Supraspinal correlates of learned activation of descending pain inhibition and its variability in humans

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SUMMARY

Chronic pain is the leading cause of disability worldwide. Great interest therefore exists in deciphering not only the causes and risk factors of chronic pain, but also in a better general understanding how the pain sensation itself is generated and modulated. Pain is a complex and highly individual sensation combining both physiological sensation and psychological experience. It is therefore no wonder that a large degree of variability exists in individual pain sensation. Endogenous pain modulation describes processes by which the body itself either increases or decreases painful sensation. A dysfunction in endogenous pain modulation has been closely associated with many chronic pain conditions. Measures of endogenous pain inhibition show large variability in healthy populations and may act as predictive factors for the chronification of pain. In this thesis, I investigate the brain activity related to the activation of endogenous pain inhibition and the factors contributing to its variability in the healthy population.

The first project presents a study aimed to discern the brain areas responsible for deliberate activation of descending pain inhibition. For this we used a longitudinal task-based fMRI design that measured the brain activity of participants before and after healthy participants learned to activate their descending pain inhibition. This was done with a previously validated biofeedback training using the RIII-reflex size as feedback parameter to develop cognitive strategies that activate descending pain inhibition. We constructed a MR-safe setup to evoke and measure the RIII-reflex as well as subjective pain rating concurrently to fMRI acquisition. This is the first study of its kind to utilize a longitudinal fMRI design to investigate brain activity when activating descending pain inhibitory systems while accounting for both between- and within-subject differences in its successful activation. We found that areas associated with pain processing showed decreased response when applying a cognitive strategy. The response to pain decreased further after training. We also found increased activity in the mPFC and thalamus when applying the strategy, suggesting their involvement in activating descending pain inhibition. Overall, we found that our biofeedback training improves willfull activation of descending pain inhibition and variability in training success is reflected in frontal area response to painful stimuli. This corroborates previous findings and theories that cognitive strategies can indeed activate descending inhibition and that frontal cortical areas are crucial in initiating this.

The next two studies in this thesis are published first-author papers in peer reviewed journals. They are concerned with explaining the contributing factors to conditioned pain modulation (CPM) variability. CPM is another measure of endogenous inhibition. Inter-individual differences in CPM are large and can predict both chronic and acute pain. Therefore factors influencing this variability are of great interest clinically. Previous studies have investigated factors including participant age, sex, psychological variables, CPM paradigms and the intensity of the painful

stimuli used. Typically, these studies used cross-sectional designs with conflicting results. We pooled current repeated measures data with previous repeated and cross-sectional CPM studies from our lab to investigate the effect of these variables on CPM variance in a large study cohort. Estimating the variance explained by age, sex, depression, anxiety, catastrophizing, CPM paradigm, conditioning stimulus intensity and "residual unexplained" inter-individual effects demonstrates that the unexplained inter-individual effect accounts for approximately three times more variance than all other effects combined. We also found that only in a repeated measures analysis does the conditioning stimulus intensity significantly predict the CPM effect, not in cross sectional studies. Our results complement the existing literature on CPM by showing that a large part of inter-individual variance remains unexplained when taking into account known individual parameters. This variance is indeed so large that it drowns out potentially significant effects of commonly accounted for factors in cross-sectional designs. We suggest that future studies on CPM influences utilize a repeated-measures design to account for these large individual differences.

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LIST OF ABBREVIATIONS

ACC	anterior cingulate cortex
CPM	conditioned pain modulation
CS	conditioning stimulus
dlPFC	dorsolateral prefrontal cortex
DMN	default mode network
EA	endogenous analgesia
fMRI	functional magnetic resonance imaging
HRF	haemodynamic response function
LC	locus coeruleus
mPFC	medial prefrontal cortex
MRI	magnetic resonance imaging
MRIQC	MRI quality control (toolbox)
PAG	periaqueductal grey
PFC	prefrontal cortex
pgACC	perigenual anterior cingulate cortex
rACC	rostral anterior cingulate cortex
RVM	rostroventral medulla
sgACC	subgenual anterior cingulate cortex
SRT	spinoreticular tract
STT	spinothalamic tract
TS	test stimulus
VPL	ventral posterolateral nucleus (of the thalamus)

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1 GENERAL INTRODUCTION

The international association for the study of pain (IASP) recently revised the definition of pain as "An unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage"[1]. This improves upon the previous definition as it defines pain as separate from nociception, i.e. the physiological response of the sensory neurons, and highlights the importance of psychological aspects in the sensation. Pain most commonly presents as a result of injury or disease and usually disappears after healing. Transient pain is a useful sensation, preventing us from engaging in behaviours or situations which result in physical harm, and may confer evolutionary advantages[2,3]. The problem with pain begins when it is no longer transient and becomes a permanent fixture in a person's life.

Chronic pain affects approximately 10% of people worldwide[4]. It presents a major disease burden on the population and chronic pain is among the leading causes of disability[5]. Pain disorders vary widely in their clinical presentation and etiology[6]. Great interest has therefore been put into understanding not only the causes of these pain disorders, but also into how pain itself is processed.

As elucidated from the IASP definition of pain, pain is a complex sensation consisting of both physiological and psychological components. It is a highly subjective experience that can change, also within an individual, with various cognitive factors such as expectation, anxiety, and experience. Although the basic anatomy of pain reception and transduction has already been understood, the transition from nociception to pain sensation and its cognitive correlates remain subject to much debate.

1.1 Anatomy of pain perception

1.1.1 From nociceptor to the brain: ascending pain pathways

Peripheral nociceptors

Pain is sensed by multiple subtypes of specialized peripheral neurons: the nociceptors. These are found throughout the body including the skin, muscles, joints, teeth and viscera[7–9]. Primary nociceptors consist of C-, Aδ, and Aβ-fibres, with C fibres being the most common[9,10]. Nociceptors vary in transmission speed, the stimulus they respond to (heat, cold, or mechanical

pain), specificity (small vs. large receptive areas) and neurochemistry[9,10]. These nociceptors transmit signals in an all-or-none fashion and are usually silent. Activation occurs once a noxious stimulus is detected by free nerve endings, transmitting the signal towards the dorsal root ganglia of the spine. Different parts of the pain sensation are thought to be transmitted by the different fibre types. For instance, the initial sharp pain sensation upon injury is transmitted by the faster Aδ-fibres, while C-fibres transmit a slower and less localized "second" pain[11].

Spinal transmission of nociceptive inputs

In the dorsal horn of the spinal cord, synapses in laminae I-V connect the primary nociceptor to secondary nociceptive neurons which send long ascending projections towards the brain. Of the secondary nociceptors, nociception-specific high-threshold secondary neurons lie more superficially (lamina I), while wide dynamic range neurons (WDR), receiving input from multiple afferent nociceptors, lie in deeper spinal laminae[11]. From the dorsal horn of the spine, two ascending pain pathways have been described: the spinothalamic (STT) and spinoreticular tracts (SRT)[12]. These tracts differ both in their targets, ascending paths, and proposed functions. The STT ascends through the contralateral white matter of the spinal cord to the thalamus, specifically the ventral posterolateral nucleus (VPL), medial and intralaminar nuclei. As this tract is somatotopically organized, it allows for localisation of the painful stimulus[13]. It is thought to primarily transfer information on the nature of the stimulus (i.e. where, what kind, how strong). The SRT on the other hand, does not cross the midline and is not somatotopically organized. It terminates in the medulla of the brainstem, with further connections within the brainstem, cerebellum and midbrain periaqueductal grey (PAG)[13]. It is thought to be involved in conveying information to higher evaluative regions.

1.1.2 Supraspinal processing of painful stimuli

All areas involved in pain processing beyond the spinal cord are part of supraspinal pain processing. Supraspinal processing of nociceptive stimuli involves a diffuse network of cortical and subcortical areas[14,15] (Figure 2). This network has been termed the "pain matrix" and involves primarily S1/S2, insula, thalamus, anterior cingulate cortex (ACC) and the prefrontal cortex (PFC)[14]. However, other areas have also been described as active in response to nociceptive input, such as M1, hypothalamus, cerebellum and basal ganglia. Due to individual differences in brain activation patterns, the full network responsible for pain evaluation is difficult to classify. Based on brain activation studies, the sensation of pain has been divided into two broad aspects: the somatosensory aspect and the affective-evaluative aspect of the stimulus[15,16].

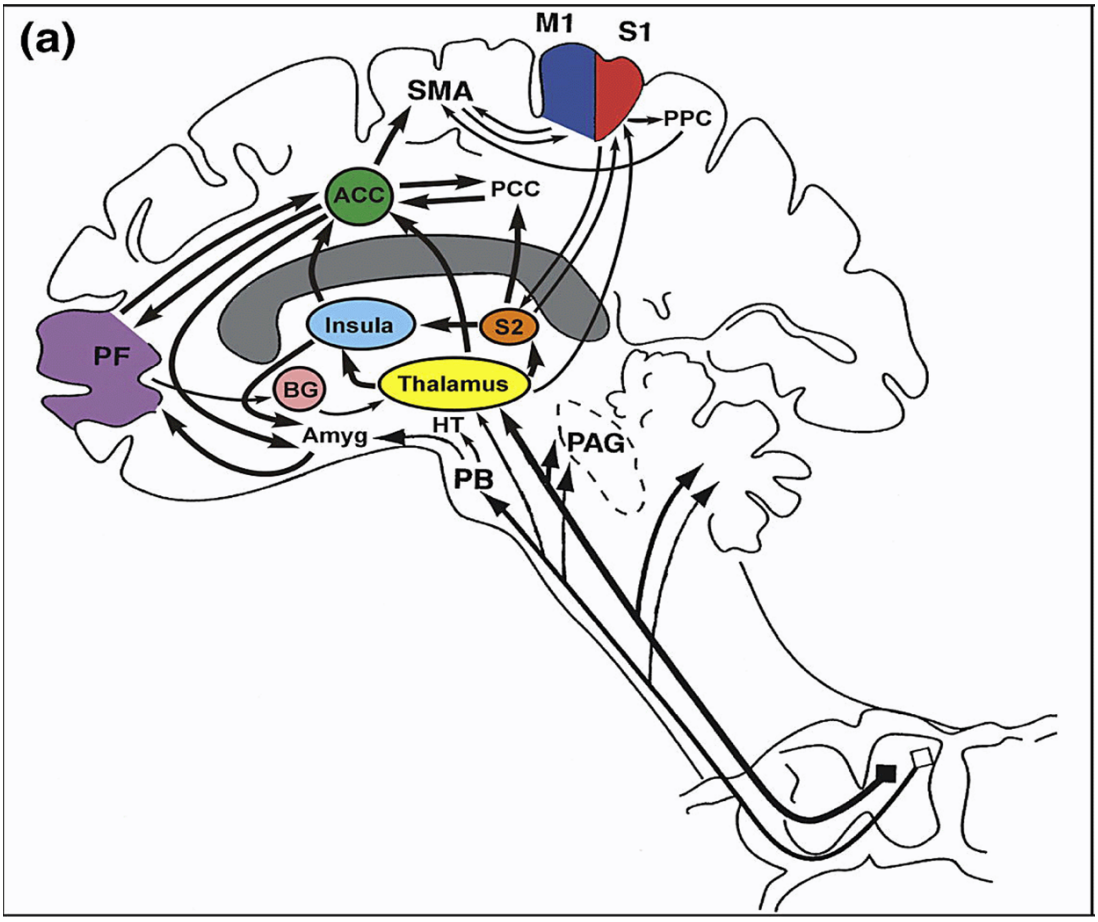

Figure 1: Areas involved in pain sensation. Coloured areas are consistently active in response to painful stimuli and are considered core constituents of the "pain matrix". Image modified from Apkarian et al. (2005)[14], first published online 11 January 2012 and reproduced here with the permission of John Wiley & Sons.

Somatosensory evaluation: the where, when, and what

Nociceptive inputs into the thalamus are transmitted to somatosensory areas (via the VPL), insular cortex and the ACC (via medial nuclei)[17]. There is evidence suggesting that S2 is the first area to receive input from the thalamus before continuing to S1[14]. In S1, somatotopic organization of the signal is conserved and allows for localization of the painful stimulus. Together, the S1, S2, insula and ACC show a graded response to painful stimulation of different intensity[18]. This suggests that response to more painful stimulation is encoded directly in the magnitude of the neural response.

Not just location and intensity, but also the nature of the stimulus is encoded. Differences in activation loci within insula, ACC and somatosensory cortices suggests that different pain modalities (e.g. heat vs. pressure pain) can be encoded by sub-regional activation differences, although the general activation pattern remains[14]. In insula and ACC, only some subareas are responsible for processing the purely somatosensory aspect. Stimulation of the posterior insula

has been shown to elicit a painful sensation, suggesting that the sensory-discriminative processing of pain occurs here[19]. Similarly, the ACC displays a functional subdivision with the anterior ACC commonly active in painful sensations.[20].

Affective-cognitive evaluation: emotions and valuation

Pain is more than just a physical sensation and carries value beyond simply "how badly does it hurt". It is common to describe painful sensations both in terms of pain intensity and pain unpleasantness[21], with intensity describing the physical part and unpleasantness describing how the stimulus is valuated cognitively and emotionally. Affective valuation is a key component of pain sensation and the integration of painful experiences. Context and internal psychological state are important factors which shape our perception of pain. Brain areas involved in the cognitive-affective evaluation of pain differ from the areas involved in somatosensory evaluation, although some overlap remains.

Investigating the somatosensory or the affective-cognitive component of pain alone has proven to be a difficult undertaking for two reasons: first, pain intensity (somatosensory) and pain unpleasantness (affective) are highly correlated. Second, the affective-cognitive valuation of painful stimuli depends on the integration of information in and from overlapping brain regions. The ACC and insula in particular seem to play a dual role in pain perception, processing both somatosensory and affective components and most likely being sites integrating both dimensions. The ACC is a key region associated with the affective component of pain. The posterior section of the ACC, for example, shows a changed response when pain unpleasantness is manipulated[21]. Similarly, the anterior insula has been shown to encode affective dimensions of pain[22].

Internal factors like mood, expectations and previous experiences, together with external factors such stress or distraction influence how much pain affects us without a change in nociceptive intensity. Studies investigating the effect of mood on pain have shown the anterior insula and the PFC to be involved in mood dependent changes of pain perception[23]. Additionally, the locus coeruleus (LC) with its connections to affective processing regions like the ACC and PFC likely also plays a role in the affective processing of pain[24]. Brain regions involved in emotional regulation and reward in general, such as the amygdala, nucleus accumbens and the basal ganglia are inconsistently activated in pain studies[14,21,23], suggesting that these regions only have partial involvement in processing pain. However, inputs from the amygdala, nucleus accumbens and basal ganglia to higher brain regions may contribute to the emotional processing of painful stimuli. Affective evaluation of painful stimuli therefore seems to involve a diverse network of brain areas that may be active depending on the context of the painful stimulus.

Pain is also affected by cognitive processes beyond emotional valuation. This integration has been shown in experiments altering the context in which pain is perceived or by changing its internal context. Cortico-limbic interactions evaluate pain with respect to previous experiences, internal state, and environmental context[21]. Catastrophizing can increase subjective pain[25], while reappraisal of pain may decrease it[26]. Environmental context such as stress can lead either to stress induced analgesia[27] or hyperalgesia[28]. The effect of attention to pain shows its importance as well, as distraction from pain decreases the subjective pain experience[29,30]. Pain is not only a combination of nociceptive and affective information, but an amalgam of effects embedded in the context of previous experience and current mental state.

To add to the complexity, areas such as the ACC and PFC are not only involved in the affective-cognitive aspects of pain, but are tightly associated with the modulation of pain at the spinal level[15]. In other words, these regions can change the strength of the nociceptive stimulus that reaches supraspinal pain processing regions. The question therefore poses itself: is the affective-cognitive processing of pain purely a part of the pain sensation, or is it a dynamic system that can modulate its own nociceptive inputs in addition to evaluating pain?

1.2 Endogenous analgesia and descending pain inhibition

The sensation of pain can be modulated in a variety of ways. These modulations can be either inhibitory (i.e. decreasing pain) or facilitatory (i.e. increasing pain) in nature. Our perception of pain is modulated cortically via expectations, attention/distraction, positive and negative thinking or emotions, as described above. Changes in pain perception also occur on a spinal level. Pain perception can be facilitated by spinal sensitization, where an increased reaction of spinal interneurons and/or ascending neurons to painful stimulation is observed. This can occur when painful stimuli are given in short succession, called “wind-up” in animals or temporal summation of pain in humans, or when normally non-painful stimuli become painful due to a sensitizing stimulus applied to another (usually adjacent) part of the body, a sensation called secondary hyperalgesia[31].

Of great interest to the field of pain and pain treatment is endogenous analgesia, which is the body’s ability to reduce pain by itself. Individual differences in endogenous analgesia have been proposed to be predictive of post-operative pain and potential pain chronification[32,33]. Indeed, a dysfunction of endogenous pain modulation is thought to be the common denominator of chronic pain states[34,35]. Phenomena such as stress-induced and placebo analgesia, which reduce pain through aversive stimuli[27] or belief of pain reduction alone[36], and conditioned pain

modulation (see 1.2.3) have been used measure endogenous analgesia. The driving mechanism behind these effects involves the descending pain inhibitory pathway, named so because the modulation is initiated supraspinally and travels down to act on the synaptic transmission of nociceptive input at the level of the dorsal horn. Endogenous analgesia may also occur supraspinally, when the cortical modulation of painful stimuli occurs without a change of spinal nociception, although this mechanism is poorly studied as of yet[37].

1.2.1 The descending pain inhibitory pathway

Supraspinal origins

The key neural regions involved in descending pain inhibition are situated in the subcortical regions of the brain. Here, two pathways modulate spinal nociception originating either from the locus coeruleus (LC) or the PAG-RVM axis. Of the two, the PAG-RVM system has received significantly more attention, probably because it relates more directly to higher cognitive control through PAG-cortical connections[38,39].

The LC is the primary noradrenergic center of the central nervous system and among its various important roles such as in arousal, is also involved in both pain sensation and pain modulation. LC is active when pain is present and in terms of modulation, it engages in feedback inhibition[40], leading to a decreased in perceived pain. A direct link from LC to spinal dorsal horn neurons has been shown using neural tracer methods in both rodents[41] and monkeys[42]. In the dorsal horn, the release of noradrenaline inhibits pain transmission via both pre- and postsynaptic action, and via activation of inhibitory interneurons[40].

Electrical stimulation or application of opioids to the PAG has been shown in animals to elicit an antinociceptive effect[30]. This effect translates to humans; electrical stimulation of the PAG results in pain relief. Reversal of the relief by naloxone treatment further confirms that the PAG elicits its effect via opioid signalling[43]. The PAG is known to have close ties with the RVM. Descending fibres of the inhibitory pathway stem entirely from the RVM[44,45]. Two distinct cell populations within the RVM, ON and OFF cells, are responsible for descending inhibition and facilitation respectively[30]. PAG stimulation of OFF cells is believed to be the mechanism behind the antinociceptive effects of PAG stimulation. The descending projections of OFF cells terminate in the dorsal horn, where they inhibit nociceptive transmission via serotonin release onto the synapse between primary and secondary nociceptive neurons.

Cortical influences on descending pain inhibition

Multiple cortical areas show top down control over pain perception[15,30,46]. The fact that pain can be modulated by various cognitive paradigms and expectations about pain demonstrates that higher cognitive processes are able to influence our perception of pain. They may modulate it both via changes in cortical reaction to pain as well as activating descending pain inhibition. Changes in PAG connectivity have repeatedly been shown to be indicative of changes in pain states[47–49], suggesting that cortical areas influence descending pain inhibition via the PAG (Figure 2).

Imaging studies have given insight into the supraspinal areas involved in descending pain inhibition. Modulatory paradigms such as reappraisal[26], distraction[39,50], placebo analgesia[51,52] and mental imagery[53] have all been shown to change both brain activity and decrease pain intensity. Here, brain activity in the affective parts of the ACC and lateral parts of the PFC increased when participants engaged in these paradigms. Somatosensory-evaluative areas such as thalamus and insula showed a decreased response to painful stimulation. Currently, both PFC and ACC are considered the driving areas of cortical influence. However, due to great variability in methodology, study populations (comparing clinical and healthy) and inter-individual differences in brain activity, discerning a mechanism of action has proven difficult. Connectivity between the PFC or ACC and the PAG has been established in multiple studies to be tightly linked to descending pain inhibition[51]. It then seems that top-down influence may be initiated via the PFC and realized through a PFC-ACC-PAG axis activating descending inhibition. The LC can also initiate descending pain inhibition via noradrenergic paths. Connections from the PFC to the LC[54] may elicit pain inhibition using the same paths previously shown to be active during feedback inhibition in response to painful stimulation[40].

In addition to ACC and PFC, areas like the thalamus and the amygdala have been implicated in descending pain inhibition, although their involvement has been shown less consistently. Loss of hypo- or analgesic effect upon chemical inhibition or lesions of the central amygdala have implicated it in pain inhibition[55]. The exact role of the thalamus in pain inhibition is still unclear, but the nucleus submedius (Sm) seems to play a role via its connections to the PFC and the PAG[56].

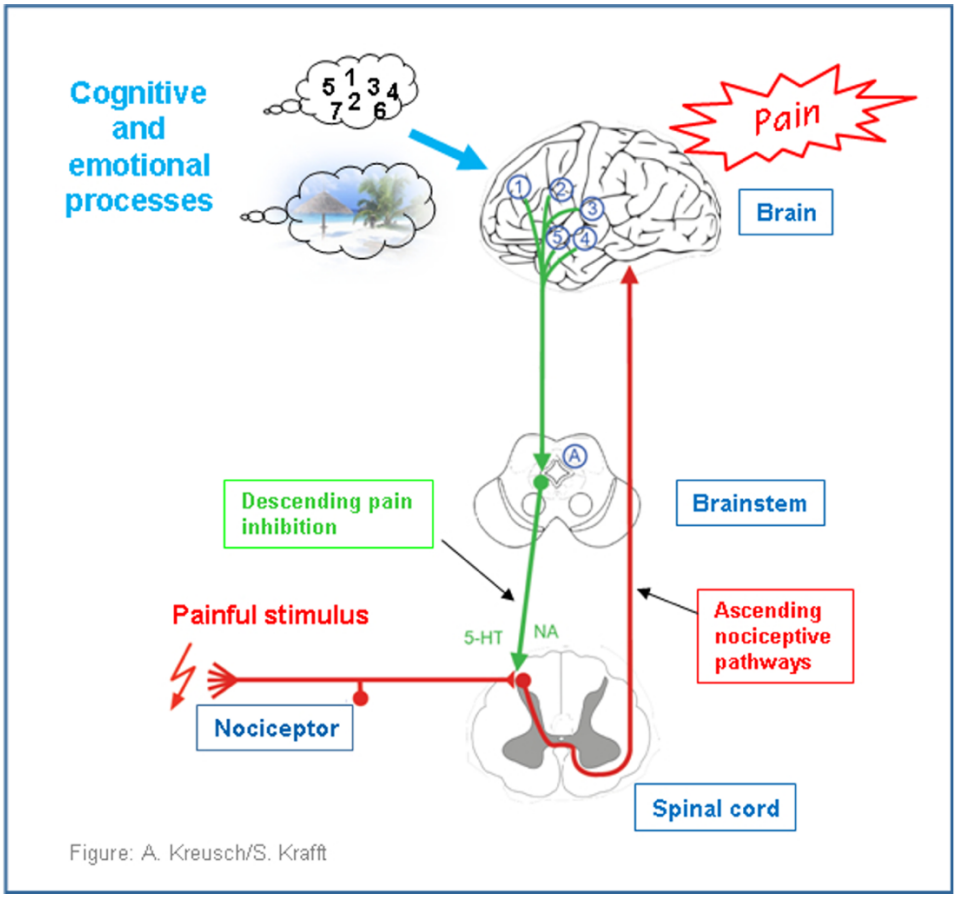

Figure 2: Sketch of the descending inhibitory pathway. Cortical regions including the PFC (1), ACC (2), and subcortical structures such as thalamus (3), hypothalamus (4) and amygdala (5) activate the PAG (a), which connects to the RVM in the brainstem. From the rvm and the lc (not explicitly shown here), long descending fibres release sertotonin (5-HT) and noradrenaline (NA) onto the dorsal horn of the spine, inhibiting pain transmission from primary to secondary nociceptor. Figure reproduced with permission of the authors.

Individual variability in endogenous inhibition

The correlation between brain activity and pain reduction suggests that variability in the pain reduction can be accounted for by variability in brain activity. Indeed, individual differences in brain activity as well as connectivity have been proposed as predictors for the effectiveness of endogenous analgesia and the resulting chronification of pain[51,57,58]. It has been suggested that certain individuals are more capable of activating their endogenous pain inhibition than others when using a variety of cognitive strategies[53], such that indeed baseline differences would exist not only in our innate pain inhibition, but also in our ability to explicitly or implicitly activate it. Not only baseline differences may account for this variability, but also the best choice of strategy in reducing pain at any time point may be different. We previously found that participants were most successful at activating descending pain inhibition utilizing different cognitive strategies[59].

Finally, variability in the brains reaction to painful stimulation itself remains as a factor that makes group-level inferences difficult.

Variability in the pain system therefore remains a major obstacle in finding a common mechanistic explanation of pain inhibition. It is also a major point of scientific and clinical interest[60]. For example, it was shown that unimodal areas involved in pain, such as S1, show less individual variability in response to pain than higher order integrative regions like the PFC[61], indicating that it may have a less conserved reaction to pain. Signal variability in pain modulatory areas is also increased in healthy control compared to migraineurs[62], suggesting that variability in frontal areas could be indicative of a healthy endogenous modulatory system. We have also seen that within a single subject, trial-by-trial fluctuations in pain decrease relate do differential brain activity[53]. Hence, we see that variability in brain activity related to pain and pain inhibition can stem from both a between- and within-subject level. Differences in individual pain inhibitory systems may prove to be an attractive avenue of investigation to determine individual risk factors for pain chronification and understanding the contributions of brain areas to these individual aspects of the pain sensation are an important component thereof.

1.2.2 Dysregulation of pain modulation in chronic pain

Previous work comparing chronic pain patients with healthy controls has brought about a large body of evidence that points towards a dysregulation of endogenous pain modulatory pathways and perception of pain in chronic pain patients. Understanding how dysregulation of endogenous pain systems relate to the development and presentation of chronic pain therefore presents one way of understanding the mechanisms behind pain modulation. Chronic pain patients display a significantly stronger reaction to temporal summation of pain, evidence of increased spinal sensitization to nociceptive input in these patients[63,64]. Additionally, chronic pain patients show generally decreased brain activity in response to painful stimulation[14]. Much of the differences involves activation decreases in primary pain sensory areas such as S1/S2 and increases in frontal areas and their connections to centres of descending inhibition[14,23]. The positive correlation between ACC activity and reported pain intensity in healthy subjects[65,66] also disappears in chronic pain, suggesting some sort of uncpoupling[14]. They also exhibit altered brain connectivity of areas like PFC, insula and ACC and altered grey matter volumes in cortical and subcortical areas [57,67–70]. Grey matter changes differ between chronic pain conditions, but decreases in the insula seem to be a common denominator[71]. Default mode network connection to prefrontal and insular cortices has been shown to be dysregulated in chronic pain, further pointing towards dysregulated connectivity[72,73].

Nonetheless, these findings demonstrate that changes in brain anatomy and function occur in chronic pain states. These changes correspond to reduced pain thresholds and increased sensation of pain[74,75]. Patients also show an altered relationship to pain, especially the affective impact of pain, as be seen by increased catastrophizing[76] and comorbid affective disorders[77]. The question remains whether these differences are brought on by chronic pain or are causative of it.

1.2.3 Measures of endogenous pain inhibition

In order to draw meaningful inferences on descending pain inhibition, one must employ methodologies which measure spinal nociception in a more objective manner. In humans, measures of spinal nociception and its modulation can only be achieved indirectly. Two methods commonly employed are conditioned pain modulation (CPM), a psychophysical paradigm whose equivalent in animals has been shown to directly affect spinal nociceptor firing rate, and measurement of the nociceptive flexor (RIII) reflex, a spinal reflex loop initiated by painful stimulation. These two paradigms are able to give insight into changes of spinal nociception either via its direct transmission strength (as with the RIII reflex size) or via its reduction of perceived pain (as with CPM).

Conditioned pain modulation

"Conditioned pain modulation (CPM) measures the component of human endogenous pain inhibition underlying the "pain inhibits pain" phenomenon, based on a noxious test stimulus (TS) being perceived as less painful if presented in combination with a painful heterotopic conditioning stimulus (CS)"[78]. CPM is believed to be the psychophysical equivalent of "diffuse noxious inhibitory control ", an extensively studied phenomenon of descending nociceptive inhibition in animals[79]. From electrophysiological evidence we know that the application of a heterotopic painful CS inhibits the response of spinal neurons to the TS[80,81].

Differences in CPM have been shown to be predictive of acute post-operative and chronic pain[32,33]. Furthermore, CPM is dysregulated in a variety of chronic pain conditions[82], further supporting the theory that it reflects human pain inhibitory systems. Interestingly, a proportion of the population does not show CPM, and another part of the population exhibits an increase, rather than a decrease in TS pain intensity[83] during the CPM paradigm. As CPM is used in clinical practice to measure endogenous inhibition, it is of great interest to elucidate the mechanisms and influences on CPM measures.

Previous studies have investigated the influence of various factors such as sex, age, CPM methodology, and psychological variables on the strength and direction of the CPM effect, with conflicting results. Some studies suggest that age[84,85] or sex[86,87] influence CPM magnitude, while others find no such relationship[88,89]. Similarly, methodological concerns, such as stimulus

intensity, have shown either no influence[90–92], or suggested some relationship with CPM magnitude[93–95]. Additionally, various CS and TS modalities, such as pressure, heat, cold or electrical pain, are used in different experiments, adding to the heterogeneity of the research body. It remains unclear if the variability of CPM responses is entirely due to differences in endogenous analgesia, or if other, controllable factors contribute to it.

Measures of spinal nociception in humans

Investigation of descending pain inhibition in humans poses some limitations in comparison to animal studies. For example, direct electrode recordings of the dorsal horn or supraspinal areas is impossible without highly invasive methods, making it not feasible for general human pain studies. However, only changes in spinal nociception are objective evidence for descending pain inhibition. Therefore, an adequate proxy measure of spinal nociception in humans must be used to investigate changes in dorsal horn synaptic transmission resulting from descending pain inhibition.

One of these measures in humans is the nociceptive flexor, or RIII, reflex. Initially described in animal models[96], it is a polysynaptic reflex of the ipsilateral flexor muscles in response to painful stimulation. The reflex has been long-established in the pain field and is usually evoked in the lower limb[97]. It can be evoked by painful electrical stimulation of the retromalleolar path of the sural nerve, which evokes a polysynaptic reflex loop in the dorsal horn activating ipsilateral motor neurons. This results in a contraction of the ipsilateral biceps femoris. This muscle contraction can be quantified by measuring the EMG response and integrating the rectified signal 90ms-150ms post-stimulus[98]. The RIII-reflex is directly related to subjective pain perception. Therefore is presents itself as a useful objective measure of spinal nociception and evoked pain in general[99].

Due to the necessity of electrical stimulation and measurement, its use in the MRI environment has been very limited[100,101]. The main reason for this is electromagnetic interactions between the MRI scanner and the stimulation setup/EMG recording. Sending electrical signals through the magnetic field of the MRI may induce artifacts in the MR image if not correctly filtered. In response, the fast fluctuations of local magnetic field can induce electrical current in the conductive electrode, producing undesired and uncontrolled stimulation unless appropriate electrical resistors are incorporated into the electrode. Similarly, the magnetic fluctuations of the MRI cause large artifacts in the EMG recording (Figure 3), making post-processing and artifact correction algorithms necessary to evaluate the RIII-reflex in MRI experiments. Nonetheless, construction of an MR-compatible setup to stimulate and record the RIII reflex as a proxy measure of spinal nociception while measuring BOLD signal via fMRI would allow us to quantify both brain activity and spinal nociception simultaneously on an individual and trial-by-trial basis.

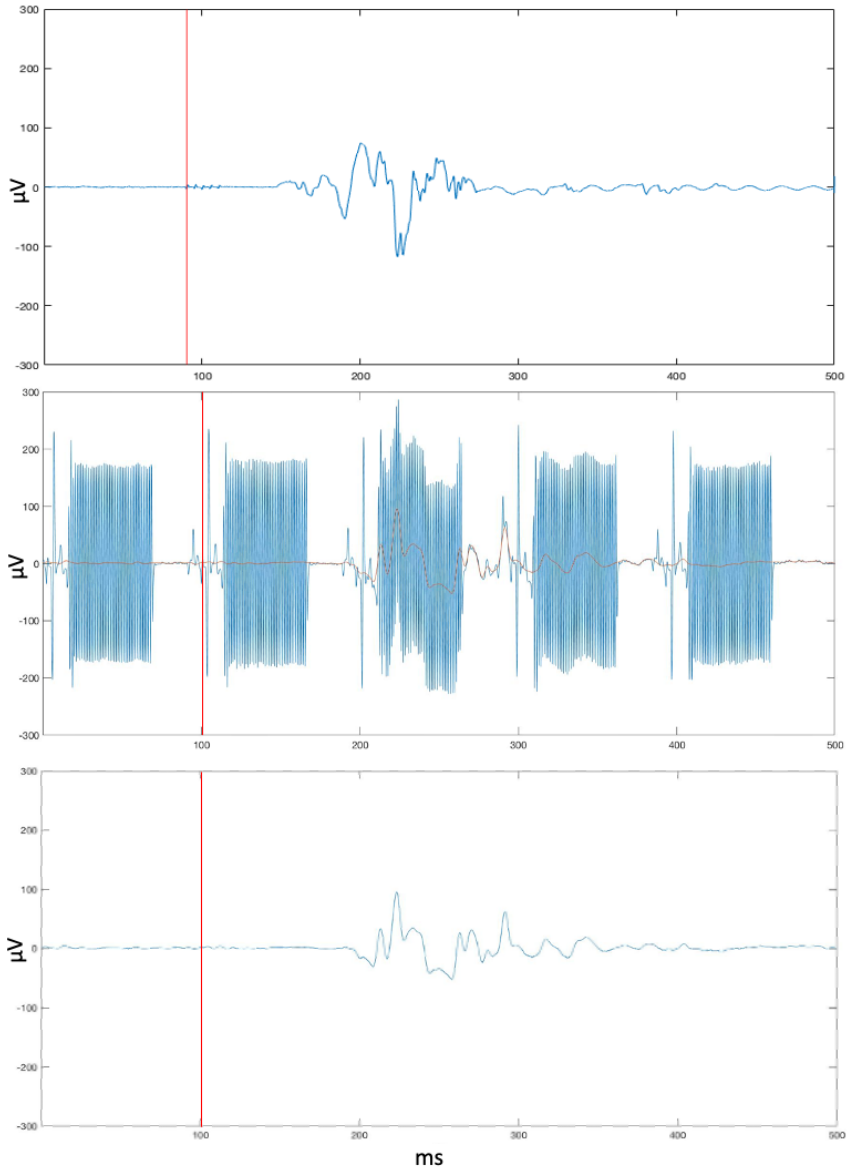

Figure 3: Example of a single RIII reflex evoked outside and inside the MRI. Painful electrical stimulation (red line) at time t = 90ms during feedback training and at t = 100ms during MRI sessions. **Top:** EMG trace outside of the MRI **Middle:** raw MRI-EMG trace including MRI artifacts. **Bottom:** post-processed MRI-EMG trace using artifact correction software.

1.3 Aim of the thesis

The goal of my thesis was to use two different measures of nociception to investigate descending pain inhibition. The aim was to understand two central aspects: First, what is the relationship between brain activity leading up and in response to painful stimulation and descending pain inhibition as quantified by a physiological measure of spinal nociception and subjective pain ratings? Furthermore, how does this brain activity change when people learn to willingly activate their descending pain inhibition? Second, what are the contributing factors to the individual variability in measures of descending pain inhibition and do these factors explain all the individual variability we see? In order to answer these questions I conducted three studies: one to investigate the former, and two to investigate the latter.

First, I investigated the brain areas involved in conscious activation of the pain inhibitory system. For this, I designed and built an MRI-compatible setup for electrically stimulating and recording the RIII-reflex during functional MRI. With this setup, I conducted the first longitudinal fMRI study on descending pain inhibition utilizing the previously established RIII-reflex feedback paradigm to teach participants to willingly activate their descending pain inhibition. With this paradigm we could directly compare brain activity between participants on a trial-by-trial basis and relate this to the degree of spinal nociception, as measured with the RIII-reflex. We could also look for group-level changes in brain activity related to training as well as how individual differences in the ability to activate descending pain inhibition is related to brain activity.

Second, I elucidated which factors can explain the degree of individual variability in CPM magnitude, another measure of descending pain inhibition. Here I wanted to investigate how fixed factors such as age, sex, and psychological scores affect CPM magnitude, and how much of the individual variability is explained by them. Additionally I examined whether methodical concerns such as CPM paradigm or stimulus strength affect the CPM magnitude. To do this I examined the effect of these fixed factors in both cross-sectional and repeated-measures designs. Repeated-measures investigation allowed me to show that the vast majority of individual differences are not explained by any of the above factors, and that the lack of effect seen by them in cross-sectional investigation likely stems from them being overshadows by the vast baseline differences in CPM magnitude. This expands on previous investigations trying to determine the causes for inter-individual variability in measures of endogenous analgesia. It further adds to the existing CPM knowledge by suggesting future investigation employ a repeated-measures design to account for underlying individual differences.

2 RESEARCH CHAPTER

This PhD thesis consists of one detailed manuscript (chapter 2.1, first authorship) and two peer-reviewed published papers (chapters 2.2 and 2.3, both first authorship).

A longitudinal task-based MRI study investigating the changes in brain activity after participating in a real-time biofeedback training aimed at teaching participants to activate their endogenous pain inhibitory system is presented in the first manuscript (see 2.1). The first publication investigates how much of variability in CPM, a measure of human endogenous analgesia (see 2.2), is explained by inter-individual differences. As a follow-up study to the first publication, the second publication expands the investigation of influences on CPM variability by examining the effect of common psychological variables measured in clinical and research practice.

2.1 Longitudinal changes in human supraspinal pain processing after RIII-feedback training to improve descending pain inhibition

Graeff P., Ruscheweyh R., Virginia L. Flanagin 2022. (in preparation)

The manuscript "Longitudinal changes in humans supraspinal pain processing after RIII-feedback training to improve descending pain inhibition" was prepared by Philipp Graeff under supervision of Virginia L. Flanagin and Ruth Ruscheweyh.

Summary

This manuscript aims to determine which areas of the brain are active when deliberately activating descending pain inhibition via a cognitive strategy, as well as the brains reaction to painful stimulation when the strategy is applied. The study employed a longitudinal fMRI design utilizing an established training paradigm based on RIII-reflex feedback to teach participants over the course of three sessions which strategy can decrease their spinal nociception. We constructed an MR-safe electrophysiological setup to evoke and record the RIII reflex and gather pain rating during fMRI acquisition for this. Participants completed feedback training with comparable success to previous studies. The training effect carried over to the MRI in pain, but not RIII-reductions. Our findings show that mPFC activity increases when participants engage in their strategy, and activity in the lateral thalamus increases post training. Reaction to painful stimulation was decreased during strategy in brainstem, thalamus, insula and frontal cortical regions, with a significant training effect in LC, thalamus, Insula and dlPFC. There findings indicate that the mPFC is integral in initiating descending pain inhibition and that the lateral thalamus may play a role in pain modulation, not just pain sensation. The decreased reaction to nociceptive stimuli in primary receptive areas of ascending pain paths and affective-evaluative regions suggest that participants could indeed reduce their pain, most likely already on a spinal level. Our study demonstrates for the first time, using a longitudinal design, the effect of learning to inhibit pain via feedback training on brain activity leading up and in response to painful stimulation.

Author contribution

The study was designed by Philipp Graeff, Virginia L. Flanagin and Ruth Ruscheweyh. MRI-hardware setup was constructed by Philipp Graeff. Data was collected by Philipp Graeff under supervision of Ruth Ruscheweyh (electrophysiological part) and Virginia L. Flanagin (MRI part). Data was analyzed by Philipp Graeff. Imaging data was analyzed and visualized by Philipp Graeff with guidance of Virginia L. Flanagin. Philipp Graeff wrote the manuscript with guidance of Ruth Ruscheweyh and Virginia L. Flanagin .

Longitudinal changes in human supraspinal pain processing after RIII-feedback training to improve descending pain inhibition

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Abstract

The human body has the ability to influence its sensation of pain by modifying the transfer of nociceptive information at the spinal level. This modulation, known as descending pain inhibition, is known to originate supraspinally and can be activated by a variety of ways including positive mental imagery. However, its exact mechanisms remain unknown. We investigated, using a longitudinal fMRI design, the brain activity leading up and in response to painful electrical stimulation when applying positive mental imagery before and after undergoing a previously established RIII-feedback paradigm. Mass univariate analysis revealed activity decreases post- compared to pre-training in prefrontal, posterior cingulate, lateral occipital cortex, precuneus and parahippocampal gyrus. ROI analysis shows that a main effect of strategy in mPFC and an interaction of strategy and time in the thalamus. Timecourse analysis of the reaction to painful stimulation shows decreased reaction post-training in brainstem and thalamus, as well as the insula and dorsolateral PFC. Our work suggests that feedback training decreases activity in brain areas related to affective processing, as well as in brain areas receiving primary nociceptive information, which points to an activation of decreased spinal nociception. We further suggest that the mPFC and the thalamus play a key role in initiating descending pain inhibition.

1. Introduction

Pain is a conscious sensation that comprises both the physiological sensory perception as well as the psychological experience of pain. While nociception refers to the processing of the somatosensory signal to noxious stimuli, the individual experience of pain is shaped by the integration of nociceptive information with affective information, personal experience/expectation, and other psychological and emotional factors. As a result, this experience varies from person to person and although it is usually transient, it can become pathological when it becomes chronic, leading to one of the major global disabilities and burdens of disease[1].

Nociceptive information enters the brain via the brainstem, reaching the thalamus and dispersing into the cortex[2]. Here, the painful stimuli are thought to be processed by somatosensory and cognitive-affective regions[3,4], including the somatosensory cortices, insula, medial operculum, anterior cingulate cortex (ACC), insula, and prefrontal cortices[3,5]. The degree to which a nociceptive stimulus is perceived as painful, can be influenced by cognitive processes. They can inhibit or reduce the amount of experienced pain, such as through distraction or positive emotions[6], or facilitate the pain through catastrophizing or attention to the painful stimulus[7,8].

These cortical areas appear to also exert a degree of top-down control over pain processing[5,9] via descending pathways that affect nociception at the spinal level. These descending paths originate in the brainstem, more specifically the rostroventral medulla (RVM)[10,11] and locus coeruleus (LC) [11,12], from which long descending fibers extend to the dorsal horn of the spinal cord, modulating nociception. The periaqueductal Grey (PAG), identified as a key area for descending pain inhibition[4,13], connects to the RVM to exert its modulatory effects [14,15]. In turn, the PAG is targeted by several cortical areas in the cognitive-emotional modulation of pain[4,16,17]. Stimulation of descending pain inhibition therefore results in a reduction of afferent nociceptive input arriving at the brainstem, and reduced activity of subcortical and cortical regions reacting to painful stimulation.

Although these connections at the subcortical and brainstem level for descending pain inhibition have been identified, no uniform relationship in terms of brain activity between pain and specific cognitive tasks has been found. The connection between brain activity and pain modulation has been studied using a variety of strategies, including catastrophizing[18], reappraisal[19], distraction[20,21], and placebo analgesia[22,23]. Evidence regarding the contribution of higher cortical areas to descending pain inhibition is inconsistent across the literature[3], also due to the high level of individual variability in experienced pain[24,25]. As a result, the cortical mechanisms for the activation of descending inhibition remain elusive.

Since descending pain inhibition acts on the dorsal horn of the spinal cord, spinal nociception can provide an objective measure of descending pain inhibition. One of the few such measures currently in humans is the nociceptive flexor, or RIII-reflex. We successfully established a feedback training method using the RIII-reflex as real-time measure of spinal nociception. Subjects learn to use cognitive-emotional strategies that reduce their RIII-reflex by activation of their descending pain inhibition[26,27]. Both healthy[27,28] and chronic pain patients[27] were able to activate their descending pain inhibition via our RIII-feedback training. How brain activity changes as a result of RIII-feedback training to activate their descending pain inhibition, and how this is reflected in the brain's reaction to painful stimuli under application of such a strategy still remain unknown.

Therefore, in this study, we investigated brain activity while participants activate their descending pain inhibitory network via a cognitive-emotional strategy before and after RIII-feedback training with a longitudinal functional MRI design. We were interested in the activity that resulted from the cognitive strategy, as well as how this strategy influences immediate pain processing in the brain. To achieve this, we presented participants with an electrical nociceptive stimulation while simultaneously measuring the RIII-reflex as an objective physiological measure of spinal nociception during fMRI. Subjective pain rating was also acquired for each trial for a psychological measure of pain. Between the two MRI acquisition days, participants underwent real-time RIII-reflex feedback training. We hypothesized that when participants learned to activate their descending pain inhibition using the RIII-feedback training, the reflex and pain rating reductions would carry over to the nearly identical setup in the MRI machine. We also hypothesized that the areas involved in pain processing (Table 1) would show a decrease in brain activity in response to a painful stimulation during strategy with a further decrease after RIII-feedback trianing. We further hypothesized that areas involved in descending pain inhibition would show increased activity during application of strategy, with a greater increase post-training.

2. Methods

2.1.Preregistration

The desired sample size, variables, hypotheses, and planned analyses were preregistered on the Open Science Framework prior to any data collection under the following link: https://osf.io/gza5n/.

2.2.Participants

This study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of the Ludwig-Maximilians-University Munich (19-903). Participants were compensated

for their time with 10€/hour. A total of 35 healthy participants were initially recruited via advertisement on the campuses of the Ludwig-Maximilians-University and the University Hospital Großhadern in Munich. Participants had to meet the following criteria for inclusion in the study: (1) age ≥18 years, (2) no severe internal, neurological or psychiatric conditions, (3) no history of chronic pain, (4) no alcohol, nicotine or drug abuse, (5) no regular medication (except hormonal contraception or thyroid hormones), (6) no pregnancy or breastfeeding at the time of participation, (7) no contraindications for MRI scans (incl. but not limited to electrically stimulating implants, medicine pumps, non-MRI-compatible implants or metallic foreign objects in soft tissues). Additionally, measurements were postponed if participants had acute pain on the day of, or used pain medication within 48h prior to, the experiment. All participants we briefed on the experimental procedure before giving written, informed consent. Thirty of the initially recruited participants were included in the experiment, four were excluded due to poor RIII-reflexes during the introductory session, and one due to an unrelated post-hoc neurological diagnosis (see Section 3.1 for gender and age statistics).

2.3.*Study design*

We were interested in the changes in brain activity after training the voluntary activation of descending pain inhibition. Therefore, we conceptualized a longitudinal experiment where participants' brain activity was measured before and after RIII-reflex feedback training. Participants attended a total of 6 sessions, which included an introductory session, the two MRI sessions and three RIII-feedback training sessions (Figure 1A). All sessions were conducted on different days, with a minimum of 72h sessions. The fMRI task was designed to be as similar as possible to the RIII-feedback training.

In the introductory session (S0), participants filled out either Beck's Depression Inventory (BDI[29]), Pain Catastrophizing Scale (PCS[30]), Pain Sensitivity Questionnaire (PSQ[31]), and the State-Trait Anxiety Inventory (STAI[32]) in addition to giving written informed consent. They were then familiarized with RIII-reflex recording, and we confirmed that a reproducible reflex (>100 µVxms) could be recorded. We kept the number of painful stimulations to a minimum and did not describe the feedback training to keep participants as naive as possible for the first MRI session. After S0, the first MRI session (MRI1) was performed, in which high resolution anatomical images and a RIII-reflex functional MRI was performed (see 2.7 fMRI task design). This served as a baseline pain response for all participants before feedback training. Then participants performed the RIII-feedback training paradigm we previously established[26–28] (S1-S3, described below). Finally, participants' brain activity was measured again in MRI2, with the identical procedure as MRI1.

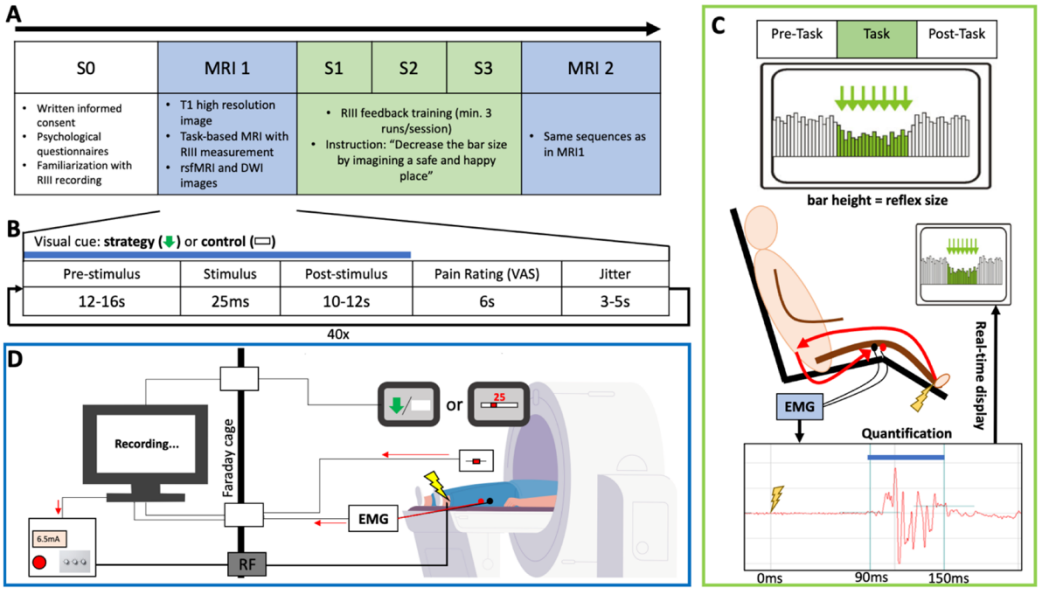

Figure 1: ***Entire study design. A*** *The longitudinal study timeline from left to right. S0 = introductory session, MRI1/MRI2 = task-based MR imaging session, pre- and post-training, S1-S3 = RIII-feedback training* ***B The timing of a single experimental trial in the fMRI experiment (MRI1 & MRI2)****. A single trial lasted between 32 and 40 seconds. Forty trials (20 control, 20 strategy) per experiment were performed, with an average total experiment time of ~26 minutes* ***C The experimental setup for RIII-feedback training sessions (S1-S3)****. Participants received real-time biofeedback of their RIII-reflex size and were instructed to apply a positive cognitive strategy during the task block* ***D The experimental setup for stimulation and RIII-reflex recording during fMRI (MRI1 & MRI2)****. The computer and stimulator were in the MR control room (outside of the faraday cage). The electrical signal from the stimulator went via the patch panel of the faraday cage and a radiofrequency filter to the stimulation electrode. The EMG signal, VAS signal and visual display signals went to and from the recording computer via waveguides. A single computer ran the experimental script that triggered electrical stimulation, produced visual cues, and simultaneously recorded EMG and VAS responses in parallel.*

2.4.RIII-recording

To acquire a physiological measure of nociception, we evoked and recorded the RIII-reflex as described previously[7,28] and according to established techniques[33,34]. The RIII-threshold was defined on an individual level for each session as the stimulus intensity that first evoked a reflex response exceeding a raw area of 100 µV*ms (from the average of 3 series with stimulation intensity increasing from 2.0mA in 0.5mA steps) using a staircase procedure described in more detail elsewhere[7,35]. The stimulation intensity for RIII-recording was set at ~150% RIII-threshold.

In non-MRI sessions (S0, S1-S3), the participant sat comfortably in a reclining chair with the recorded leg flexed at ~150°. Stimulation and recording were performed with a Keypoint Portable EMG System (Medtonic, Natus, Planegg, Germany). Stimulation and recording sites were prepared by degreasing and lightly abrading the skin prior to attachment of electrodes. Constant current stimulation,

consisting of 5x1ms electrical pulses at 200Hz (21ms total duration), was applied to the retromalleolar pathway of the sural nerve with a bipolar bar electrode with an interelectrode distance of 23mm (Natus Europe, Planegg, Germany). RIII-reflex responses were recorded from the short head of the biceps femoris, ipsilateral to the stimulation site via a pair of Ag/AgCl electrodes placed 4-5cm apart on the muscle belly. Signals were amplified (up to 10000 times) and band-pass filtered (20-500 Hz). The segment 90ms before to 410ms after the stimulation was digitized at 24kHz and used for reflex analysis and feedback.

During MRI sessions, the participant lay on the scanner bed with the recorded leg flexed at ~150°. Stimulation and recording sites were located and prepared in the same manner as above. Stimulation was delivered via custom-made MR-compatible electrodes (interelectrode distance, 23mm) and a Digitimer DS7A constant current stimulator (Digitimer Ltd, Welwyn Garden City, UK) triggered by an Arduino UNO microprocessor to achieve the same stimulation pattern as in training (i.e., 5 x 1ms pulses at 200Hz). The stimulator was equipped with an RF-filter (Mini-Circuits, Camberley, UK) to prevent high frequency interactions in the MRI data. Here, the entire EMG signal was recorded with an MRI-compatible ExG recording system (BrainProducts, Munich, Germany), digitized at 5kHz, and stored for offline artefact correction and segmentation. MRI induced artefacts in the EMG-trace were corrected using the MR-correction tool in BrainVision Analyzer (BrainProducts, Munich, Germany) with the following settings: baseline correction for average trace, template drift compensation, no downsampling, IIR filter with slope = 48 and cutoff frequency = 150 Hz. Baseline reflexes recorded before MRI-acquisition were low-pass filtered with an 8th-order Butterworth filter (cut-off frequency 150Hz) to be congruent with the MR-artefact corrected traces and used to normalize reflex sizes before entering them as parametric modulators into the statistical model (see Section 2.11).

For quantification of RIII-reflex areas, EMG signals were rectified, and the area under the curve in the analysis window (90-150ms post-stimulus) was obtained and corrected for average baseline area (90-30ms before stimulation).

2.5.Pain ratings and pain thresholds

In addition to the electrophysiological reflex signals, we acquired pain ratings to quantify experienced pain, and set individual pain thresholds. This was done to compare not only the objective marker of spinal nociception (RIII-reflex), but also the subjective pain experienced by each participant. Participants rated the intensity of the electrical stimulation on a numerical rating scale (NRS) from 0 (no pain) to 10 (strongest pain imaginable). The pain threshold was defined as the stimulation intensity that

first evoked a NRS rating >1 during three ascending series starting from 2mA and increasing in 0.5mA steps. For suprathreshold stimulation in sessions S0 and S1-3, participants rated the pain intensity verbally after each 2 min stimulation block on the NRS. During MRI sessions, participants rated each stimulus haptically on a MR-compatible sliding scale. The responses were digitized and stored for analysis.

2.6.RIII-feedback training

We performed nociceptive RIII-reflex feedback training as it has been done in our previous successful feedback training studies with both patients and healthy individuals[26–28]. During each feedback session, subjects were given the opportunity to optimize a strategy for RIII-reduction during three to four feedback runs per session. We asked all participants to use the same positive mental imagery ("Imagine a safe and happy place") strategy, which was one of the most successful strategies in our previous studies[26]. RIII-reflexes were evoked in random intervals every 8-12s, with a run consisting of 4 blocks of 12 stimuli each. Block 1 served to stabilize the RIII-reflex and was not analysed. Blocks 2 and 4 were pre- and post-task blocks (Figure 1c), respectively, in which participants were asked to simply observe the pain, but not think of anything specific. Block 3 was the task block, in which participants were asked to use and optimize the aforementioned cognitive-emotional strategy to actively decrease the size of their RIII-reflexes, which were analyzed online and displayed as consecutive bars on a feedback monitor. The task block was cued with a green downward arrow appearing on the monitor. Pain intensity was rated at the end of each block as the average pain intensity of the preceding 5 stimuli.

2.7. fMRI task design

Before entering the MRI machine, participants were briefed again about the experimental procedures. We explained that the task was not going to be presented in 2 min blocks as in the behavioral sessions, but as single trials in blocks of 5 with individual visual cues. This was done to optimize the design for measuring the hemodynamic response function, while remaining as close as possible to the feedback training. In the first MRI session, participant had no information about the cognitive-emotional strategy they would later use for nociceptive bio-feedback training. Therefore, the instructions regarding the visual cues were simply "Don't think of anything in particular" during control blocks (cue: white bar) or "Imagine a safe and happy place" during strategy blocks (cue: green downward arrow). In MRI2, post-feedback training, participants were instructed to "Apply the strategy that you developed during the feedback training" during strategy blocks. The functional MRI experiment was coded in Matlab (version 2016a,

Mathworks) with visual presentation in PsychToolBox3.0 (Version 3.0.11) connected to MR-compatible goggles (NordicNeuroLab, Bergen, Norway).

The experiment was conducted in a single session consisting of 40 trials (20 strategy, 20 control), structured into 8 blocks of 5 trials. The visual cue presented remained constant for the duration of the block, but the cue order between blocks was random, such that each participant received their own randomized block order. A single trial consisted of the following (Figure 1 B): Cue presentation, followed by a pseudorandom delay of 12-16s after which participants received the RIII-reflex evoking electrical stimulus, followed by another pseudo-random interval of 10-12s post-stimulus. The task cue was continuously presented throughout these 22-28s, and partipants were asked to apply their strategy or control intervention continuously while the cue was on. After that they were asked to rate the pain intensity of the stimulus on a 0-100 scale (0= no pain, 100 = worst pain imaginable, steps of 5); rating time was 6s. The rating was presented visually, and participants used a slider to determine the pain rating. The round concluded with a 4-6 second jitter without cue presentation. After each block, participants were asked to rate how well they could employ the strategy or "think of nothing in particular" on a scale of 0-100 (0 = not at all, 100 = very well, steps of 5).

2.8.RIII reflex and pain rating: statistical analysis

For RIII-analyses, we computed RIII-size as a percent of the average RIII-size during the control condition in the MRI and of the average RIII-size of the corresponding pre-task block in feedback training. For RIII-feedback training sessions, RIII-sizes and pain ratings were analysed with a repeated measures ANOVA with factors block (pre, task, post) and session (S1 and S3) in R (RStudio, version 3.6.3). For the MRI sessions, RIII-reflex reductions and pain reductions were analysed by repeated measures ANOVA with factors session and condition. p-values < 0.05 were considered significant. Repeated measures ANOVA was performed using the lmer() function (lme4[36], version 1.1-26) with significance tested by the Anova() function (car[37], version 3.0-10) and post-hoc tests performed using emmeans() (emmeans, version 1.7.2)

2.9.MRI data acquisition

MRI images were acquired on a 3T Siemens MAGNETOM Prisma scanner (Erlangen, Germany). For each session, a high resolution T1-weighted anatomical image (TR = 2060 ms, TE = 2.17 ms, flip angle = 12 deg., FoV = 240mm , 256 slices, 0.75mm isotropic voxel resolution, A-P phase encoding, GRAPPA = 2), and a field map (TR = 760ms, TE1/TE2 = 4.92ms/7.38ms, dTE = 2.46ms, flip angle = 45 deg., FoV = 240mm, 74 slices, 2.5mm isotropic voxel resolution, A-P Phase encoding) were acquired. Functional images were

collected with a 2D multiband EPI sequence with the following parameters: TR = 900ms, TE = 33ms, flip angle = 45 deg., FoV 210mm, 54 slices, 2.5mm isotropic voxel resolution, multiband acceleration factor = 6, A-P phase encoding. The EPI sequence covered the entire brain down to the base of the PONS in all participants. We did not additionally accelerate the sequence or use a 3D sequence to simplify artifact correction in the EMG signal. An additional resting state fMRI sequence and a diffusion weighted MRI sequence were acquired but were not analysed here.

2.10. fMRI Preprocessing

Data preprocessing was performed by FMRIPREP version stable[38][RRID:SCR_016216], a Nipype[39] [RRID:SCR_002502] based tool. Each T1w (T1-weighted) volume was corrected for INU (intensity non-uniformity) using N4BiasFieldCorrectionv2.1.0[40] and skull-stripped using antsBrainExtraction.shv2.1.0 (using the OASIS template). Brain surfaces were reconstructed using recon-allfrom FreeSurfer v6.0.1[41] [RRID:SCR_001847], and the brain mask estimated previously was refined with a custom variation of the method to reconcile ANTs-derived and FreeSurfer-derived segmentations of the cortical gray-matter of Mindboggle[42] [RRID:SCR_002438]. Spatial normalization to the ICBM 152 Nonlinear Asymmetrical template version 2009c[43] [RRID:SCR_008796] was performed through nonlinear registration with the antsRegistrationtool of ANTs v2.1.0[44] [RRID:SCR_004757], using brain-extracted versions of both T1w volume and template. Brain tissue segmentation of cerebrospinal fluid (CSF), white-matter (WM) and gray-matter (GM) was performed on the brain-extracted T1w using fast [45] (FSL v5.0.9, RRID:SCR_002823). Functional data was slice time corrected using 3dTshiftfrom AFNI v16.2.07[46] [RRID:SCR_005927] and motion corrected using mcflirt[47](FSL v5.0.9). This was followed by co-registration to the corresponding T1w using boundary-based registration[48] with six degrees of freedom, using bbregister (FreeSurfer v6.0.1). Motion correcting transformations, BOLD-to-T1w transformation and T1w-to-template (MNI) warp were concatenated and applied in a single step using antsApplyTransforms (ANTs v2.1.0) using Lanczos interpolation. Physiological noise regressors were extracted applying CompCor[49]. Principal components were estimated for the two CompCor variants: temporal (tCompCor) and anatomical (aCompCor). A mask to exclude signal with cortical origin was obtained by eroding the brain mask, ensuring it only contains subcortical structures. Six tCompCor components were then calculated including only the top 5% variable voxels within that subcortical mask. For aCompCor, six components were calculated within the intersection of the subcortical mask and the union of CSF and WM masks calculated in T1w space, after their projection to the native space of each functional run. Frame-wise displacement[50] will be calculated for each functional run using the implementation of Nipype. ICA-based Automatic Removal Of Motion

Artifacts (AROMA) was used to generate aggressive noise regressors as well as to create a variant of data that is non-aggressively denoised[51]. As ICA-AROMA already smooths data, we did not perform any additional spatial smoothing. The non-aggressively denoised AROMA images were inclusion-masked using the anatomical brain mask and used as input for first level analysis.

We reduced our preregistered exclusion criteria of a global tSNR of less than 40 in our functional images, as the pilot data was obtained using a much shorter total scanning duration (less than a minute compared to 26 minutes) and tSNR is known to decrease over time[52]. Instead, our mean global tSNR of the raw fMRI images was 35 before preprocessing. After ICA-AROMA correction for movement, we calculated the tSNR for our ROIs and found a mean tSNR of 147 ± 23. We therefore did not exclude any participants based on tSNR (see Supplementary Table 1 for tSNR values of individual ROIs). 3 participants were excluded due to excessive movement (over ten trials with a fd >0.9mm) that was directly associated with the painful stimulus.

2.11. fMRI statistical analysis

We were interested in the specific activity in predefined structures known to be involved in pain and descending pain inhibition. However, as additional regions may be involved in the RIII-training and altered response to painful stimulation we performed a whole-brain analysis in addition to the ROI analysis. The whole-brain analysis was performed using SPM12 (Version 7771) for Matlab. The single-subject generalized linear model included the painful electrical stimulus (**Stimulation**) as an event-predictor of length 0 convolved with the canonical HRF and its first two derivatives. We modelled the derivatives as opposed to the preregistered HRF only, to better account for temporal variation in such a short stimulus. Application of the cognitive strategy or the control was modelled as a boxcar regressor (**Task)** spanning the interval during which the task cue was presented convolved with the canonical HRF. Both stimulation and task were additionally linearly modulated by the relative RIII-size and pain intensity. These regressors were termed RIII-modulated and pain-modulated, respectively. The time the subjects rated their pain via VAS was added as a boxcar regressor of no interest. Single volumes in which subjects had a fd>0.9mm were added as nuisance regressors. As ICA-AROMA preprocessing already removed movement related artifacts, we did not add movement parameters as nuisance regressors. Serial autocorrelation of the BOLD time series was modelled with a first-order autoregressive model and low-frequency fluctuations were removed via SPM's DCT with 100s cut-off. Contrasts of interest were constructed for each session by subtracting control from strategy regressors (e.g., $Task_{Strategy}$-$Task_{Control}$) to

obtain contrasts for task, stimulation HRF and derivatives, pain modulated task and pain modulated stimulation. The negative contrast (i.e. control – strategy) was created at the group-level analysis.

Group-level analyses were conducted with the Sandwich Estimator (SwE) toolbox[53] for SPM. This toolbox constructs mixed-effects models which takes all random effects into account by using an unstructured covariance structure and as such provides a better estimate of longitudinal and repeated measures data than the classical group-level SPM analysis. We constructed our model with the "classic" SwE type, which estimates the covariance matrix for each subject and session separately, using small sample adjustment type C2. One model per contrast of interest was constructed, inputting the contrasts from MRI1 and MRI2 for each subject. Using non-parametric wild bootstrapping[54] with 5000 permutations and small sample adjustment type C2 with an unrestricted sandwich estimator to compare contrasts between MRI1 and MRI2. FWE <0.05 was considered significant. Type C2 was used as opposed to Type III (defined in the preregistration) as it was published as the newest recommended correction after writing of the preregistration.

Two different approaches were used to analyse our regions of interest. First, average beta values for the regressors of interest were extracted from each ROI (Table 1) for further analysis. We performed four 2x2repeated measures ANOVAs for each ROI separately using R (RStudio, version 3.6.3). ANOVAs were constructed using the lmer() function of the lme4 package, with condition (strategy/control) and session (MRI1/MRI2) as factors and participant as random effect, using the Anova() function of the car package to test for significance.

Table 1: Regions of interest and how they were constructed. *Centroid are in MNI coordinates are in MNI space.*

ROI region	**ROI definition**	**Centroid (mm)**		
		X	Y	Z
Rostroventral Medulla (RVM)	Manual construction of a 40 voxel (1mm isotropic) sheet at z= 49.9mm, covering most of the Medulla	-1	-39	-49
Locus coeruleus (LC)	Harvard Ascending Arousal Network[55]	0	-38	-28
Periaqueductal grey (PAG)	Harvard Ascending Arousal Network[55]	0	-33	-12
Anterior congulate cortex (ACC)	FSL Harvard-Oxford Cortical Atlas (threshold = 75)	-1	19	24
subgenual	5mm spheres based on coordinates by Zhou et al., 2016[56]	-2	24	-10
perigenual		-2	46	10
rostral		-2	34	28
Thalamus (R)	FSL Harvard-Oxford Subcortical Atlas (threshold = 75)	10	-19	7
Thalamus (L)		-11	-18	7
Hypothalamus	5mm sphere based on coordinates by Karlsson et al., 2010[57]	0	-8	-8

Insula (R)	FSL MNI Structural Atlas (threshold = 50)	37	4	-38
Insula (L)		-38	7	2
dlPFC (R)	Combining 'pars opercularis' and 'pars	53	20	11
dlPFC (L)	triangularis' from the FSL Harvard-Oxford Cortical Atlas (threshold = 50)	-55	21	11
mPFC (R)	Combinig 'Rectus' and	6	43	-15
mPFC (L)	'Frontal_Mid_Orb' regions of the AAL3 Atlas[58]	-8	43	-14

Second, we performed a time course analysis on the average signal from each ROI in addition to our preregistered ROI analysis. This was done for two reasons. First, both the task and the stimulation temporal dynamics that may be difficult to capture in a classical general linear model. The stimulation because of its short duration and the task because such a highly cognitive and introspective task will not have clear starting and ending points, even with a visual cue. Second, the haemodynamics in particular in subcortical and brainstem areas likely differ from the rest of the brain[59] and the signal-to-noise ratio is smaller due to proximity to major blood vessels, CSF flow and breathing artifacts. Because we measured the hemodynamic signal at a temporal resolution of below one second, a time course analysis has the ability to capture additional information about the course of the hemodynamic response function that a coarser resolution would not find. We therefore extracted and z-transformed the raw time courses of our ROIs from the time point of stimulation until 14 volumes post-stimulation. We analysed the time courses using a repeated measures ANOVA with the factors condition and session while controlling for timepoint in R. This gives an impression of the signal change which is unconstrained by how well the haemodynamics fit the canonical HRF used by SPM.

Correlation analysis of beta estimate differences between MRI1 and MRI2 and improvement in pain reduction between MRI1 and MRI2 were performed by computing the Pearson correlation coefficient with the cor_test() function (rstatix, R version 0.7.0)

After Bonferroni correction for multiple comparisons, $p \leq 0.004$ was considered significant for ROI, time course and correlation analyses.

3. Results

3.1.Participants

A total of 30 (17 female) participants were included in the study, 27 of these were included in the MRI-analyses. The three participants that were not used for the MRI analyses had excessive movement (see fMRI preprocessing). Mean age of all 30 participants was 25 ± 4 years (range: 18-35). Mean

questionnaire scores were 4 ± 4 (BDI), 12 ± 8 (PCS), 3.0 ± 1.2 (PSQ), 32 ± 8 (STAI-State), and 33 ± 9 (STAI-Trait), indicating our study population is in the normal psychological range. The average time between first and second MRI session was 44+-23 days (range 14-105). Mean age of the 27 participants (15 female) included in the MRI-analyses was 25 ± 4 years (range: 18-35)

3.2.Feedback training was successful and indicative of experienced pain reduction during fMRI

During feedback training, the average RIII- and pain thresholds across all participants were 8 ± 4 mA and 7 ± 3 mA, respectively. Training success, i.e., the reduction in RIII-reflex during S3 was on average to 84 ± 14 % of control. Success ranged from a reduction to 57 % to an increase to 124 % of control. This means that although on average participants were able to reduce their RIII-reflex, some individuals in fact had larger reflex sizes when using their pain reduction strategy. There was a main effect of task on both RIII-reflex areas ($F_{2,29}$ = 13.7, p < .001) and pain ratings ($F_{2,29}$ = 5.7, p < .001) (Figure 2 A). When using all participants, no interaction between session and block was found for RIII-reflex area ($F_{2,29}$ = 0.7, p = .258) or pain rating ($F_{2,29}$ = 0.1, p = .892). However, if we look at only those participants who showed an RIII-reduction of at least 90% of control in S3 (n = 22), there was a significant interaction between task and block ($F_{2,21}$ = 1.8, p = .027), with greater reduction of RIII reflex area during task in S3 compared to S1 (p < .01), but still no interaction for pain ratings. These findings are in line with previous results; successful participants express a significant improvement in reduction of RIII-size pre- vs. post training, but not necessarily in pain reduction[26–28].

During the MRI sessions, reduction of RIII-reflex size during strategy was to 94±12% of control (range: 71%-114%) in MRI1 and to 97±13% (range: 75%-135%) in MRI2. There was a significant main effect of task ($F_{1,26}$ = 8.2, p = 0.004) but no significant difference in reflex reduction between MRI sessions ($F_{1,26}$ = 0.637, p = .425). In contrast, experienced pain was significantly reduced during strategy in both MRI sessions ($F_{1,26}$ = 5.1, p < .001) (Figure 2B) and it was significantly ($F_{1,26}$ = 5.1, p < .05) more reduced in MRI2 (78 ± 11 %, range: 51 % - 99 %) than in MRI1 (87 ± 19 %, range: 56 % - 135 %). This suggests that feedback training success did not transfer directly to a RIII-reduction during MRI imaging, but to a change in experienced pain. This was reflected in a significant positive correlation (coefficient: 0.40, p =0.03) between pain reduction in S3 and improvement of pain reduction between the MRI sessions (Figure 2C).

In summary, 73 % of participants were able to increasingly reduce their RIII-reflex using the strategy 'think about nice things/imagine a safe and happy place' over 3 sessions of real-time biofeedback about the magnitude of their RIII-reflex. Participants were able to transfer their training to the functional MRI session with a reduction in their experienced pain, but not in their reflex size. Those individuals with

greater achieved pain reduction in RIII-feedback training also reduced their experienced pain more in MRI2, indicating that the psychological component of pain reduction during training carried over to the MRI experiment.

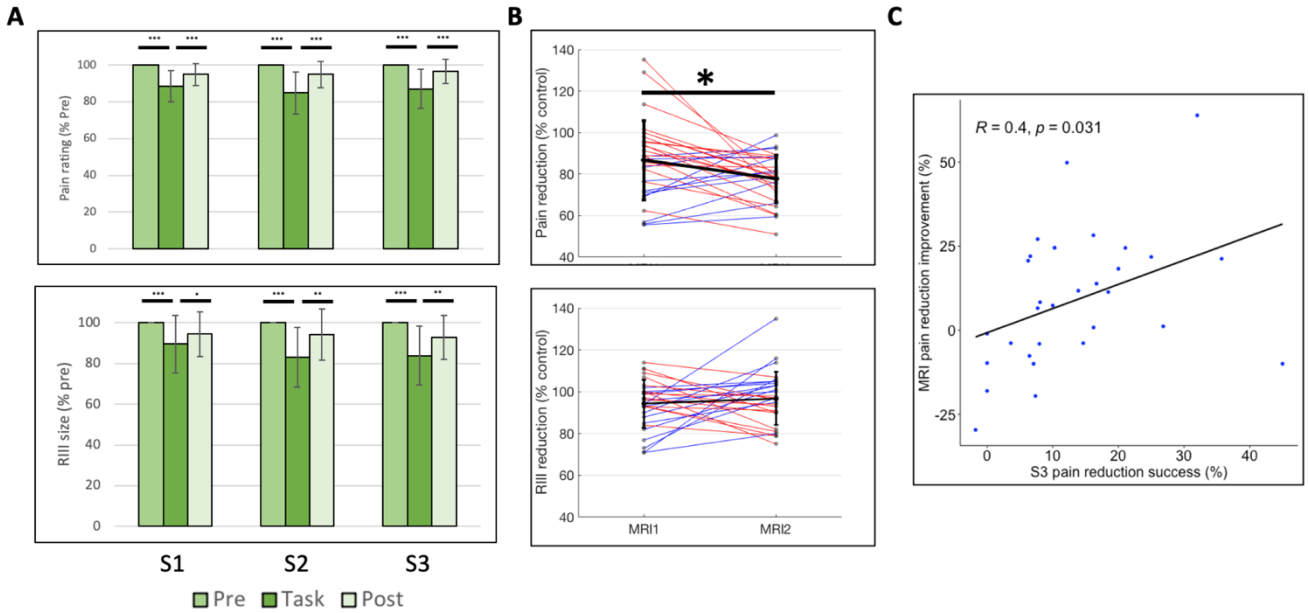

Figure 2: Behavioural and electrophysiological results. A) RIII-feedback training significantly reduces pain ratings (top) and RIII-reflex sizes (bottom). *There is a significant main effect of task (*** p< 0.001, ** p<0.01, * p<0.05), but no interaction between session and condition. The subset of participants that successfully achieved more than 10% RIII-reduction in the last session (S3) exhibit significant improvement in RIII-reduction between S1 and S3 (p<0.02)(not shown).* ***B) Pain rating (top) and RIII-reflex (bottom) during MRI sessions.*** *Participants showed a significant pain reduction between MRI1 and MRI2 (p= 0.02), but no difference in the RIII-reflex. Both MRI1 and MRI2 show a significant reducting in pain rating (p<0.001) during strategy, but no difference was found in individual MRI sessions for the RIII-reflex. Individual changes are shown based on whether their pain or RIII-reduction improved (red) or worsened (blue)* ***C) Correlation between training success and increased pain reduction in MRI2 compared to MRI1.***

3.3.*Consistent pain-related haemodynamic activity across task conditions*

Painful electrical stimulation evoked responses in the classical pain matrix, including the insula, S1/S2, ACC, and thalamus (Supplementary Figure 1, Supplementary Table 2). Investigation of brain activity during task application (i.e. the entire time when participants should apply their cognitive strategy, Figure 1B) and stimulation (i.e. response to painful electrical stimulation) showed that on a whole-brain level the response to the painful stimulation is consistent across session (MRI1 vs. MRI2) and task (strategy vs. control). No task-based differences in the reaction to the painful stimulus, over all three basis functions combined or individually, were found between sessions. Also, no pain-modulated effects between MRI1 and MRI2 were found. The same pattern of activity was seen in the ROI analysis. There was no difference

in the mean parameter estimates for the stimulation regressor between strategy and control or between MRI1 and MRI2. In short, the effect of strategy in reducing experienced pain was not reflected in the average activity during painful stimulation when analysed with the general linear model.

3.4. Longitudinal changes in haemodynamic activity upon task application

In the whole-brain longitudinal group analysis, we found a significant interaction between task and session for the block-regressor of task application, that is to the entire time when participants either used their cognitive strategy or did nothing (control) (Figure 3). The interaction was significant in occipital, precuneus/posterior ACC, superior parietal, paracingulate and lateral frontal areas (Table 2), but not in any of the predefined regions traditionally associated with pain processing. To determine the direction of the changes, the beta-value of the central voxel was extracted for each cluster and averaged over all clusters. This demonstrated that the change between MRI1 and MRI2 was driven by a decrease in haemodynamic activity during strategy (Figure 3 – bar graphs). It is important to note here that the absolute beta-values extracted do not have meaning in themselves, but it is the relative difference between strategy and control that has meaning.

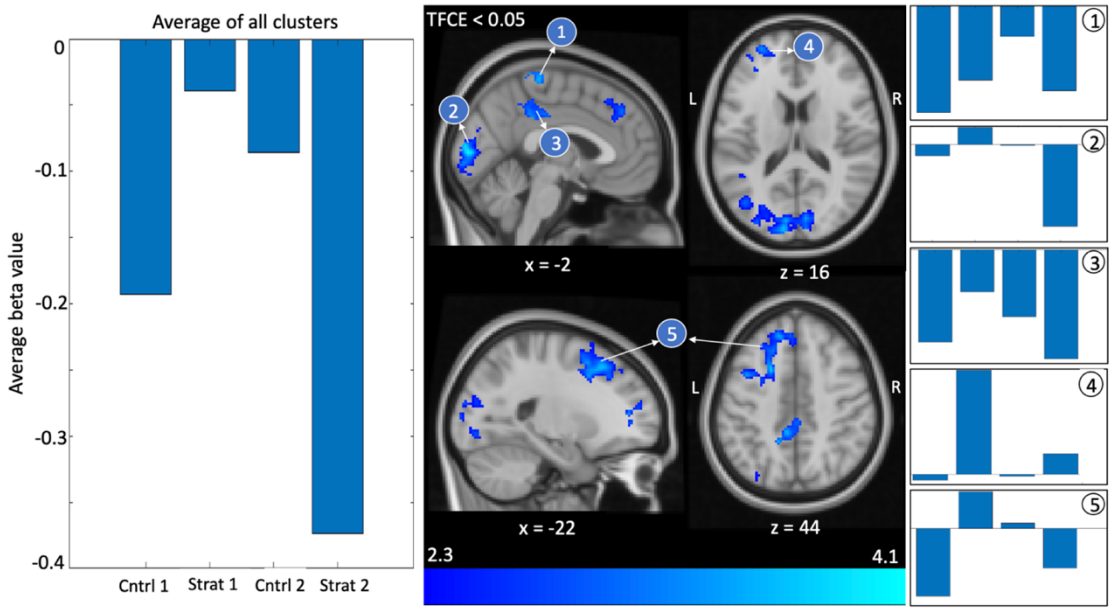

Figure 3: Longitudinal effect of cognitive strategy on brain activity during task. *Effects were significant with TFCE, 5000 bootstraps. The decreases were driven by a decrease of activity during strategy (middle bottom panel) between MRI1 and MRI2. For demonstration purposes the beta-weight of the centroid of five different ROIs are plotted on the right. Note that relative differences within a session are of interest; absolute beta values have no meaning in this model. 1 = pre-/postcentral gyrus, 2 = occipital lobe, 3 = precuneus/posterior cingulum, 4 = lateral frontal pole, 5 = superior/middle frontal gyrus*

Table 2: Table of whole-brain clusters showing changes in haemodynamic activity between task conditions in the longitudinal analysis. *Anatomical labels were extracted the Harvard oxford cortical atlas in FSL. Cluster size refers to the number of voxels of the current cluster, voxel size is 2mm isotropic. Coordinates are in MNI space.*

Anatomical region	p-value	Cluster size	Peak coordinates (mm)		
	FWE-TFCE	voxel	x	y	z
Pre-/postcentral gyrus (L)	0.037	113	-4	-30	68
Occipital Lobe (pole & lateral)	0.020	3195	-2	-90	6
Precuneus/posterior cingulum	0.037	371	-6	-40	44
Lateral frontal pole (L)	0.042	232	-28	56	16
Superior/middle frontal gyrus (L)	0.028	1383	-14	40	34
Parahippocampal gyrus (L)	0.042	66	-28	-40	-12

Although none of the predefined regions of interest showed significant task or training-related activity at the whole-brain level, the ROI analysis led to a significant main effect of condition for the average parameter estimate of task ($F_{1,26} = 15.2$, $p < .001$) in medial prefrontal cortex. In this region, parameter estimates were higher during strategy than control (Figure 4). The thalamus showed significant interaction between session and condition ($F_{1,26} = 4.3$, $p < .002$). The parameter estimates for control did not differ between MRI sessions, while they increased in strategy from MRI1 to MRI2. (Figure 4). Neither region showed laterality effects.

Both the medial prefrontal cortex and the thalamus are subdivided into components that have differential functions in relation to pain modulation. Therefore, for visualization purposes, we plotted the difference in t-values within these two ROIs. For the mPFC, we plotted strategy > control averaged over both MRI sessions, for the thalamus we display the difference of the (strategy - control) contrast between MRI2 and MRI1 (Figure 4). We can see that the frontal mPFC and the ventral (VP) portions of the thalamus contribute the most to the effects found.

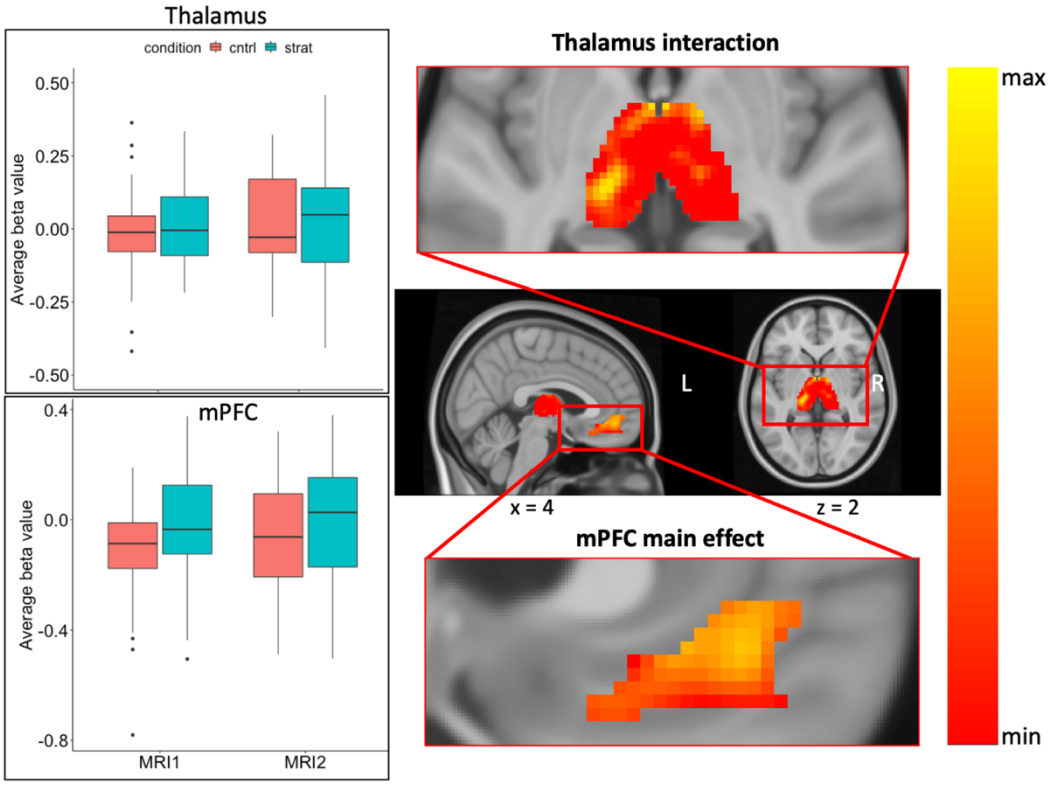

Figure 4: Significant results of ROI analysis in task condition. *Significant interaction of condition and session in Thalamus ($p < 0.002$) and significant main effect of condition in mPFC ($p < 0.001$). Unthresholded graphs of activity show that the activity increase in strategy relative to control is primarily located in medial and lateral parts of the thalamus, and ventromedial and subgenual parts of the mPFC.*

3.5. Task-related time course differences during stimulation in subcortical and cortical structures

To examine more subtle differences in the timing and strength of the haemodynamic activity, we performed a time course analysis on the mean activity in our regions of interest. This time course analysis revealed significant results that we were unable to find with our parameter estimates, suggesting that the classical HRF and its derivatives did not entirely reflect the response to the very short painful stimulation. We found a significant main effect of condition in the rostroventral medulla ($F_{1,26} = 11.4$, $p < .001$), locus coeruleus ($F_{1,26} = 11.0$, $p < .001$)), PAG ($F_{1,26} = 11.9$, $p < .001$ and thalamus ($F_{1,26} = 23.0$, $p < .0001$) .In all these areas, the haemodynamic response was lower when participants used their RIII-reflex reduction strategy compared to the control task. We furthermore found a significant interaction between MRI session and condition in the dorsolateral prefrontal cortex ($F_{1,26} = 22.1$, $p < .0001$), insula ($F_{1,26} = 10.6$, $p = .001$), thalamus ($F_{1,26} = 26.0$, $p < .0001$) and locus coeruleus ($F_{1,26} = 12.3$, $p<0.001$). In these regions the

relative decrease in signal during the reflex reduction strategy was more in MRI2 compared to MRI1. The time courses for all four conditions in two exemplary subcortical ROIs is shown in Figure 5. Additional timecourses can be found in Supplementary Figure 2.

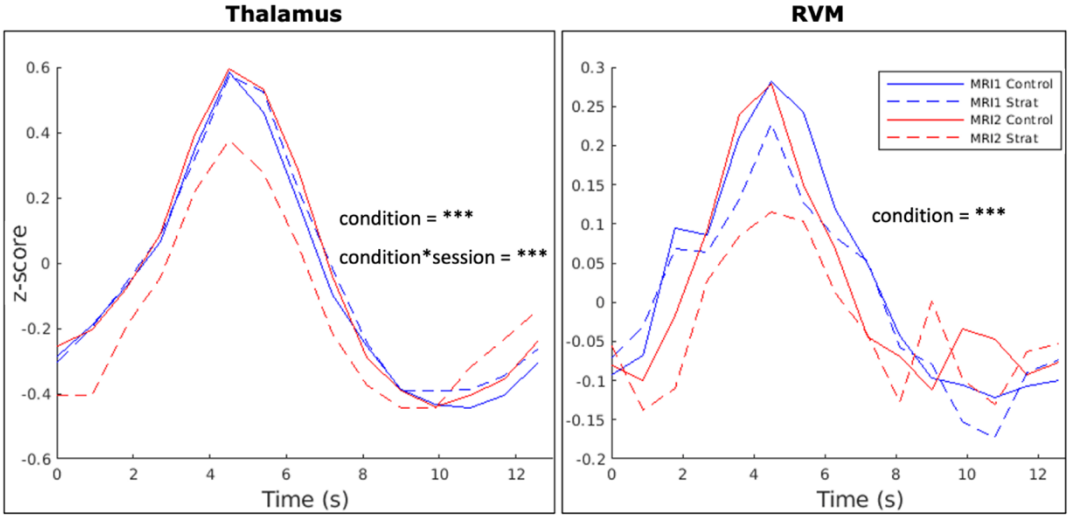

Figure 5: Timecourse of z-transformed raw data after painful stimulation (given at scan 0). *A greater decrease in signal relative to control is observed after undergoing feedback training. Some decrease is already observed pre-training in RVM, but not thalamus. There was a significant effect of condition (strategy vs. control) in both thalamus and RVM, and a significant interaction of condition and session in thalamus*

3.6.Activity in rostral frontal ROIs is related to experienced pain

We found no significant correlation between training success (RIII reduction in S3) and brain activity in both the whole-brain and ROI analyses. Correlation between the difference in pain reduction from MRI1 to MRI2 and the difference in beta values from MRI1 and MRI2 revealed a significant correlation between improved pain reduction and a decrease in the relative (i.e. strategy-control) beta value of the canonical HRF of the painful stimulus in the perigenual (coefficient: 0.42, $p<0.05$) and rostral ACC (coefficient: 0.47, $p<0.02$), mPFC (coefficient: 0.49, $p< 0.01$) and a significant negative correlation between improved pain reduction and the pain stimulus temporal derivative in rostral ACC (coefficient: -0.45, $p<0.02$).

4. Discussion

4.1. Feedback training successfully reduces physiological and psychological measures of pain

Participants were, on average, successful in their ability to reduce their RIII-reflex via RIII-feedback training. The size of the reduction in both pain and RIII-reflex size during the use of a cognitive-emotional strategy was in line with our previous studies[26–28], as was the improvement in the reduction of RIII-size, but not pain, over the course of training. As expected, we observed a high individual variability in training success, both for RIII-size and pain. This variability was also reflected in the behaviour during MRI.

During functional MRI, participants had lower RIII-sizes and pain ratings while using their cognitive-emotional pain reduction strategy, evidence that the strategy affected descending inhibitory circuits. RIII-feedback training reduced experienced pain, demonstrating the influence of training on pain at a psychological level. Training did not have an impact on the RIII-reflex size, which we believe is less related to an actual lack of effect but is rather a result of the challenges measuring EMG in the MRI and a different body position between MRI and training (see Section 4.6). Indeed, the psychophysical pain reduction was correlated to the pain reduction achieved during the last feedback session (Figure 2C), i.e., participants that could better reduce their pain in S3 had a greater improvement in pain reduction in the second MRI session. Supporting the notion that the participants were still successfully applying their strategy in MRI2 are studies showing that even four months post-training, participants can still successfully apply their strategy without biofeedback[60].

4.2. Subcortical and frontal regions show a reduction in haemodynamic response to painful stimulation when the cognitive-emotional strategy is applied

Using the classical model of the HRF, together with its basis functions, and a general linear model approach, we could capture all the cortical and subcortical regions known to be involved in pain processing, down to the brainstem. However, no differences in brain activity in response to painful stimulation were revealed with this mass univariate and classical HRF approach. This is not unsurprising, as we would expect more subtle changes in the size of their response to painful stimulation within the same regions, rather than the recruitment of additional regions that is more easily captured with this approach. Indeed, by analysing the timecourse of the MRI signal, we observed that our predefined regions of interest all responded with an increase in haemodynamic activity to painful stimulation and that the size of the response differed between tasks and across sessions. Differences in brain activity relating to descending pain inhibition were seen from the brainstem up to the frontal cortical pain processing regions.

In the RVM, PAG and thalamus, regions that receive direct nociceptive input from the spinoreticular and spinothalamic tracts[2], the reaction to a painful stimulus was reduced during the application of the cognitive-emotional pain reduction strategy. In the thalamus, along with the LC in the brainstem, the haemodynamic response was further reduced during strategy after training. Although the RVM and PAG did not show a significant interaction, we can observe the same trend. This suggests that the increase in descending pain inhibition through strategy use and RIII-feedback training results in a decrease in hemodynamic activity in subcortical and brainstem pain processing areas. As the thalamus is the primary terminus for ascending pain pathways[2] one interpretation of our results is that during pain inhibition, there is decreased nociceptive input arriving in the primary recipient structures of ascending pain paths.

The same pattern is reflected in the insula and dlPFC. These areas showed a weaker haemodynamic response to painful stimulation when participants applied their strategy post-training. The insula is one of the primary areas responsible for both the somatosensory and emotional-evaluative experience of pain[3,4]. The role of the dlPFC in pain perception is not fully understood, but it activates in response to acute pain[61,62] . Based upon the proposed functions of these regions we suggest that we not only see a decreased nociceptive input into the brain, but also a reduction in its valuation on a cortical level. Given that participants exhibited a greater pain reduction post-training, the observed decrease in these regions would explain the psychophysical behaviour.

4.3. Greater improvement of pain reduction correlates to a change in heamodynamic response to painful stimulation in frontal regions

In addition to observing haemodynamic changes in response to painful stimulation post-training, pain reduction differences between MRI sessions were correlated with a reduced hemodynamic response to the painful stimulus in frontal areas, specifically the frontal parts of the ACC and the mPFC. Participants with greater improvement in pain reduction observed a positive correlation with the reduction of canonical HRF in mPFC, rostral and perigenual ACC. Both the mPFC and the ACC participate in the processing painful stimuli, specifically in affective and evaluative aspects thereof[63]. A decreased beta estimate for the HRF suggests a decreased reaction to the painful stimulus.

A negative correlation was found in the rostral ACC with the first derivative of the HRF. This shows that improved subjective pain reduction indeed translated to a reduced reaction to painful stimulation in mPFC and ACC. The additional negative correlation in rostral ACC likely stems from a shift in haemodynamic response, i.e., the reaction to the stimulus is faster after training.

4.4. After feedback training the brain is more efficient at application of the cognitive emotional strategy

On a whole brain level, our predefined pain-related regions of interest did not show a difference in brain activity while participants used their cognitive strategy. Instead, cortical regions not traditionally related to pain processing showed less activity post training during strategy. These cortical areas included the occipital, frontal and precuneus/posterior cingulate cortices. The precuneus, anterior cingulate and prefrontal cortex are core regions of the default mode network[64], suggesting that the training of the cognitive emotional strategy decreases default mode activity while applying this strategy. The default mode network is thought to decrease connectivity when cognitively demanding tasks are performed[65]. However, our cognitive task was imagery, which is introspective in nature. Introspective or imagery tasks have been associated with increased DMN activity[66,67]. This suggest that our task is not purely introspective, at least after feedback training. We cannot differentiate which parts of our task are related to introspective imagery, and which parts are related to pain inhibition. Nonetheless, some change in the application of the strategy occurred over training which decreased DMN activity. A possibility is that participants can apply their task in a more focused manner post training. Activity of the DMN is also shown to be associated with mind wandering[68,69]. Before learning an effective strategy, it stands to reason that participants engage in more mind wandering than after training when they have developed a strategy proper. The decreased activity in DMN associated areas may therefore stem from decreased mind wandering, which hints to a more focused application of their cognitive strategy.

In the ROI analysis, only thalamus and mPFC showed an effect of cognitive strategy unaccompanied by painful stimulation. Activity in mPFC was increased during strategy compared to control but did not change with training. Thalamus activity increased during strategy, but only after training. The effects were most pronounced in the frontal regions of the mPFC and the left ventroposterolateral (VPL) and posterior portions of the thalamus. Activity in the mPFC is commonly associated with cognitive tasks[70] so increased activity during the cognitive emotional strategy is expected. Increases in thalamic activity, in the absence of painful stimulation is less expected as the VPL is most commonly associated with the direct processing of painful information[71]. Our results indicate that activity in the VPL of the thalamus increases while participants use their strategy post-training. Similarly, a previous study found increases in thalamic activity during distraction from pain[21]. The activation of descending inhibition via mental imagery may activate similar areas as the distraction from pain investigated in that study. It may therefore be that the VPL is not only involved in the direct processing of a nociceptive stimulus, but may also be involved in the downregulation and modulation of pain.

Our results indicate that the mPFC and thalamus play a key role in the strategy used to decrease pain perception. The thalamus, which is known to connect not only to mPFC, but also other areas related to descending pain inhibiton[72,73], may act as a crossover point, integrating and relaying signals between these areas while the mPFC is involved in the mental imagery. Additionally, feedback training likely makes participants more efficient at applying their strategy and by consequence at activating their descending pain inhibition.

4.5. Individual variability in descending pain inhibition may impact finding group-level effects

We expected a stronger group-level effect of strategy use on a whole-brain level, and in particular in our ROIs. However, the choice of cognitive strategy ("think of a safe and happy place") may not have been ideal for all participants. Previous studies have shown that although it is the most successful strategy for most participants, not all individuals are successful at using it[28]. Using different strategies may lead to a stronger general pain inhibition success but also results in differential brain activity for each strategy[24] and a large degree of individual variability remains. In fact, the large variability in both training success, as well as in brain activity for any given strategy will make finding common effects difficult. This variability in responses across participants is consistent with findings in both pain imaging[74,75] and other measures of descending pain inhibition, such as CPM[76,77]. To compound the issue, the effect size of applying a cognitive strategy is likely small enough that differences between conditions may be too subtle to detect. Imaging research in mindfulness and other mental imagery tasks shows equally small effect sizes[78–81].

In addition to the differences in the cognitive strategy, we find variability in multiple aspects of the study that may impact our imaging findings. One expected source of variability was the individual training success. Some participants were able to reduce their RIII-reflex size more than others, and some could not do so at all. This of course introduces variability in the desired effect and most likely also reduces the effect size of the brain activity. Additionally, we observe some participants which could already reduce their RIII-reflex in the first feedback session. This suggests that even without RIII-feedback, these individuals can already control their descending pain inhibition while using the proposed cognitive strategy. Longitudinal comparisons are more difficult if one subpopulation presents with the desired training effect, while other subpopulations show no effect at all and a third does not require training. Our current study population is too small to stratify into subpopulations and still draw meaningful inferences, however the differences between them would present an interesting investigation of underlying individual differences in descending pain inhibition.

Underlying differences in brain anatomy and connectivity have previously been shown to influence pain inhibition. Here, functional connectivity of areas such as the PAG and RVM is shown to be indicative of pain and pain reduction[82–84]. Connectivity between the ACC and the PFC has been associated with the ability to modulate pain[84,85]. These functional connections are further supported by studies investigating anatomical connectivity using tractography. PAG connections with the ACC and PFC have been shown to vary between individuals and to be linked to placebo analgesia[86]. Taking together the functional and anatomical connectivity we can see that underlying differences in brain connections can influence descending pain inhibition. We also performed both resting state functional and diffusion weighted imaging, however these measures are outside the scope of this study. It may well be that differences between functional or anatomical connectivity are better predictors of training's success, or a general ability to activate descending pain inhibition than brain activation.

Although we set the stimulation intensity for each individual to 150% of their personal reflex threshold, to reliably evoke a reflex with every stimulus, there was still a large degree of variability in the absolute pain ratings of the stimulus during fMRI. Although we designed the experiment such that the pain rating was normalized to the control task, different absolute pain intensities may lead to differences in brain activation. Another potentially meaningful and oftentimes overlooked factor when studying pain in the MRI, is the participants' disposition to the MRI environment. Our study population included MRI-naïve and MRI-experienced participants. Some participants perceived the MRI environment as more stressful than others. As stress is known to affect pain, pain inhibition[87,88] and cognitive performance[89], we cannot preclude that baseline differences in stress level did not influence participants' pain or their ability to concentrate on their strategy. However, none of our participants suffered from stress or panic disorders, such as claustrophobia, and the longitudinal design should take into account baseline differences, so we believe this effect to be small.

4.6. Proposal – the effect of feedback training on descending pain inhibition

Taking all results together, we propose that feedback training improves the activation of descending pain inhibition in the following way: An increased activity during strategy in the mPFC starts a top-down process that goes through the thalamus to the brainstem. This activation results in decreased response to nociceptive input in primary receptive regions of both spinothalamic (thalamus) and spinoreticular (RVM and PAG) tracts of pain transmission which propagates to a reduced response in emotional-evaluative cortical regions such as the insula and dlPFC, and a resulting decrease in experienced pain. Increased activity of the dlPFC has been linked to pain inhibition[90], suggesting its involvement in

modulation beyond pain evaluation. It has also been shown to modulate other cortical areas to modulate pain[91]. This suggests that it, along with the mPFC, is a critical prefrontal contributor to descending pain inhibition. The ACC, repeatedly shown to be involved in pain inhibition[3,4], is known to connect to the PAG as well. Including the existing evidence regarding the ACC and dlPFC, we further suggest that these areas are24nvolveed in initiating descending inhibition, either via cortico-cortical interactions with each other and the mPFC, or through direct connections to the PAG.

The mPFC is a key area in placebo and expectation based analgesia[92,93], and it provides the primary cortical input to the PAG[94,95]. We found higher activity in the mPFC during strategy in both MRI sessions, where participants report a decrease in subjective pain. As mPFC activity does not change post-training, we propose that training led to an improved efficiency in strategy use, as seen by the reduction in default mode network activity. The increased activity in the thalamus post training supports the notion that participants more effectively apply their cognitive strategy. Whether the application of a cognitive strategy only activates cortical and thalamic pain-related centers, or whether the individual variability in training success is preventing us from detecting more subtle subcortical effects cannot be resolved at this time.

The immediate response to painful stimulation shows a more robust training effect. The decreased haemodynamic response within primary receptive areas of ascending pain pathways indicates that nociceptive input is already decreased upon reaching the brain, either via a reduction in the first brainstem relay centers or a decrease in nociceptive transmission already on a spinal level. We also see a decreased reaction to the painful stimulation in higher evaluative regions of the brain including the insula and the dlPFC. This may stem from two mechanisms: decreased nociceptive input into the cortex may lead to a proportionately lower reaction in cortical areas, or the cognitive strategy decreases reactivity in these areas, causing a reduced reaction to painful stimuli of the same magnitude. Which mechanism is contributing to the results we see cannot be resolved with this study. However, a combination is likely to occur. Improved pain reduction was correlated with reduced hemodynamic activity in frontal ACC areas in pain evaluation[96]. Variability in the cortical response has also been previously reported to correlate with pain intensity[75,97], suggesting that a decreased cortical activity is indicative of less experienced pain. Our findings support this hypothesis and additionally suggest that a reduction in and speeding up of hemodynamic activity in these frontal evaluative regions is what is relevant for reducing experienced pain during nociceptive stimulation.

4.7. Improvements and future directions

The biggest strength of this study was its longitudinal design. By measuring each participant before and after feedback training, we could create a direct, within-subject comparison of brain activity before and after they learned to activate descending pain inhibition. This is particularly relevant in pain research as experienced pain is a highly individual and variable phenomenon. We also read-out both physiological and psychological measures of pain, by constructing a hardware setup for the MRI environment with simultaneous EMG recording and electrical nociceptive stimulation. The MRI setup resembled the feedback training as closely as possible so that task and design could be as congruent as possible. In the analyses, we accounted for potential discrepancies between canonical HRF and the actual haemodynamic response by conducting a timecourse analysis. This allowed us to detect differences in subcortical and cortical regions where classical analyses often fail.

Nonetheless there are important methodological lessons we can learn here. Measuring the RIII-reflex is inherently difficult in an MRI environment. We cannot exclude the possibility that the MRI environment itself had an effect on the RIII-reflex that rendered it less effective at measuring spinal nociception. Mutual interference of electrical stimulation, MRI, and electrophysiological recordings added the need for additional safeguards in the MRI environment including higher resistance electrodes, on-line filtering of electrical signals, and post-hoc processing with artifact correction, which likely interfered with the reflex signal itself. In addition, the body position of the participant made a direct transfer between training and MRI sessions challenging. Participants were not in a supine position during training, which may change the nature of the reflex signal[98]. Also, training is performed in a much more quiet and less confined environment than the MRI and participants are inherently less relaxed in the MRI environment, which may affect EMG recordings. The lack of transfer from RIII-reflex training success to a RIII-reduction during MR imaging prevents us from making direct inferences regarding spinal nociception. The decreased reaction to stimulation in nociceptive brainstem areas are still a good indicator of decreased ascending nociceptive input. Finally, we cannot determine when participants started and for how long they could effectively apply their cognitive emotional strategy. Although visual cues and careful instructions were given, the timing-based regressor used to analyze this effect does not adequately capture the true application time of the strategy or its haemodynamic effect.

5. Conclusion

The present study shows that learning a cognitive strategy to activate descending pain inhibition via RIII-feedback training leads to decreased nociceptive processing in the brainstem, thalamus and affective-processing areas. We believe that the pain reduction by strategy is caused by a combination of decreased ascending nociceptive information combined with decreased pain processing in both somatosensory and affective-cognitive dimensions. It further highlights the importance of mPFC activity in mental imagery for pain inhibition and suggests that lateral thalamic nuclei play a role in pain modulation in addition to their role in pain processing.

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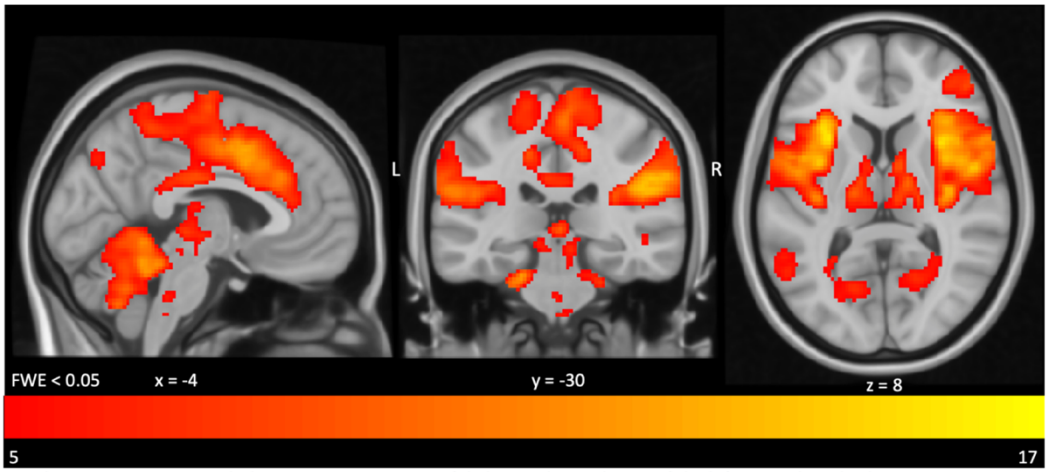
Supplementary Data

Supplementary Table 1: tSNR values of the individual ROIs define in Table 1*. tSNR was calculated after ICA-AROMA preprocessing and motion correction.*

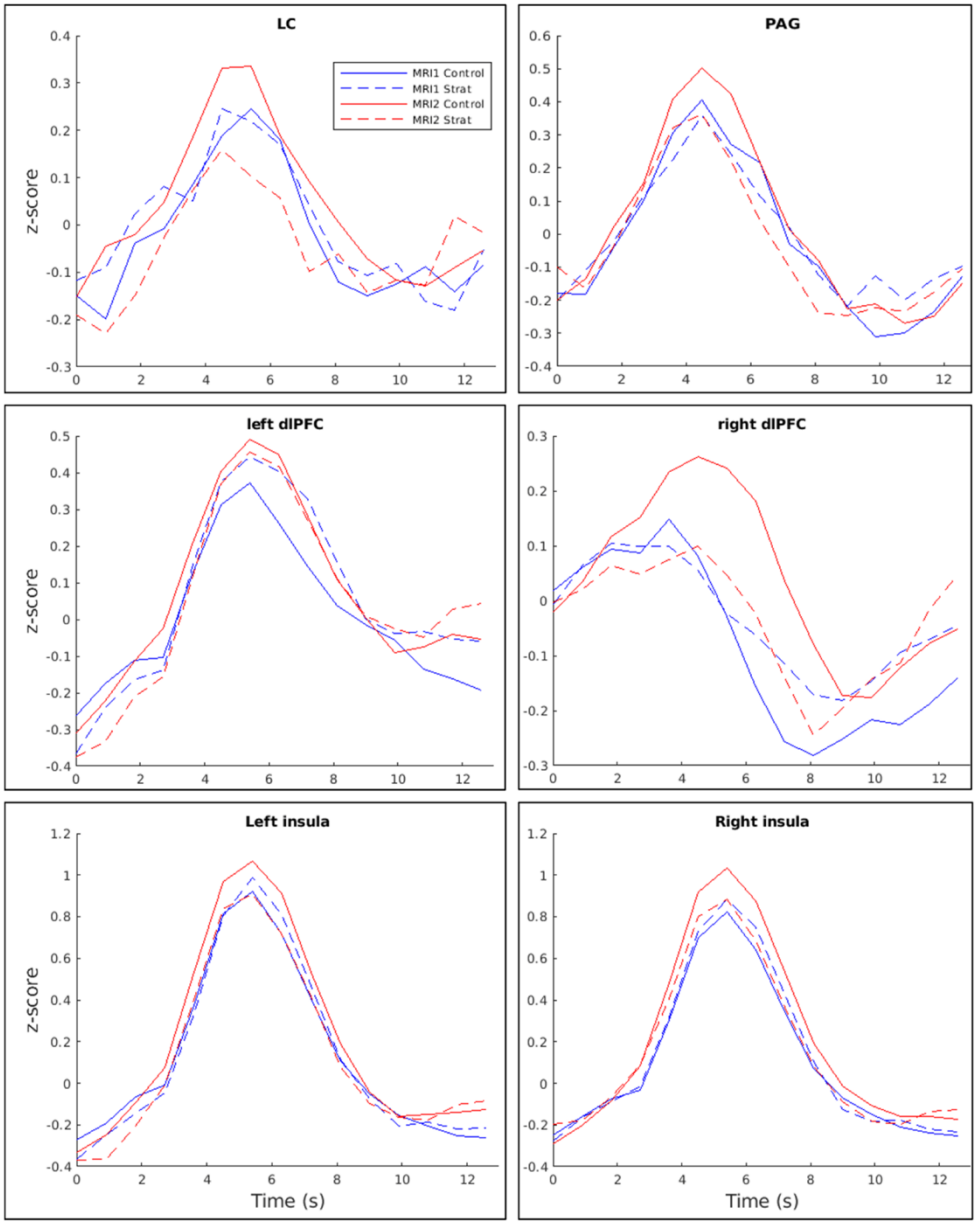
ROI	tSNR
RVM	155 ± 24
Locus coeruleus	164 ± 21
PAG	155 ± 21
Thalamus	152 ± 19
Hypothalamus	108 ± 31
ACC	149 ± 25
Insula	165 ± 25
mPFC	103 ± 22
dlPFC	172 ± 28

Supplementary Table 2: Cluster size and location for the general response to painful electrical stimulation. *Anatomical labels were extracted the Harvard oxford cortical atlas in FSL. Cluster size refers to the number of voxels of the current cluster, voxel size is 2mm isotropic. Coordinates are in MNI space.*

Anatomical region	p-value	Cluster size	Peak coordinates (mm)		
	FWE	voxel	x	y	z
Right insula	< .001	7838	44	2	2
Left insula	< .001	6686	-32	16	10
Cerebellum/Thalamus/Brainstem	< .001	7306	-20	-32	-26
SMA / ACC / PCC / paracingulate gyrus / postcentral gyrus	< .001	13295	6	24	36
(lateral) frontal pole (R)	< .001	926	46	44	6
(lateral) frontal pole (L)	< .001	637	-32	40	28
Intracalcarine / suprecalcarine / cuneal / precuneal cortex (R)	< .001	908	18	-64	12
Intracalcarine / supracalcarine coretex (L)	< .001	436	-14	-70	6
Cuneal / precuneal cortex (L)	< .001	290	-12	-72	34
Middle temporal gyrus (left temporooccipital part)	< .001	155	-52	-60	6



Supplementary Figure 1: Whole Brain reaction to painful electrical stimulation of the left n. suralis in both MRI sessions. *Areas activated include areas commonly active during pain such as contralateral S1, bilateral insulae, anterior and midcingulate cortices, supplementary motor area, thalamus, brainstem and cerebellum. The contrasts investigated were the combined regressors of painful stimulation in both control and strategy over both MRI sessions.*



Supplementary Figure 2: ***Timecourses of z-transformed raw MRI signal****. The is a significant main effect of condition (strategy < control) in PAG (p < 0.001) and locus coeruleus (p < 0.001) and a significant interaction between condition and session in LC (p < 0.001), insula (p = 0.001) and dorsolateral prefrontal cortex (p < 0.0001), with a greater decrease during strategy relative to control in MRI2.*

2.2 Inter-individual differences explain more variance in conditioned pain modulation than age, sex and conditioning stimulus intensity combined.

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Summary

In this publication we estimated the relative variance in CPM explained by residual inter-individual differences compared to age, sex, and CS physical and pain intensity. We constructed linear and mixed effect models on pooled data from 171 participants from several studies, of which 97 participants had repeated measures. By applying variance decomposition estimations to our repeated measures data for the first time, we were able to investigate the contribution of known factors (age, sex, CS intensity, or CPM paradigm) and the unexplained individual differences to the CPM variability. Cross-sectional analyses showed no significant effect of age, sex or CS intensity, while repeated measures analyses revealed a significant effect of CS physical intensity. Variance decomposition showed that inter-individual differences accounted for between 24% to 34% of the variance in CPM while age, sex, and CS intensity together explained <3% to 12%. This demonstrates variance in CPM explained by inter-individual differences largely exceeds that of commonly considered factors such as age, sex and CS intensity. We show that the conflicting predictive capability of these factors in the literature may be due to baseline differences in the population. We suggest that future investigations should account for the individual variability by employing repeated measures designs or statistical analyses which take into account the individual as a factor.

Author contribution

All authors designed the study, with the analysis design being conceptualized by Philipp Graeff and Ruth Ruscheweyh. Data was collected by Alina Itter, Katharina Wach and Philipp Graeff under the supervision of Ruth Ruscheweyh. Analysis was performed by Philipp Graeff. Philipp Graeff and Ruth Ruscheweyh wrote the paper with input from all authors.

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Article

Inter-Individual Differences Explain More Variance in Conditioned Pain Modulation Than Age, Sex and Conditioning Stimulus Intensity Combined

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Abstract: Conditioned pain modulation (CPM) describes the reduction in pain evoked by a test stimulus (TS) when presented together with a heterotopic painful conditioning stimulus (CS). CPM has been proposed to reflect inter-individual differences in endogenous pain modulation, which may predict susceptibility for acute and chronic pain. Here, we aimed to estimate the relative variance in CPM explained by inter-individual differences compared to age, sex, and CS physical and pain intensity. We constructed linear and mixed effect models on pooled data from 171 participants of several studies, of which 97 had repeated measures. Cross-sectional analyses showed no significant effect of age, sex or CS intensity. Repeated measures analyses revealed a significant effect of CS physical intensity ($p = 0.002$) but not CS pain intensity ($p = 0.159$). Variance decomposition showed that inter-individual differences accounted for 24% to 34% of the variance in CPM while age, sex, and CS intensity together explained <3% to 12%. In conclusion, the variance in CPM explained by inter-individual differences largely exceeds that of commonly considered factors such as age, sex and CS intensity. This may explain why predictive capability of these factors has had conflicting results and suggests that future models investigating them should account for inter-individual differences.

Keywords: conditioned pain modulation; endogenous analgesia; conditioning stimulus; interindividual factors; CPM variability

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1. Introduction

Conditioned pain modulation (CPM) paradigms measure the component of human endogenous pain inhibition underlying the "pain inhibits pain" phenomenon [1], based on a noxious test stimulus (TS) being perceived as less painful if presented in combination with a painful heterotopic conditioning stimulus (CS). CPM magnitude is reduced in a variety of chronic pain conditions, pointing towards dysregulation of endogenous pain inhibition in these patients [2].

Individual differences in CPM are considerable, and have been proposed to predict susceptibility to acute and chronic pain [3,4]. Some individual factors influencing CPM magnitude have been identified: e.g., some studies have found a larger CPM effect in males than females [5,6] and in younger compared to older subjects [7,8]. An effect of pre-existing psychological factors has been discussed, but a recent study has not shown a clear relation to the CPM effect [9]. It is currently not known how much individual variance remains after accounting for the effects of age and sex.

In addition, many different experimental paradigms have been used [10] which may also influence CPM magnitude., e.g., the role of conditioning stimulus intensity has been

investigated repeatedly with inconsistent results. While some studies find no effect [11–13], others find larger CPM with stronger conditioning stimuli [14–16]. It has been proposed that as long as it is clearly painful, further increases in conditioning stimulus intensity do not increase CPM magnitude [12,13]. It might also occur that conditioning stimulus intensity does have an effect in within-subject designs [14,17], but that the effect is small compared to inter-individual differences, which makes it difficult to detect in cross-sectional designs. In addition, it is worth considering whether physical stimulus intensity or rather subjective pain perception of the conditioning stimulus is related to CPM magnitude.

It would therefore be useful to estimate the relative importance of the various factors influencing the CPM effect. There are now methods to estimate variance contributions of both fixed effects (such as sex, age and conditioning stimulus intensity) and random effects (such as remaining individual differences) within the same model [18,19], in addition to the relative variance contributions of the different fixed effects [20,21].

Here, we used pooled datasets from various studies measuring the CPM effect in healthy individuals once or multiple times to assess the relationship between CPM effect and age, sex, and conditioning stimulus physical or pain intensity in both cross-sectional and repeated measures settings, and estimated the relative variance in CPM magnitude explained by remaining inter-individual differences vs. age, sex, and conditioning stimulus intensity.

2. Materials and Methods

2.1. Pooled Data

Data was pooled from seven separate studies performed by our group, which investigated different aspects of endogenous pain inhibition in healthy participants, including at least one measurement of the conditioning pain modulation effect. Four studies included repeated measures gathered on different days. In total, data was pooled from 171 participants for cross-sectional analysis and from 97 participants for repeated-measures analysis. Of the repeated measures data pool, 83 participants had two repeated measures and 14 participants had three repeated measures.

Pooled data included three types of test stimulus: a 30 s or 60 s heat stimulus or electrical stimulation of the sural nerve. Conditioning stimulus in all studies was a cold pressor test of varying length (60 s, 90 s, 120 s). An overview of the studies can be found in Table 1.

Table 1. Overview of studies used for pooled data in this analysis. N = 171 participants total. Repeated measures: n = 97 participants, n = 208 observations.

Study	Age	M/F	Conditioning Stimulus	Test Stimulus	Repeated Measures	Citation
1	25 ± 6	18/12	Cold water (120 s)	Electrical	Yes	Unpublished
2	23 ± 4	15/5	Cold water (60 s)	Contact heat (30 s)	Yes	Unpublished
3	27 ± 6	14/9	Cold water (60 s)	Contact heat (30 s)	Yes	[22]
4	47 ± 10	27/0	Cold water (90 s)	Contact heat (60 s)	No	[23]
5	23 ± 5	17/9	Cold water (60 s)	Contact heat (30 s)	Yes	Unpublished
6	25 ± 5	9/19	Cold water (60 s)	Contact heat (30 s)	No	Unpublished
7	25 ± 3	7/10	Cold water (60 s)	Contact heat (30 s)	No	Unpublished

2.2. Participants

All studies were conducted in accordance with the Declaration of Helsinki and were approved by the ethics committee of the Ludwig-Maximilian University, Munich. Healthy

participants were recruited by announcements on the university campus and gave written informed consent. Participants had to meet the following criteria (which apply to all our studies with healthy participants): (1) age ≥18 years, (2) sufficient knowledge of German, (3) no severe internal, neurological or psychiatric conditions, (4) no history of chronic pain, (5) no alcohol, nicotine or drug abuse, (6) no regular medication (except hormonal contraception or thyroid hormones), (7) not pregnant or breastfeeding, (8) no acute pain and no use of pain medication within the previous 48 h, (9) Beck's depression inventory score <13.

2.3. Conditioned Pain Modulation

The study data collected utilized three different CPM paradigms (combinations of test stimulus and conditioning stimulus).

The conditioning stimulus in all studies was immersion of the contralateral (in regard to the test stimulus) hand into a Styrofoam box filled with cold water for 60–120 s. Pain intensity ratings were collected on an 11-point numeric rating scale (NRS, 0 = no pain, 10 = most intense pain imaginable). According to the results of Granot [12], we aimed at a conditioning stimulus intensity that was clearly painful (usually ≥3 on the NRS) but could be tolerated for the planned stimulus duration. To achieve this, in a pre-test, water temperature was individually adjusted starting at 10 °C.

Test stimuli were either painful heat (30 s or 60 s) or electrical stimulation of the sural nerve.

Heat stimulation was applied via a thermode (Pathway system, Medoc, Israel) to the volar side of the forearm at a temperature individually tailored to evoke a pain intensity rating of approximately 6 on the NRS, resulting in temperatures of 46.3 ± 1.2 °C, range: 43–49 °C. Heat pain intensity ratings were collected every 10 s for the stimulus duration. The heat stimulus was first presented in isolation (baseline) and then 30 s following the start of the conditioning stimulus. A ≥ 5 min break was taken between baseline and conditioning measures and the thermode was shifted between measurements to avoid habituation.

Painful electrical stimulation of the sural nerve was performed as described previously [23,24]. Electrical stimuli were applied every 8–12 s for three consecutive 2 min blocks, each block containing 12 stimuli. Conditioning stimulus was present during the second block (120 s). Pain intensity rating of the test stimulus was collected at the end of each block as the average pain intensity of the last five stimuli.

CPM effect was calculated as the percentage difference between average test stimulus NRS rating at baseline (NRSts(baseline)) and during conditioning stimulation (NRSts(cond)), where a more negative result denotes a stronger CPM effect:

$$\text{CPM effect} = \frac{\text{NRSts(cond)} - \text{NRSts(baseline)}}{\text{NRSts(baseline)}}$$

2.4. Statistical Analysis

All statistical analysis was performed in R [25]. $p < 0.05$ (two-sided) was considered statistically significant.

Linear regression: linear regression analyses were performed using the *lm()* function of the *stats* package [25]. The linear regression models used for the cross-sectional population analysis of the relationship between CPM effect and age, sex, paradigm and conditioning stimulus pain or physical intensity were:

$$\text{CPM effect} \sim \text{NRS}_{\text{cond}} + \text{age} + \text{sex} + \text{paradigm} \quad (1)$$

$$\text{CPM effect} \sim \text{temperature}_{\text{cond}} + \text{age} + \text{sex} + \text{paradigm} \quad (2)$$

NRS_{cond} describes the CS pain intensity on the NRS (0–10) immediately after the test stimulus, $\text{temperature}_{\text{cond}}$ describes the physical intensity (cold water temperature) of the

CS and paradigm describes the CPM paradigm as a factor. The three paradigms included were 60 s heat/90 s cold (reference), 30 s heat/60 s cold, and Electrical/120 s cold.

Mixed models: linear mixed model analysis was performed on the pooled repeated measures data using the lme4 [26] and car [27] packages in R. From the repeated measures population, we extracted those participants in which the CS pain intensity differed by at least 0.5 points on the NRS between measurements (85 participants, 184 observations) and those participants in which the CS physical intensity (temperature) differed by at least 0.5 °C between measurements (52 participants, 118 observations). Linear mixed models were constructed for the pain intensity rating variable, and the temperature variable subgroup using the *lmer()* function of lme4 [26]:

$$\text{CPM effect} \sim \text{NRS}_{\text{cond}} + \text{age} + \text{sex} + \text{paradigm} + \text{repeat} + (1 \mid \text{participant}) \quad (3)$$

$$\text{CPM effect} \sim \text{temperature}_{\text{cond}} + \text{age} + \text{sex} + \text{paradigm} + \text{repeat} + (1 \mid \text{participant}) \quad (4)$$

CPM effect, NRS_{cond} and $\text{temperature}_{\text{cond}}$ are as described above; paradigm describes the two CPM paradigms included in the dataset: 30 s heat/60 s cold and Electrical/120s cold (reference). Repeat describes the measurement repeat (i.e., first, second, or third measurement for that participant). The random effect (1 | participant) allows for variable intercept for each participant. Significance of each fixed effect was tested using Wald's Chi-squared test implemented via the *Anova()* function of the *car* package [27].

Fixed vs. random effects variance contribution: in order to determine the inter-individual variability of the CPM effect not explained by age and sex we calculated the percent variance in CPM effect explained as contributed by fixed and random effects. To do this we calculated the marginal and conditional coefficient of determination as described in Nakagawa et al. [18,19] using the *r.squaredGLMM()* function of the *MuMIn* package [28] on the mixed models described above. Marginal R^2 describes the variance explained by all fixed effects and conditional R^2 describes the variance explained by both fixed and random effects combined. Variance explained by inter-individual variability was calculated by subtracting the marginal from the conditional R^2.

Variance contribution of the fixed effects: in order to determine the relative contribution of each fixed effect factor to the variance in the CPM effect, the *calc.relimp()* function of the *relaimpo* package was used [29]. We utilized the "lmg" option of R^2 variance decomposition, which averages the R^2 contribution of each factor over all orderings as described by Lindeman, Merenda and Gold [21] and Chevan and Sutherland [20].

As *calc.relimp()* cannot handle mixed model input, we constructed the following linear models using the *lm()* function to reflect the fixed effects of the mixed model 3 and 4 and used them as input to the *calc.relimp()* function:

$$\text{CPM effect} \sim \text{NRS}_{\text{cond}} + \text{age} + \text{sex} + \text{paradigm} + \text{repeat} \quad (5)$$

$$\text{CPM effect} \sim \text{temperature}_{\text{cond}} + \text{age} + \text{sex} + \text{paradigm} + \text{repeat} \quad (6)$$

3. Results

3.1. Cross-Sectional Analysis

The mean age of the cross-sectional sample was 29 ± 11 (n = 171, 64 women). The average CPM effect was significant ($p < 0.001$) and amounted to -16.9 ± 23.9%. The average pain rating of the conditioning stimulus was 4.5 ± 1.8 (range: 1.0–9.0) on the NRS and the average temperature of the conditioning stimulus was 7.9 ± 3.4 °C (range: 0.1–16.2).

Multiple linear regression was calculated to predict CPM effect from participant age, sex, CPM protocol and either CS pain or physical intensity (models 1 and 2, Table 2). There was no significant relation of the CPM effect with CS pain intensity (NRS_{cond}) or CS physical intensity ($\text{temperature}_{\text{cond}}$) (Figure 1). There also was no significant relation of age, sex or CPM paradigm with the CPM effect. Proportions of variance in CPM effect explained by all predictors together were low (1.1% for model 1 and 1.9% for model 2). Results for the individual predictors are given in Table 2.

Table 2. Results of linear regression analysis of the cross-sectional data (n = 171). See Methods for construction of models 1 and 3. NRS_{cond}, CS pain intensity rating on the NRS [0–10]. $Temperature_{cond}$, temperature of the CS (cold water bath). Fixed effects of sex and paradigm were compared to a reference (male and 30 s heat/60 s cold, respectively).

Model	Predictor	Estimate	Std. Error	*p*-Value	Multiple R^2
Model 1	Intercept	−17.385	9.933	0.082	0.0109
	NRS_{cond}	−0.916	1.101	0.407	
	Age	−0.053	0.288	0.854	
	Sex	4.181	4.080	0.307	
	30 s heat/60 s cold	3.643	8.853	0.681	
	Electrical/120 s cold	−1.190	4.988	0.812	
Model 2	Intercept	−27.890	10.237	0.007	0.0194
	$Temperature_{cond}$	0.866	0.594	0.147	
	Age	−0.102	0.288	0.723	
	Sex	4.818	4.096	0.242	
	Heat30 s/Cold60 s	1.825	8.715	0.834	
	Electrical/120s cold	0.470	5.141	0.927	

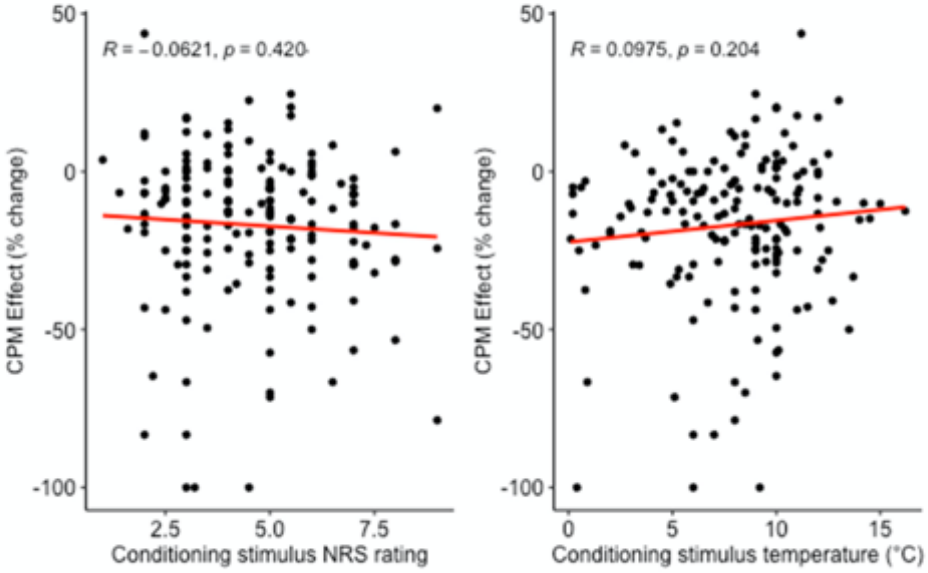

Figure 1. Relation between CPM effect and conditioning stimulus pain intensity or conditioning stimulus temperature in the cross-sectional analysis. Conditioning stimulus was hand immersion in cold water. More negative CPM effect designates a larger reduction of test pain rating by the conditioning stimulus.

3.2. Repeated Measures Analysis of Linear Mixed Models

The average CPM effect in the repeated measures sample (Model 3: 184 observations, Model 4: 118 observations) was significant (both $p < 0.001$) and amounted to $-17.6 \pm 24.6\%$ and $-16.9 \pm 21.2\%$, respectively. Mean difference in NRS rating of CS between observations in Model 3 was 1.9 ± 1.2. Mean difference in CS temperature between observations in Model 4 was 4.3 ± 2.6 °C.

Linear mixed models were constructed in order to analyze the contributions of the different fixed effects to the CPM effect (Table 3). A larger (i.e., more negative) CPM effect was significantly related to a lower CS temperature ($p = 0.001$, −1.5% change in CPM effect per °C temperature decrease) in Model 4.

The remaining relations were all non-significant (Table 3): in Model 3, CPM effect increased (i.e., was more negative) by −1.5% per NRS point with increasing CS pain intensity ($p = 0.159$). CPM effect decreased non significantly by 0.26% per year of age in both models. CPM effect in women was 2.5% lower in Model 3, and 1.9% larger in Model 4 compared to men (both n.s.). The 30 s heat/60 s cold protocol produced a 7.8% (Model 3) and 8.5% (Model 4) larger CPM effect than the electrical/120 s cold protocol (both n.s.).

Table 3. Relation between CPM effect and CS pain intensity or physical intensity (temperature) in the repeated measures analysis. Linear mixed effect analysis of Model 3 (n = 85 participants, 184 observations) and Model 4 (n = 52 participants, 118 observations), see Methods for model specification. *p*-values were obtained by Wald's Chi-Square test on the fitted mixed models. NRScond, CS pain intensity rating on the NRS [0–10]. Temperaturecond, temperature of the CS (cold water bath). Paradigm, 30 s heat/60 s cold as opposed to Electrical/cold120s (reference). Repeat refers to the measurement repeat and is used to control for order of measurement. Significant effects are marked in bold.

Model	Predictor	Estimate	Std. Error	*p*-Value	REML Criterium at Convergence
Model 3	Intercept	−11.685	13.461	-	1649.6
	NRS_{cond}	−1.485	1.056	0.159	
	Age	0.257	0.388	0.506	
	Sex	2.452	4.402	0.578	
	Paradigm	−7.815	4.808	0.104	
	Repeat	−2.617	2.637	0.321	
Model 4	Intercept	−21.712	13.935	-	1015.8
	$Temperature_{cond}$	**1.532**	**0.482**	**0.002**	
	Age	0.258	0.428	0.546	
	Sex	−1.860	4.831	0.700	
	Paradigm	−8.468	4.910	0.085	
	Repeat	−3.073	2.343	0.190	

3.3. Repeated Measures Analysis: Decomposition of Explained Variance

To determine the relative variance explained by fixed effects vs. inter-individual differences we decomposed the total R^2 into conditional (fixed effects) and marginal (fixed effects + inter-individual factors) R^2. Conditional R^2 and marginal R^2 were 3.4% and 27.4% in Model 3, and 11.5% and 45.8% in Model 4, respectively. Therefore, in Model 3, all fixed effects together explained 3.4% of the variance in the CPM effect, while the remaining inter-individual differences explained 24.0%. In Model 4, all fixed effects together explained 11.5% of the variance in the CPM effect and remaining inter-individual differences explained 34.3%.

Finally, in order to further decompose the variance explained by the different fixed effects, we constructed linear models including only the fixed effects (Models 5 and 6, Table 4). CS pain intensity explained 0.7% of the variance (model 5) while CS physical intensity (cold water temperature) explained 4.7% (Model 6). The type of CPM paradigm used explained 1.5% and 3.0% of the variance in Model 5 and 6, respectively. Age, sex and measurement repeat made only small contributions to the explained variance (<1% each). Variance breakdown of the significant model (Model 4) and the relative variances of its fixed effect are seen in Figure 2. It is important to note that the fixed effects' variance in Models 5 and 6 does not sum to the variance explained by fixed effects in Models 3 and 4, respectively. This is due to the fact that the models used differ, resulting in slightly different fits. Additionally the statistical methods used for variance decomposition in the two types of analysis are different, which will further lead to discrepancies.

Table 4. Variance in the CPM effect explained by fixed effects, including CS pain and physical intensity in the repeated measures analysis. Model 5: n = 85 participants, 184 observations; model 6: n = 52 participants, 118 observations, see Methods for model specifications. R^2 indicates the total variance explained by each individual predictor as calculated by the *calc.relimp()* (relaimpo package) in R. NRScond, pain intensity rating of conditioning stimulus on the NRS [0–10]. Temperaturecond, temperature of the conditioning stimulus (cold water bath). Paradigm, 30 s heat/60 s cold as opposed to Electrical/120 s cold (reference). Repeat refers to the measurement repeat and is used to control for order of measurement.

Model 5		Model 6	
Predictor	R^2	Predictor	R^2
NRS_{cond}	0.00681	$Temperature_{cond}$	0.04650
Age	0.00438	Age	0.00886
Sex	0.00559	Sex	0.00091
Paradigm	0.01491	Paradigm	0.03010
Repeat	0.00325	Repeat	0.00871

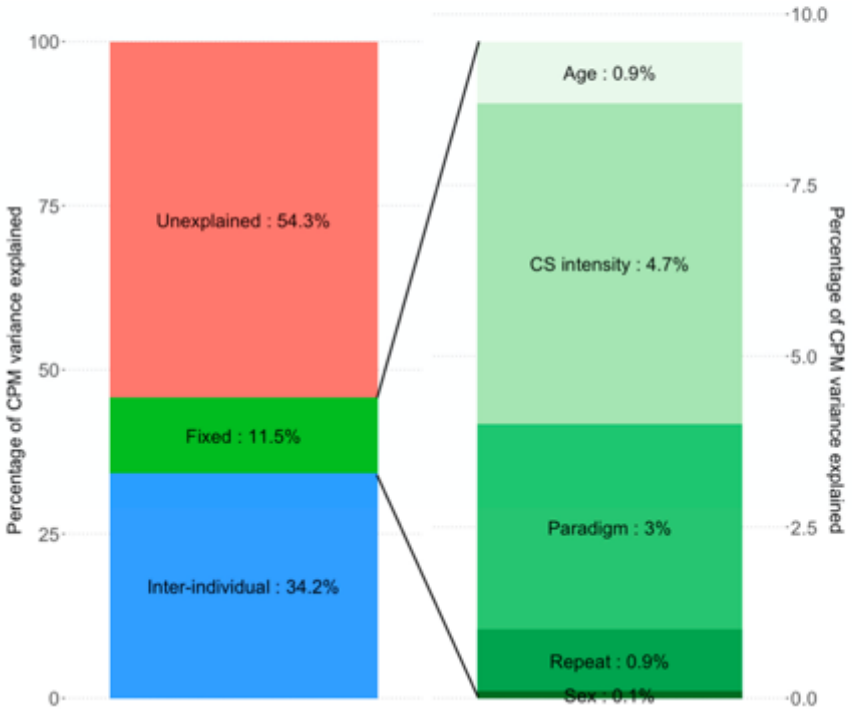

Figure 2. Proportions of variance in CPM explained by the CS physical intensity models (models 4 and 6). Inter-individual differences explain substantially more CPM variance than the fixed effects of age, sex, CS intensity or the nuisance regressors CPM paradigm or measurement repeat (34.2% vs. 11.5%, respectively). Of the fixed effects, CS intensity explains the most variance (4.65%), followed by CPM paradigm (3.01%). Age (0.89%), Sex (0.09%), and measurement repeat (0.87%) explain negligible amounts. Due to different model types (model 4: linear mixed effects model; Model 6: multiple linear regression model) and different statistical methods needed to estimate partial variance explained, the sum of fixed effects variance explained in Model 6 does not equal exactly the estimated variance of combined fixed effects in Model 4.

4. Discussion

Main results of the present study were:

(i) In a large cross-sectional analysis, neither CS physical intensity nor CS pain intensity predicted the CPM effect. In contrast, in a repeated measures analysis, CS physical intensity, but not CS pain intensity predicted the CPM effect.

(ii) Inter-individual differences explained a large proportion of CPM variance (24.0% to 34.2%) while all fixed effects together (CS pain or physical intensity, age, sex, CPM paradigm, measurement repeat) predicted only 3.4% to 11.5% of CPM variance.

4.1. Conditioning Stimulus Physical Intensity and Pain Intensity

Previous results on the dependence of CPM magnitude on CS intensity are inconsistent, ranging from no effect [11–13] to a significantly increased CPM effect with higher CS intensity [14–16]. Our study confirms and extends previous cross-sectional results [12] showing no significant relation between CPM magnitude and either CS physical intensity or CS pain rating in a large cross-sectional sample (n = 171). In contrast, the repeated measures analysis revealed a significant relation between CPM magnitude and CS physical intensity, while the relation with CS pain intensity remained non-significant. This raises two interesting points.

First, as CPM is a psychophysical measure, one could assume that the subjective pain experience would have a larger influence on the CPM effect than the CS physical intensity. Indeed, some studies have shown a relation between CS pain intensity and CPM magnitude [15,16]. In addition, placebo-induced reduction of perceived CS pain was related to a reduced CPM effect [30] and CS-induced supraspinal activation correlated with CPM magnitude [31]. On the other hand, some processes underlying CPM seem to be independent of the subjective pain experience [32]. In spite of the above cited supraspinal influences, a spino-bulbo-spinal pathway is thought to be the main circuitry responsible for CPM [33,34]. This may be one possible explanation for CS physical intensity being a larger determinant than CS pain intensity. Consistently, some previous studies have shown a relation between CS physical intensity and CPM effect [14,35]. However, since CS physical and pain intensity are highly correlated, only studies that investigate both parameters over a range of different values will be able to show which correlation is larger. The present study conducted such a direct comparison and found a preferential relation with CS physical intensity. Notably, it may be both a strength and a limitation of the present study that variability in CS physical and/or pain intensity was mostly random and not due to a dedicated study design. This point would clearly merit further investigation, systematically and independently varying both CS physical and pain intensity, ideally over more than two to three observations per subject.

Second, the significant relation between CPM magnitude and CS physical intensity was detected in the repeated measures but not in the cross-sectional analysis. This suggests that within a given subject, there is a dependence of CPM magnitude on CS physical intensity, which however is small compared to inter-individual variability in the CPM effect. Analysis of explained variance indeed showed that the variance due to inter-individual differences is much larger than the variance explained by CS physical intensity (see below).

4.2. Age, Sex, Measurement Eepeat and CPM Paradigm

While some previous findings have suggests less efficient CPM with increasing age [7,8,36], we did not find such a relationship. This may be due to the limited age range present in our dataset, as most of our participants were young. We also found no difference in CPM effect between men and women, which is consistent with some previous findings [37,38], but larger CPM effects in men compared to women have also been reported [6,39]. We found no significant effect of the CPM paradigm used, suggesting no inherent difference between paradigms. However, we did not aim to investigate the effect of paradigm and included it as a regressor solely to control for any potential

paradigm-related differences. Our data stemmed from only three different paradigms, and all used cold pain as CS. Measurement repeat was also included for control, not revealing any significant effects.

4.3. Variance Explained by Inter-Individual Differences vs. Fixed Variables

To our knowledge, our study is the first to estimate the relative importance of intra-individual differences (other than age or sex) vs. fixed variables for the CPM effect. By including only participant as random effect, we could estimate only the variance attributed to each individual participant, i.e., the inter-individual contribution to CPM variance. It resulted that the variance explained by inter-individual differences was large (24.0% to 34.2%) compared to that of all fixed effects combined (3.4% to 11.5%). Among the fixed effects, while the contribution of CS pain intensity, age, sex and measurement repeat were all <1%, only CS physical intensity (4.7%) and CPM paradigm (1.5% to 3.0%) made a somewhat larger contribution. This again raises two important points for discussion.

First, the large effect of inter-individual differences provides an important basis for recent attempts to use inter-individual differences as a predictor for acute or chronic pain states [3,40–42]. In comparison, methodological effects such as CPM paradigm and CS stimulus intensity explain much less variance. Even the contribution of commonly included factors age and sex seems to be small in comparison, with the caveat that our data were not optimally suited to detect age effects.

Second, as suggested above, the large inter-individual differences may be the reason why the relation between CS intensity and CPM effect might be difficult to detect in cross-sectional studies. Such inter-individual differences could be due to genetic, epigenetic, developmental and/or behavioral differences. Indeed, Lindstedt et al. [43] showed that genetic variation in a serotonin transporter gene is related to CPM magnitude. Cardiovascular reactivity to pain also seems to be related to CPM magnitude [44]. Psychological traits such as anxiety, depression, or catastrophizing might also contribute to inter-individual differences. Although a meta-analysis by Nahman-Averbuch et al. [9] found no link between psychological traits and overall CPM effect in healthy subjects, they did show a modality-specific relation with psychological scores of depression, anxiety and catastrophizing. Acute changes in catastrophizing and mood have been shown to influence endogenous pain inhibition [45,46]. Moreover, the role of psychological factors might be more important in clinical populations who tend to have more pronounced psychological traits. These considerations give ample room for further studies to dissect the nature of individual differences in CPM magnitude., e.g., twins studies for the role of genetic differences, and studies looking at inter-individual differences in clinical populations using a similar methodology, including psychological factors, while accounting for inter-individual differences as random factors to discern how much variance these traits account for.

Nonetheless, standardized methods are clearly desirable, and controlling for inter-individual differences in repeated measures designs may allow researchers to detect other, smaller contributing factors that would otherwise go undetected.

4.4. Unexplained Variance

In the present analysis, with 3.4% to 11.5% accounted for by fixed effects and 24.0% to 34.3% by inter-individual variability; this leaves 54.2% to 72.6% of the variance in CPM magnitude unexplained. Multiple factors may contribute to this. Test–retest reliability of CPM yields intraclass correlation coefficients between 0.21 and 0.82 [14,47,48] (reviewed in [49]), showing that even under constant experimental conditions, there is still a significant amount of variability between measurements. This variability may be explained in part by measurement error, which is expected when dealing with subjective pain reports. In addition, there might also be something such as the "daily form" of the subject., e.g., transient psychological states such as acute anxiety or catastrophizing might influence CPM magnitude, especially in clinical populations. Additionally, tiredness, physical activity, menstrual phase, distraction, and previous experiences and expectations might

influence the CPM effect differently between sessions. Indeed, it has been shown that factors such as distraction, catastrophizing and even voluntary mental strategies can acutely change the activity of endogenous pain inhibitory systems [24,45,50]. In addition, experimental conditions not considered in the present study, such as time of the day, gender and personality of the experimenter, or use of pain ratings compared to pain thresholds for the test stimulus might be factors that remain to be investigated.

4.5. Strengths and Limitations

The major strengths of our study are (1) the inclusion of a relatively large sample that allowed the comparison of cross-sectional and repeated measures effects for both CS physical and pain intensity, and (2) the use of new techniques for variance decomposition that allowed the estimation of the variance contribution of inter-individual differences and fixed effects within the same model. There are also some major limitations. First, our analysis did not include a comprehensive sample of different CPM paradigms. Therefore, it remains to be confirmed if these results translate to other CPM paradigms, especially those which assess pain thresholds instead of pain ratings for the test stimulus. Second, in our repeated measures analysis the majority of our participants only had two repeats. Repeating our analysis using multiple CS intensities and pain levels would potentially lead to more robust results. Third, our repeat analysis did not include a broad age-range, possibly precluding detection of an age effect. Lastly, our data is derived exclusively from healthy participants. It remains to be determined if our findings can be applied to clinical populations, such as chronic pain patients or patients undergoing painful medical procedures.

5. Conclusions

The present data emphasize the role of inter-individual differences in CPM magnitude, providing a basis for investigating these differences in clinical populations and using CPM as a predictive tool to individualize medicine by giving insight into the individuals' endogenous pain modulation system. They also show that in comparison, CS intensity makes a minor contribution to CPM magnitude. In repeated measures designs, to further reduce methodological effects on CPM measurement, keeping CS physical intensity constant seems to be more important than CS pain intensity.

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Institutional Review Board Statement: All studies involved were conducted according to the guidelines of the Declaration of Helsinki, and approved by the local Ethics Committees at the University Hospital, Ludwig-Maximilian University Munich, Munich, Germany.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data is available upon reasonable request from the corresponding author.

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Conflicts of Interest: The authors declare no conflict of interest.

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2.3 The contribution of psychological factors to inter-individual variability in conditioned pain modulation is limited in young healthy participants

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Summary

In this publication, we expand our findings from the previous chapter (see 2.2) by investigating the contribution of psychological scores to CPM variability. As many potential factors could help explain the contributions of inter-individual differences to CPM, we used the same pool of participants and investigated how their psychological scores contribute to explained CPM variance. Here, the psychological scores we used were trait depression, anxiety and catastrophizing. We also followed up on a previous meta-analysis[89] suggesting that psychological traits show an effect in some CPM paradigms and not in others. We employed the same methodology to our previous publication (see 2.2) to analyse the repeated measures data. Neither psychological scores, nor their interactions with the CPM paradigm could significantly predict the CPM effect. Including psychological traits did not increase the explained variance of fixed effects, suggesting that they do not contribute appreciably to CPM variance. However, the interaction between depression and CPM paradigm explained a significant amount (3.0%) of the variance of the CPM effect. The interaction of CPM paradigm with either trait catastrophizing or trait anxiety explained <0.1% each. This suggests that, at least in a healthy population, the contribution of psychological factors to CPM variability is limited. However, our findings lend support to a previous study suggesting that the CPM paradigm may be selectively affected by trait depression.

Author contribution

The study/analysis was designed by Philipp Graeff and Ruth Ruscheweyh. Data was collected by Regina Stacheneder, Laura Alt and Philipp Graeff under the supervision of Ruth Ruscheweyh. Analysis was performed by Philipp Graeff. Philipp Graeff and Ruth Ruscheweyh wrote the paper with input from all authors.

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The Contribution of Psychological Factors to Inter-Individual Variability in Conditioned Pain Modulation Is Limited in Young Healthy Subjects

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Abstract: Conditioned pain modulation (CPM) describes the decrease in pain perception of a test stimulus (TS) when presented together with a heterotopic painful conditioning stimulus (CS). Inter-individual differences in CPM are large and have been suggested to reflect differences in endogenous pain modulation. In a previous analysis, we demonstrated that in young, healthy participants, inter-individual differences account for about one-third of CPM variance, with age and sex together explaining only 1%. Here, we investigated if psychological factors explain significant amounts of inter-individual variance in CPM. Using the same dataset as before, we performed both cross-sectional (n = 126) and repeated measures (n = 52, 118 observations) analysis and the corresponding variance decompositions, using results of psychological questionnaires assessing depression, trait anxiety and pain catastrophizing. Psychological factors did not significantly predict CPM magnitude, neither directly nor when interactions with the CPM paradigm were assessed; however, the interaction between depression and the paradigm approached significance. Variance decomposition showed that the interaction between depression and the CPM paradigm explained an appreciable amount of variance (3.0%), but this proportion seems small when compared to the residual inter-individual differences (35.4%). The main effects of the psychological factors and the interactions of anxiety or catastrophizing with the CPM paradigm are explained at <0.1% each. These results show that the contribution of psychological factors to inter-individual CPM differences in healthy participants is limited and that the large inter-individual variability in the CPM effect remains largely unexplained.

Keywords: conditioned pain modulation; endogenous analgesia; inter-individual differences; psychological factors; CPM variability

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1. Introduction

Conditioned pain modulation (CPM) describes a phenomenon of human endogenous pain inhibition thought to be the psychophysical equivalent to the "diffuse noxious inhibitory controls" (DNIC) described in animal experiments [1]. During CPM testing, a noxious test stimulus (TS) is presented in parallel with, or directly after, a heterotopic noxious conditioning stimulus (CS), with the underlying principle being summarized as "pain inhibits pain" [2]. When presented with the CS, the TS is perceived as less painful compared to the presentation without CS [1]. Inter-individual differences in CPM magnitude are substantial and can predict an individual's susceptibility to acute or chronic pain [3,4]. The basis for these inter-individual differences has not yet been understood, although age and sex may make a contribution [5,6]. In addition to inter-individual differences,

experimental factors such as CS intensity and the CPM paradigm may also influence the CPM effect [7,8].

In a previous study on healthy young individuals, we used repeated measures analysis to identify the amount of variance in the CPM effect explained by the inter-individual differences (above age and sex), the experimental factors CPM paradigm, CS intensity and measurement repeat. It resulted that residual inter-individual differences accounted for 34.2% while age and sex accounted for only 1.0% and the other experimental factors together explained 10.5% of the variance [9]. This shows that inter-individual differences in the CPM effect are large and largely unexplained.

Psychological factors might explain a part of these residual inter-individual differences. Pain perception has been shown to increase with higher scores of anxiety, depression and pain catastrophizing [10–12]. However, discrepancies in the literature exist, with other studies finding no such association [13–15]. CPM is reduced in a variety of chronic pain conditions [16], which often shows increased scores for depression, anxiety and catastrophizing [3–5]. Therefore, one could hypothesize that increased scores for these psychological factors could be associated with a decreased CPM effect. A previous meta-analysis of cross-sectional data did not find an overall relation between CPM magnitude and psychological factors but found paradigm-specific relations, i.e., of depression with heat-based CPM [17]. Repeated measures investigations may increase sensitivity by reducing the influence of session-specific factors and can provide direct information on how much of the inter-individual variance in CPM is explained by psychological factors. In addition, discerning the contribution of psychological factors to inter-individual CPM variance in a healthy population may establish a normative baseline to which their effect in chronic pain populations can be compared.

We, therefore, followed up on our previous analysis [9] and used repeated measures analysis to investigate if depression, anxiety and pain catastrophizing scores explained a significant amount of the inter-individual differences in the CPM effect. For this, we used a subsample of our previous cohort, for which all three scores were present.

2. Materials and Methods

2.1. Pooled Data

Data were pooled from the same seven studies used previously [9], resulting in 126 participants for cross-sectional analysis and 52 participants (with 118 observations) for repeated measures analysis. Only participants with Beck's Depression Inventory (BDI [18]), State-Trait Anxiety Inventory (trait subscale, STAI-T [19]) and Pain Catastrophizing Scale (PCS [20]) scores available (collected during the first session of the respective study) were included. These are three self-rating questionnaires that are reliable and valid for the assessment of depression, anxiety and pain catastrophizing and are widely used in pain research.

Details of CPM measurement are reported in [9]. Briefly, the conditioning stimulus (CS) was hand immersion into cold water for 60–120 s, targeting a pain intensity $\geq$3 on a 10-point NRS (0 = no pain, 10 = strongest pain imaginable). The test stimulus (TS) was either contact heat for 60–90 s or electrical stimulation of the sural nerve. TS was applied once alone and once during the CS. The three paradigms used were (TS/CS): (1) electrical/120 s cold, (2) 60 s heat/90 s cold, and (3) 30 s heat/60 s cold. The repeated measures sample included only paradigms 1 and 3.

The CPM effect was calculated as the percentage difference between the test stimulus rating at baseline ($\mathbf{NRS}_{\mathrm{TS(baseline)}}$) and during conditioning stimulation ($\mathbf{NRS}_{\mathrm{TS(cond)}}$), where a more negative result denotes a stronger CPM effect:

$$\text{CPM effect} = \frac{\mathbf{NRS}_{\mathrm{TS(cond)}} - \mathbf{NRS}_{\mathrm{TS(baseline)}}}{\mathbf{NRS}_{\mathrm{TS(baseline)}}} \times 100$$

2.2. Statistical Models

For cross-sectional analysis, linear regression models were constructed using the *lm()* function of the *stats* package in R [21]. For repeated measures analysis, mixed models were constructed using the *lmer()* function of the *lme4* package [22]. Based on our previous research, we included age, sex, CS temperature, measurement repeat and the CPM paradigm in the models [9].

We first investigated the main effect of the psychological factors in a linear model (Model 1). For repeated measures analysis, we constructed mixed models with and without the inclusion of psychological factors as the main effects, to allow for a comparison of explained variance (Models 2 and 3). (1 | participant) denotes the participant as a random effect.

$$\text{CPM effect} \sim CS_{temp} + \text{age} + \text{sex} + \text{BDI} + \text{STAI Trait} + \text{PCS} + \text{paradigm} \quad (1)$$

$$\text{CPM effect} \sim CS_{temp} + \text{age} + \text{sex} + \text{paradigm} + \text{repeat} + (1 \mid \text{participant}) \quad (2)$$

$$\text{CPM effect} \sim CS_{temp} + \text{age} + \text{sex} + \text{BDI} + \text{STAI Trait} + \text{PCS} + \text{paradigm} + \text{repeat} + (1 \mid \text{participant}) \quad (3)$$

In the next step, we included interaction terms between the CPM paradigm and the psychological factors in both the cross-sectional (linear) and repeated measures (mixed model) analyses. Note, however, that in R notation, '*' indicates that both the main effects and the respective interactions are included in the model.

$$\text{CPM effect} \sim CS_{temp} + \text{age} + \text{sex} + \text{BDI*paradigm} + \text{STAI Trait*paradigm} + \text{PCS*paradigm} \quad (4)$$

$$\text{CPM effect} \sim CS_{temp} + \text{age} + \text{sex} + \text{BDI*paradigm} + \text{STAI Trait*paradigm} + \text{PCS*paradigm} + \text{repeat} + (1 \mid \text{participant}) \quad (5)$$

Significance was tested by the *lm()* function (linear models), and by the *Anova()* function (Wald's chi-square test) (*car* package [23]) for mixed models. A $p < 0.05$ was considered significant.

Variance decomposition of the repeated measures analysis was performed as described in detail in our previous article [9]. Briefly, we used the *r.squaredGLMM()* function of the *MuMIn* package [24] on the mixed Models 2, 3 and 5 to determine the variance explained by the fixed effects and residual inter-individual variance, and the *calc.relimp()* function of *relaimpo* package [25] on the following linear models to further decompose the fixed effects variance of Models 3 and 5:

$$\text{CPM effect} \sim CS_{temp} + \text{age} + \text{sex} + \text{BDI} + \text{STAI Trait} + \text{PCS} + \text{paradigm} + \text{repeat} \quad (6)$$

$$\text{CPM effect} \sim CS_{temp} + \text{age} + \text{sex} + \text{BDI*paradigm} + \text{STAI Trait*paradigm} + \text{PCS*paradigm} + \text{repeat} \quad (7)$$

3. Results

In the cross-sectional sample (n = 126, 91 females), the mean age was 29 ± 12 years and the mean CPM effect was significant at −19.5 ± 25.9% ($p < 0.001$). Mean BDI, STAI Trait and PCS scores were 3 ± 4 (range: 0–17), 37 ± 9 (21–56) and 14 ± 9 (0–33), respectively. In the repeated measures sample (52 subjects/28 females, 118 experiments), the mean age was 24 ± 6 years and the mean CPM effect was −16.9 ± 21.2% ($p < 0.001$). Mean BDI, STAI Trait and PCS scores were 4 ± 4 (range: 0–17), 38 ± 9 (21–56) and 14 ± 8 (0–33), respectively.

3.1. Main Effects and Interactions

In the cross-sectional sample, none of the psychological factors was a significant predictor of CPM (Model 1, Table 1). CS physical intensity, age, CPM paradigm or sex were also non-significant.

Table 1. Cross-sectional analysis (linear regression, Model 1, $n = 126$). Multiple $R^2 = 4.1\%$, $p = 0.757$. CS_{Temp} = conditioning stimulus temperature in °C. Sex and paradigms compared to a reference (male and 30 s heat/60 s cold, respectively). Paradigm 1 = heat 60 s/cold 90 s, Paradigm 2 = electrical/cold 120 s. CS_{Temp} = conditioning stimulus temperature in °C, BDI = Beck's Depression Inventory score, STAI Trait = State-Trait Anxiety Inventory score (trait subscale), PCS = Pain Catastrophizing Scale score.

Predictor	Estimate	*p*-Value
CSTEMP	1.35	0.085
AGE	−0.07	0.840
SEX	1.35	0.817
BDI	−0.14	0.861
STAI TRAIT	−0.08	0.821
PCS	−0.30	0.289
PARADIGM 1	3.43	0.753
PARADIGM 2	5.05	0.442

The repeated measures analysis also revealed no significant main effect of any psychological factor on the CPM effect (Model 3, Table 2). As in our previous analysis [9], CS physical intensity (i.e., cold water bath temperature) was a significant predictor of CPM size in both Models 2 and 3 (both $p < 0.01$), but age, sex, paradigm or measurement repeat were not significant (Table 2).

Table 2. Repeated measures analysis (mixed Models 2 and 3, 54 participants, 118 observations). Model 2: REML criterion at convergence = 1015.8. Model 3: REML criterion at convergence = 1014.7. *p*-values were obtained by Wald's chi-square test on Models 2 and 3. Sex and paradigm compared to a reference (male and electrical/120 s cold, respectively). Significant effects are marked in bold. CS_{Temp} = conditioning stimulus temperature in °C, BDI = Beck's Depression Inventory score, STAI Trait = State-Trait Anxiety Inventory score (trait subscale), PCS = Pain Catastrophizing Scale score.

Model	Predictor	Estimate	*p*-Value
	CS_{Temp}	**1.54**	**0.001**
	Age	0.26	0.546
	Sex	−1.86	0.700
	Paradigm	−8.47	0.085
	Repeat	−3.07	0.190
MODEL 3	**CS_{Temp}**	**1.55**	**0.002**
	Age	0.19	0.679
	Sex	−1.57	0.755
	BDI	−0.48	0.599
	STAI Trait	0.02	0.965
	PCS	0.00	0.999
	Paradigm	−7.60	0.197
	Repeat	−3.04	0.195

The results of Nahman-Averbuch et al. [17] prompted us to look for paradigm-specific relations between the CPM effect and psychological factors. However, in our cross-sectional analysis (Model 4) none of the psychological factors exhibited a significant interaction with the paradigm (Supplementary Table S1). Similarly, repeated measures analysis (Model 5) showed no significant interactions. Of the three interactions tested, paradigm*BDI was the largest, although non-significant at $p = 0.130$ (Supplementary Table S2).

3.2. Analysis of Explained Variance

In a complementary approach, we investigated the possible contribution of psychological factors to inter-individual CPM differences through analysing the explained variance by adding psychological factors or their interactions to the models. A meaningful effect of psychological factors on CPM magnitude would be expected to show as an increase in

the fixed effects variance with a parallel decrease in the residual inter-individual variance. Including psychological factors as main effects (Model 2 vs. Model 3) did not increase the variance explained by the fixed effects (11.5% vs. 11.6%) and increased (rather than decreased) the variance explained by residual inter-individual effects (34.2% vs. 36.2%).

However, including the interactions between psychological factors and the CPM paradigm (Model 5 vs. Model 3) resulted in an appreciable increase in fixed effects variance (14.3% vs. 11.6%), combined with a small decrease in residual inter-individual variance (35.4% vs. 36.2%, Figure 1). This suggests that paradigm-specific interactions can explain some of the inter-individual variances of the CPM effect. To determine which interaction(s) were responsible for this change, we decomposed fixed effect variance contributions (Model 7, Supplementary Table S3). This revealed that only the BDI*paradigm interaction closely approached significance ($p = 0.053$), while the interactions with STAI Trait or PCS were non-significant at $p = 0.708$ and $p = 0.561$, respectively. Indeed, BDI*paradigm explained 3.0% of the fixed effects variance, while CS physical intensity explained 4.5%, followed by the CPM paradigm (2.1%) and measurement repeat (1.0%) (Figure 1 and Supplementary Table S3).

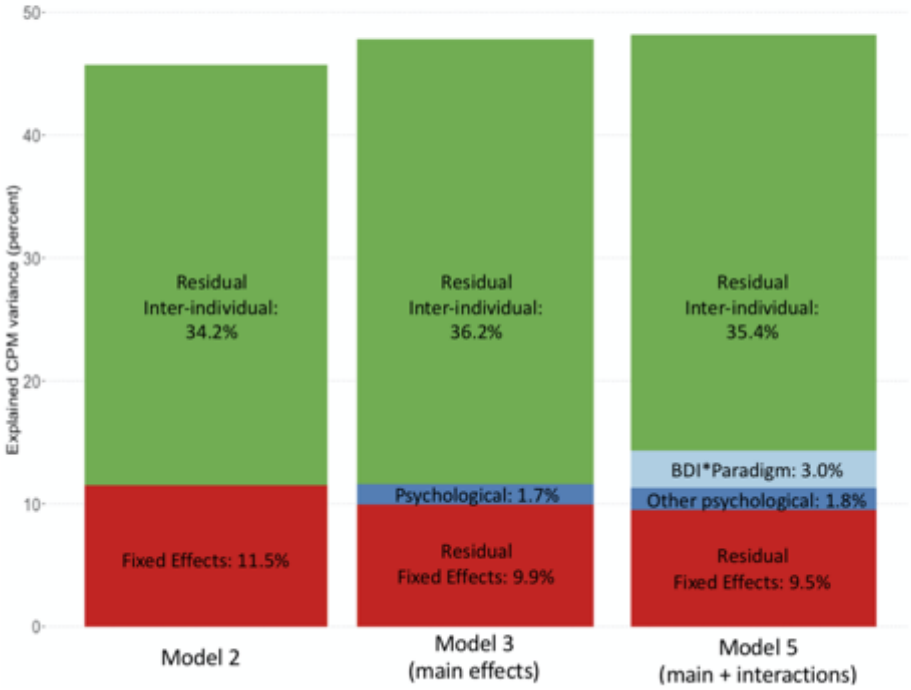

Figure 1. Variance decomposition while adding psychological factors to the model. Variance in CPM magnitude explained by Model 2 (including age, sex, repeat, CPM paradigm and CS intensity, but no psychological factors), Model 3 (additionally including psychological factors as main effects), and Model 5 (additionally including interactions of psychological factors with CPM paradigm). "*" denotes the interaction effect of two variables. Only inclusion of the interaction terms increased variance explained by the fixed effects, which was mainly due to the BDI*paradigm interaction. The variance explained by psychological factors in Models 3 and 5 was determined using Models 6 and 7, respectively (see Supplementary Table S3 for a full breakdown of fixed effects variance). Note that the figure only illustrates the variance explained by the fixed effects and the residual inter-individual variance. The remaining variance is unexplained and may be due, e.g., to between-session differences.

When investigating the direction of the interaction, we found a decrease in the CPM effect with increased BDI values in the electrical/120 s cold paradigm (coefficient = 0.16)

and the opposite in the 30 s heat/60 s cold paradigm (coefficient = −0.18, Supplementary Figure S1).

4. Discussion

This follow-up investigation shows that in young healthy subjects:

(i) Psychological factors, such as depression, anxiety or pain catastrophizing, do not significantly predict the CPM effect when different CPM paradigms are pooled.
(ii) Depression can explain some amount of inter-individual CPM variance dependent on the CPM paradigm. However, this contribution remains small (3.0%) when compared to the residual inter-individual variance (35.4%).

Our previous investigation [9] showed that inter-individual differences account for approximately one-third of the variance in CPM magnitude, with age and sex contributing only ~1% combined. In the present analysis, we set out to determine if part of the residual, unexplained inter-individual differences can be explained by psychological factors. Indeed, it has been shown before that pain perception and some measures of endogenous pain modulation may be dysregulated (i.e., increased pain perception and/or reduced endogenous pain inhibition) in populations suffering from depressive [11,26] or anxiety disorders [10], or when healthy subjects engage in acute catastrophizing thoughts [27] or experience unpleasant emotions or fear [28,29]. However, it must be mentioned that other studies find no such relationship between pain perception and anxiety [14], catastrophizing [15] and depression [13] in healthy individuals.

In the present analysis, depression, trait anxiety and pain catastrophizing scores did not significantly predict the CPM effect, neither in the cross-sectional nor in the more sensitive repeated measures analysis. A previous meta-analysis of cross-sectional studies [17], investigating the association of various psychological scores with CPM magnitude, also found no overall effect. As our analysis contained different CPM paradigms (involving different modalities as test stimuli) and the previous meta-analysis found paradigm/modality-specific relations between psychological factors and CPM magnitude [17], we investigated if including the interaction between psychological factors and the CPM paradigm significantly improved our models. This was not the case, although, in the mixed model analysis, the interaction between depression scores and the CPM paradigm approached significance.

One advantage of repeated measures analysis is that novel techniques [30,31] allow to estimate the contribution of (residual) inter-individual differences to a variable—in this case, CPM magnitude—and compare it to the variance explained by known (fixed) effects. It turned out that the interaction between depression scores and the CPM paradigm increased the variance explained by the fixed effects while decreasing the residual inter-individual variance. The interaction term again closely approached significance. When analysing the direction of interaction, the CPM effect decreased with increased depression scores in the electrical/cold paradigm, while the effect was opposite in the heat/cold paradigm.

These results support the previous findings [17] that the relation between CPM magnitude and psychological factors can be dependent on the CPM paradigm (especially on test stimulus modality). However, the specifics of the single interactions were different, as the previous study found a significant positive relation between depression and the CPM effect (i.e., more depression, less effective CPM) when heat was used as the TS. We found a positive relationship between depression and electrical CPM (i.e., more depression, less effective CPM), while the relation was negative between depression and heat CPM (i.e., more depression, more effective CPM). In addition, we did not find an interaction between pain catastrophizing and the CPM paradigm, while the previous study found such a relation when electrical pain was used as TS. The specific methodological differences of the CPM paradigms used may contribute to these differences.

Together, the present and previous [9,17] results emphasize that the CPM paradigm can make an important contribution to CPM magnitude, not only directly, but possibly also by its interaction with psychological factors. However, it must be recognized that the additional contribution of the psychological factors investigated here and their interactions

with the CPM paradigm to the variance explained was small (3.0%) when compared to the residual inter-individual variance (35.4%). Therefore, further investigations will be necessary to address the basis of the large inter-individual differences in CPM magnitude.

4.1. Future Directions

Several additional factors could contribute to inter-individual differences in CPM. First, there may be other psychological factors, transient or not, that could influence the CPM effect. For example, active cognitive strategies have been shown to influence descending pain inhibition [32,33], and intrinsic attention to pain, i.e., an individual's tendency to attend to painful stimuli is related to CPM [34]. Stressful tasks have also been shown to inhibit the CPM effect [35]. Expectations towards the direction and magnitude of CPM may be individually different and can affect CPM magnitude [36]. Second, allelic differences in certain genes have an effect on both pain perception and CPM [37,38]. Third, Ibancos-Losada et al. [39] suggested that individual differences in perceived unpleasantness of certain pain modalities over others may influence their CPM effect. Fourth, cardiovascular reactivity to pain may also affect CPM [40]. There may be many more factors not mentioned here. It remains to be determined how much of the inter-individual differences in the CPM effect are explained by these factors, both alone and in combination. Repeated measures analysis with a determination of explained variance, as performed here, may aid to perform these investigations.

In addition, it is possible that we found little relation between psychological factors and the CPM effect because our study population was healthy. CPM may not be affected by psychological factors if they are within a fairly narrow and low range, as they were within our study. However, this is a necessary first step in investigating the relation between psychological factors and CPM and also to establish a normative baseline for a healthy population. The next steps will include the investigation of clinical populations. A previous meta-analysis also suggested no association between the CPM effect and psychological factors in chronic pain patients [17]. However, the situation is complex. Chronic pain patients often have psychological comorbidities, resulting in elevated scores for depression, anxiety and/or catastrophizing [3–5], and they also exhibit a relationship between these scores and increased pain perception and/or a reduced CPM effect [41–43]. Therefore, to dissect the relation between CPM, psychological factors and chronic pain, at least three different groups of patients will have to be compared: patients with chronic pain but without psychological comorbidity, patients with increased depression, anxiety and/or catastrophizing scores but without chronic pain, and patients with chronic pain and psychological comorbidities. Moreover, chronic pain populations can be very heterogeneous regarding the type and cause of chronic pain. Limiting investigation to one of the major types of chronic pain known to be associated with reduced CPM effect might be a good starting point.

Moreover, our present and previous [9,17] data show that the CPM paradigm has an effect on CPM magnitude and also on the relation between CPM and psychological factors. Future studies should take this into account and either use a single paradigm or ideally compare multiple paradigms, including paradigms not examined in this study, e.g., pressure pain.

4.2. Strengths and Limitations

The most important strength of our study is that it used repeated measures analysis to directly assess the CPM variance accounted for by inter-individual differences. An important limitation of our study is that the number of included subjects and experiments was limited because not all subjects included in our previous investigation had psychological scores available. This might have been the reason that only a trend but no significance was found for the interaction between depression and the CPM paradigm. Moreover, results cannot be generalized to chronic pain patients, who exhibit a broader range of psychological scores and will need to be studied separately (see Section 4.1). In addition, our study

only examined two CPM paradigms. The addition of a pressure pain paradigm could help further investigate the paradigm-specific interactions with psychological factors.

5. Conclusions

The psychological factors depression, anxiety and pain catastrophizing did not make a significant contribution to explaining inter-individual variance in the CPM effect of healthy young subjects. Interaction analysis suggested that depression scores may have a modality-specific effect on CPM ($p = 0.053$). However, compared to the residual inter-individual variance (35.4%), the variance explained by the interaction between depression and the CPM paradigm was small (3.0%), as was the variance explained by age and sex (<1%). In conclusion, up to now, most of the inter-individual variance in the CPM effect remains unexplained.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/brainsci12050623/s1, Figure S1: Interaction plot of the effect of BDI scores on CPM magnitude in CPM paradigms using electrical and heat stimulation as test stimuli, respectively; Table S1: Cross-sectional analysis including interactions between psychological factors and CPM paradigm (linear regression, Model 4, n = 126) for psychological factor interaction with paradigm; Table S2: Repeated measures analysis including interactions between psychological factors and CPM paradigm (mixed model analysis, Model 5, 52 participations, 118 observations); Table S3: Significance and variance explained by fixed effects in Models 6 and 7 (n = 52, 118 observations).

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3 DISCUSSION

3.1 SUMMARY OF THE MAIN RESULTS

In this thesis I examined both internal and external factors influencing the ability of individuals to engage descending pain inhibition. First, I investigated supraspinal activity patterns when activating the descending pain inhibitory system before and after feedback training. Second, I examined the degree to which inter-individual and methodological effects contribute to the variability of CPM, a measure of endogenous pain inhibition. In this section I will present in brief the general findings of both projects, which I will then discuss both in more detail and in context to the broader literature in the subsequent sections.

In the first project we used a longitudinal fMRI study design to compare the brain activity before and after a previously established biofeedback training where participants learned to use cognitive strategies to activate their descending pain inhibition. We investigated specifically the activity patterns during application of this cognitive strategy and in reaction to short painful electrical stimuli . The results of the feedback training were in line with our expectations based upon our previous studies that established and utilized the training paradigm. Participants were able to reduce both their perceived pain and spinal nociception, evidence for descending pain inhibition, when applying a positive mental imagery. This could be improved through RIII-biofeedback training. Inter-participant variability in training success was large, as was the variability in the RIII-reflex reductions during MRI sessions. The fMRI analysis revealed a decreased default mode activity post-training, indicating that participants become more efficient at applying a mental strategy through training. Utilization of the mental strategy activated the mPFC, a core frontal area involved in descending inhibition, as well as the thalamus, a region more often related to pain processing than pain modulation. Thalamic activity increased further through training, while the mPFC showed no session-specific change in activity. Significantly reduced reactions to painful stimulation were not only seen in cortical but also in brainstem areas responsible for pain sensation, supporting the idea that the cognitive strategy indeed decreases nociceptive input already on a spinal level. Larger decreases post-training in brainstem, thalamus and cortical areas show that training was effective in increasing descending inhibition. Lastly, participant pain reduction improvement was correlated with changes in haemodynamic response in frontal cortical areas, supporting previous theories of their involvement in both pain sensation and pain modulation.

To better understand the impact of inter-individual differences in descending pain inhibition, I then switched paradigms to investigate conditioned pain modulation, where I took previous data and collected new data to determine the proportion of variance in the CPM effect explained by controllable and uncontrollable inter-individual factors. Our findings clearly demonstrate that large inter-individual effects in CPM variance exist and that the majority of these remain unexplained. We show that methodology-specific effects such as CS intensity and CPM paradigm explain more variance than any other fixed-effect we investigated (including age, sex, and psychological scores). "Unexplained" inter-individual effects accounted for approximately 10 times more variance than the largest measured contributor, CS intensity. Indeed, a major reason why the effect of methodological factors has been conflicting in the literature could be because their relatively small effects are drowned out by the underling individual variance. We showed this by demonstrating a significant predictive effect of CS intensity only when accounting for the individual in repeated measures analysis, while no such effect was found in the cross-sectional analysis. This adds to the existing literature by suggesting baseline differences need to be accounted for if contributions of other factors are to be investigated. If this is not done, a study would run the risk of having the result of the independent variably drowned out by the residual individual variance.

3.2 LEARNED ACTIVATION OF DESCENDING PAIN INHIBITION CHANGES THE BRAIN ACTIVITY

3.2.1 POSITIVE COGNITIVE-EMOTIONAL STRATEGIES ACTIVATE HIGHER COGNITIVE AREAS OF DESCENDING PAIN INHIBITION

When participants applied positive mental imagery to decrease their RIII-reflex two principle pain-related areas were activated: the mPFC and the Thalamus. The mPFC exhibited an increase in activity during task, but its activity did not change with training. Activation of the mPFC can be expected as it is known to be involved in higher cognition, imagery and episodic memory recall[103]. Increases in mPFC activity have also been shown in anticipation of decreased pain[52,104]. The increase we found, along with the pain reduction experienced by the participants during strategy indicates that the activity is related to pain inhibition. Participants were already asked in the pre-training MRI session to apply mental imagery (albeit untrained) of nice things. We believe the mPFC activity did not change for mental imagery post-training because of an improved efficiency in mental imagery resulting in a larger psychophysical effect without a concomitant increase of the haemodynamic response in the brain. This is supported by the additional decrease in default mode network (DMN) activity post-training. DMN activity is known to decrease when

applying cognitively demanding tasks[105]. DMN activity is indicative of introspective mentation, or mind wandering[106,107]. Participants learn to employ a specific mental strategy over the course of training, leading to a more focused mental imagery in MRI2 compared to MRI1. It stands to reason that they engage in less mind wandering during strategy post training, which would explain the decreases in DMN activity.

The increase in thalamic activity during application of the strategy was primarily observed post-training, showing that this increase is a training effect. When qualitatively observing what subregions of the thalamus show the greatest effect, we found that the VPL and posterior parts of the thalamus changed their activity the most, along with a small anterior part of the thalamus. Activity increases during strategy, independent of the stimulation, were unexpected here. The role of the VPL is in the relay and processing of ascending nociception[56]. However, as the thalamus serves as a relay and integration centre for many cortical and subcortical regions, one could hypothesize that the increase in thalamic activity during task are of an integratory nature. That is to say, information from cortical areas related to pain inhibition and with connections to the thalamus, such as ACC and PFC, flow to the thalamus, thereby increasing its activity. Interestingly, the role of the thalamus in the modulation of pain is not well explored but the medial nuclei are believed to play a role. Our findings do not confirm this theory. They rather support a finding by Valet et al., who discovered increased lateral thalamic activity during distraction from pain[39]. Whether the increase we find stems from participants having found a strategy that better distracts them, or whether the thalamus is more involved in pain modulation than previously thought, cannot be dissociated with our study.

Overall our findings suggest that the mPFC is a key region involved in decreasing experienced pain via positive mental imagery. RIII-feedback training leads to a general improvement in efficiency in the mPFC and in DMN network regions, which in turn increases thalamus activity. Targeted connectivity analyses between the mPFC, DMN and the thalamus would help us to understand how these activity patterns are specifically involved in descending pain inhibition.

3.2.2 APPLICATION OF A LEARNED PAIN REDUCTION STRATEGY DECREASES THE CORTICAL AND SUBCORTICAL RESPONSE TO PAINFUL STIMULATION

The brain's reaction to painful stimulation showed more robust training-related effects. These effects were not revealed through the classical linear regression analysis using the canonical hemodynamic response function (HRF) and its derivatives as basis functions. One reason for this is likely the very short (21 ms) stimulus used does not fit well enough to the

canonical HRF used by SPM to detect subtle differences. Large differences in the parameter estimates between the HRF and its derivatives in our experiment support this. The HRF depends on the brain area and stimulus speed used[108]. The brainstem is notorious for its increased physiological noise and different haemodynamics. As a result the general linear model and resulting whole-brain and ROI-analysis do not adequately capture our response. We did not expect participants to recruit entirely new brain regions post-training, but rather to change the levels of activity in the already present areas. These small differences were difficult to capture using traditional GLM estimation if the fit of the HRF is not good. A more sensitive method was therefore needed. Fortunately, the brain regions that respond to pain are generally well known and so we could extract the time courses of these regions for further analysis, and our sub-second fast repetition time provided a better temporal resolution than is often possible with fMRI. Visual inspection of the timecourses of the normalized intensity values within our ROIs confirmed a general increase in activity in response to the painful stimulus with deviations from the canonical HRF, supporting "non-canonical" responses to pain stimulation in our ROIs.

The timecourse analysis revealed lower activation in response to painful stimulation while participants used their cognitive strategy in all investigated brainstem ROIs (RVM, LC and PAG) in addition to insula, dlPFC and thalamus. This is in support of the psychophysical results of a pain intensity reduction during strategy, showing that lower brain activity reflects lower experienced pain. Areas such as insula and dlPFC are involved in the evaluation of painful stimuli in both affective and sensory dimensions. Along with the thalamus and LC, they exhibit an interaction between session and condition, showing a further reduction in pain-related activity post training. The decrease in the thalamus, the first supraspinal region of the lateral pain system to receive nociceptive input, suggests that feedback training does decrease nociception, likely by improving descending pain inhibition. The brainstem areas, which are part of the medial pain system, show a similar decrease in activity during the use of the cognitive strategy for pain reduction. Both the PAG and the RVM also present a trend of a further decrease in activity post-training. In summary, these decreases in initial processing areas strongly suggest that nociception is already decreased when ascending to the brain. Unfortunately, while the RIII reflex was decreased during application of the strategy in the feedback training sessions as expected, the RIII-reflex decreases with strategy in the MRI sessions were too small to make clear inferences with the data. The RIII reflex is sensitive to leg position and muscle tension, normally being recorded in a relaxed half sitting position with the leg flexed at 150°. The supine position in the scanner together with the non-relaxing surroundings may have precluded detection of RIII reflex modulation in the MRI sessions.

The decreased nociceptive input into basal regions was reflected in the activation pattern of two higher evaluative regions, the insula and the dlPFC. Taking these results together, we can say that our cohort of participants were already able to decrease their experienced pain and spinal

nociception before feedback training. Nonetheless, training additionally helped to reduce nociception. Brainstem and thalamic activity suggest that the decrease is in spinal nociception is propagated to higher pain processing centres, leading to a reduced pain sensation in addition to a reduced spinal nociception. We have also shown that the supraspinal reaction to short, painful electrical stimulation does not follow the canonical HRF, and therefore alternative methods for analysis should be considered.

3.2.3 RESULTS IN CONTEXT: PROPOSAL FOR THE DESCENDING PAIN INHIBITORY PATHWAY

Based on our results and what is already known about the pain inhibitory paths I propose the following mechanism for descending pain inhibition via cognitive strategies and RIII-reflex feedback training. Positive mental imagery activates the medial PFC. Other frontal areas often implicated in descending inhibition and cognitive control such as the ACC and the dlPFC may additionally be involved through direct activation[50,109] or as a result of cortico-cortical crosstalk[110,111] between these areas. The mPFC signals the PAG via cortical projections and perhaps also to through the thalamus where the PAG-RVM axis inhibits spinal nociception via descending inhibitory projections[56,58,112,113]. If the thalamus is involved it likely provides an integrative role[114], combining the input from the multiple cortical regions, including limbic system input on emotional state. From the thalamus, integrated signals can also travel to the PAG[113,115]. The primary neurochemical mechanism is most likely opioid signalling, as it is implicated to be the driving pathway of the PAG.[44,116]

When a painful stimulus is then applied, serotonin-mediated inhibition of the synapse between primary and secondary nociceptor in the dorsal horn of the spinal cord stunts transmission[117] and reduces spinal nociception. As less nociceptive input is transferred to ascending paths the areas receiving the nociceptive input, namely brainstem and thalamus, have a decreased response to said stimulus, leading to a lower cortical response to an already decreased nociceptive input.

Through the RIII-reflex training the driving activity in the mPFC becomes more efficient at activating descending pain inhibition, which means the same level of activity leads to more descending pain inhibition. This is accompanied by a decrease in introspective default mode network activity. Although we did not analyse this here, we would expect structural and functional connectivity changes between the mPFC and the PAG to be accompanied by successful training.

The same mechanisms which activate descending pain inhibition may prime higher evaluative areas in the brain to respond less severely to painful stimuli. This would lead to not

only decreased overall intensity of pain perception, but it would mean a modulation of the affective valuation, or unpleasantness, of the pain. These mechanisms are transient, i.e. pain reduction only happens when applying the strategy, and we cannot say with our experiments how it relates to the chronification of pain.

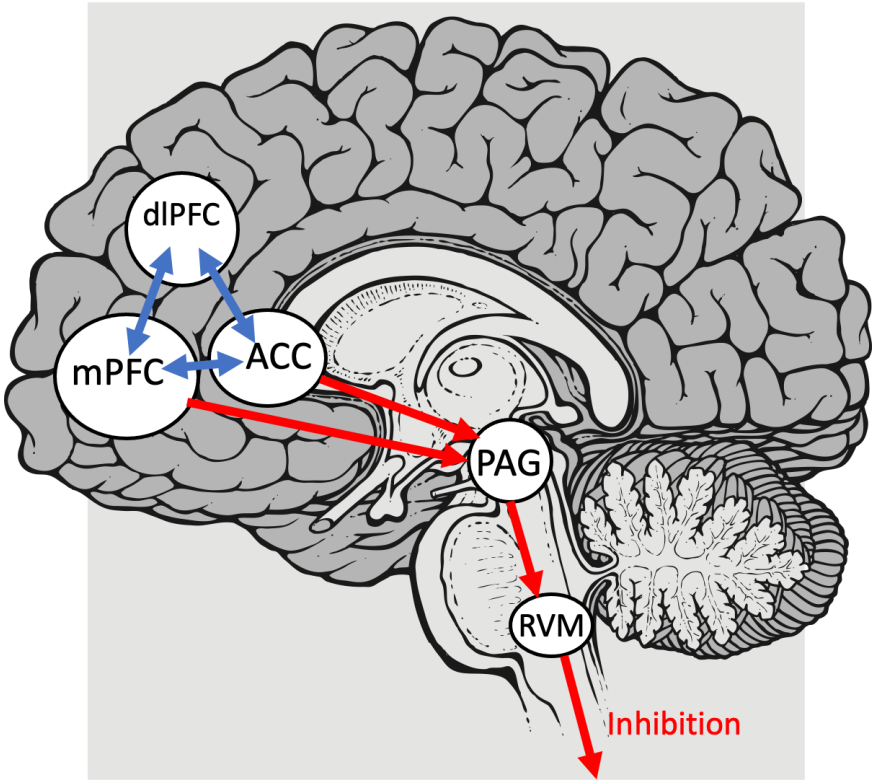

FIGURE 4: Proposed mechanism of initiating descending pain inhibition. Increased cortical activity in mPFC, dlPFC and ACC starts the process, with potential cortico-cortical communication (blue) facilitating/strengthening the effect. Descending projections (red) from the mPFC and ACC to the PAG activate opioid-dependent pain inhibitory circuitry, resulting in serotonin-based descending inhibition from the RVM.

3.3 INTER-INDIVIDUAL VARIABILITY IN THE HUMAN ENDOGENOUS PAIN INHIBITORY SYSTEM

3.3.1 INTER-INDIVIDUAL DIFFERENCES IN ENDOGENOUS ANALGESIA REMAIN LARGELY UNEXPLAINED

In all projects presented, we found large variability in descending pain inhibition. In the first project, the achieved RIII and subjective pain reduction presented a broad range in both the MRI and the feedback training itself. Indeed, already in the first training session we observed that some participants could significantly decrease their RIII-reflex. We also saw a stratification of the participant pool into participants that could learn to use a cognitive strategy to activate descending pain inhibition and those who even after 3 feedback session could not decrease their

RIII reflex appreciably. This, along with the participants that were already capable of decreasing the RIII size in the first session, suggests that baseline differences in the ability to activate descending pain inhibition using mental imagery exist between these participants. A similar stratification into responders and non-responders has been suggested by Kennedy et al.[83] They examined differences in the CPM effect and suggested that these differences are caused by underlying differences in the endogenous inhibitory system. However, it is entirely possible that the "non-responders" we found simply could not utilize the "positive mental imagery" strategy to activate endogenous inhibition. In previous experiments, we have shown that different participants achieve greater success using other strategies like meditation/relaxation techniques or mental arithmatic[59,118]. However, non-responders were also present in these studies. A study by Schulz et al., investigating the effect of multiple techniques on pain reduction between individuals, proposes that no single strategy is ideal for a participant, but rather that a participant with good pain reduction using one technique is likely also be successful using others[53]. They also found large inter-individual differences in success and brain activity. This studies together with ours provide strong evidence for the existence of baseline differences regardless of the mental strategy used.

The second and third studies tried to elucidate what measurable interindividual factors may influence these interindividual differences by examining conditioned pain modulation (CPM). We compared the variance explained by measurable parameters such as age, sex and the strength of the conditioning stimulus to "residual" or "unexplained" CPM variance. We see that factors that have previously been proposed to affect CPM such as age and sex explained less than 1% of CPM variance. Even a factor like CS intensity, which could significantly predict CPM effect and explained the largest amount of variance, explained 10 times less variance than these unexplained effects. Further support of the importance of accounting for individual differences was given when comparing the repeated-measures to the cross sectional designs: None of the factors we included could significantly predict CPM effect in the cross-sectional analysis, while we found CS intensity to be significant in repeated measures. This suggests that these unexplained inter-individual differences are large enough to prevent the detection of potentially significant effects.

3.3.2 LARGE VARIANCE IN FEEDBACK-TRAINING EFFECT IS LIKELY A DRIVER OF SMALL EFFECT SIZES

Most fMRI analyses investigate population-level effects. In our fMRI study we saw large between-participant variability in the pain ratings and pain reductions during the fMRI sessions. Different levels of pain are known to evoke different levels of activity in the brain[18], and differences in pain reduction has been linked to differential brain activity[119]. We can therefore

assume that our population effects include baseline differences between participants' brain activity. This increased variability in brain activity makes it harder to detect effects in group-level analyses. In addition, the utilization of a longitudinal design presents additional challenges. Feedback training success also showed large variability, which was in turn reflected in pain reductions between the MRI sessions, thereby introducing additional variability in the brain activity on top of the individual variability in baseline responses. Our utilization of a method that estimates within-subject and between session variability partially alleviates this issue with longitudinal comparisons, more than traditional GLM analyses[120].

These effects we still believe are the reason we see no widespread effects of strategy in the brain. In particular the following issues likely played a role: 1) the effect sizes of applying cognitive strategies themselves, compared to a baseline task of thinking of nothing in particular, are likely very small, compared to e.g., the response to a painful stimulation, resulting in a lower power than originally estimated for the preregistration and sample size estimation. 2) the cognitive strategy may not change activity through training enough between the first and second MRI to detect the effects. This is seen for example in the lack of a difference in mPFC activity between pre and post training MRI sessions. 3) The individual participant variability during strategy use may be so large that it precludes the detection of effects. We believe this variability stem from two potential sources: a) Differences in training success. Not all our participants were successful, with some achieving no reduction whatsoever. And b) even when using identical stimuli and/or tasks, brain activity patterns may differ quite significantly between individuals, especially for such an introspective task[121].

One way to ameliorate the high degree of variability would have been to collect a larger cohort and only investigate those participants that showed successful training. Investigating only successful participants would decrease the variability between participants, allowing for detection of smaller effects. In order to achieve this, we would need to either expand the spectrum of strategies participants were allowed to use or recruit a much larger cohort and include only successful participants. Allowing participants to use a variety of strategies would create a circular problem: it would potentially decrease the general overlap in activity due to the nature of the strategy, while increasing success rate. Looking for common activation patterns between strategies may help discern the common denominator in descending inhibition. However, results of a previous study suggest that some participants are simply better at activating their descending inhibition regardless of strategy[53], which would mean that adding different strategies would not entirely alleviate the individual variability. Therefore, our current knowledge including the results of this thesis would argue for the use of a large study cohort and a longitudinal within-participant design would be the best way to further investigate descending pain inhibition in humans,

although this would require a concerted effort as participants could only be excluded after training.

3.3.3 VARIABILITY IN TRAINING SUCCESS IS CORRELATED TO CHANGES IN HAEMODYNAMIC RESPONSE

One benefit of behavioural variability is that we can use it to look for correlates in brain activity. Indeed, we found that the lower the activity after training in the ACC in response to the painful stimulation, the greater the training success. This suggests a direct role of the ACC in successful RIII-reflex training, which is one reason I believe it to play a central role in my proposed mechanism (see Section 3.2.3). Frontal ACC areas are involved in the evaluation of painful stimuli[14,21], supporting the hypothesis that more successful participants have a decreased pain response while using their strategy after training.

Frontal ACC areas are also involved in descending pain inhibition[15]. Activation of the mPFC-ACC-PAG axis likely initiates descending pain inhibition. Once the inhibited painful stimulus is transmitted to the brain, the reaction to it in these areas is then decreased. This could be due to the fact that nociceptive input is lower. The haemodynamic response to painful stimuli is known to fluctuate depending on stimulus intensity[18], and decreased spinal nociception would lead to decreased nociceptive input into the brain and consequently a decreased haemodynamic response. Alternatively, active descending inhibition may cause a decreased haemodynamic response to a stimulus of the same intensity due to less signal being transduced.

Interestingly, we only find a correlation with training in frontal areas more associated with higher cognitive evaluation of pain, and not in more basal areas like thalamus or the brainstem that we would expect to be more involved in descending pain inhibition. Our findings therefore indicate that training success is indicative of changes in cognitive and/or integrative evaluation of the painful stimulus, but not activity of brain areas associated with descending inhibition during the application of the strategy itself. However, as mentioned previously, the HRF does not fit well to the hemodynamics, especially in the brainstem[108] and we did not, due to the complexity of the analysis look for correlations with our time course analysis. Therefore, we may just be missing the effect in these regions as of yet.

3.3.4 CONTRIBUTING FACTORS TO INTER-INDIVIDUAL VARIABILITY IN ENDOGENOUS PAIN MODULATION

Across all the studies in this thesis, inter-individual variability played a large role in the results found. In the last two studies we specifically addressed the topic of variability but none of the factors we measured explained the large variability found. What other factors could contribute to the individual differences of endogenous analgesia that were not measured in these studies? Here I will present the current evidence for genetic, psychological, anatomic and connectivity differences that may underly these individual differences.

Genetic heterogeneity within a population is normal and desirable. However, certain genotypes predispose people to certain diseases or change phenotypes. Animal models of pain processing show that certain strains, i.e. certain genetic lineages, can predispose pain phenotypes. The field of pain genetics has received considerable attention over the last 20 years[122], and nearly 100 genes have been associated with some aspect of pain[123]. These genes range widely in function and phenotype from ion channels, neurotransmitter signalling, junction proteins, enzymatic function to direct implication in pain sensing, such as capsaicin receptor genes. Endogenous analgesia is known to act in part via opioid (PAG), serotonin (RVM) and noradrenalin (LC) signalling, therefore allelic differences in related genes may cause the individual differences found. In fact, differences in serotonin transporter genes have already been shown to relate to CPM magnitude[124], supporting the hypotheses that baseline differences in endogenous pain inhibition efficacy exist and contribute to individual variability .

There has long been a debate on whether pain perception and endogenous analgesia is affected by male or female gender. So far, the results are conflicting, with some studies finding effects of sex on pain thresholds and endogenous analgesia, and others finding no such effect. However, one has to be aware that sex differences may also stem from psychological/sociological reasons, for instance, men are generally less likely to admit pain[125], than from genetic or biological differences. In our study, the sex differences in CPM effect were minimal. We believe that sex differences are not particularly important contributors to variability.

Anatomical differences may also contribute to why we observe variation in pain responses and endogenous inhibition. Differences in gray matter volume are present in multiple chronic pain conditions[126–128], suggesting an involvement of brain anatomy in pain perception. Chronic pain patients have smaller gray matter volumes in pain evaluative regions, primarily the cingulate cortex, prefrontal cortex, insulae, and thalamus. Investigations in healthy populations have shown that the larger regional gray matter volume of the insula is associated with lower pain sensation[129]. Gray matter volume in cingulate and prefrontal areas could influence descending inhibition, such that more gray matter would correspond to more “processing power”. Advances

in diffusion weighted imaging and tractography have allowed researchers to investigate the correlation between brain connectivity and our ability to decrease pain. Here, the connections between the PAG and other cortical and subcortical regions demonstrate the clearest effects. For example, white matter connectivity between dlPFC, ACC and PAG is associated with improved placebo analgesia response[130] and another study showed a relationship between PFC-PAG connectivity and greater CPM response[131]. A multitude of studies have also found white matter differences in chronic pain conditions (for examples see [132–134]) suggesting that either changes in white matter are symptomatic of dysregulated pain inhibition, or that white matter differences are a potential cause of chronification leading to poorer inhibition. The latter theory is supported by results showing that differences in anatomical connectivity are risk factors of pain chronification[57,135].

Functional connectivity has also revealed differences in functional coupling between these regions related to pain processing. Resting-state fMRI studies have shown that resting functional connectivity between brainstem/PAG and cortical regions is predictive of pain sensitivity[136,137] and pain modulation[136]. The corticocortical functional connectivity of frontal areas is also related to pain modulation[104], furthering the notion that intracortical communication plays a key role in the descending inhibitory network. Specifically, intrinsic functional connectivity[138] between ACC, PAG and RVM suggests that there indeed exists a ACC-PAG-RVM axis through which pain modulation can occur. In summary, both anatomical and functional connectivity between the brainstem and the frontal cortical areas associated with pain inhibition exerts an influence on behaviour. It stands to reason that these differences, either in isolation or combination, alter an individuals' ability to modulate pain.

Many psychological factors are also thought to influence pain modulation. These factors could be either traits (e.g., a person is more likely to catastrophize), learned behaviours or current states. Psychological comorbidities, such as depression, are common in chronic pain conditions[77], and psychologically distressed individuals (e.g., individuals with increased trait depression, anxiety, catastrophizing) show signs of dysregulated pain perception and modulation[139–141]. However, it is not clear whether psychological distress is a cause or consequence of altered pain processing. Additionally, there may be a threshold above which psychological variables influence pain, but below which (i.e. within healthy ranges) they do not. The results of my two CPM studies confirmed the findings of a meta-analysis investigating the effect of psychological scores on the CPM effect[102], suggesting that within healthy populations depression, anxiety and catastrophizing have no influence on endogenous analgesia in general, but may affect it in a paradigm specific manner. Although the psychological scores for depression, catastrophizing or anxiety do not appear to affect endogenous analgesia, acute behaviours that can modify our thoughts on pain are able to influence nociception. We have previously shown that acute catastrophizing increases

spinal nociception, i.e., activates descending pain facilitation[25]. Additionally, participants may subconsciously apply coping mechanisms when receiving painful stimuli. As researchers we try to control for such things with explicit and clear instructions, but the possibility remains that participants automatically fall into mental patterns of either catastrophizing or supressing pain. This is supported by an association between CPM effect and individual affinity to pain modality[142]. That is to say pain modulation is dependent on the individual preference of pain modality (i.e. "I prefer cold over heat"). This shows that the perceived salience of a stimulus may not only be dependent on stimulus type, but vary between individuals. This methodological concern cannot be altered in study design, as one would want to keep the same stimulus modality across participants, but could perhaps be accounted for by the choice of participants. Lastly, expectations and internal bias towards pain or effectiveness of pain modulation will be different between participants. The power of expectation is demonstrated time and again with placebo studies [36,143], and such internal biases, especially if subconscious, are hard to account for and particularly relevant in pain research.

3.4 STRENGTHS

3.4.1 METHODOLOGICAL STRENGTHS OF THE LONGITUDINAL MRI STUDY

The biggest strengths of the first project were 1) its longitudinal design of task-based functional imaging (as opposed to structural or resting state) with inclusion of an intervention (i.e. feedback training) and 2) measurement of both objective (RIII reflex) and subjective pain markers parallel to fMRI acquisition, 3) implementation of a feedback paradigm to train deliberate activation of descending pain inhibition, and 4) usage of timecourse analysis to investigate the haemodynamic response to painful electrical stimulation.

The longitudinal design and utilization of the Sandwich Estimator to estimate individual covariance matrices allowed us to account for individual differences in brain activity when investigating the effect of training. Longitudinal studies in functional imaging can be difficult as standard SPM assumes all participants to have the same covariance matrix. This is of course not the case in interventional studies, such as the one we did, as we presume the brain activity to change between the first and second MRI session. The high quality of our fMRI data, with multi-band imaging and a 64-channel head coil also allowed us to investigate deep regions of the brain, which are usually hard to image.

The measurement of the RIII-reflex within the MRI has only been published in two previous studies[100,101], although electrical stimulation of the sural nerve has been performed more often. Although the RIII-reflex results did not meet our expectation, potentially due to remaining experimental constraints, such as body positioning in the MRI, signal filtering, or electrode resistances, construction of a hardware setup to measure spinal nociception is nonetheless an attractive tool to use in future pain investigations.

The usage of feedback training to teach participants to activate their descending pain inhibition adds a direct intervention between the MRI sessions. With this we could measure the brain activity in participants before and after they could activate descending pain inhibition. This allowed for a direct within-subject comparison of the pain inhibitory system and the reaction to painful stimuli at different levels (or ability) of pain inhibition.

Lastly, our usage of timecourse analysis of the normalized raw MRI signal allowed us to investigate haemodynamic changes not captured by the classical regression analysis using the canonical HRF. The HRF did not adequately capture the haemodynamic signal changes in response to painful electrical stimulation. Haemodynamics have been known to differ in the brainstem, a region that is important to capture for pain modulation, therefore not relying solely on regression of the canonical HRF and its derivatives gave us better insight into the changes per vs. post training. Through this we could identify decreases in activity in both subcortical and cortical regions not apparent via classical regression. The results of our study suggest that, although there is a large degree of variability between participants, a few key regions are consistently activated or deactivated during descending pain inhibition across the population.

3.4.2 STRENGTHS OF THE REPEATED MEASURES INVESTIGATIONS OF CPM VARIABILITY

The most important strength of the second project was its combination of cross-sectional and repeated measures design to allow for a comparison of the effects when accounting for individual variability. This allowed us to better measure the effect of different factors thought to influence CPM in more sensitive analyses, informing the statistical model of the variability between subjects. Additionally, the inclusion of novel statistical estimation algorithms to discern the relative contribution of the fixed effects, as well as total contribution of unaccounted-for inter-individual effects showed for the first time how much more yet-unexplained inter-individual differences contribute to CPM variability. Lastly, we confirmed our hypothesis that these differences are large enough to drown out any potentially significant effects of controllable variables such as CS intensity in cross sectional analyses.

3.5 FUTURE DIRECTIONS: DISCOVERING THE BASIS OF INTER-INDIVIDUAL DIFFERENCES IN PAIN

The basis for the inter-individual variability in both sensing and modulating pain remains poorly understood. The second part of this thesis provided some suggestions about the methodologies suitable to investigate CPM, but they are equally as applicable to other studies investigating the brains response to either differently intense pain or different pain modalities. It is apparent that the differences between individuals contribute greatly to the individualized sensation of pain, and any attempt to discerns facets of the sensation must take this variability into account. Future studies, be they neuroimaging, psychophysical, electrophysiological or any combination thereof should be aware that manipulation of a variable may best be analyzed in a repeated measure or mixed-model fashion. Additionally, researchers should be aware that a lack of detected effect may be due to a large population variability, or that the effect may only be seen in a certain subgroup.

The reasons for the variability in our training success for example may be due to differences in underlying brain connectivity. Indeed, we collected both resting state functional and diffusion weighted images before and after training. Unfortunately, time-constraints did not allow us to analyze these data up until now. However, previous literature indicates that an increased baseline activity between PAG and frontal cortical areas may be the reason why some participants were inherently better at activating their descending pain inhibitory system. It will also be interesting to see if feedback training resulted in any baseline changes in anatomical or functional connectivity. Another analysis that may help investigate the relationship between brain activity and training success would be a psychophysical interaction analysis investigating the functional coupling of the PAG with our ROI and relating it to either feedback training success or the pain reduction achieved during that particular session. Especially the latter may give insight into how transient functional differences affect our endogenous modulation capacity. In a similar vein, transient changes in resting state connectivity before experiments investigating descending inhibition could elucidate this further. In our current analyses, we found a population level effect. We believe that with a more targeted approach towards investigating functional connectivity and individual variability we can gain greater insight into the mechanisms of descending pain inhibition in humans.

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6 LIST OF PUBLICATIONS

Publications:

1. **Graeff P**, Itter A, Wach K, Ruscheweyh R. Inter-Individual Differences Explain More Variance in Conditioned Pain Modulation Than Age, Sex and Conditioning Stimulus Intensity Combined. *Brain Sci*. 2021 Sep 9;**11**(9):1186. doi: 10.3390/brainsci11091186. PMID: 34573207; PMCID: PMC8468738.
2. **Graeff P**, Stacheneder R, Alt L, Ruscheweyh R. The Contribution of Psychological Factors to Inter-Individual Variability in Conditioned Pain Modulation Is Limited in Young Healthy Subjects. *Brain Sci.* 2022 May 10;**12**(5):623. doi: 10.3390/brainsci12050623. PMID: 35625010; PMCID: PMC9139004.

Posters:

1. **Graeff P.**, Itter, A., Wach, K., Ruscheweyh, R. Effekt der Intensität des konditionierenden Schmerzreizes auf die Größe des CPM Effekts beim Menschen. *Deutscher Schmerzkongress*, 19-23 October 2021, Mannheim, Germany

Talks/Presentations:

1. **Graeff P.** Supraspinale Korrelate der erlenten, willentlich Aktivierung der absteigenden Schmerzhemmung. *Winterschool der detuschen Schmerzgesellschafft,* 19-20 March 2021, online
2. **Graeff P.** Supraspinale Korrelate der erlenten, willentlich Aktivierung der absteigenden Schmerzhemmung. *Juniorakademie der deutschen Schmerzgesellschafft*. 25-26 November 2021, Berlin, Germany
3. **Graeff P.** Supraspinal correlates of learned willing activation of descending pain inhibition *IASP European Pain Winter School*, 13-17 December 2021, online

7 DECLARATION OF AUTHOR CONTRIBUTION

Chapter 2.1 **Longitudinal changes in humans supraspinal pain processing after RIII-feedback training to improve descending pain inhibition**

Graeff P., Ruscheweyh R., Virginia L. Flanagin 2022. (in preparation)

The study was designed by Philipp Graeff, Virginia L. Flanagin and Ruth Ruscheweyh. MRI-hardware setup was constructed by Philipp Graeff. Data was collected by Philipp Graeff under supervision of Ruth Ruscheweyh (electrophysiological part) and Virginia L. Flanagin (MRI part). Data was analyzed by Philipp Graeff. Imaging data was analyzed and visualized by Philipp Graeff with guidance of Virginia L. Flanagin. Philipp Graeff wrote the manuscript with contributions from Ruth Ruscheweyh and Virginia L. Flanagin .

Chapter 2.2 **Inter-individual differences explain more variance in conditioned pain modulation than age, sex and conditioning stimulus intensity combined.**

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All authors designed the study, with the analysis design being conceptualized by Philipp Graeff and Ruth Ruscheweyh. Data was collected by Alina Itter, Katharina Wach and Philipp Graeff under the supervision of Ruth Ruscheweyh. Analysis was performed by Philipp Graeff. Philipp Graeff and Ruth Ruscheweyh wrote the paper with input from all authors.

Chapter 2.3 **The contribution of psychological factors to inter-individual variability in conditioned pain modulation is limited in young healthy participants**

Graeff P, Stacheneder R, Alt L, Ruscheweyh R. *Brain Sciences.* 2022; 12(5):623.

The study/analysis was designed by Philipp Graeff and Ruth Ruscheweyh. Data was collected by Regina Stacheneder, Laura Alt and Philipp Graeff under the supervision of Ruth Ruscheweyh. Analysis was performed by Philipp Graeff. Philipp Graeff and Ruth Ruscheweyh wrote the paper with input from all authors.

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