibrated test stimulus temperatures, we confirmed in healthy humans what we had previously observed in

rodents: test stimulus intensity can determine the direction of CPM, with higher-intensity stimuli producing analgesia and lower-intensity stimuli producing hyperalgesia. Furthermore, we observed no significant evidence for either analgesic CPM or hyperalgesic CPM (anti-CPM) in FM patients, suggesting that the mechanism(s) subserving both phenomena are dysfunctional.

### *3.4.1. Animal model data*

Our hypothesis was borne of an incidental observation in a study on emotional contagion in mice [38], in which mice experiencing abdominal pain and tested for heat pain withdrawal thresholds unexpectedly displayed hypersensitivity to the thermal noxious stimulus, in opposition to what DNIC (now proposed to be called descending control of nociception, or DCN [6]) would predict. To follow up, we performed a series of parametric studies using multiple conditioning stimuli, test stimuli, and rodent genotypes [74]. The intensity of the conditioning stimulus determined the magnitude of DCN, as has been known since the phenomenon was discovered [42], but the intensity of the test stimulus determined both its magnitude and its direction. For example, using intra-abdominal acetic acid as the conditioning stimulus, Hargreaves' (hind paw-directed radiant heat) test intensities >95 mW/mm2 produced analgesia, those <60 mW/mm2 produced hyperalgesia, and intermediate intensities produced no change.

In an attempt to determine the neurochemical mediation of anti DCN, we administered agonists and antagonists of norepinephrine and serotonin receptors to mice using both DCN (analgesic) and anti-DCN (hyperalgesic) parameters [51]. We observed that both phenomena were mediated by α2-adrenergic and 5-HT7 receptors, but in opposite directions, such that DCN was blocked by antagonists of these receptors, as has been reported [5; 7; 8] but anti-DCN was blocked by agonists instead. The conversion of analgesic DCN to hyperalgesic anti DCN—dubbed "descending facilitation"—is well known after injury [57; 78]. For example, in a recent finding

directly relevant to anti-DCN neurochemistry, activation of μ-opioid receptors by the selective agonist, [D-Ala2,N-Me-Phe4,Gly5-ol]-enkephalin (DAMGO) enhanced DCN analgesia in normal rats, but produced frank anti-DCN in rats with monoarthritis [61]. These rodent models serve as the foundation for confirmational human studies, demonstrating the continued value of pre-clinical models of human pain phenomena.

### *3.4.2 Stimulus intensity effects on CPM*

A large literature has been amassed on the effects of conditioned stimulus intensity on CPM magnitude. Conditioning stimulus intensity produces mixed effects on the degree of CPM response, with some studies showing a positive correlation and some studies showing no correlation between the intensity of the conditioning stimulus and the magnitude of CPM effect [4; 20; 27; 56; 67; 70]. Mild or non-painful conditioning stimuli have been shown to produce some CPM [9; 39; 40], although this is not always the case [79].

We were surprised to discover that there is almost no existing evidence on the effects of test stimulus intensity in human CPM protocols. A recent study by Lie and colleagues [44] demonstrated that the use of a tonic heat test stimulus (120 seconds) produced larger CPM than a phasic heat test stimulus (three plateaus of 5 seconds separated by 10 seconds). Although not remarked upon by the authors or analyzed statistically, the tonic stimulus was rated more painful than the phasic one. To our knowledge, ours is the first study to have directly manipulated the effect of test stimulus intensity on CPM in humans.

The current observations suggest that test stimuli of low intensity predispose participants to hyperalgesia, at least in HCs, both as a function of the objective threshold-derived stimulus intensity, but also as a function of the participant's individual, subjective pain intensity. Within and across test stimulus groups, those with less sensitivity in the form of higher pain thresholds

(Fig. 4A) and/or lower pain ratings (Fig. 5A) were less likely to display CPM and more likely to display anti-CPM. This positive correlation between perceived noxious intensity and (analgesic) CPM magnitude has been demonstrated previously [28; 71].

The use of a subthreshold stimulus in this study adds to its novelty. Stimulations at -1 °C, below participants' previously determined pain thresholds, would be expected to elicit pain ratings of zero. However, this did not occur in any participant, with HCs supplying an average rating of 15/100 and FMs supplying an average rating of 58/100. This increase in pre-CPT ratings is likely a form of nocebo, given that participants following instructions believed that this was the point in the CPM protocol at which a frankly "noxious" stimulus would be applied. Despite these initial increases, participant pain ratings of this sub-threshold stimulus still primarily increased after the CPT. Despite arguments for both calibration and non-calibration of stimulus intensities in a CPM protocol [1], we believe that variability in the painfulness of the test stimulus can explain CPM response variability and anti-CPM responses seen in the literature.

#### *3.4.3. 'Anti-CPM' in humans*

Many CPM studies only report the average CPM effects without disclosing the proportion of non responders. This may be because no consensus exists on how best to determine a meaningful CPM effect: either any decrease compared to baseline is considered CPM, or CPM is defined with respect to a decrease over and above some threshold defined by observed variability around the baseline [2; 81]. In the past decade, however, reporting on percentage of CPM non-responders has become more common, and numerous studies have reported hyperalgesic CPM outcomes in many healthy participants [e.g., 2; 3; 15; 19; 23-25; 30; 35; 46; 47; 49; 50; 55; 65; 68; 75; 77].

For example, Potvin and colleagues [65] showed "pain facilitation during the CPM procedure" in 21% of healthy controls. Kennedy et al. [35] reported that ~20% of participants have facilitated

or unchanged pain when using a heat conditioning stimulus. Mertens et al. [49] and Oono et al. [55] observed anywhere from 9–36% and 0–33% of CPM non-responders, respectively, depending on the type and location of the conditioning stimulus. Locke et al. [46] reported that the percentage of those experiencing "no CPM" increased over time from 7.2% during CPT, to 34.4% two minutes post-CPT, and finally 40.2% five minutes post-CPT. Vaegter et al. [75] stated that across their five different test stimuli, 11.5–46.2% of participants were classified as CPM non-responders. Firouzian et al. [19] observed this phenomenon in 50% of healthy participants, describing it as "no-CPM". Finally, Rabey et al. [68] reported that 32.8% of healthy controls experienced no change in pain and 31.2% experienced pain facilitation during a CPM protocol.

#### *3.4.4. CPM in FMs*

Several researchers have observed that the analgesic efficacy of CPM is reduced in individuals with fibromyalgia compared to healthy individuals [16; 32]. An excellent review from O'Brien and colleagues [54] considered 13 studies examining CPM outcomes in FMs. Three of them used mechanical pressure pain and display mixed results [14; 29; 48]; the rest used thermal stimuli. Kosek and Hansson [36] found that FM patients had less CPM responses to an ischemic conditioning stimulus, and Lautenbacher and Rollman [39] observed that FM patients did not increase their electrical pain thresholds after a thermal test stimulus. Many studies used a thermal test stimuli and CPT conditioning stimuli and reported less effective CPM in FM patients [11; 12; 53; 58; 63; 64]. Potvin and Marchand [65] found that a full 47% of FM patients showed pain facilitation (i.e., hyperalgesia) after CPM. Finally, one study using a thermal test stimulus and hot water conditioning stimulus showed no differences in CPM between FMs and female HCs [72]. Thus, there is overwhelming evidence that CPM mechanisms are dysfunctional in FMs, and our study shows that their anti CPM is similarly dysfunctional. We note that our initial hypothesis was

that FMs would lack CPM but would display even more anti-CPM, and this might explain their pain hypersensitivity. Our results suggest that this is not the case. Interestingly, a recent study found that FMs are less responsive to nocebo hyperalgesia manipulations [33], which is perhaps in line with our observations here.

#### *3.4.5. Limitations and conclusions*

Despite a large literature suggesting HCs have higher heat pain thresholds than participants with FM [13; 31; 58; 63], we did not see significant differences between groups. This may have been due to concurrent medication use by FM participants. Conclusions regarding the gender related effects and trends observed were hampered by small sample size and deserve further attention.

It is widely recognized that variability in CPM research protocols is a barrier for meta-analyses, and standardized protocols have been recommended [81]. We believe that the present observations should help in this regard. Further research is clearly needed in this area, with the aim of methodological standardization across the field.

### 3.5. Acknowledgments

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***Table 1. Participant Characteristics***

| **Demographics** | **Healthy (HC) Participants (n=60)** | **Fibromyalgia (FM) Participants (n=39)** |
|---|---|---|
| **Age** (Range, Mean ± SD) | 18–35, 21 ± 3.1 | 19–69, 41 ± 13.4 |
| **Sex** | | |
| Male | 6 | 0 |
| Female | 54 | 39 |
| **Gender Identity** | | |
| Man | 6 | 0 |
| Woman | 54 | 34 |
| Non-Binary/None/Other | 0 | 5 |
| **Ethnic Background** | | |
| Arab | 1 | 0 |
| Black | 3 | 2 |
| East Asian | 24 | 0 |
| Indigenous | 0 | 1 |
| Latin American | 2 | 1 |
| Mixed Race | 2 | 2 |
| South Asian | 1 | 0 |
| Southeast Asian | 1 | 1 |
| White | 25 | 30 |
| Unspecified | 1 | 2 |
| **Psychometrics** | | |
| PHQ-4 | 0-11, 2.9 ± 2.5 | 0-12, 4.9 ± 3.7 |
| PCS | 0-46, 18.5 ± 10.3 | 5-48, 23.4 ± 10.9 |
| **Pain** | | |
| Threshold Temperature (Range, Mean ± SD) | 37.6 °C -48.7 °C, 43.6 °C ± 2.9 °C | 38.5 °C -48.1 °C, 43.5 °C ± 2.7 °C |
| Chronic Pain Rating: Now | - | 5-85, 41.5 ± 21.5 |
| Chronic Pain Rating: Average | - | 5-95, 55 ± 22.1 |
| **Regular Exercise** (Range, Mean ± SD) | None: 20; Yes: 40 0.08-16 hrs/week, 3.6 ± 2.7 | None: 16, Yes: 23 0.5-7.5 hrs/week, 3.4 ± 2.4 |
| **Smoking Behaviour** | None: 57, Yes: 3 | None: 31, Yes: 8 |
| **Prescription Medications Taken** | None: 50, Yes: 10 (Oral Contraceptives: 5, SSRI/SNRI: 5) | None: 6, Yes: 33 (Various) |

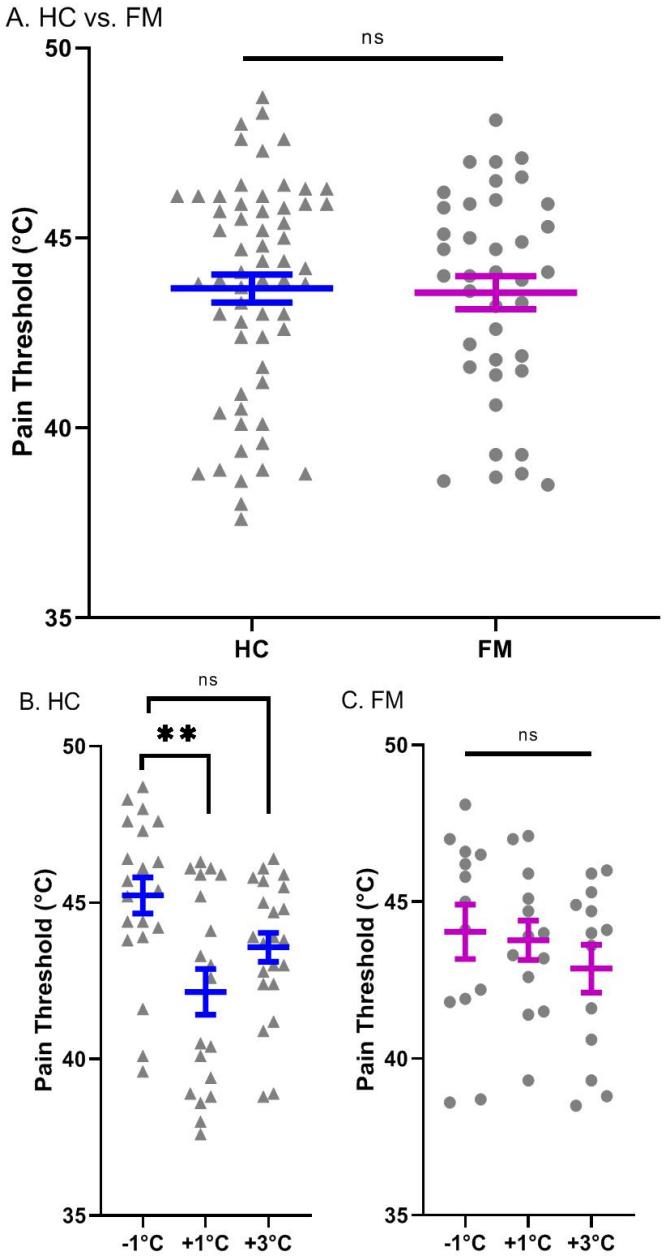



**Fig. 1.** Heat pain thresholds in healthy control (HC) and fibromyalgia patients (FM). A) Pain thresholds in all participants. B, C) Thresholds in HC (B) and FM (C) participants assigned to the -1 °C, +1 °C, and +3°C groups. Lines represent mean ± SEM. **p<0.01; n.s., not significant.

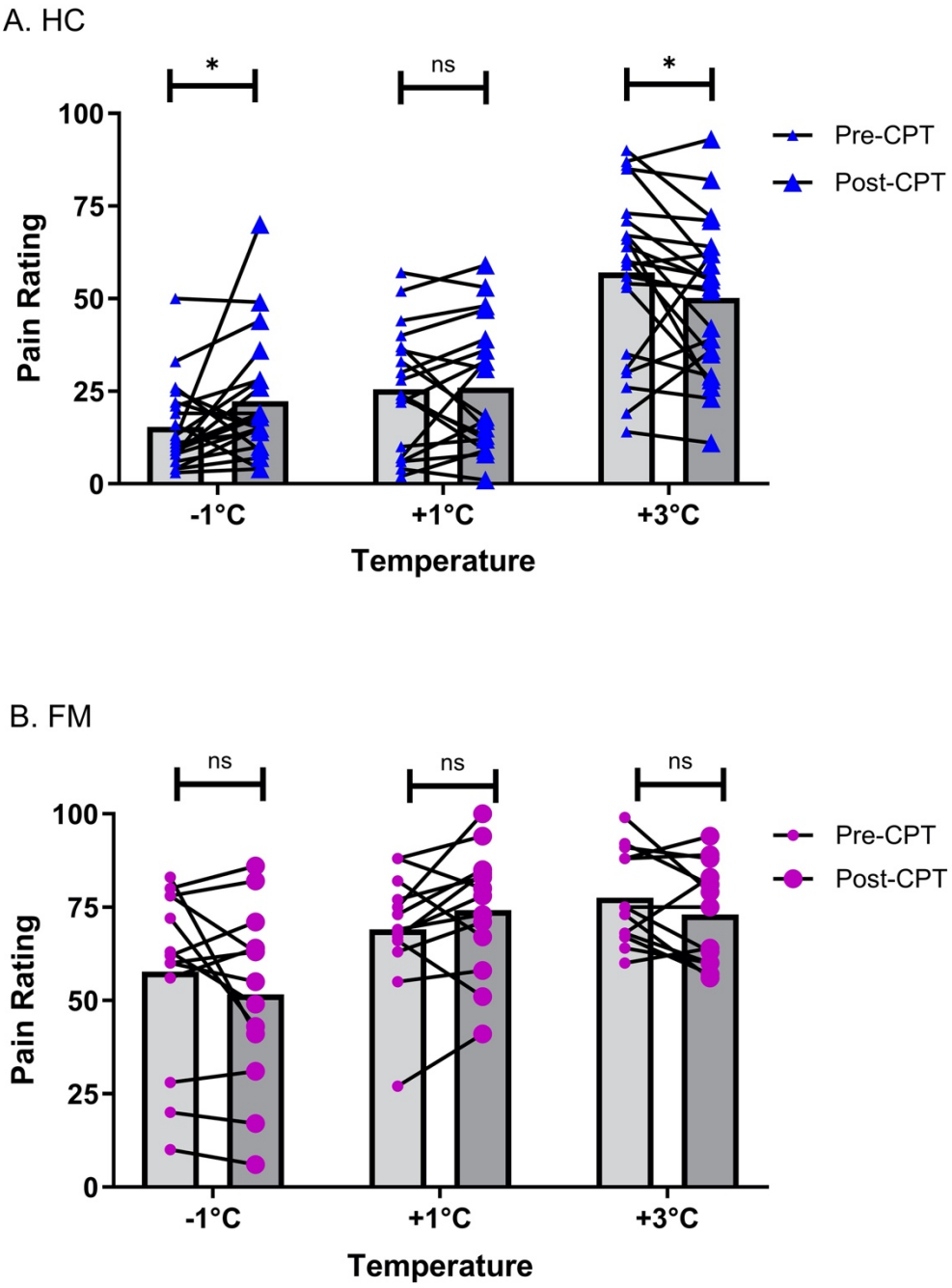



**Fig. 2.** Conditioned pain modulation in healthy control (HC; A) and fibromyalgia patients (FM; B). Bars represent mean pain ratings pre- (light bars) and post- (dark bars) experiencing a cold pressor test (CPT). *$p<0.05$; n.s., not significant.

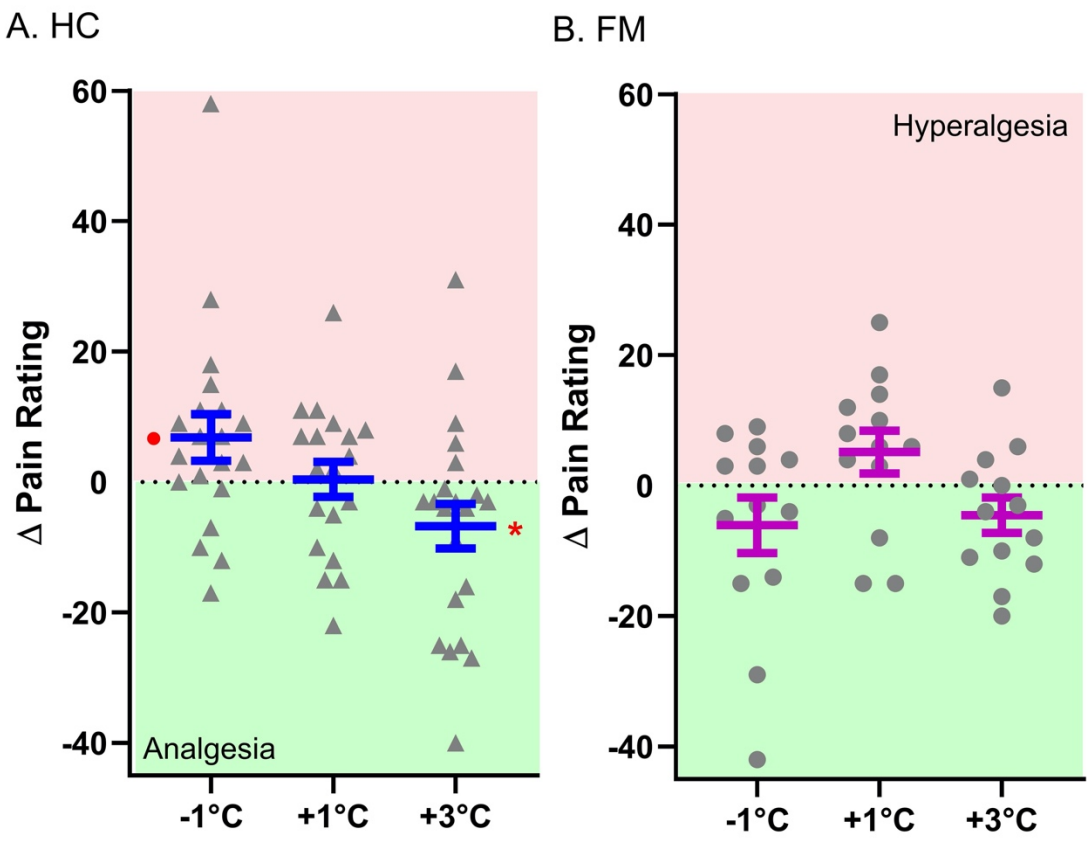



**Fig. 3.** Conditioned pain modulation data expressed as pain rating difference scores in healthy control (HC; A) and fibromyalgia patients (FM; B). Lines represent mean ± SEM difference in pain ratings (pre-CPT – post-CPT in Fig. 2). •$p<0.05$ above zero (one-sample t-test); *$p<0.05$ below zero (one-sample t-test).

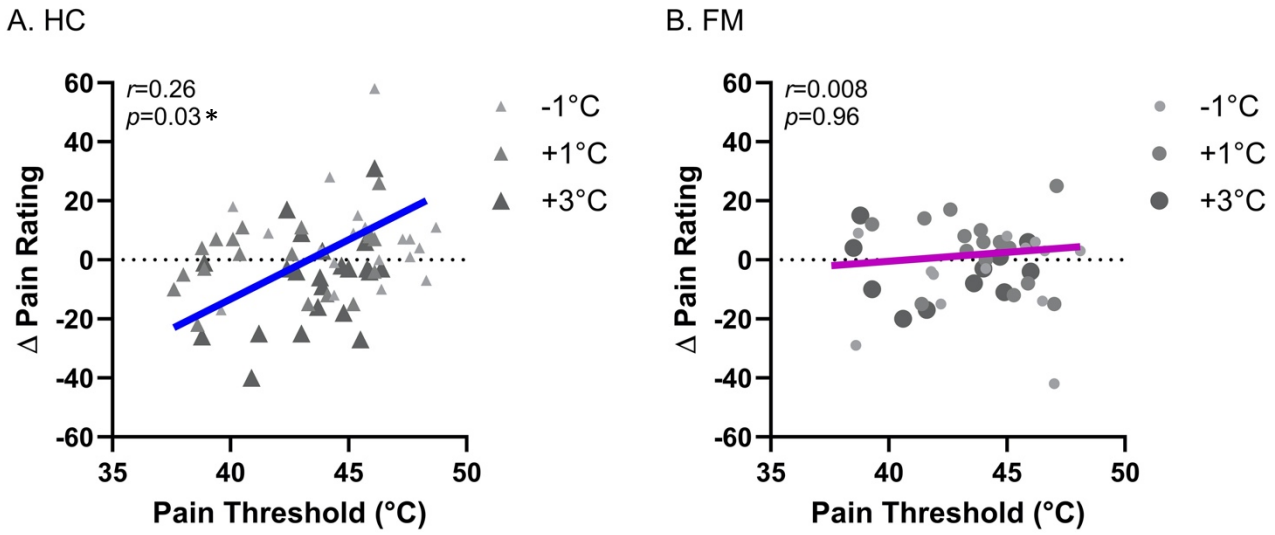



**Fig. 4.** Correlations between heat pain thresholds and conditioned pain modulation (expressed as pain rating difference scores) in healthy control (HC; A) and fibromyalgia patients (FM; B). Group allocation is indicated by symbol size; linear regression lines are shown.

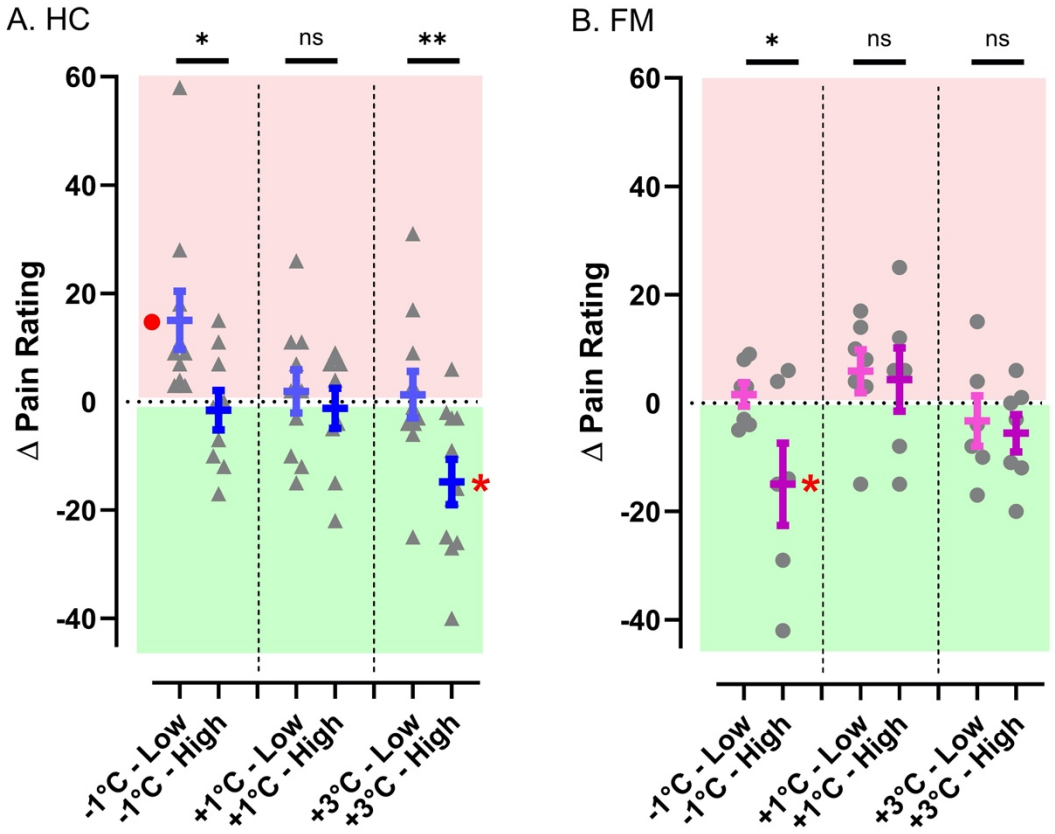
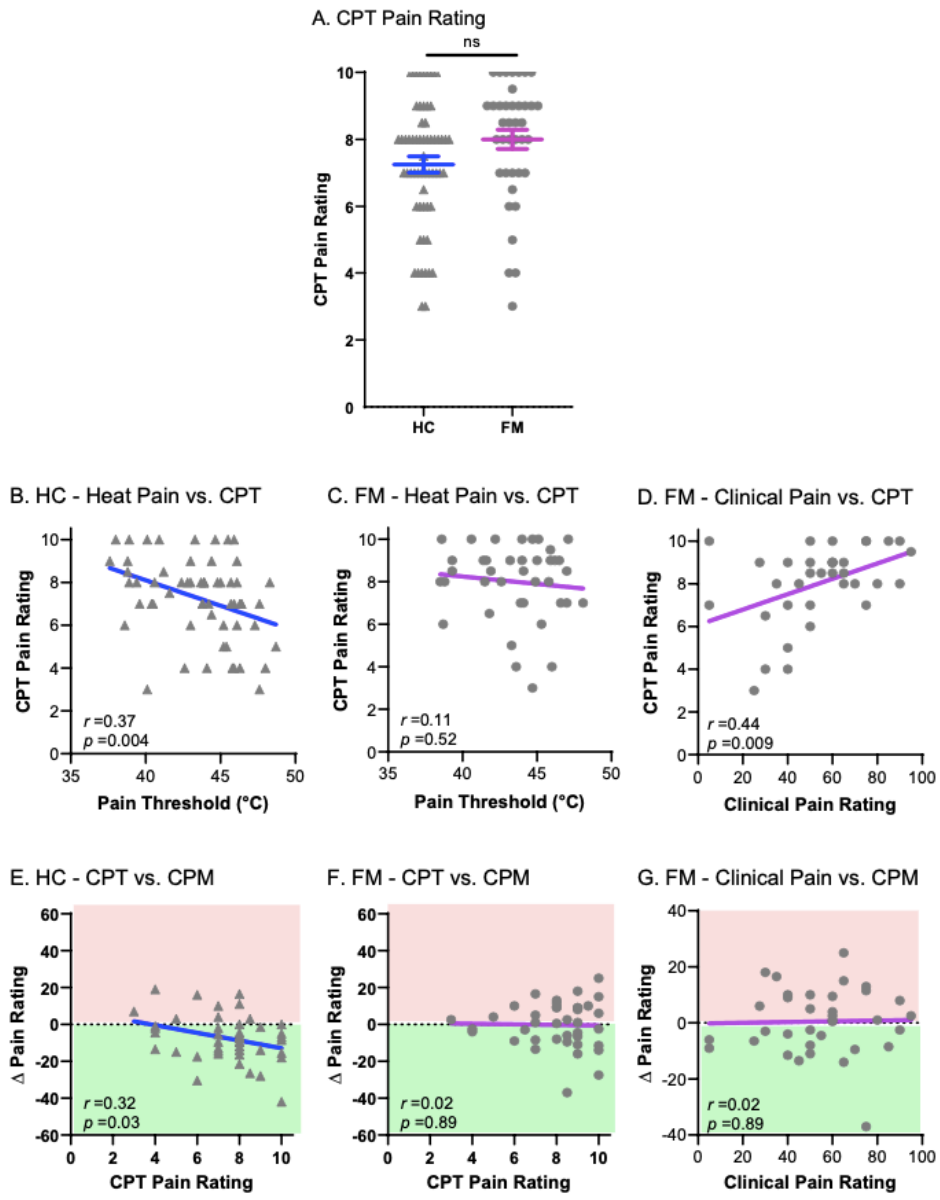
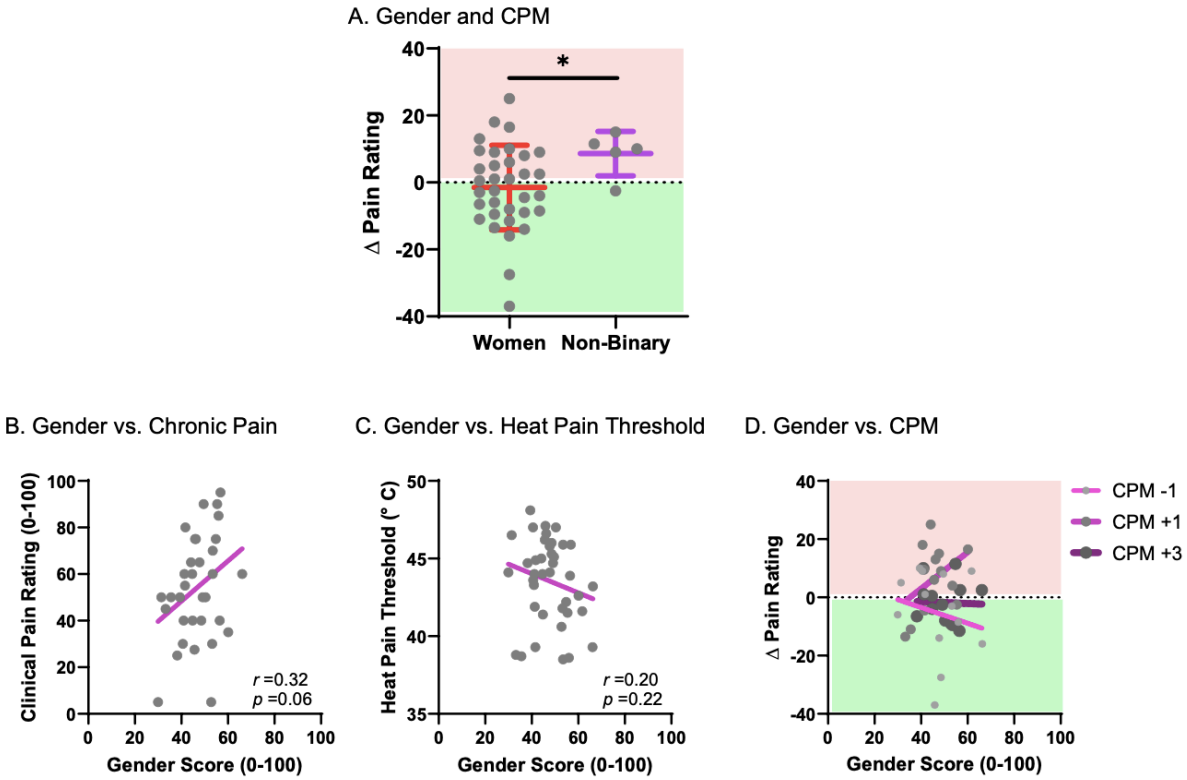


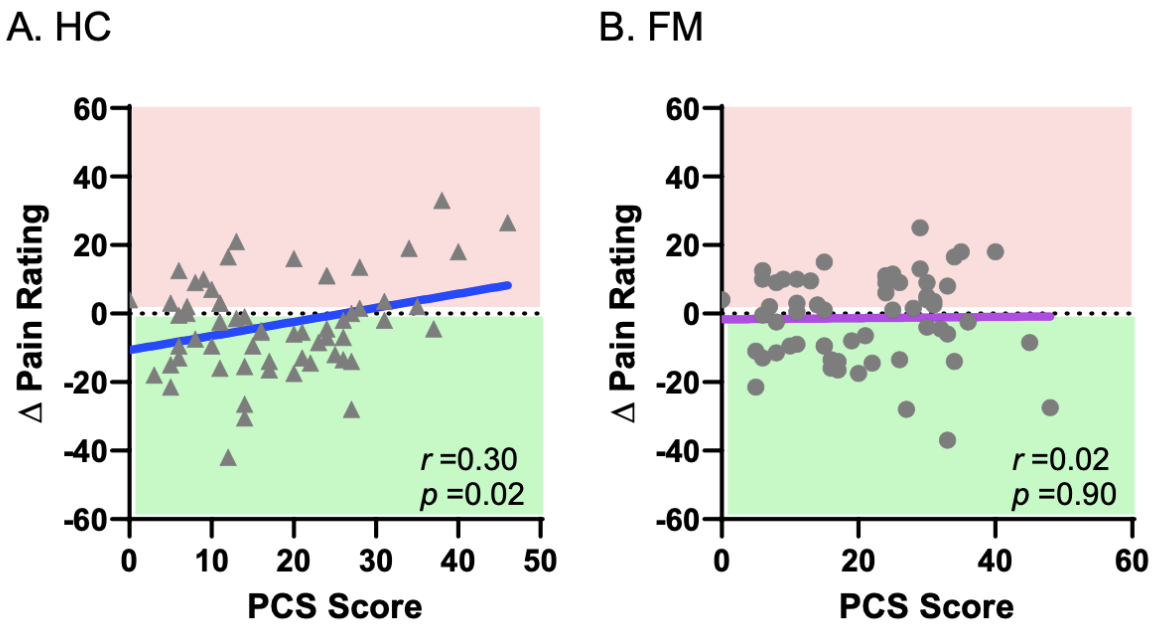

**Fig. 5.** Conditioned pain modulation data expressed as pain rating difference scores in healthy control (HC; A) and fibromyalgia patients (FM; B) separated by a median split of baseline pain ratings into low and high subgroups. Lines (lighter colors for low subgroups; darker colors for high subgroups) represent mean ± SEM. *p<0.05; **p<0.01; n.s., not significant.




**Supp. Fig. 1** Cold pressor test (CPT; the conditioning stimulus) pain ratings and CPT correlations. A) CPT ratings in healthy control (HC) and fibromyalgic (FM) participants. Lines represent mean ± SEM. B–C) Correlations between heat pain thresholds and CPT pain ratings (20 s after immersion) in HC (B) and FM (C) participants. D) Correlations between FM participants' rating of their clinical pain and CPT pain ratings. E–F) Correlations between CPT pain ratings and CPM in HCs (E) and FMs (F), expressed as difference scores. G) Correlation between clinical pain and CPM in FM participants.




**Supp. Fig. 2.** Effect of gender in FM participants. A) FM participants self-identifying as non-binary exhibited significantly more conditioned pain modulation (CPM), as expressed as pain rating difference scores. Lines represent mean ± SEM. B–D) Correlations between gender (GENESIS-PRAXY Gender Questionnaire score) and clinical pain (B), heat pain threshold (C), and CPM (C). Linear regression lines are shown; no relationships are statistically significant.




**Supp. Fig. 3.** Correlations between pain catastrophizing (using Pain Catastrophizing Scale [PCS] scores; [1]) and conditioned pain modulation data expressed as pain rating difference scores in healthy control (HC; A) and fibromyalgia patients (FM; B). Linear regression lines are shown.

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### 3.7. Bridging text

Despite its common use in the pain field, the mechanisms underlying CPM are still incompletely understood. Due to the fact that common pain modalities used to elicit CPM rely on blood pressure/flow (e.g., ischemic cuff, heat pain, CPT) it is possible that these mechanisms are autonomic in nature. Because CPM is impaired in patients with chronic pain, and autonomic dysfunction is also common in this group, it is possible, and even likely, that these two phenomena interact to propagate chronic pain [39]. Better understanding of the intersection of autonomic function and CPM will allow us to better treat certain chronic pain disorders, as well as their cardiovascular comorbidities.

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# Chapter 4

## Pain and autonomic function:

## the relationship between conditioned pain modulation and baroreflex sensitivity

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**Abstract**

Conditioned pain modulation (CPM) is considered to measure the "capacity" of human endogenous pain inhibitory mechanisms and is elicited using a 'pain inhibits pain' paradigm. Alternatively, stress can also inhibit pain. Evidence suggests that blood pressure (BP)-regulating baroreceptors mediate stress-induced analgesia: naturally or experimentally increased BP stimulates baroreceptors, producing descending pain inhibition. Given this evidence, we can assume that baroreceptor mechanisms (i.e., baroreflex) are involved in pain modulation, yet it is not known if the baroreflex is involved in CPM specifically. Therefore, the purpose of this study was to determine the relationship between the baroreflex and CPM. Participants (n=8) were instrumented to measure heart rate (HR), BP, and muscle sympathetic nerve activity (MSNA), underwent 10 min of baseline quiet rest, then lastly a CPM protocol consisting of two suprathreshold heat stimulations, followed by a 30-s cold pressor test, then a final heat stimulation. Baseline systolic BP (SBP) and HR values were continuously measured to calculate cardiovagal baroreflex sensitivity (BRS) via the sequence method. Up-sequences, a marker for parasympathetic activation, and down-sequences, a marker for parasympathetic withdrawal were quantified. BRS was then stratified by up- and down-sequences and regressed against values of CPM. In a subset of participants (n=5), baseline diastolic BP (DBP) and MSNA were also recorded to calculate sympathetic BRS. DBP values associated with MSNA bursts were calculated as a ratio of all DBP values in the sample, graphed, and these slope values were regressed against CPM values. Greater CPM was significantly related to less sensitive parasympathetic withdrawal ($r=0.66$, $p=0.01$). MSNA nor sensitivity of sympathetic mechanisms were significantly related to CPM. Taken together this implies that parasympathetic withdrawal sensitivity may be negatively related to CPM efficiency and may serve as a valuable autonomic target for chronic pain therapies.

## 4.1. Introduction

Interactions between pain and the autonomic nervous system (ANS) are complicated and vary depending on the ANS branch and the temporal nature of the pain experience. Acute pain can induce a stress response, and acute stress suppresses pain, a phenomenon called stress-induced analgesia [5; 20; 30; 47]. Contrarily, chronic stress and continuous activation of the ANS are considered to be linked to the development and maintenance of chronic pain [3; 27]. The connection between pain and the ANS is likely mediated through the baroreceptor reflex, which influences pain sensitivity by activating pain-inhibitory pathways [42]. Evidence from normotensive humans suggests that baroreceptors play a role in mediating the relationship between resting blood pressure (BP) and acute pain sensitivity: BP increases during stressful tasks resulted in naturally increasing baroreceptor stimulation, producing reduced pain sensitivity, or stress-induced analgesia [1; 22]. Moreover, direct experimental activation of baroreceptors using external suction of the carotid artery produced diminished acute pain sensitivity [2; 13; 15; 16; 32; 43; 44]. Overall, this evidence underlines the possible contribution of baroreflex in the modulation of pain [15; 17].

The baroreflex, which includes the cardiovagal/parasympathetic (cardiac control) and sympathetic (vasculature control) components, plays an important role in both short-term and long-term regulation of blood pressure in humans [24; 31]. Given the baroreflex is an essential function of the ANS, which is dysfunctional in some chronic pain conditions [33], it is possible, and even likely, that baroreflex plays a role in the mediation of pain. As the prevailing theory for pain chronification is that it arises from the failure of descending pain modulatory mechanisms, as opposed to increased pain transmission, it is also likely that the baroreflex dysfunction is associated with dysfunction of these descending pain mechanisms [6; 8].

Conditioned pain modulation is a psychophysical phenomenon considered to be a biomarker of the functional status of descending endogenous pain inhibitory mechanisms in humans [21]. Experimental demonstration of CPM uses a "pain inhibits pain" paradigm, whereby two painful stimuli (i.e., the test stimulus and the conditioning stimulus) are applied to different regions of the body, reducing an individual's pain perception of the initial test stimulus. The immersion of the arm in cold water (i.e., cold pressor test, CPT), is one of the most widely utilized and effective conditioning stimuli used to elicit CPM [41]. The CPT is also used to elicit sympathetic reactivity during cardiovascular testing [25; 50-52], therefore it is possible that sympathetic activation— which can be directly measured via muscle sympathetic nerve activity (MSNA)—is involved in CPM efficacy, much like in stress-induced analgesia.

Because autonomic dysfunction is a common comorbidity of various types of chronic pain [33], and there is some evidence that the altered relationship between baroreceptor dysfunction and chronic pain gradually progresses in tandem [4], better understanding the relationship between the baroreflex and pain inhibition/propagation mechanisms may help in the treatment and prevention of chronic pain. Therefore, the aim of this study is to investigate the relationship between cardiovagal BRS, sympathetic BRS, and conditioned pain modulation.

## 4.2. Methods

### *4.2.1 Participants*

We recruited 8 healthy participants, 4 men and 4 women, all cisgender, free from cognitive impairment as well as any cardiovascular, neurological, and chronic pain disorders. All participants provided written, informed consent prior to participation. All participants underwent the search process for MSNA and the CPM protocol, but we were only able to obtain MSNA data

in a subset of n=5 (2 men, 3 women). This study was conducted in accordance with the Declaration of Helsinki and was approved by the Faculty of Medicine Institutional Review Board at McGill University (24-01-026).

### *4.2.2. Experimental Design*

On a day previous to testing, participants were consented and familiarized with all non-invasive aspects of testing. On the test day, all participants arrived at the laboratory having fasted and abstained from caffeine, strenuous exercise, alcohol, and analgesics for a minimum of 3 hours. On arrival, participants were instructed to void their bladders. Participants were then positioned supine on a padded table for instrumentation. After ~15 min of stable supine rest, manual sphygmomanometry was used to obtain three manual BP values that were used to calibrate finger photoplethysmography values. Participants then underwent a heat pain threshold assessment. Microneurography was then conducted to obtain an MSNA signal. Following the attainment of an adequate MSNA site or the 60-min search time cutoff, 10 min of baseline quiet rest was recorded. A CPM protocol was then performed, consisting of two suprathreshold heat stimulations, placement of the participant's hand up to their wrist in ice water (i.e., CPT; ~4 °C), and a final heat stimulation following the CPT. Participants reported pain ratings verbally on a numerical rating scale (0–10) after each heat stimulation, and at the end of the CPT.

### *4.2.3. Instrumentation*

Heart rate (HR) was measured using a standard 3-lead electrocardiogram (ECG). BP was measured on a beat-by-beat basis using finger photoplethysmography (Finometer Pro, Finapres, Amsterdam, Netherlands), which involved the placement of a small cuff around the participant's third or fourth finger on the hand contralateral to the CPT. HR and continuous BP signals were sampled continuously at a frequency of 1.0 kHz and saved for offline analysis (PowerLab and

LabChart v8, ADInstruments). Microneurography was used to record multiunit postganglionic MSNA from the common peroneal nerve (NeuroAmp EX, ADInstruments) [24]. Briefly, an insulated tungsten recording electrode (35 mm in length, 200 μm in diameter, 2 ± 0.4 MΩ impedance) was inserted transcutaneously into the peroneal nerve, and a reference electrode was inserted subcutaneously 1–3 cm away from the recording site. An adequate MSNA signal consisted of pulse-synchronous bursts of activity that increased in firing frequency during voluntary apnea and remained unchanged during arousal to a loud noise [14]. The raw sympathetic signal was amplified 100× by a head-stage and the total amplification was 20,000×. The signal was then band-pass filtered (700–2000 Hz), full wave rectified, and integrated (time constant 0.1s). Sympathetic activity was recorded at 10.0 kHz.

#### *4.2.4 Heat Pain Threshold Assessments*

To determine each participant's heat pain threshold, heat stimuli were delivered to the volar forearm using a contact thermode and probe (QST Lab T14, Strasbourg, France). Participants received four successive temperature ramps, separated by 30 s, and were asked to indicate when the stimulus first became painful. The average was then taken of these four temperatures, to establish each participant's individual heat pain threshold [41].

#### *4.2.5. CPM Protocol*

Temperatures used in the CPM trial were calculated based on each participant's individual heat pain threshold. Participants received stimulations at +3 °C above their previously determined individual threshold. During the entire protocol, participants remained supine with their right volar forearm placed on the heat probe [10]. Two pre-CPT 10-s heat stimulations (1-s ramp and 9-s plateau, 10-s interval) were administered. Participants were asked to verbally rate their level of pain on a 0–10 numerical rating scale (NRS) after each of these stimulations. Within 30 s of the

second pain rating, the participant began a 30-s CPT, immersing their left hand in an ice-water bath (~4 °C). Participants were asked to verbally rate the intensity of the cold pain on a 0–10 NRS upon withdrawing their hand. Immediately following the CPT, a single 10-s heat pain stimulation (at the same previous temperature) was administered to the right volar forearm, and participants were asked to rate their level of pain a final time on a 0–10 NRS. CPM was defined as the difference between the post-CPT pain rating and the average of the pre-CPT pain ratings [10].

#### *4.2.6. Data Analyses*

ECG and calibrated BP waveforms were analyzed to determine HR, interval between R-waves, systolic BP (SBP), Diastolic BP (DBP). Bursts of MSNA were detected using a semi-automated peak detection algorithm (LabChart V8) based on a 3:1 signal-to-noise ratio and confirmed by a trained microneurographer following a shift of the MSNA signal to account for the neural conduction latency within each subject, aligning each sympathetic burst with the cardiac cycle that initiated it [24].

Cardiovagal BRS was determined through alignment of BP waves with ECG, and extraction of every SBP per RR interval. The sequence method was then applied [38]. The sequence method calls for identification of three or more consecutive heartbeats in which SBP is increasing and the RR interval is lengthening (i.e., HR is slowing), or in which SBP is decreasing and the RR interval is shortening (i.e., HR is increasing). The former sequences are referred to as up-sequences and represents parasympathetic nervous system (PNS) activation, and the latter are referred to as down-sequences and represent PNS withdrawal. The threshold values for including beat-to-beat SBP and RR interval changes in a sequence were set at 0.5 mmHg and 6 ms, respectively [31]. SBP values were then graphed against corresponding HR, and the slope of the line was then used to determine one cardiovagal BRS value. All cardiovagal BRS values taken

during the 10 min baseline period were then averaged to create one BRS slope for up-sequences and one BRS slope for down-sequences.

Bursts of MSNA were quantified for baseline and the CPM protocol and expressed as bursts per 30 s (e.g., Pre-CPT heat stimulations, CPT, Post-CPT). Sympathetic BRS was determined through alignment of BP waves with bursts of MSNA [25]. DBP values and burst times from baseline quiet rest were then extracted per RR interval. The number of DBP values corresponding with bursts were then divided by the number of overall DBP values and stratified by increments of 2 mmHg [12; 51]. Burst probability per 2 mmHg increment was then expressed as a percentage and graphed against DBP. The slope of this line was then used to determine sympathetic BRS.

#### *4.2.7. Statistical Analyses*

Statistical analyses were performed, and figures created using GraphPad Prism v.9 (La Jolla, CA). A paired Student's t-test was used to compare changes in pain. A one-way repeated measures ANOVA was used to assess differences in MSNA across time. Linear regressions were used to compare the relationships between pain, CPM, PNS activation, PNS withdrawal, and sympathetic activation. Correlations were assessed using Pearson's r statistics. In all cases an $\alpha$ criterion of 0.05 was adopted.

## 4.3. Results

### *4.3.1 Participant Characteristics*

Participant characteristics are displayed in Table 1. Temperature thresholds were within the average range for healthy participants [8; 30; 41; 42].

### *4.3.2 Changes in CPM and MSNA over time*

Pain ratings of the heat stimulus significantly decreased from pre-CPT to post-CPT (p=0.01; Fig. 1A). Mean MSNA gradually increased from baseline (BSL) to pre-CPT heat stimulus, during the CPT, and finally post-CPT heat stimulus, but these changes were not statistically significant (p=0.12; Fig. 1B)

### *4.3.2 Relationship between CPM and MSNA variables*

The relationship between changes in pain (i.e., CPM) and changes in burst frequency from pre- to post-CPT was not significant (r=0.28, p=0.65; Fig. 2A). Sympathetic BRS was extracted from 10 min of baseline quiet rest; sensitivity of sympathetic activation mechanisms was not significantly related to CPM (r=0.26, p=0.66; Fig. 2B). Sympathetic BRS also did not show any relationships with pre-CPT pain ratings or post-CPT pain ratings (see Supplementary Fig. 1C, 1F).

### *4.3.2 Relationship between CPM and cardiovagal BRS*

PNS activation-related up-sequences showed no relationship with CPM (r=0.53, p=0.17; Fig. 3A). However, PNS withdrawal-related down sequences showed a significant relationship with CPM (r=0.81, p=0.01; Fig. 3B): greater CPM was related to less sensitive PNS withdrawal (i.e., a negative relationship). Cardiovagal BRS did not show any significant relationships with pre-CPT pain ratings or post-CPT pain ratings (see Supplementary Fig. 1A, 1B, 1D, 1E), although a trend is visible between PNS withdrawal and post-CPT pain ratings (r=0.54, p=0.16).

## 4.4. Discussion

Through physiological monitoring during a CPM protocol, we were able to assess autonomic factors associated with CPM. Understanding autonomic function and how it interacts

with pain in healthy participants provides a baseline from which to gauge dysfunction, and ultimately treat autonomically mediated chronic pain conditions.

### *4.4.1 CPM and sympathetic activity*

Even with our small sample size, we observed significant differences in pain ratings when comparing pre- and post-CPT, implying robust CPM in our participants. We also observed a trend of gradual increases in MSNA from pre-CPT timepoints. As previously described, MSNA is a measure of purely sympathetic activity, specifically sympathetic efferent activity of neurons synapsing to smooth skeletal muscle surrounding blood vessels. Therefore, it is directly responsible for neurovascular control of BP. Because MSNA is slowly increasing, and there is previous evidence that BP is related to CPM [6; 8], it is reasonable to assume that CPM outcomes are directly related to MSNA. This could imply that, when induced via CPT, CPM is akin to stress-induced analgesia. Interestingly, CPM-related changes in pain and changes in MSNA burst frequency did not display a significant relationship. Additionally, the resting efficiency or 'sensitivity' of homeostatic baroreflex mechanisms that trigger bursts of sympathetic activity were also not related to CPM. Thus, our evidence does not support the idea that the modulation of pain via CPM is sympathetic in nature.

### *4.4.2 CPM and parasympathetic activity*

Cardiovagal BRS measures the efficiency of PNS activity as a function of the baroreflex, by primarily assessing the change in HR directly influenced by the vagus nerve [9]. Up sequences are defined as an increase in the interval between ECG R-waves in response to increases in SBP. This implies that HR is slowing in response to BP, a function of the "rest and digest" PNS. Activation of this system did not show a significant relationship with CPM. However, down sequences—or SBP decreases triggering a decrease in RR interval (i.e., HR increase)— maintained

a significant negative relationship with CPM. In other terms, greater efficiency of PNS withdrawal mechanism was related to less efficient CPM, and greater pain. This was supported by the trend between PNS withdrawal and post-CPT pain ratings (see Supplementary Fig. 1E). Although surprising and a bit perplexing, this would imply that slower withdrawal, or prolonged activity, of the PNS, likely promotes CPM, thereby reducing pain, despite CPM and PNS activation not being related. However, this is not the first time such a relationship has been observed. Low HRV (i.e., PNS withdrawal) has been associated with lower CPM [50] and linked with the development esophageal hyperalgesia [47]. Additionally, Chalaye and colleagues [7] found that PNS withdrawal was associated with inefficient pain inhibition in fibromyalgia and irritable bowel syndrome, and finally, Sowder et al. [48] associated low cardiac vagal tone (i.e., PNS withdrawal) with chronic pain conditions in children.

The PNS is primarily controlled via the release and binding of the acetylcholine (ACh)[11]. Parasympathetic agonists that promote activation of the PNS bind to muscarinic receptors. These are referred to as direct-acting parasympathomimetics [39]. Indirect-acting parasympathomimetics increase synaptic levels of ACh by inhibiting its breakdown by acetylcholinesterase, thereby prolonging the activation of the PNS; these are aptly named acetylcholinesterase inhibitors (AChEIs) [39]. Finally, parasympathetic antagonists block the activation of muscarinic receptors [36], preventing activation of the PNS or promoting its withdrawal. When examined in the context of our findings, prolongation of PNS activity via AChEIs would likely promote CPM. Despite literature describing the analgesic efficacy of AChEIs on neuropathic pain in mice [15], in patients with fibromyalgia [28], and as a therapeutic resource for chronic pain [19], there is currently no literature on the interaction of CPM and AChEIs. Future research would benefit from examination of these two together in both healthy humans and patients with chronic pain.

Finally, because the vagus nerve is involved in both the processing of PNS information and pain, and is the main driver behind cardiovagal BRS, being able to obtain highly specific microneurographic recordings from the vagus nerve during pain and/or CPM would allow us to better understand the mechanisms we see at play. In vivo human vagus nerve recordings are a brand-new methodology and are currently being performed in only one lab in the world [20; 37], but have already been used alongside vagus nerve stimulation in epileptic patients to assess downstream function [40].

### *4.4.3 Limitations and Conclusion*

When interpreting the results of the current study, some limitations must be considered. First, due to our small sample size, the effect of possible confounds is quite large. Second, the limited number of participants included in this study prevented us from exploring the potential modulating effects of sex. This highlights the need for future studies to compare the relationship between males and females. Third, two of eight participants were researchers in the pain field, and therefore may have had their CPM responses affected by expectation bias, given their knowledge of undergoing a CPM protocol. These two participants were also the two oldest in our sample, and because baroreflex sensitivity decreases with age, it is possible that these underlying factors may contribute to our findings.

Despite this, we believe this study provides a solid foundation for further exploration of the role of autonomic mechanisms in chronic pain and could open the door to several new theoretical and therapeutic options.

**Table 1. Participant Characteristics**

| **Demographics** | **(n=8)** |
|---|---|
| **Age**<br>(Range, Mean ± SD) | 23–58, 30.8 ±11.6 |
| **Sex** | |
| Male | 4 |
| Female | 4 |
| **Gender Identity** | |
| Man | 4 |
| Woman | 4 |
| **Pain** | |
| Threshold Temperature<br>(Range, Mean ± SD) | 44.4°C-47.7°C<br>46.6°C ± 1.2 |
| Average Pre-CPT pain rating | 7-10, 8.1 ± 1.1 |
| Average CPM Pain reduction | 0-2, 0.89 ± 0.78 |

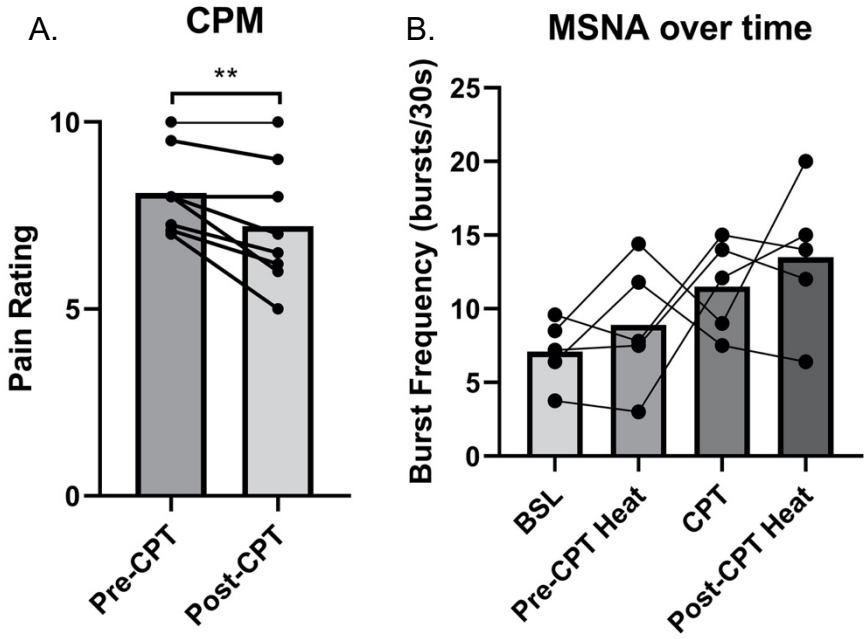



**Fig. 1.** Changes in pain and MSNA from to pre- to post- cold pressor test (CPT) (A, Conditioned Pain Modulation [CPM]; B, Muscle Sympathetic Nerve Activity [MSNA]). Data were analyzed using a paired t-test (1A), and a one-way repeated measures ANOVA (1B).**$p \leq 0.01$.

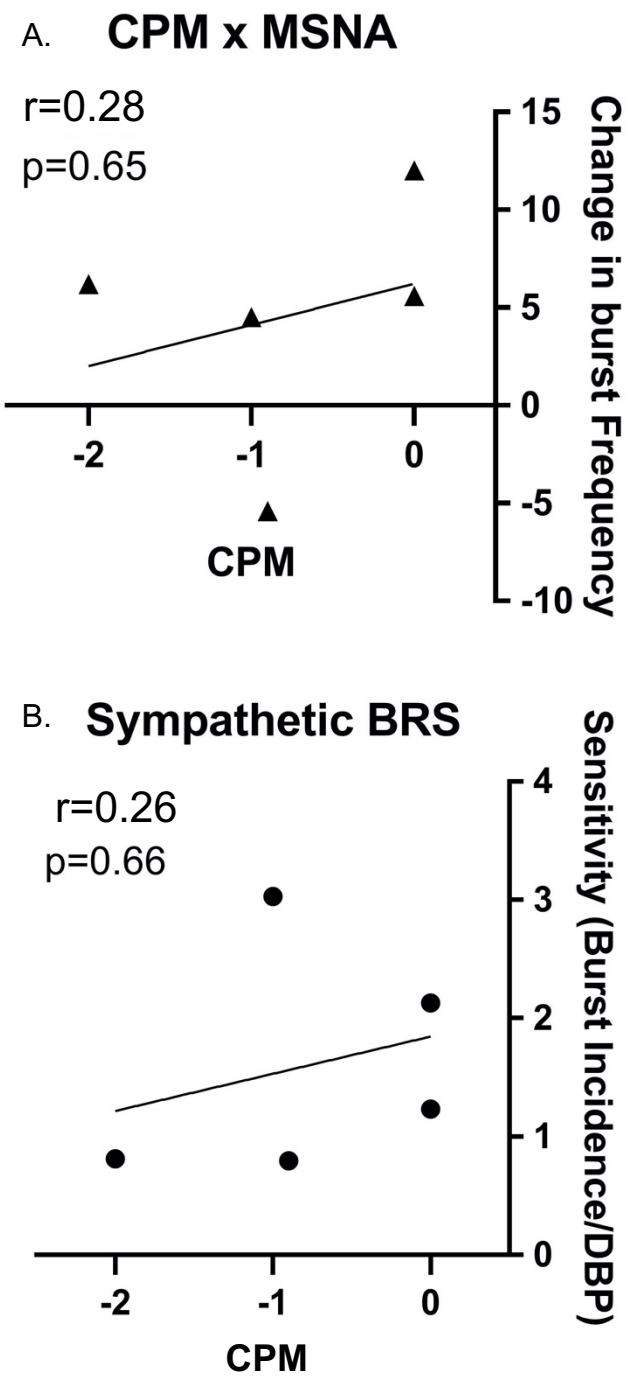



**Fig. 2.** Relationships between conditioned pain modulation (CPM) and muscle sympathetic nerve activity (MSNA) variables (A, change in burst frequency from pre-CPT to post-CPT; B, sympathetic baroreflex sensitivity [burst probability/diastolic blood pressure; DBP]). Data were analyzed using Pearson's correlation and lines represent linear regressions.

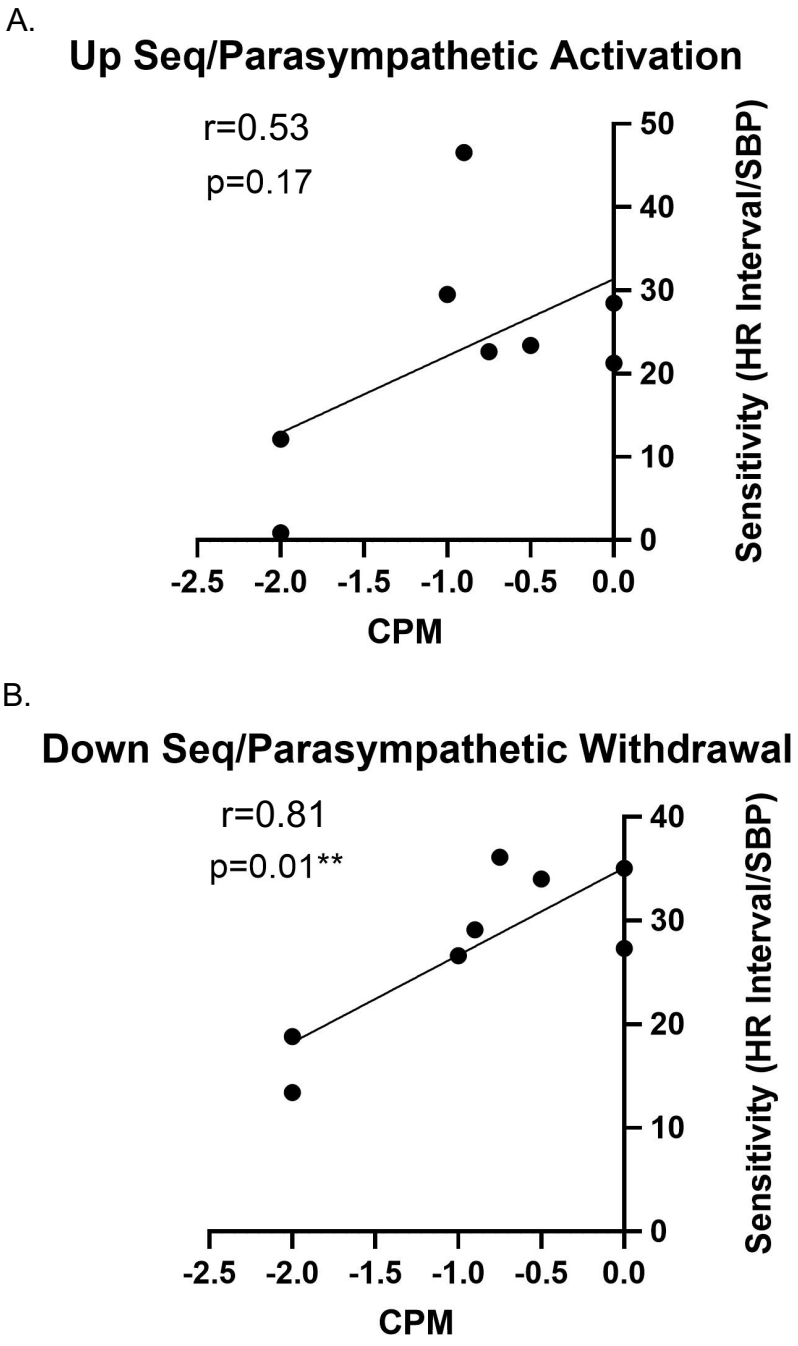
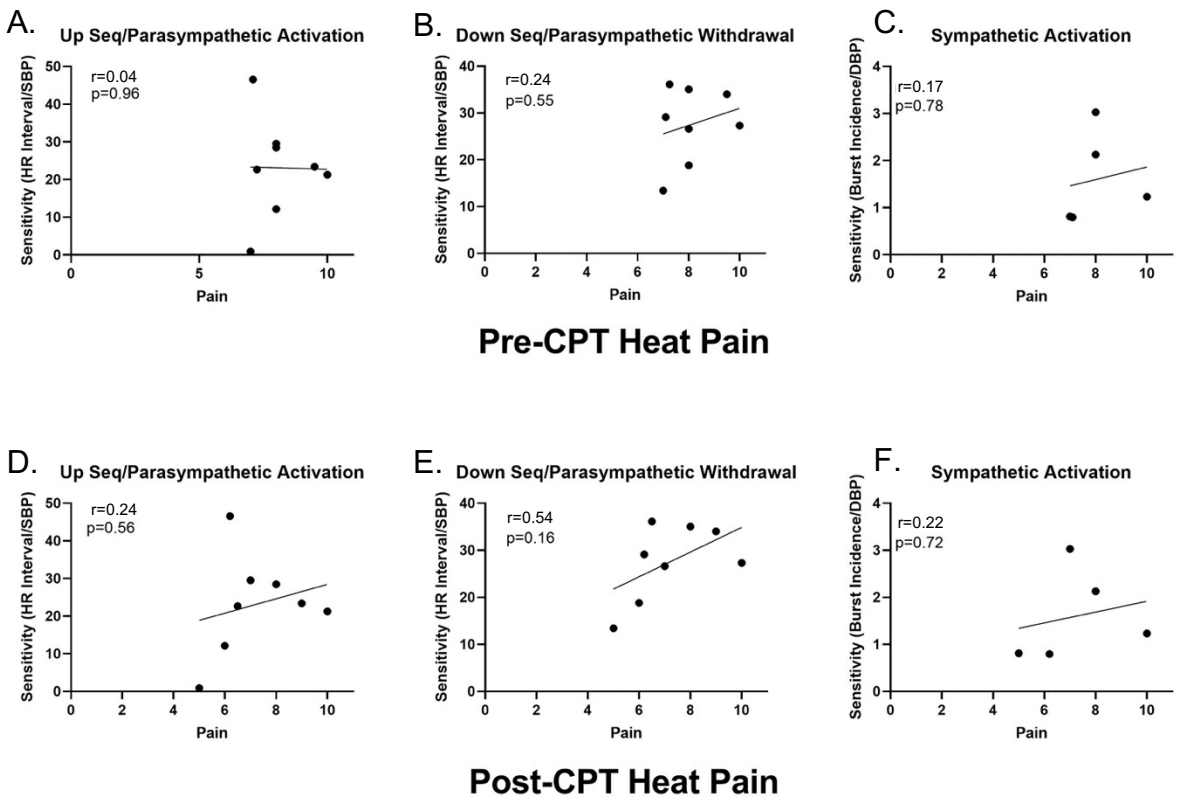



**Fig. 3.** Relationships between conditioned pain modulation (CPM) and cardiovagal baroreflex sensitivity variables (A, up sequences representing sensitivity [heart rate/systolic blood pressure] of parasympathetic activation mechanisms; B, down sequences representing sensitivity of parasympathetic withdrawal) Data were analyzed using Pearson's correlation and lines represent linear regressions. $^{**}p \leq 0.01$.




**Supplementary Fig. 1.** Relationships between pre- and post-CPT pain and baroreflex sensitivity variables (A, D, up sequences representing cardiovagal sensitivity [heart rate/systolic blood pressure] of parasympathetic activation; B, E, down sequences representing cardiovagal sensitivity of parasympathetic withdrawal; C, F, sympathetic baroreflex sensitivity [burst probability/diastolic blood pressure; DBP]). Data were analyzed using Pearson's correlation and lines represent linear regressions**.**

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# Chapter 5: Comprehensive Discussion

The primary goals of the research projects in this doctoral thesis were to investigate mechanisms and parameters affecting pain, pain inhibition, and autonomic activity in healthy individuals and women with fibromyalgia. The aim was to deepen our understanding of the function of pain modulation mechanisms. Specifically, we focused on CPM and MSNA. The following sections discuss the theoretical and clinical implications of our findings.

## 5.1 Summary of Findings

### *5.1.1 Sex differences in the relationship between pain and autonomic outflow during a cold pressor test*

This study examined the relationship between pain ratings and MSNA during a 6-min CPT. We found that pain, HR, and BP increased in the first 30 s of the CPT, but most variables stayed fairly constant afterwards. Throughout the CPT, females exhibited a greater increase in MSNA burst frequency when compared to males. Additionally, we observed various significant relationships between pain and autonomic indices over time; in males, pain was positively correlated with HR, while in females, pain was positively correlated with MSNA burst frequency and negatively correlated with MSNA burst amplitude. In summary, males displayed a strong relationship between tonic pain and parasympathetic HR, and females displayed strong relationships between tonic pain and purely sympathetic MSNA variables, suggesting sex differences in autonomic mechanisms during long-lasting pain.

### *5.1.2 The direction and magnitude of conditioned pain modulation is dependent on test-stimulus intensity in healthy participants but not in those with fibromyalgia*

This study aimed to determine if test stimulus intensity was related to CPM outcomes in healthy pain-free participants (HC), and if this phenomenon differed in fibromyalgia patients (FM). Test stimulus intensity was calibrated to heat pain threshold at stimulation temperatures -1, +1, or +3 °C below/above participants' threshold. In HC, stimulation temperature significantly influenced CPM outcomes, with hyperalgesia observed at -1°C and analgesic CPM at +3°C. In contrast, FM showed no significant differences across stimulation temperatures, resulting in a significant group-by-temperature interaction. In addition, HC participants also displayed a positive relationship between pain threshold temperature and CPM, where those with higher pain thresholds experienced more post-CPT hyperalgesia. Similarly, a median split analysis revealed that, in HC, lower pain rating of the pre-CPT stimulus was more likely to result in "anti-CPM". Pain catastrophizing also positively predicted CPM in HC, but not in FM. These findings suggest that anti-CPM is a function of CPM with low-intensity test stimuli, and that FM hyperalgesia is not related to anti-CPM.

**5.1.2.1 Preliminary gender findings.** Given the limited research on pain and non-binary gender, we conducted two distinct gender analyses. First, an unequal variances t-test examining CPM in women and non-binary FM revealed that non-binary FM displayed significantly greater hyperalgesia than FM women, which may indicate that gender minority stress may interact with and exacerbate chronic pain. Second, we examined the relationship between a gender percentile score and various pain measurements, finding a strong trend suggesting that gender percentile scores may predict recent chronic pain ratings.

### *5.1.3 Pain and autonomic function: the relationship between conditioned pain modulation and baroreflex sensitivity*

This study assessed autonomic activity, specifically cardiovagal baroreflex sensitivity (BRS) and MSNA, during a CPM protocol in healthy participants. Despite the presence of significant CPM, and a trending increase in MSNA after the CPT, pre-CPT to post-CPT changes in pain and MSNA were not related to each other. CPM was also not related to resting mechanisms of sympathetic activation. However, interestingly, CPM was related to resting parasympathetic mechanisms, maintaining a significant negative relationship with parasympathetic withdrawal. This relationship was further supported by a negative trend between parasympathetic withdrawal and post-CPT pain ratings.

## 5.2 Contributions to the Literature

### *5.2.1 Sex differences in the relationship between pain and autonomic outflow during a cold pressor test*

*Study 1* is one of only a few studies to investigate the relationship between prolonged pain and MSNA, and the first to assess pain at multiple timepoints during the stimulus, allowing for a more dynamic view of pain-autonomic interactions. This is also the only prolonged pain and MSNA study to recruit enough men and women to conduct a sex-based analysis. Sex differences in autonomic activity [68; 108], and pain [167-170] have both been established individually, but there is very little evidence of sex differences in the relationship between pain and autonomic activity. This study determined that females are more reliant on sympathetic and vascular mechanisms when responding to tonic pain, showing a positive relationship between pain and MSNA burst frequency, and an offsetting negative relationship between pain and burst amplitude.

Females also showed a non-significant positive trend with pain and HR. Males are more reliant on parasympathetic mechanisms when responding to tonic pain, displaying a positive relationship between overall pain and HR. Indeed, parasympathetic HRV variables has been shown to be more closely tied to pain modulation in men, but not in women [174]. As autonomic explanations for pain gain popularity [37; 39; 239], we must integrate our knowledge that females are more likely to suffer from chronic pain, as well as have worse cardiovascular disease outcomes [136]. It is very likely that both of these phenomena are affected by sex (or gender)-specific processing of pain and autonomic information. Overall, understanding these sex-specific autonomic responses could help shape personalized pain management strategies and enhance interventions aimed at addressing both chronic pain and cardiovascular health disparities.

### *5.2.2 The direction and magnitude of conditioned pain modulation is dependent on test-stimulus intensity in healthy participants but not in those with fibromyalgia*

*Study 2* is the first study to specifically examine and compare CPM outcomes in response to various test stimulus intensities. In addition to confirming this finding from a previous mouse study, we also observed that CPM efficacy was positively related to pain threshold temperature and pre-CPT pain ratings. Taken together this implies that people with higher pain sensitivity were less likely to experience hyperalgesia, and this trend has been shown before [81]. Therefore, the *perceived* intensity of the test stimulus has a great effect on both the direction and magnitude of CPM, further supporting evidence for the use of threshold-calibrated stimuli. This study was also one of the first forays into non-binary gender assessments in pain. Gender percentile scores calculated from the Genesis-Praxy allow us to assess gender on a spectrum, independent of sex. These scores show that gender may predict chronic pain severity. Finally, given that the only

participants that identified as non-binary in our sample were those with fibromyalgia, and there is evidence that gender non-conforming individuals experience higher rates of chronic pain [235], this data is valuable in building a foundation for understanding how gender-minority stress may interact with chronic pain.

#### *5.2.3 Pain and autonomic function: the relationship between conditioned pain modulation and baroreflex sensitivity*

*Study 3* is the first to dissociate baroreflex activation and withdrawal mechanisms and relate them with pain. This is also one of the first studies to assess CPM and MSNA, sympathetic BRS and pain, and the only study assessing the relationship between sympathetic BRS and CPM. We discovered that MSNA increases gradually over a CPM protocol, but, surprisingly, is not related to decreases in pain. Instead, sensitivity of resting parasympathetic withdrawal mechanisms showed a negative relationship with CPM and a positive trend with post-CPT pain, implying that prolongation of parasympathetic activity is related to CPM. These findings further elucidate the role of the autonomic system in pain propagation and modulation.

### 5.3 Theoretical perspectives, limitations, and future directions

#### *5.3.1 Parasympathetic withdrawal and pain*

Both *Study 1* and *Study 3* displayed findings supporting the role of parasympathetic withdrawal and pain. *Study 1* showed that increased HR (i.e., parasympathetic withdrawal) was related to greater pain in males (with a similar trend in females). *Study 3* showed that parasympathetic withdrawal was negatively related to CPM, implying that parasympathetic withdrawal was related to greater pain. It has long been known that acute stress is associated with

reductions in pain [34], and chronic stress with increased pain [97]. This has primarily been attributed to sympathetic activation mechanisms, but given our findings, it is possible that some pain propagation could be attributed to parasympathetic withdrawal.

**5.3.1.1 Limitations.** These parasympathetic findings were, of course, in pain-free participants so it is not known if the direction of these findings would be the same in individuals with chronic pain. More generally, our sample consisted of young, healthy participants and therefore, this limits generalizability to a wider population. However, there is a body of evidence to support the theory of parasympathetic dysfunction in individuals with chronic pain [242]; further research on the implications of these findings in a chronic pain population is needed.

### *5.3.2 Conditioned pain modulation and stress*

In *Study 2,* like many studies before ours [181], we confirmed that individuals with fibromyalgia have defective endogenous pain modulation presenting itself as reduced CPM efficacy. CPM likely involves multiple pain modulation mechanisms, including both inhibitory and facilitatory processes [273]. Whether the reduced efficacy of CPM in fibromyalgia is linked to abnormal baroreflex function or decreased activity in descending inhibitory pathways (e.g., opioids, norepinephrine, serotonin) is still unknown.

Pain catastrophizing—a psychological tendency to focus excessively on pain and amplify its threat—has been shown to be negatively correlated with CPM effectiveness [258]. Yet it is still unclear whether pain catastrophizing directly causes a reduction in CPM effectiveness or whether it is a result of it. People with higher pain catastrophizing levels also exhibit diminished cardiovascular reactivity during emotional stress [270], suggesting that emotional states could modify pain sensitivity by altering autonomic responses.

**5.3.2.1 Limitations.** As *Study 2* was not a physiological study, we were unable to examine cardiovascular variables. This would have allowed us the opportunity to assess some autonomic aspects of *Study 1* and *Study 3* in a chronic pain sample, to see how they differ from HC, as well as examine the three-way interaction of pain catastrophizing, CPM, and cardiovascular activity in HC and FM

**5.3.2.3 Future directions.** Administration of naloxone in a repetition of *Study 2* and *Study 3* could yield interesting in findings. In *Study 2,* naloxone could be used to block the effects of CPM [105; 263] and to determine if anti-CPM is also reliant on opioidergic mechanisms. In *Study* 3, naloxone would block the effects of CPM, but we could examine how it would affect our various other outcomes, as it has also been shown to reduce cardiovascular responses (BP, HR, and baroreflex sensitivity) to acute stress [64]. Also, pharmacological intervention with parasympathomimetics such as AChEIs could modulate the CPM response. Additionally, pain catastrophizing data was collected for *Study 3,* but insufficiently powered for analysis. Further data collection and analysis in this area would aid in our understanding of how psychological factors interact with pain, CPM, and cardiovascular activity. Finally, repetition or continuation of *Study 3* in a chronic pain sample, specifically fibromyalgia, would allow us a better understand how autonomic dysfunction works alongside widespread chronic pain.

### *5.3.3 Fibromyalgia and stress*

The etiology of fibromyalgia remains unclear, though several theories suggest that dysfunction in pain modulation mechanisms plays a major role in the disorder [104; 226]. Additionally, ANS dysfunction has been proposed as a contributing factor to symptoms such as chronic pain [157]; stress is thought to play a role in its onset and symptom maintenance. Stress

can be either physiological or psychological (e.g., relationship issues, job loss) [155]. Some individuals experience a worsening of fibromyalgia symptoms during stressful periods [39; 97]. Healthy individuals exhibit stress-induced analgesia to acute stress, but this effect is less pronounced in fibromyalgia patients [177; 178]. Additionally, fibromyalgia patients show a blunted cardiovascular response to stress, with a noted correlation between this response and the development of stress-related pain [178].

**5.3.3.1 Fibromyalgia and stress management.** Various stress reduction techniques are commonly used in the management of fibromyalgia [83]. Relaxation methods and slow, deep breathing are frequently taught to fibromyalgia patients to alleviate stress, anxiety, and muscle tension. Slow, deep breathing, in particular, has demonstrated analgesic potential [33; 38]. This simple technique significantly activates the baroreflex arc, increases parasympathetic cardiac activity, and improves baroreflex sensitivity [16]. Slow, deep breathing has been shown to reduce experimental pain in fibromyalgia patients, though the effect is less pronounced compared to healthy individuals [278], and fibromyalgia patients often exhibit decreased baroreflex sensitivity, which correlates with pain levels [55]. Taken together, these suggest that the relationship between the parasympathetic activity and pain is less direct or efficient in individuals with fibromyalgia than in pain-free counterparts.

**5.3.3.2 Fibromyalgia and MSNA.** There is a small but significant literature surrounding microneurographic MSNA in individuals with fibromyalgia. The first study to examine MSNA in fibromyalgia, was by Elam et al. [61] in 1992, which found no differences in MSNA burst frequency between fibromyalgia and healthy controls at rest or during CPT reactivity. However, a more recent study by Zamunér et al. [277] found that MSNA burst frequency was directly related to the magnitude of chronic pain, and recommended the use of anti-adrenergic agents in the

treatment of fibromyalgia. This aligns with evidence that fibromyalgia pain is suppressed by sympathetic blockade and rekindled by norepinephrine injections [156]. However, in *Study 1,* we saw the same direct relationship between MSNA burst frequency and tonic pain in healthy females, implying that this relationship between MSNA burst frequency and pain may not truly be dysfunctional (but could provide a clue to why fibromyalgia is primarily prevalent in women). However, the offsetting relationship of MSNA burst amplitude observed in healthy females remains to be examined in fibromyalgia patients; therefore, further research is needed in this regard.

### 5.4. Conclusion

The body has various mechanisms that facilitate or inhibit pain perception. A dysregulation of pain-inhibiting mechanisms seems to facilitate chronic pain, likely involving structures that also play a role in autonomic regulation. We sought to further investigate the mechanisms of CPM, how interactions between pain and the autonomic system could contribute to pain propagation or modulation, and how these phenomena might differ between sexes and/or genders. *Study 1* showed us that despite acute pain and MSNA being related in both sexes, tonic pain was related to changes in sympathetically-mediated MSNA burst frequency and amplitude in females, whereas tonic pain was related to changes in parasympathetically-mediated HR in males. As the sympathetic and parasympathetic nervous system are involved in modulation of pain, these findings imply sex differences in the autonomic mechanisms of pain modulation. *Study 2* found that hyperalgesic responses to CPM were related to test-stimulus intensity in healthy humans, offering a possible explanation for pain facilitation in healthy controls in many CPM studies. This knowledge allows us to better elicit CPM during experimental protocols, allowing us to study pain inhibition

mechanisms more effectively. Finally, *Study 3* found that MSNA burst frequency increases throughout a CPM protocol but is not related to the magnitude of CPM. Instead, parasympathetic withdrawal mechanisms showed a negative relationship with CPM magnitude, implying that prolongation of parasympathetic activity may promote CPM. We believe the findings from these research projects contribute new knowledge to the field of pain, aid in our understanding of autonomic upregulation and downregulation of pain, providing opportunities for better experimental research and clinical treatment of chronic pain disorders like fibromyalgia.

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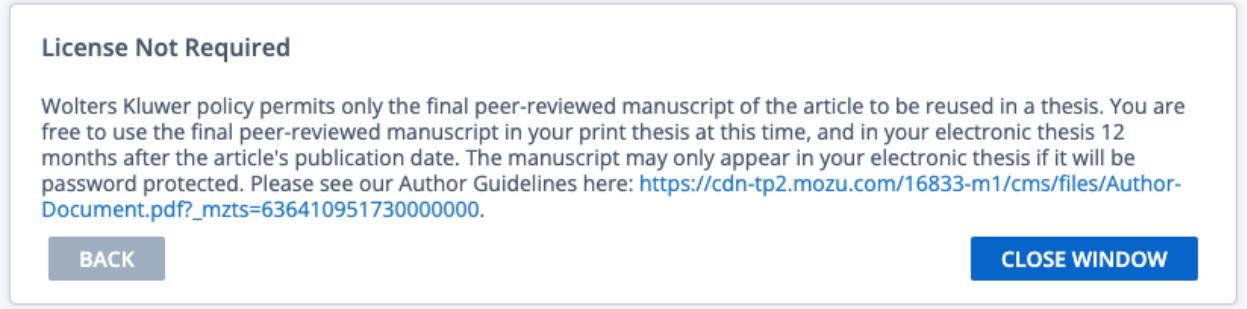

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