Bachelor Thesis

No Pain, No Gain: Are Men In Fact More Tough Than Women?

An Investigation Into The Complexity Of Pain And Several Factors That Affect It

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Abstract

Background: Pain is a complex phenomenon, and the experience can be affected by a plethora of genetic, physiological, psychological, and environmental factors. The peripheral and central nervous systems (PNS and CNS) form the core of pain perception, as these are the places where stimuli are perceived and processed. One factor affecting pain perception is conditioned pain modulation (CPM), a phenomenon in which a noxious stimulus inhibits the pain caused by a different noxious stimulus. This factor is studied extensively in one of the experiments. This study aims to identify factors influencing pain experience in healthy visitors of a festival in two separate experiments. Additionally, this study proposes a more complete method to compute an individual's CPM.

Methods: In the first experiment performed during the Betweter Festival in Utrecht, menthol and capsaicin creams were applied on the forearms of subjects, who after 5 minutes described the pain and rated it on a 0 to 10 numeric rating scale (NRS). Using data on sex, age, and self-reported pain threshold of the subjects, a logistic regression model was created.

In the second experiment, CPM was investigated in subjects using cuff algometry on the lower legs. The cuff was inflated (~1 kPa/s), during which the subject rated pain on a 10-cm visual analogue scale (VAS). The pressure at which the subject started sliding was defined as the pressure pain detection threshold (PDT), the pressure at which the experiment was stopped was extracted as the pressure pain tolerance threshold (PTT). Additionally, the subjects rated pain on a 0 to 10 NRS after the test. The methods to calculate CPM were based on literature and newly developed by the study team. A logistic regression model was created to study the relation between sex, age, chronic pain, pain threshold, activity level and alcohol consumption, and an efficient CPM system.

Results: In the first experiment, 76% of the individuals (*n = 181*) receiving the capsaicin cream and menthol cream, were between 21 and 40 years old, and 63% were female. Overall, the capsaicin cream was considered to be more painful than the menthol cream. Being female or having a high self-reported pain threshold significantly reduced the chance of experiencing discomfort. For the menthol cream, no variable significantly affected the experience of discomfort.

In the second experiment, 82% of the individuals (*n = 49*) undergoing the cuff algometry study, were between 21 and 40 years old, and 61% were female. Especially at the lower pressures, there were significant differences in pressures at which a certain VAS score was reached between the baseline and conditioning tests. At the higher pressures these differences did not exist, but VAS scores were considerably lower in the conditioning test. Being female significantly increased the odds of having a good CPM mechanism.

Conclusion: Women have a smaller chance of experiencing pain following capsaicin application, and a greater chance of having a good CPM mechanism. This opens the door to the possibility that current insights on gender and sex differences in pain may be outdated. In addition, this study proposes a new method to calculate the effectiveness of CPM mechanisms, which can be improved in further research.

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1. Introduction

1.1 What is pain?

The experience of pain is personal and can be experienced and influenced in a variety of ways. It is a very vague term describing a subjective phenomenon, that may be difficult to be put into words. For the longest time, mankind had therefore not a clue as to what pain actually is, and how it works. An example of this is the description of pain by Descartes in his book *L'Homme*, in which he states the following: "Particles of heat activate a spot of skin attached by a fine thread to a valve in the brain where this cavity opens the valve, allowing animal spirits to flow from a cavity into the muscle causing them to flinch from the stimulus, turn the head and eyes toward the affected body part, and move the hand and turn the body protectively."[2] In the 358 years since the publication of L'Homme, we have gained a better understanding of the human body and pain, and we now know that pain is not associated with the flow of animal spirits, but with the flow of action potentials through neurons. Our definition of pain has thus changed too, and the International Association for the Study of Pain (IASP) has in 2020 redefined pain as "An unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage."[1,3] This change – for the first time since 1979 – was deemed necessary as the previous definition of pain, "An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.", was lacking some context. The revised definition takes six important aspects into account that the previous did not (Box 1).

- Pain is always a personal experience that is influenced to varying degrees by biological, psychological, and social factors.
- Pain and nociception are different phenomena. Pain cannot be inferred solely from activity in sensory neurons.
- Through their life experiences, individuals learn the concept of pain.
- A person's report of an experience as pain should be respected.
- Although pain usually serves an adaptive role, it may have adverse effects on function and social and psychological well-being.
- Verbal description is only one of several behaviors to express pain; inability to communicate does not negate the possibility that a human or a nonhuman animal experiences pain.

Box 1: The six key notes used for the revision of the definition of pain by the IAPS.[1]

As the IASP states clearly, pain and nociception are different phenomena, and there is an important distinction. Nociception is defined as "the physiological process of activation of neural pathways by stimuli that are potentially or actually damaging to tissue."[4] It thus refers solely to physiological aspect of what is happening in or to the body. Nociceptive pain is essential to the human body, and not a clinical problem, even though it hurts. A lack of nociceptive pain may eventually lead to a variety of problems, such as bone fractures, scars, unintentional self-mutilation, joint deformities, amputations, and even early death.[5] Nociceptive pain is therefore, no matter how annoying it may be, crucial to maintaining body integrity.[6]

Nociception is thus a key aspect of pain, and in many cases the main determinant, but it is important to realise that it does not tell the whole story. According to the IASP, nociceptive pain is thus just one of three types of pain that one can experience. The others are neuropathic pain and nociplastic pain.[7] In contrast to nociceptive pain, neuropathic pain and nociplastic pain are not protective to the body and have no physiological function. Neuropathic pain arises as a result of damage to the somatosensory nervous system. When nociplastic pain arises in the body, it does so from no clear cause. There is altered nociception, but no damage to any tissue, nor to the nervous system.

Neuropathic pain illustrates the importance of the central nervous system (CNS) on the perception of pain, as well as its ability to influence this perception. Pain is actively regulated by both inhibitory and excitatory circuits, which is mainly controlled by nuclei in the brainstem. These can strengthen or weaken the sensation of pain depending on past experiences, cognitive function, or mood.[8] A common example is the placebo effect, in which your brain will believe that you have been given a treatment, and accordingly, while in reality no active compound has been admitted.

The challenge of studying pain is thus that it is multifaceted, and subjective. It should come as no surprise then, that objective measurements of pain are indeed complex and difficult to achieve.[9] Pain and pain scores are also highly dependent on personal experiences of individuals, and this creates a problem for both clinical practice as well as research. Nonetheless, while pain is often a protective mechanism, for the hundreds of millions of people suffering from chronic pain, it is not.[10] According to some studies, up to 30% of the world population is affected by chronic pain in some way, shape or form, carrying a massive burden on these individuals and on society itself.[11,12] By gaining a better understanding of the factors

affecting pain, and the mechanisms of pain, the quality of life of these people could be improved.

1.2 TRPV1 and TRPM8

The human body is able to sense temperatures between a range of about 8 to 52 °C and detect changes of as little as 1 °C.[13,14] Below ~15 °C and above ~42 °C, temperature becomes noxious and potentially harmful.[15] Central to the ability to detect this is the peripheral nervous system (PNS). Four heat-activated channels (TRPV1 – 4) inform the body of temperatures over ~25 °C, and two cold-activated channels (TRPM8 and TRPA1) inform us about temperatures below ~25 °C.[13,16,17] Two of these, TRPV1 and TRPM8 have been extensively researched, in great part due to their natural agonists; capsaicin for TRPV1 and menthol for TRPM8, respectively.[15] These substances have added significantly to the study of pain, as those channels operate, at least partially, in the noxious temperature zones.

TRPV1 is activated by temperatures greater than ~42 °C, which is the point where heat starts to become noxious.[15] Like all TRP channels, TRPV1 is a transmembrane ion channel. TRPV1 is polymodal, and as mentioned, can also be activated by capsaicin, which stabilizes the open state of the receptor by interaction with subdomains S4 and S5.[18] Capsaicin is the ingredient of chili peppers and it is responsible for the burning and tingling sensation of spicy foods.[19] It is this correlation between the heat sensor of the human body and spicy food which causes people to sweat when eating spicy food; the same pathways are activated and thus the same information is conveyed to the brain. Another activator is low pH, and inflammatory factors – nerve growth factor, bradykinin, lipids, prostaglandins, protein kinases A and C, and ATP – are also known to potentiate the receptor.[20]

TRPM8 on the other hand is activated at temperatures from ~28 °C to ~8 °C. Cold becomes noxious from about 15 °C.[15] One natural agonist of TRPM8 is menthol, and the receptor is, similar to TRPV1, polymodal, with other activators being voltage, pressure, and hyperosmolarity.[21] Menthol, the cooling component from mint, induces temporal conformational changes in the S6 subsection of TRPM8 upon binding, causing the receptor to open up and the cell to depolarize.[22] It has been shown that at very high concentrations, menthol can induce a painful sensation.[23–25] Cold can induce a painful sensation too, especially so in chronic pain patients suffering from cold allodynia.[26] They will perceive cold as excruciatingly painful and thus have to avoid it. Although menthol is the most well-known

natural agonist for TRPM8, and has been crucial in the discovery of the receptor, ilicin, a toxic substance of holly, is a more potent stimulus, which activates the channel in a manner that is different to menthol.[17] Extracellular calcium is required for ilicin to activate TRPM8.[27]

For both channels, the transmembrane voltage is a defining factor for the temperature sensitivity.[28] When the temperature changes, the voltage dependence of voltage activation gradually shifts, making the channel either more or less likely to be activated. Specifically, the opening rate α of TRPV1 shows a steep temperature dependence, while the temperature dependence of the closing rate β was shown to be shallow.[28] The opposite is true for TRPM8, where the opening rate α showed a shallow temperature dependence and the closing rate β was steeply temperature dependent. This model, albeit simplified suggests that the differences in thermosensitivity of TRPV1 and TRPM8 are a result of different activation energies for the voltage-dependent opening and closing of the channels.[17,28] Findings regarding TRPV1 were rewarded with a Nobel Prize in the past year, as it helped the field understand pain perception much better.

1.3 Conditioned Pain Modulation

Next to the PNS mechanism, the CNS can, unconsciously, alter the perception of pain. A well-known example of this is how one may get injured while doing sports, and it does not hurt that badly until one stops. Then the adrenaline levels in the blood decrease and it turns out the situation is a little worse than anticipated. A more general process of pain alleviation by the CNS was first known as diffuse noxious inhibitory controls (DNIC). In this phenomenon, the pain caused by one noxious stimulus is reduced by another noxious stimulus.[29] It has been shown that this process is a central phenomenon, as only animals with an intact brain possess it.[30] The descending pathways must be activated in the brain, which then allows for a reduction of pain. However, since specific CNS mechanisms cannot be perceived in humans, it was recommended to refer to the process as conditioned pain modulation (CPM) rather than DNIC when related to humans.[31] CPM is clinically important, as an insufficient CPM mechanism is related to prolonged operative pain, chronic pain, and an increase in pain and decrease in physical functioning in healthy subjects.[29,32,33] In the study of CPM there is a test stimulus, which evokes the pain, and a conditioning stimulus, which should reduce the pain.[31,34]

Various parameters are thought to influence CPM efficiency. These include unmodifiable patient related factors, such as age, sex, ethnicity, genetic makeup, and hormones,

but also psychological factors such as anxiety, depression, and catastrophizing. Underlying medical problems may also have an influence, as do methodological and procedural factors when CPM is studied in a research setting.[35] The CPM effect appears to be more efficient in younger people, and decline with age.[36,37] Research is less conclusive about the effect of sex, with some suggesting that men have a more efficient CPM mechanism, and others suggesting that there is no difference.[38,39] It must be noted that menstrual cycles seem to have an effect on CPM, with pain modulation being more effective when oestradiol levels are high, and progesterone levels are low, as is the case in the ovulatory phase.[40,41] The effect of chronic pain on CPM in research largely depends on whether the subjects are using medication, and what kind.[35] Exercise, especially aerobic and isometric, reduces pain sensitivity in muscles, both at rest and during exercise, suggesting that pain inhibitory mechanisms are stronger in people who frequently do sports.[42] However, in people who already suffer from widespread pain, a high pain sensitivity, or an impaired CPM mechanism, isometric and aerobic exercises may cause an increase in pain sensitivity.[42]

1.4 Creation of models

The aim of this study is to create several linear and logistic regression models, with which we will be able to predict the influence that various factors may have on the experience of pain. Many studies show that women are more likely to experience more severe levels of pain, experience the pain for a longer amount of time, have pain more frequently, and have a higher risk of chronic pain.[39,43,44] It is thus expected that the present study comes to similar conclusions. Another factor that has been studied extensively in relation to pain is alcohol. While alcohol is able to inhibit pain, and moderate drinking may be positively related to pain sensitivity[45], extensive alcohol use and abuse is related to chronic pain and greater pain severity.[45–47] We thus expect that the individuals who have consumed alcohol at the time of the experiment to show a better pain inhibition, but it does highly depend on the everyday drinking habits of the participants. Age has been found to have an influence on the experience of pain too, with older people being more likely to suffer from the negative aspects of pain.[48] With these factors in mind, we will predict the likelihood of the experience of pain with capsaicin and menthol creams, and the relation of these factors with one's CPM mechanism.

2. Methods

2.1 Subjects

Subjects attending the Betweter Festival 2021 were eligible for inclusion in this study. The Betweter Festival is an event organised by Utrecht University, where researchers and interested visitors meet in a festival setting. At this festival, researchers from the UMC Utrecht had set up a stand with these experiments. The participants all voluntarily took part in either one of the experiments, or both. The Medical Research Ethics Committee of UMC Utrecht (The Netherlands) approved this study and waived the need to obtain informed consent from the participants (protocol 21/397).

2.2 Experimental procedure

2.2.1 Cream experimental procedure

A capsaicin cream was applied on the left forearm of the participants, and a menthol cream on the right side (figure 1A). The participants were then asked to give the pain of both a score on a numeric rating scale (NRS) from 0 to 10, where 0 is 'no pain at all', and 10 is 'maximum pain'. At an NRS score of 4 or higher, the cream was defined to have induced discomfort. They were also asked the describe the sensation, and comment on whether they thought the creams were painful. For the participants in which either of the creams induced irritation on the skin, the circumference of the affected area was measured and noted down.

After the experiment participants were asked to fill in a short questionnaire, in which they commented on their sex, age group, and self-reported pain tolerance. This information was used to divide the participants in groups: male or female; below the age of 40 years, or above the age of 40 years; low, medium, or high pain tolerance. These groups were to be used in the creation of a logistic and linear regression model later.

2.2.2 CPM experimental procedure

The participants were placed in a relaxed, seated position (figure 1B). The experiment consisted out of three parts: a baseline test, a baseline test for the conditioning stimulus, and a conditioning test. During the baseline test, the cuff on the left lower leg was inflated, either until it was fully inflated, or until the participant stopped the experiment because of an intolerable pain sensation. This was the baseline measurement. In the baseline test for the

conditioning stimulus, the same procedure was repeated on the right leg. This measurement provided the conditioning stimulus for the last test, in which both cuffs were inflated. In this conditioning test, the cuff on the right leg was first inflated to 70% of the maximal pressure that was reached in the baseline test for the conditioning stimulus. About eight second after the cuff on the right leg started inflation, the cuff on the left leg was inflated. Again, this proceeded until it was fully inflated, or until the participant stopped the experiment. This was the conditioning measurement. After each test, the participant was asked to rate the pain on a numeric rating scale (NRS) from 0, signifying 'no pain', to 10, signifying 'maximum pain'.

Following the experiment, the participants were asked to fill in a short questionnaire. They were asked about their sex, age group, presence of chronic pain, self-reported pain tolerance, how frequently they exercise per week, and whether they had consumed alcohol during the festival. If the answer to the last question was yes, their blood alcohol content was measured using a Breathalyzer. The information collected was used to divide the participants into several groups: male or female; below the age of 40 years or above the age of 40 years; chronic pain or no chronic pain; exercised more than twice per week or not; low, medium or high pain tolerance; had consumed alcohol or had not consumed alcohol. These groups were later used to create a logistic and linear regression model.

A computer-controlled cuff algometer with a 13-cm wide tourniquet cuff was used for the experiment. The cuffs were connected to the compressor and wrapped around the lower legs, at the level of the largest circumference of the gastrocnemius muscle. The cuff around the right leg formed the conditioning stimulus, while the left leg was tested for CPM. The cuffs were not moved or released over the course of the experiment. The pressure in the cuff was increased by ~0.97 kPa/s. The maximum time limit was set at 100 seconds, at which point the pressure would reach 96 to 97 kPa. Participants were instructed to continuously rate the pain during the inflation, starting from the point when the pressure was perceived as painful.

In order to continuously record the pain score, the participants were given a 10-cm electronic visual analogue scale (VAS), as well as a button to release the inflation. The participants were instructed to press this button when the pain became unbearable, which determined the pressure pain tolerance threshold (PTT). If the participant did not stop the experiment, the max pressure readout was taken as the PTT, which varied between 96.08 kPa and 96.86 kPa. 0 cm on the VAS was defined as 'no pain at all', while 10 cm was defined as 'maximum pain'. The pressure pain detection threshold (PDT) was defined to be the pressure

when the VAS score became greater than 0, i.e., when the participant started sliding the VAS slider. At this point, the pain can be referred to as mild.[49] The pressure at which the VAS score exceeded 4 was defined as the point where the pain became moderate (PVAS4). The PVAS7 was taken as a supra-pain threshold measure, when the pain became severe.[49] Due to the nature of the experiment in the festival setting, each test was performed only once per participant.

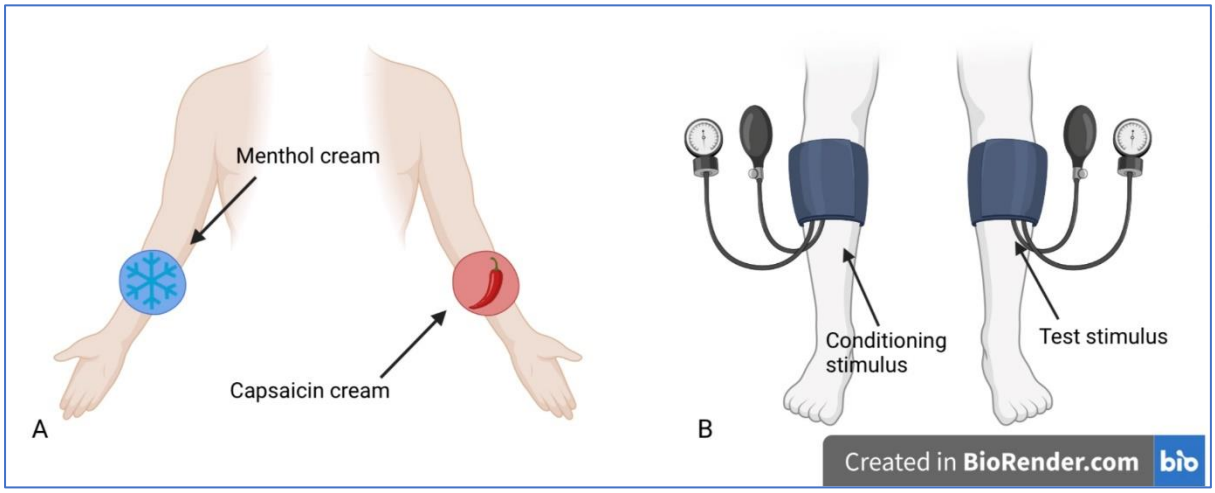

Figure 1: (A) Experimental set-up of the cream experiment. A capsaicin cream was applied on the left lower arm of the participant, and a menthol cream on the right lower arm. (B) Experimental set-up of the CPM experiment. A cuff was placed on each lower leg of the participant. Two test stimuli runs were performed, one on each side, plus a conditioning stimulus test on both legs, where the conditioning stimulus was 70% of the max test stimulus on the right leg. Image created in BioRender.

2.3 CPM definition

To determine a score for the CPM, the trajectory of the Pressure x VAS data was graphed out and integrated, giving us an area under the curve (AUC) value. This value was divided by the PTT, in order to account for any differences in that area. We referred to the values that we derived here as the base scores, and they were obtained for the baseline and the conditioning test. The CPM was then defined as the difference between the base score for baseline and the base score for conditioning.

$$CPM = \frac{AUC_{baseline}}{PTT_{baseline}} - \frac{AUC_{conditioning}}{PTT_{conditioning}}$$

With this formula, a positive value denotes a good, efficient CPM, while a negative value denotes a poor, inefficient CPM.

2.4 Statistics

Descriptive statistics were performed for all variables and were presented as mean and standard deviation (SD). Variances in all matching scores were analysed. Independent samples t-tests were then performed to assess any differences across the dependent variables in relation to the independent variables. For the cream experiment this meant that the NRS scores for capsaicin and menthol were compared, as well as the individual creams for age group and sex. Following this, logistic and linear regression models were created to predict the odds that a parameter influences the perception of discomfort, or a higher NRS score.

In the CPM experiment, differences in PDT, PVAS4, PVAS7, and PTT between baseline and conditioning tests were assessed using independent samples t-tests. Differences in base scores and CPM scores were also assessed using independent samples t-tests, with sex, age group, physical activity level, chronic pain, and alcohol consumption as independent variables. Logistic regression models were created to predict the odds that various factors have on having a good CPM efficiency. Linear regression models predicted the influence of various factors on the CPM score. The factors available for the models were sex, age group, presence of chronic pain, pain tolerance, physical activity level, and alcohol consumption.

The analyses were performed using R version 4.0.3.[50] The significance level was set at $p \leq 0.05$ for all analyses.

3. Results

3.1 Cream experiment

A total of 181 individuals participated in this study (table 1). There is 0.55% missing data, as one person did not comment on their pain tolerance, one did not report any pain scores for either cream, and two only reported a score for one of the creams. A total of 179 individuals were included in the regression analysis. The sex category 'other/unknown' is not included in the regression analysis, as this sample size is too small.

Table 1: Demographics of subjects in the cream experiment

	Sex			*Age*							*Pain tolerance*		
	Female	Male	Other/unknown	11 – 20 years	21 – 30 years	31 – 40 years	41 – 50 years	51 – 60 years	61 – 70 years	> 70 years	Low	Medium	High
N	114	65	2	4	88	45	23	15	3	3	14	106	60

Overall, the capsaicin cream was perceived to cause more discomfort than the menthol cream (table 2). This is reflected both by higher numbers of subjects with an NRS ≥ 4, reflecting a perception of discomfort, and the average NRS being higher for every subgroup (tables S1 and S2, figure S1). Only 32 subjects rated the menthol cream as more painful than the capsaicin cream. Still, the vast majority of subjects did not perceive either of the creams as painful, nor as inducing a lot of discomfort. Both for the capsaicin (72 times) and the menthol (125 times) cream, the most common NRS score was 1 (figure 2). The NRS was significantly higher for the capsaicin cream than the menthol cream for the overall group, and also specifically in male subjects, in subjects below 40 years, and in subjects with a

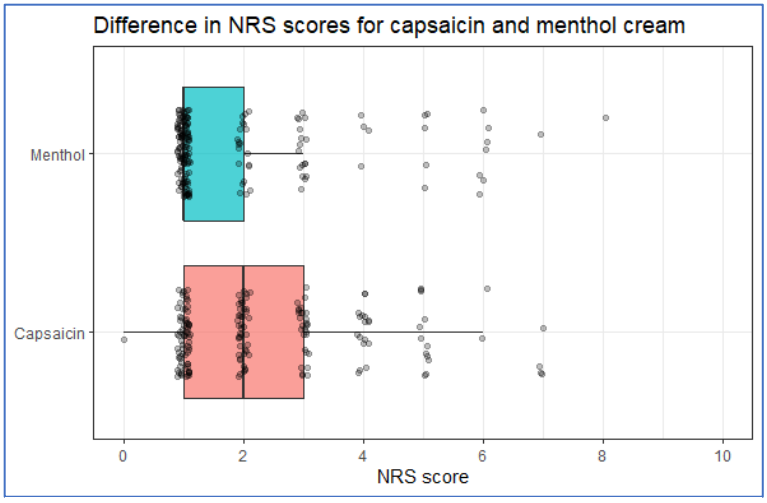

Figure 2: Distribution of the reported NRS scores for the capsaicin and menthol creams.

medium self-reported pain tolerance (p = 0.0004, 0.0004, 0.0024, and 0.0003, respectively). For women the capsaicin cream induced less discomfort than for men (15% vs. 26%), but they experienced more discomfort as a result of the application of the menthol cream (11% vs. 7%) (tables S1 and S2). Among the categories of self-reported pain tolerance, the subjects with a low pain tolerance perceived both creams to cause relatively more discomfort, while the subjects with a high pain tolerance did not perceive the creams to induce a lot of discomfort.

In 19 cases (11%), there was irritation caused by the capsaicin cream (table S3). Of these, 14 were confined to an area with a circumference of one to four cm. Three times the circumference went beyond 10 cm. The average circumference was 4.42 (± 3.75) cm. The menthol cream caused no irritation in any of the subjects.

Table 2: Perception of capsaicin and menthol creams by all subjects

	Capsaicin			***Menthol***			
	Discomfort[1]	Mean NRS	Pain[2]	Discomfort[1]	Mean NRS	Pain[2]	p-value[3]
All subjects	35 (20%)	2.29 ± 1.49	10 (6%)	18 (10%)	1.75 ± 1.42	9 (5%)	0.0004*

[1] We denoted an NRS score ≥ 4 to reflect discomfort.

[2] Pain refers to the whether the subjects said that they experienced the creams to be painful.

[3] p-value of the independent samples t-test performed to compare the average NRS score of the capsaicin and menthol cream

3.1.1 Predictive models

Following our results shown in tables S1 and S2, we created a model predicting the odds of experiencing discomfort following the application of capsaicin cream or menthol cream, respectively. Both models contained the variables of sex, age group, and self-reported pain tolerance. For the capsaicin cream, being male or having a low or medium pain tolerance significantly increased the odds of experiencing discomfort, by 2.3 and 3.8 times, respectively. The age category played no significant role in this prediction. The model was significant with p = 0.008 and has an R^2 of 0.08. For menthol on the other hand, no variable was found which significantly increased or decreased the chance of the perception of discomfort.

The linear model, made to predict what factors have an influence on a change in the NRS score, contained sex, age group, and pain tolerance as covariates. For the capsaicin cream, having a low or medium pain tolerance significantly increased NRS scores by 2 points. Being male increased the NRS with 1.1 point, but this was not a significant result. For menthol, the

biggest contributor was having a low pain tolerance, which increased the NRS score by 1.9 point, but this was not significant.

3.2 CPM experiment

A total of 49 individuals participated in this study (table 3).

Table 3A: Demographics of subjects in the CPM experiment.

	Sex		*Age*					*Pain tolerance*			*Chronic pain*	
	Female	Male	11 – 20 years	21 – 30 years	31 – 40 years	41 – 50 years	51 – 60 years	Low	Medium	High	Yes	No
N	30	19	2	26	12	5	4	4	27	18	10	39

Table 3B: Demographics of subjects in the CPM experiment.

	N	Mean	SD
Exercise			
< twice per week	31	1.45	0.72
> twice per week	18	4.00	1.14
Alcohol consumption			
Yes	23	0.35 ‰	0.29
No	26		

In the baseline experiment, the individuals younger than 40 years gave a significantly lower VAS score at a pressure of 75 kPa than those over 40 years (5.15 vs 7.28, table S4). This was the only significant difference in the baseline test. Between baseline and conditioning, there is no real difference in the number of people that reached the max end pressure of > 96 kPa as their PTT (figure S3). The PTT

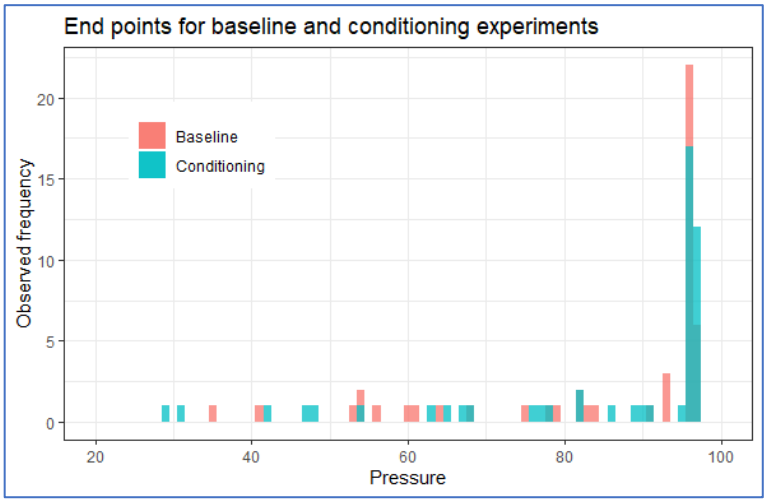

Figure 3: Distribution of end pressures for baseline and conditioning experiments.

remained unchanged between baseline and conditioning for 27 subjects. Ten subjects ended the conditioning experiment later than the baseline, whereas 12 ended it earlier.

Table 4 compares the baseline and conditioning tests at various VAS and pressure points. The PDT of the conditioning experiment was significantly higher than the baseline conditions. At a VAS score of 7 (PVAS7), the mean pressure when it was reached was lower in the conditioning experiment, but the total number of subjects reaching a VAS of 7 was down too, with five. There is no difference in PTT, both the VAS score and reported NRS score were slightly lower for the conditioning condition, albeit not quite significantly. VAS scores were considerably lower during conditioning compared to baseline at pressures of 50 kPa and 75 kPa, respectively.

Table 4: Pressures and VAS scores for baseline and conditioning, at several conditions.

	Baseline	***Conditioning***	***p-value[1]***
PDT	23.38 kPa (*n = 49*)	33.84 kPa (*n = 48*)	0.005*
PVAS4	52.81 kPa (*n = 43*)	61.21 kPa (*n = 41*)	0.110
PVAS7	67.17 kPa (*n = 22*)	64.71 kPa (*n = 17*)	0.638
PTT	85.15 kPa (*n = 49*)	84.93 kPa (*n = 49*)	0.953
VAS at 25 kPa	1.13 (*n = 49*)	0.75 (*n = 49*)	0.216
VAS at 50 kPa	3.48 (*n = 47*)	1.95 (*n = 44*)	0.002*
VAS at 75 kPa	5.48 (*n = 38*)	3.88 (*n = 39*)	0.008*
VAS at PTT	6.50 (*n = 49*)	5.42 (*n = 49*)	0.071
NRS at PTT	6.10 (*n = 49*)	5.37 (*n = 49*)	0.084

[1] p-value of the independent samples t-test performed to compare the average pressure or VAS score of the baseline and conditioning test

PDT = pressure pain detection threshold, PVAS4 = pressure at which the VAS exceeded 4, PVAS7 = pressure at which the VAS exceeded 7, PTT = pressure pain tolerance

3.2.1 Graphing out of trajectories

The trajectories of the VAS were plotted against the pressure, for both the conditions. As an example, case A showed a good CPM mechanism (figure 4A). They reached a similar VAS score for the baseline and conditioning experiments (9.88 vs. 9.80) but did so at vastly different pressures (63.53 kPa vs. 82.35 kPa). In addition, the PDT came much earlier in the baseline condition (4.71 kPa vs. 22.35 kPa). They also rated the baseline condition to be more painful

than the conditioning, with an NRS score of 8 and 7, respectively. The AUC values were 290.62 (abs error = 0.02) for baseline and 211.71 (abs error = 0.02) for conditioning.

Another example, case B, on the other hand, did not show a good CPM mechanism (figure 4B). The PTT they reached was very similar for the baseline and conditioning experiments (96.08 kPa vs. 96.47 kPa, meaning they finished the experiment both times). The VAS score was higher for the conditioning experiment however (6.20 vs. 4.67). Further, the PDT was reached a lot earlier in the conditioning experiment (45.10 kPa vs. 12.94 kPa). Both conditions were rated to be equally painful, at an NRS score of 4. The AUC values were 163.25 (abs error = 0.002) for baseline and 346.69 (abs error = 0.02) for conditioning.

Using the CPM formula, a good CPM was defined as positive, whereas a bad CPM will be negative. For case A this meant that they had a good CPM score of 2.00. Case B comes to a poor CPM score of -1.90.

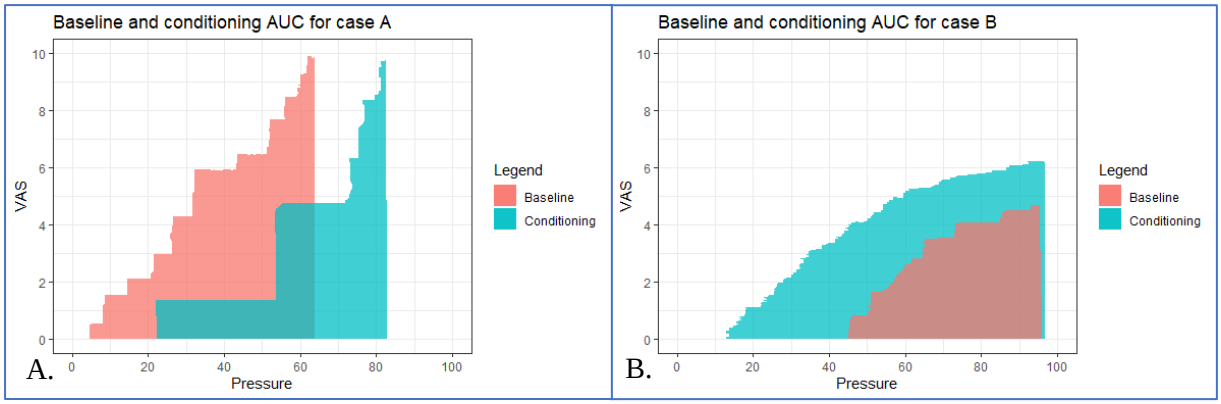

Figure 4: (A) The trajectories and AUC for the baseline and conditioning experiments graphed out for a randomly chosen subject (case A), to illustrate a good CPM mechanism. (B) The trajectories and AUC for the baseline and conditioning experiments graphed out for another randomly chosen subject (case B), to illustrate a poor CPM mechanism.

Figure 5 shows the merged trajectories of all 49 subjects. The average AUC for conditioning was significantly smaller than the one for baseline (160.65 vs. 242.46), indicating that the overall group showed a good CPM. The mean CPM was positive at 0.93, which aligns with what the numbers and graph show.

We found some significant differences between certain groups. For the conditioning experiment, the AUC was significantly smaller for women compared to men (127.2 vs. 213.4, p = 0.048), suggesting that the cuff induces less pain in women. Remarkably, the AUC in the conditioning condition was significantly higher for the individuals that had consumed alcohol, compared to those who had not (200.0 vs. 125.9, p = 0.050). Furthermore, the mean PDT during the conditioning test was significantly higher for the group that had not consumed alcohol (40.7

kPa vs. 26.4 kPa, p = 0.017). In addition, the PTT was significantly higher for the exercising group in the conditioning experiment (91.3 kPa vs. 81.3 kPa, p = 0.042).

The only factor that significantly affected the chance of having a good CPM was being female, which increased the odds of having a good CPM with 8.7 times. This model has an R^2 of 0.29, and a p-value of 0.025. In the linear model, being female was again the only factor that significantly contributes to having a higher CPM score.

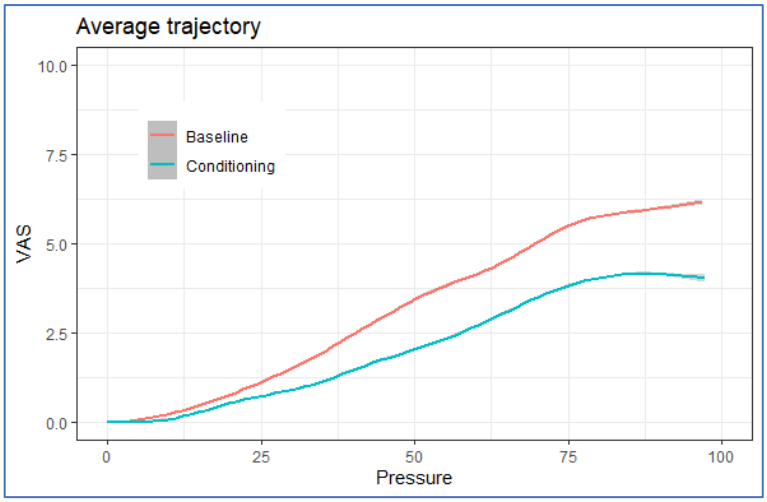

Figure 5: Average trajectories of baseline and conditioning experiments, for all subjects.

4. Discussion

4.1 Hot and cold pain

This study shows another example of the difference in how the human body perceives heat and cold stimuli. Almost twice as many participants experienced the ‘hot’ capsaicin cream to induce discomfort than the ‘cold’ menthol cream did. Men found the menthol cream to be inducing substantially less discomfort than the capsaicin cream, dropping from 26% to 8%. Women on the other hand, showed only a minor drop, from 15% to 11%. Among all subgroups, these are the largest and second smallest drops in percentage. Men gave the capsaicin cream the second highest average NRS score, whereas women did the same for the menthol cream. Similar findings are seen on the other side of the spectrum; women have the second lowest average NRS score for the capsaicin cream, while men have the lowest average NRS score for the menthol cream.

It thus appears that women can handle hot stimuli exceptionally well, whereas men struggle the most with this. In line with these sex differences we found, the models show us that being female reduce the discomfort experienced by the capsaicin cream, as does having a high self-reported pain tolerance. Previous research has largely indicated the opposite.[51–54] However, it must be noted that while these studies were more elaborate than this one, with multiple testing areas, in none of the studies did the number of participants exceed 30. Further, it has been shown that progesterone can decrease TRPV1-dependent pain by acting as an antagonist for Sig-1R, a receptor that modulates calcium signaling.[55] Since progesterone is more present in women than in men, even though it elevates and falls with the menstrual cycle, this mechanism is expected to be stronger in women than in men. When it comes to cold stimuli induced by the menthol cream, women tend to find it relatively more painful, while men appear to handle cold stimuli very well. This is in line with the finding that testosterone is an inhibitor of TRPM8-mediated cold sensitivity, by activation of the androgen receptor on the cell surface.[56] This inhibition is more present in men than in women, which is a possible explanation for the difference in responses to the menthol cream.

Leaving aside some exceptions, neither of the creams induced considerable pain or discomfort in the participants. Both creams generated heavily right-skewed data distribution, making the creation of models more difficult. It therefore seems likely that due to hardly any

individuals experiencing discomfort following menthol cream application to begin with, those models are not significant.

4.2 CPM

This study provides a new method to determine CPM in a user-independent cuff algometry experiment. By using this method to determine the CPM, the entire trajectories are used, rather than just one or a few data points, as is seen in other papers.

4.2.1 Factors that influence CPM

While a plethora of factors have been found to have an influence on the efficiency of one's CPM mechanism, and several came close to being significant in the present work, being female was the only that significantly increased the odds of having a good CPM. Likewise, with the findings of the capsaicin cream, our findings were not in line with previous research.[57–61] However, these other studies used either hot and cold pressors as testing and conditioning stimuli, of which we have shown that there are gender differences too, or they solely looked at PTT, or both. If we had done the latter too, we also would have found that men have a better CPM than women do, but this method ignores the VAS score.

Other factors that had an impact were age, activity level, and alcohol consumption. Curiously, being young, exercising more than twice per week, and having consumed alcohol all decreased the odds of having a good CPM. Since most of the participants (40/49) were under 40 years of age, and thus classified as young, the distribution was skewed. The defining factor however is likely to have been the fact that eight out of the nine participants over 40 had a good CPM. So, this finding is perhaps a result of the small sample size. Regarding the exercise, we found that the difference between the baseline and conditioning tests were not big for those who exercise a lot, as they did extremely well in both. Their ability to handle pain may have influenced their CPM score here. Future research with different stimuli may confirm or deny this. It also appeared that in this study the negative pain-related outcomes of alcohol were stronger. This is not surprising, as we expect those who drink regularly to also drink at the festival, while those who do not drink alcohol often, are more likely to stay away from it during the festival. Future studies exploring the drinking habits of the participants may shine more light on this distinction. There is growing evidence that an impaired descending pain

modulation mechanism is related to chronic pain[62], but this remains difficult to study if medication is used.

4.2.2 Comparison to other CPM methods

Smith and Pedler (2017) used a different method to study CPM. In their study, CPM was calculated as follows: $CPM\ (\%) = (PPT_{conditioning} - PPT_{baseline})/PPT_{baseline} * 100$, where the PPT was determined by asking the participant to press a button at the moment the sensation of pressure became painful.[63] Petersen et al. (2016) defined CPM as the difference between PDT during and before conditioned pain, i.e. $CPM = PDT_{conditioning} - PDT_{baseline}$.[34] The PDT was referred to here as the pressure at which the VAS score exceeded 2 cm (i.e., PVAS2).

In the present study we propose a different method to calculate CPM using the AUC: $CPM = AUC_{baseline}/PTT_{baseline} - AUC_{conditioning}/PTT_{conditioning}$, as the methods described above may induce false positive or false negative results. We replace PPT by PDT in the method used by Smith and Pedler (2017)[63], as we considered the point where the participant started sliding the VAS to be the moment when the sensation of pressure became painful. One subject (case C, figure S4A) had a baseline PDT of 20.39 kPa and a conditioning PDT of 16.08 kPa. Following the method of Smith and Pedler (2017), this subject had a CPM score of – 21%, which was poor. The entire trajectory shows us that they have a lower VAS score at a higher PTT for the conditioning test, however.

In a second example, when the PDT was taken to be the point when the VAS score surpasses two, as is seen in the method used by Petersen et al. (2016)[34], similar discrepancies come forward. Another subject (case D, figure S4B) had a PVAS2 of 34.51 kPa for the baseline test and a PVAS2 of 55.29 kPa for the conditioning test. This gives a CPM score of 20.78, per Petersen et al. (2016). However, despite this, the subject ended up with a higher VAS score in the conditioning test.

In a 2017 paper, Graven-Nielsen et al. determined the CPM by looking at the difference in scores between the baseline and conditioning tests for PDT, PVAS6, PTT, and manual PPT, giving four different CPM scores per subject.[64] This method excels by being able to deduce the efficiency of the CPM mechanism at different pain or pressure scores. This however is also possible in our method, as one can easily alter the lower and upper limits which are used for the integration. The downside is that this method gives multiple CPM scores, which makes it

challenging to summarize the CPM mechanism in one single value. In addition, not everyone reaches a VAS score of 6, so there will be some missing CPM scores.

4.3 Sex differences

Our findings, especially regarding sex, contradict many of the conclusions made by previous studies. The reason for this may be that it is extremely challenging to study differences between sexes without gender biases.[65] Gender norms are being spoon-fed by society to most children, who will learn that men must be tough and women must be sensitive.[66] These expectations, related to both gender and sex, directly influence pain perception and responses.[67] One particular study has shown that if expectations are removed prior to the experiment, there are no sex differences in pain sensitivity, tolerance, and score.[68] Further, it has been shown that men who score high on masculinity were able to tolerate more pain, whereas women who scored high on femininity were more sensitive to pain.[69] These specific examples show how gender expectations can unintentionally affect studies on pain experiences.

These expectations also trickle through into hospitals, or not, when talking about men. One participant in a British study said the following about help-seeking and masculinity: "You don't like to make a fuss because it's a macho thing just to say you're being the strong and silent type … You'll endure it, you can take it. So, if there is something wrong you won't talk to anyone about it. You have to be bed-ridden or half dead before you'll go [to the doctor's]."[70] In clinical studies, men are often seen as stoic, and they will tolerate pain, sometimes even to the point where it becomes harmful.[65] Often, if they do experience pain, they have no desire to talk about it and would rather push it down.[71,72] Pain, especially when chronic, is perceived as a threat to a man's identity – with good reason, as men with chronic pain are seen as less masculine and more feminine.[73]

On the other hand, many studies show that women are more sensitive to pain and more willing to report that they are in pain.[65] As a result of these gender expectations and prejudices, women are more often told to 'be careful' by their health professionals, whereas men are told that 'pain comes with heavy work'.[74] Furthermore, chronic pain in women is more often diagnosed to be 'in the head' rather than somatic, and women tend to feel mistrusted by their health professionals.[65]

With this in mind, and knowing that gender equality has become a large topic of interest in young people, we argue that the results found in this study make a lot of sense. Nowadays,

gender stereotypes are being challenged left, right and center, especially by the younger generation. This influences research too, and urges to reevaluate what we think we know. As most of our sample was born after the start of The Second Feminist Wave in the 1960s[75], it cannot be ruled out that many are aware of gender stereotypes and these thus had a smaller impact on this research.

4.4 Limitations

There is quite some disparity on what cut-off points on a VAS, or NRS for that matter, are defined as mild, moderate and severe pain.[76] We followed one definition described by Boonstra et al. (2014)[49], but using a different one may alter the results. Uniformity is crucial if we want to rely on pain scores more.[76] Regardless of the cut-off points one uses, someone's experiences and personality control for a large part how they rate painful sensations. In addition, someone's own perception on their pain tolerance is often not in line with reality.[77]

The study of CPM has risen tremendously in the past decade or so and is thus fairly young. It is starting to become an important biomarker for pain and is therefore generating a lot of interest. This comes with some troubles. There is very little standardization in the research of CPM, both in the techniques which are used to measure CPM and the methodologies used.[35] This allows us to introduce a new method, which looks at the full picture of the testing and conditioning stimuli. As it is new, it still needs improvements and optimization. Currently, the model predicts the odds of having a good or bad CPM, but tells us little about the efficiency of the CPM mechanism.

5. Conclusion

This study proposes that women show less pain sensitivity upon application of a capsaicin cream than men, and that women have a more efficient CPM mechanism in a cuff algometry experiment. On the other hand, men show less pain sensitivity when menthol cream is applied. We thus show that sex is a discriminating factor for pain perception, both on the level of the PNS, and the level of the CNS.

The proposed method for the calculation of CPM scores can be taken and improved in further research, so that it can be used to create a scale of efficiency. Extending this research will improve data collection and knowledge on which factors contribute to CPM and pain modulation.

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Appendix

Table S1: Demographics for the capsaicin cream

Capsaicin

	Discomfort[1]	Mean NRS	Pain[2]
All subjects (n = 181)	35 (19.55%)	2.29 ± 1.49	10 (5.59%)
Women (n = 114)	17 (14.91%)	2.18 ± 1.44	4 (3.57%)
Men (n = 65)	17 (26.15%)	2.49 ± 1.55	6 (9.23%)
< 40 years (n = 137)	26 (18.98%)	2.32 ± 1.48	7 (5.11%)
> 40 years (n = 44)	9 (20.45%)	2.21 ± 1.52	3 (7.14%)
Low pain tolerance (n = 14)	5 (35.71%)	3.00 ± 2.04	4 (28.57%)
Medium pain tolerance (n = 106)	25 (23.58%)	2.49 ± 1.53	4 (3.77%)
High pain tolerance (n = 60)	5 (8.33%)	1.80 ± 1.10	2 (3.49%)

[1] We denoted an NRS score ≥ 4 to reflect discomfort.

[2] Pain refers to the whether the subjects experienced the creams to be painful.

Table S2: Demographics for the menthol cream

Menthol

	Discomfort[1]	Mean NRS	Pain[2]
All subjects (n = 181)	18 (10.06%)	1.75 ± 1.42	9 (5.03%)
Women (n = 114)	13 (11.40%)	1.86 ± 1.49	7 (6.25%)
Men (n = 65)	5 (7.69%)	1.57 ± 1.30	2 (3.08%)
< 40 years (n = 137)	14 (10.21%)	1.78 ± 1.43	6 (4.41%)
> 40 years (n = 44)	4 (9.09%)	1.64 ± 1.40	3 (6.98%)
Low pain tolerance (n = 14)	3 (21.42%)	2.43 ± 1.95	2 (14.29%)
Medium pain tolerance (n = 106)	11 (10.38%)	1.75 ± 1.38	6 (5.7%)
High pain tolerance (n = 60)	4 (6.67%)	1.60 ± 1.34	1 (1.70%)

[1] We denoted an NRS score ≥ 4 to reflect discomfort.

[2] Pain refers to the whether the subjects experienced the creams to be painful.

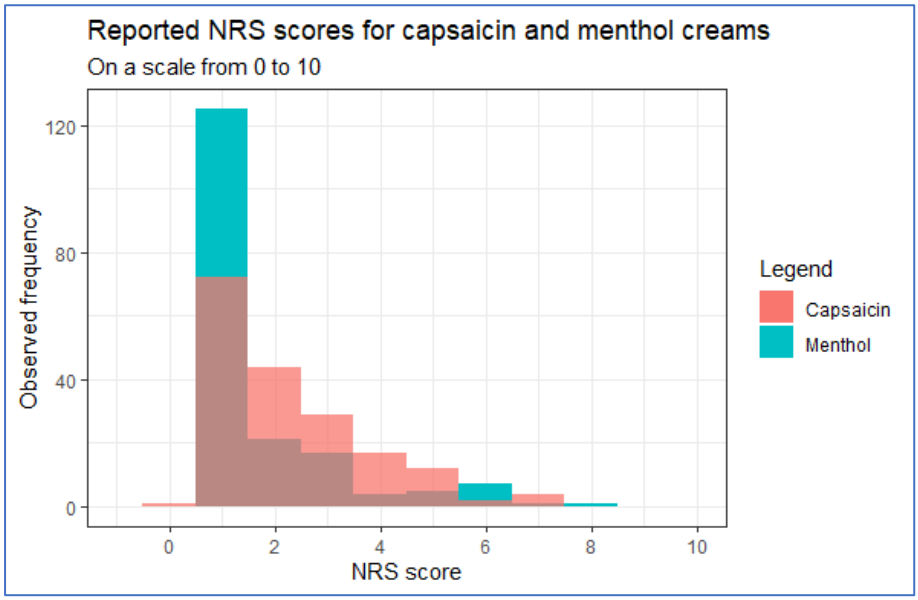

Figure S1: Reported NRS scores for capsaicin and menthol creams.

Table S3: Observed frequency of irritation caused by the capsaicin cream.

Circumference	*0 cm*	*1 cm*	*2 cm*	*3 cm*	*4 cm*	*5 cm*	*6 cm*	*10 cm*	*16 cm*
Count	162	1	5	6	2	1	1	2	1

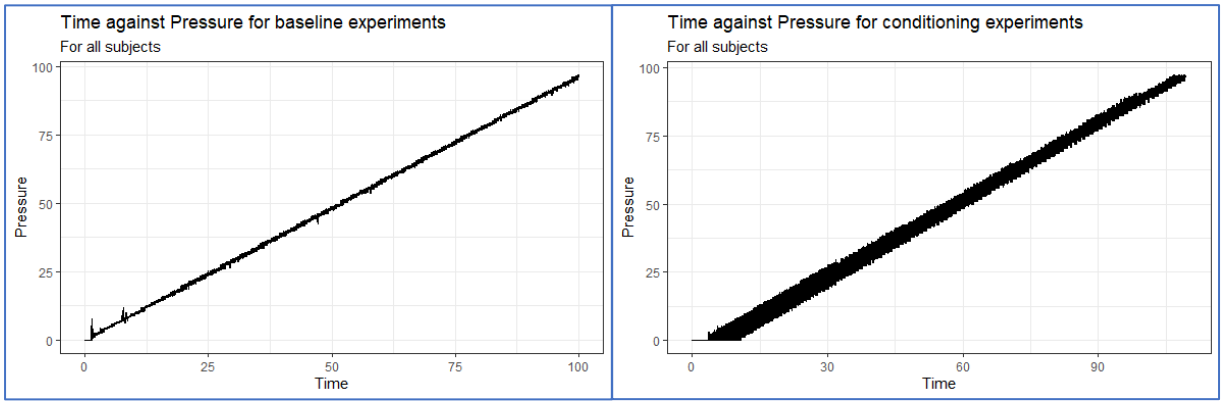

Figure S2: Time against pressure build up for baseline and conditioning experiments.

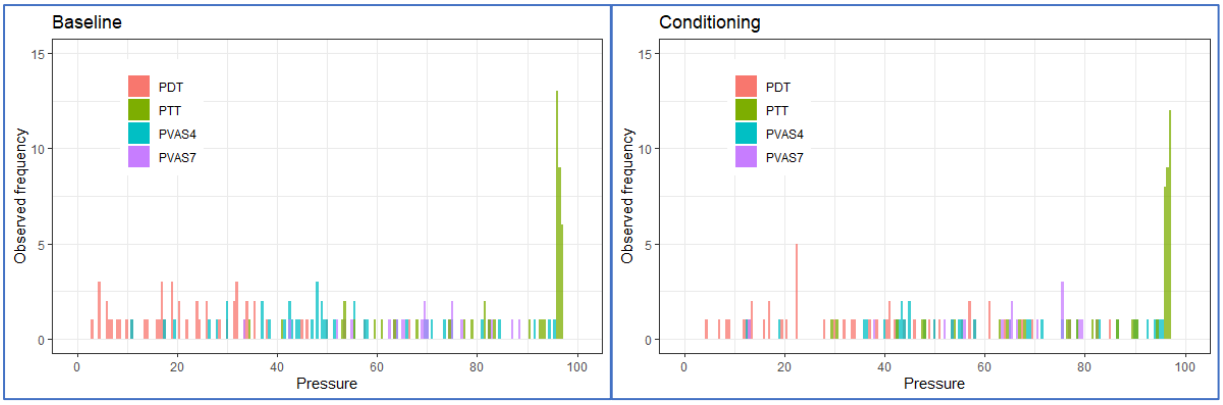

Figure S3: Observed frequencies for PDT, PVAS4, PVAS7 and PTT in the baseline (left) and conditioning (right) experiments.

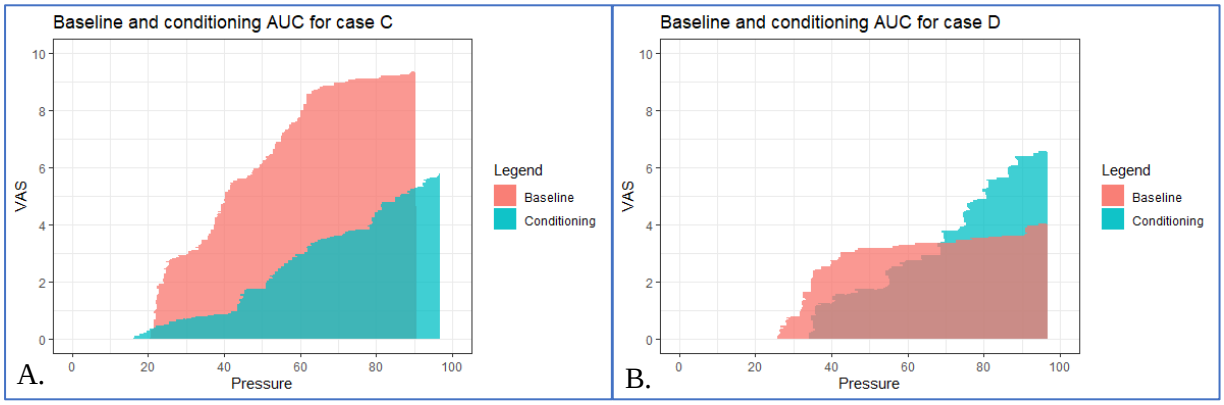

Figure S4: (A) The trajectories and AUC for the baseline and conditioning experiments graphed out for a subject (case C), to illustrate a good CPM mechanism, despite a low PDT for the conditioning test. (B) The trajectories and AUC for the baseline and conditioning experiments graphed out for another subject (case D), to illustrate a poor CPM mechanism, despite a higher PVAS2 for the conditioning test.

Table S4: Overview of mean scores for various subgroups in the CPM experiment.

	Baseline						*Conditioning*						
	Mean PDT in kPa	Mean PVAS4[1] in kPa	Mean PVAS7[1] in kPa	Mean PTT in kPa	Mean VAS at PTT	Mean NRS at PTT	Mean PDT in kPa	Mean PVAS4 in kPa	Mean PVAS7 in kPa	Mean PTT in kPa	Mean VAS at PTT	Mean NRS at PTT	Mean CPM
All subjects (n = 49)	23.38 ± 14.41	52.81 ± 20.81	67.17 ± 13.85	85.15 ± 17.30	6.50 ± 2.67	6.10 ± 1.86	30.84 ± 20.87	61.21 ± 23.34	64.71 ± 17.49	84.93 ± 19.03	5.42 ± 3.17	5.37 ± 2.28	0.93 ± 1.32
Women (n = 30)	24.30 ± 15.94	55.89 ± 20.20	67.09 ± 15.30	84.92 ± 17.70	6.41 ± 2.84	6.23 ± 1.61	35.28 ± 18.57	64.40 ± 21.73	63.73 ± 7.00	82.39 ± 20.44	5.14 ± 3.12	5.43 ± 2.03	1.18 ± 1.19
Men (n = 19)	21.92 ± 11.87	47.27 ± 21.43	67.25 ± 12.70	85.51 ± 17.13	6.66 ± 2.43	5.89 ± 2.23	31.64 ± 24.34	56.89 ± 25.54	65.58 ± 23.81	88.94 ± 16.27	5.87 ± 3.28	5.26 ± 2.68	0.53 ± 1.46
Below 40 years (n = 40)	23.66 ± 14.70	55.52 ± 20.87	66.96 ± 15.16	86.80 ± 16.86	6.41 ± 2.54	6.18 ± 1.65	33.44 ± 20.17	62.41 ± 24.76	61.69 ± 18.93	85.47 ± 19.27	5.42 ± 3.06	5.55 ± 2.23	0.91 ± 1.41
Above 40 years (n = 9)	22.14 ± 13.78	41.27 ± 17.15	67.71 ± 10.75	77.78 ± 18.34	6.91 ± 3.32	5.78 ± 2.73	35.56 ± 24.96	55.82 ± 16.01	74.51 ± 5.47	82.53 ± 18.80	5.45 ± 3.81	4.56 ± 2.46	1.01 ± 0.95
Low pain tolerance (n = 4)	28.73 ± 28.42	70.72 ± 20.03	49.22 ± 22.46	85.79 ± 21.37	6.58 ± 3.88	6.25 ± 2.22	35.30 ± 22.56	24.32 ± 16.64	34.51 ± 30.51	89.41 ± 14.39	5.36 ± 4.50	4.75 ± 2.06	0.33 ± 1.31
Medium pain tolerance (n = 27)	19.46 ± 12.18	47.72 ± 18.13	67.96 ± 13.44	81.74 ± 18.62	6.57 ± 2.64	6.33 ± 1.86	27.78 ± 18.26	61.26 ± 21.87	67.65 ± 12.06	80.73 ± 21.40	5.75 ± 3.20	5.96 ± 2.36	1.07 ± 1.39
High pain tolerance (n = 18)	28.06 ± 12.74	58.85 ± 23.49	70.81 ± 10.09	90.11 ± 13.78	6.39 ± 2.60	5.72 ± 1.84	42.27 ± 22.18	67.84 ± 22.07	70.90 ± 11.72	90.24 ± 14.92	4.95 ± 2.95	4.61 ± 2.03	0.85 ± 1.25
No chronic pain (n = 39)	21.09 ± 12.90	53.92 ± 20.41	65.95 ± 15.14	84.40 ± 17.97	6.50 ± 2.62	6.21 ± 1.84	34.36 ± 21.30	60.26 ± 24.44	63.59 ± 18.91	85.17 ± 18.58	5.38 ± 3.29	5.49 ± 2.26	0.97 ± 1.32
Chronic pain (n = 10)	32.31 ± 17.13	48.76 ± 22.98	71.29 ± 7.92	88.04 ± 14.89	6.54 ± 2.98	5.70 ± 2.00	31.88 ± 20.09	64.76 ± 20.00	69.94 ± 8.52	84.00 ± 21.72	5.58 ± 2.80	4.90 ± 2.42	0.74 ± 1.41
No alcohol consumption (n = 26)	22.29 ± 12.39	51.20 ± 25.08	61.74 ± 15.46	81.27 ± 19.10	6.33 ± 2.54	6.00 ± 2.10	40.69 ± 19.10	63.33 ± 23.03	64.02 ± 21.97	82.53 ± 22.22	4.72 ± 3.35	5.00 ± 2.53	1.19 ± 1.23
Alcohol consumption (n = 23)	24.60 ± 16.60	54.42 ± 15.90	70.92 ± 11.80	89.53 ± 14.16	6.70 ± 2.84	6.22 ± 1.59	26.40 ± 20.52	59.65 ± 24.08	65.32 ± 13.72	87.64 ± 14.63	6.22 ± 2.81	5.78 ± 1.93	0.63 ± 1.39
No sports (n = 31)[2]	24.34 ± 15.19	48.34 ± 20.58	70.39 ± 12.39	84.02 ± 18.96	6.76 ± 2.79	5.90 ± 2.02	29.61 ± 18.40	60.57 ± 23.23	67.60 ± 12.14	81.25 ± 21.40	5.71 ± 3.36	5.42 ± 2.61	0.90 ± 1.41
Sports (n = 18)[2]	21.72 ± 13.21	60.07 ± 19.67	58.56 ± 14.92	87.08 ± 14.31	6.06 ± 2.45	6.44 ± 1.54	40.89 ± 23.39	62.50 ± 24.65	55.29 ± 29.77	91.26 ± 12.11	4.93 ± 2.83	5.28 ± 1.64	0.97 ± 1.20

[1]Not all subjects reached VAS scores of 4 or 7, so the count in the first column may not apply to these.
[2]We took a self-reported activity level of 3 as the cut-off value between sports and no sports.