"Novel approaches to investigate central sensitization : from experimental models to clinical perspectives"

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ABSTRACT

Chronic pain is a debilitating condition affecting millions worldwide. It is thought to be associated with central sensitization, a phenomenon where the central nervous system amplifies pain signals, leading to increased sensitivity and prolonged pain perception. Through experimental electrical skin stimulation, this thesis investigates innovative approaches to reliably induce central sensitization in humans, evaluates potential biomarkers for its assessment, and examines psychological factors that influence its modulation. From a clinical perspective, this research assesses how these findings translate to predicting persistent post-surgical pain in patients, offering promising avenues for targeted interventions.

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Université Catholique de Louvain

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Novel approaches to investigate central sensitization: from experimental models to clinical perspectives

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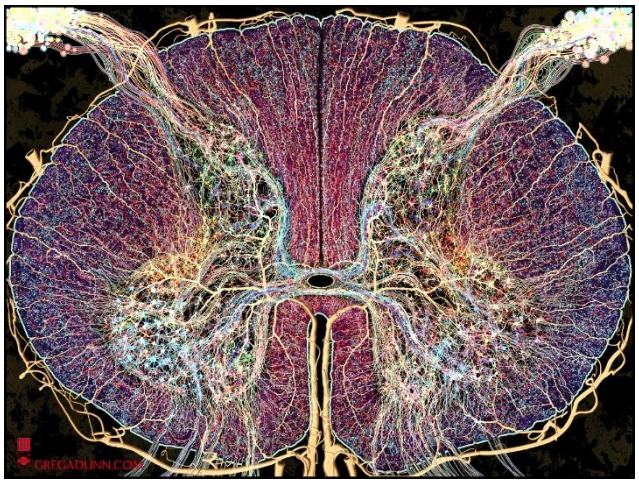

The spinal cord, with its symmetrical structure, evokes the image of a butterfly, a delicate yet essential figure that bridges sensation and motion. Its wings represent the balance of inputs and outputs, with the dorsal and ventral roots that maintain our body in harmony. However, in the context of chronic pain, this balance is disrupted, and the butterfly's graceful flight becomes heavier, burdened by the echoes of amplified pain signals.

Central sensitization is the storm that surrounds the butterfly, turning its serene journey into a turbulent struggle. What was once a gentle breeze, becomes a constant wind, amplifying even the lightest touch into a harsh and overwhelming gust. The dorsal horn, the core of the spinal cord, is thought to be the epicentre of this transformation, altering how signals are processed and perceived.

In this state, the butterfly's wings, symbols of resilience, and freedom are shadowed by the weight of persistent pain. The beauty of the spinal cord symmetry and its role in orchestrating sensation and motion remain, but the balance is lost. It is a call to unravel the mysteries of central sensitization, offering hope to restore the butterfly's ability to fly freely once again.

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It's true that the cover might resemble that of an album. And while this manuscript is certainly less rock'n'roll in its form, the doctoral journey itself doesn't lack rhythm and improvisation! Fortunately, I was surrounded by many kind people throughout the whole process. I am deeply grateful to all those who contributed to this thesis and supported me in more ways than I can count. Below, I would like to express my thanks to some of them:

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“Science isn’t finished until it’s communicated.”

Sir Mark Walport

I. Chapter 1. General introduction

I.1. Pain, beyond a physical symptom

Pain is a universal intricate human experience transcending language, culture, and time. The International Association for the Study of Pain (IASP) defines pain as "an unpleasant sensory and emotional experience associated with, or resembling, that associated with, actual or potential tissue damage"[1]. While transient acute pain serves as a vital warning signal in response to potential tissue damage, chronic pain persists for months and sometimes even years after its protective purpose has been achieved[2]. Pain is usually defined as chronic when it lasts for at least three months[3, 4]. It is well established that chronic pain affects not only physical, but also mental health, significantly impacting an individual's quality of life[5]. In addition, chronic pain imposes a substantial societal burden through decreased work productivity and even sometimes work-quitting[6].

Before the 1970s, this so-called symptom was poorly understood, and misunderstandings led to dismissive attitudes and inadequate treatment[7]. Specifically, it was commonly believed that pain was directly and proportionally linked to a specific disease or physical condition[8]. Therefore, a significant misconception was the belief that chronic pain, in the absence of specific tissue lesions or pathology, was psychological or linked to a patient's mental condition[9]. This reductionist view failed to recognize the intricate interplay between the brain, nervous system, and various physiological processes in the experience of chronic pain. Obviously, it

limited the effectiveness of treatments and importantly, underestimated the impact of pain on the individual's overall well-being.

In the 1980s, Georges Libman Engel described a biopsychosocial model, that accounted for the complex interplay between biological, psychological, and social factors[10]. Engel's model highlighted the importance of understanding the patient as a whole, rather than simply focusing on the physical aspects of their pain. It was necessary to work under a holistic and comprehensive treatment strategy for chronic pain, addressing not only the physical aspects, but also the environmental, cognitive, and social components of the patient's pain experience. However, while Engel's biopsychosocial model offers a more holistic approach to understand and treat chronic pain, it is not without some limitations[11]. One of the main issues is the complexity of the model, which can make it difficult to apply in clinical settings where time and resources are limited[12]. Another limitation of this model is its inability to provide tools to establish causal relationships. This does not allow us to differentiate between genuine cause-and-effect and coincidental associations, making it difficult to determine which explanations are theoretically sound. In summary, while the biopsychosocial model serves as a 'conceptual framework,' it should not be considered as an explanatory model of disease[13]. In parallel with these conceptual developments, neuroscience research began uncovering specific physiological processes involved in pain modulation, particularly those related to neural plasticity.

I.2. Central sensitization: A nociplastic phenomenon amplifying pain signals

I.2.1. General concepts

A significant breakthrough in the field of molecular mechanisms of plasticity was the first description of long-term potentiation (LTP) in the hippocampus by Bliss and Lømo in 1973[14]. They discovered that brief high-frequency inputs of perforant path fibers to the dentate area of the hippocampus led to a sustained increase in synapse strength, confirming Lømo's 1971 findings[15, 16]. Similar to how declarative memory is processed in the hippocampus under LTP processes, dorsal horn neurons may amplify nociceptive signals through a similar mechanism[17, 18].

Animal models show that sustained stimulation or strong nociceptive input, such as that encountered during surgical operations, can induce sensitization in the peripheral nerve endings of first-order nociceptive neurons[19]. This nociceptive input causes a large release of inflammatory mediators that lead to cascades of phosphorylation events within the synapse, which depolarizes the membrane of the nociceptive neuron[20-22]. Then, the action potential generated at the nociceptive fiber propagates towards the synaptic cleft in the dorsal horn, where second-order neurons are located[20, 23]. Neurotransmitters, such as glutamate, substance P, and brain-derived neurotrophic factor (BDNF), are released and activate their associated receptors on second order neuron[24-26]. This constant release of neurotransmitters can lead to the excitation and strengthening of the synapse through homosynaptic LTP, which is believed to be responsible for

primary hyperalgesia and is the result of both peripheral and central sensitization[24]. Consequently, this leads to an increased sensitivity and flare response at the site of tissue damage, which are hallmark features of primary hyperalgesia[27-30].

Nearby, glial cells can detect this activity and release signalling molecules like BDNF and cytokines[31-33]. These signalling molecules can activate various receptors on neighbouring synapses, constituted by other nociceptive fibers that were not stimulated at their periphery[31]. As a result, these unstimulated fibers enhance their sensitivity to neurotransmitters and amplify overall pain signal processing in the spinal cord[24]. This process, known as heterosynaptic LTP, is thought to contribute to secondary hyperalgesia, an increase in pain sensitivity in the surrounding non-injured skin, as a result of central sensitization[18, 31].

I.2.2. From semantics to science: Central sensitization's terminological odyssey

In the 1940s, Sir Thomas Lewis introduced the concept of hyperalgesia by stimulating his own forearm with a continuous painful electrical current[34]. At the end of the stimulation, there was little or no pain in the surrounding skin. After a few minutes, the soreness increased accompanied by a reduction in the threshold for pricking stimulation and gradually spread along the forearm: a phenomenon we now call secondary mechanical hyperalgesia. Nonetheless, he hypothesized that this "*excitation of the skin*" was due to peripheral mechanisms rather than central. Ten years later, Hardy et al.

(1950) conducted various psychophysical experiments, and suggested that the mechanisms underlying the soreness surrounding the skin after electrical stimulation described by Lewis was instead the result of a central excitatory state (**Figure 1**)[35].

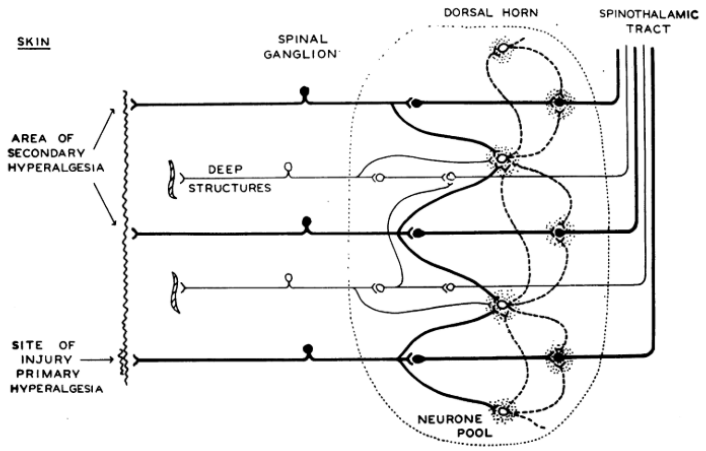


Figure 1. From Hardy et al. (1950). Schematic diagram of pain fiber connections within the neuron pool showing foci of excitation (stippled areas) resulting from the continuous barrage of noxious impulses from the site of injury.

While the term "central sensitization" may not have been established at this time, ideas and precursor concepts started emerging during that era. This was corroborated by Woolf, who showed through experiments on rodents that when a noxious (thermal or chemical irritant) stimulus is delivered to the skin, it induces functional changes, such as reduced threshold and expansion of the receptive field in motor neurons[36-38].

In his 1983 Nature paper, Woolf illustrated the neurophysiological mechanisms underlying these changes, demonstrating how transient nociceptive stimulation can lead to prolonged periods of increased

excitability within the central nervous system (CNS). This increased excitability was characterized by enhanced synaptic efficacy in neurons within the dorsal horn of the spinal cord, a process Woolf posited as a potential neural basis for the persistent pain states observed clinically[36]. This indicates that these changes take place within the CNS, specifically in the spinal cord, rather than being driven by peripheral factors. Simone et al. (1991) followed that idea by investigating the role of spinothalamic tract (STT) neurons and found that the sensitization responses of wide dynamic range (WDR) neurons to thermal stimuli following capsaicin administration in primates showed a strong correlation with the hyperalgesic responses to heat observed in humans post-capsaicin injection[39]. These effects are believed to stem from increased neuronal excitability in the dorsal horn of the spinal cord. They also showed that both WDR and high-threshold (HT) neurons responded more strongly to mechanical pinprick stimuli after capsaicin injection. In the same year, they conducted other psychophysical studies to explore what they termed "neurogenic hyperalgesia," a process wherein a group of neurons becomes sensitized due to the activation of another neuronal group, resulting in secondary mechanical hyperalgesia[39]. They discovered that administering a short-duration anaesthetic could diminish or remove secondary mechanical hyperalgesia. This suggests that the neurons sensitized during neurogenic hyperalgesia are situated within the CNS rather than in the peripheral nervous system. Thus, aligning with Woolf's group findings, they concluded that the sensitization of nociceptive neurons is influenced not only by peripheral factors, but also by interactions within the CNS. However, the relative influence of peripheral versus central activity in this process remains to be fully understood[29].

In 2008, the International Association of Study of Pain (IASP) defined central sensitization as an “increased responsiveness of nociceptive neurons in the central nervous system to their normal or subthreshold afferent input”[40].

A nociceptive neuron is defined as one that is "able to encode a noxious stimulus," while a noxious stimulus is defined as stimulus that "damages or threatens to damage normal tissues"[41]. Thus, all stimuli that have the potential to injure a body part are often referred to as "noxious," such as those causing cutaneous injury, but also extremely bright lights that could cause visual impairments or loud noises that could create persistent tinnitus. That is why, when we examine this definition more closely, though, we find that it makes no specific mention of the connection to pain hypersensitivity.

Despite Woolf's (2011) attempt to refine the concept of central sensitization as "an amplification of neural signalling within the CNS that elicits pain hypersensitivity," this definition faces applicability issues in both humans and animals[42]. In humans, the challenge lies in the direct recording of neural amplification, which remains currently unfeasible. Meanwhile, the concept of pain hypersensitivity in animals is problematic due to their inability to verbally report such experiences, leaving researchers to rely solely on indirect methods of measurement[43]. Moreover, despite studies showing CNS structures (referring as “pain matrix”) correlated with the pain perception in humans, research suggests that the responses within this "pain matrix" are strongly influenced by the context within which the nociceptive stimuli appear and importantly, that non-nociceptive stimuli can elicit similar cortical responses[44]. Currently, no regions of the brain have been identified as being exclusively dedicated to pain processing.

A recent review questions the IASP definition, pointing out that while animal studies have shown central sensitization occurs in response to acute and chronic pain models, there is still no evidence that it directly causes chronic pain, or occurs in humans[45]. Given the lack of a precise definition for central sensitization and the somewhat complex nature of the concept, practitioners and researchers often rely on the definition provided by the IASP, which covers a spectrum of pain syndromes, each potentially necessitating distinct therapeutic approaches[46].

In 2019, den Boer and colleagues conducted a systematic review of the diverse definitions of central sensitization and reached a consensus that hyperexcitability of the CNS is the central mechanism in the definition of central sensitization[47]. While this conclusion aligns with the IASP's definition and may not be incorrect, its practical contribution to the patient's care raises questions. The broad application of this definition may lead to an overclassification of individuals as having central sensitization and promote a one-size-fits-all treatment approach, potentially overlooking the nuanced differences in the underlying mechanisms of various chronic conditions. In a commentary published following this review, van den Broeke (2019) referred to this consensus about hyperexcitability of the CNS as the "lowest common denominator," highlighting it as a broad concept that includes all pain syndromes, as well as a variety of other symptoms that may not be directly related to pain[48].

One can recognize that if the meaning of central sensitization is employed overly broadly for various pain diseases, it does not help clinicians to set a clear diagnostic and appropriate treatments for each patient[46]. For instance,

the treatment strategies for migraines may not be suitable for postoperative persistent pain, despite both potentially falling under the umbrella of central sensitization[49].

A tool commonly used as an eventual indicator of central sensitization is *The Central Sensitization Inventory* (CSI)[50, 51]. However, the absence of a universally accepted standard for detecting central sensitization in humans raises questions on the content validity of the CSI, which remains unverifiable. Consequently, this questionnaire does not so much identify patients with central sensitization but rather assesses a plethora of symptoms related to the condition, including for example fatigue, depression, or anxiety. This questionnaire serves more effectively as a screening tool, enabling the tracking of symptoms over time, with each symptom being considered and monitored individually[51].

However, the confusion occurring at the clinical (and research) level is understandable since the term "central" refers to all the compartments of the CNS, including the brain and the various structures associated. Therefore, one could expect that it may encompass various chronic syndromes[52, 53]. This highlights the importance of developing more specific criteria or subcategories, to tailor treatment plans more effectively and improve patient outcomes[54].

Recently, van den Broeke et al. (2024) proposed to redefine sensitization as its original meaning which is an "enhanced behavioural responsiveness that results from repeated or prolonged exposure to the same stimulus", which is applicable both in humans and animals. This behavioural approach of

sensitization reflects defensive responses to a potentially harmful stimulation, which serve to protect the integrity of the body[55].

I.2.3. Disclaimer

It is important to note that the following chapters mention neural and molecular mechanisms demonstrated in animal pain models that have not yet been directly validated in humans. However, in this thesis, I posit that the central mechanisms of sensitization in humans share certain similarities to those observed in animal models, given the conservation of fundamental biological processes across species, and importantly, the shared aspects of behavioural outcomes. While a complete revision of the definition of central sensitization falls outside the scope of this thesis, I justify its use by defining it as "an increased behavioural response extending to non-stimulated areas resulting from sustained nociceptive repeated stimulation", which aligns with van den Broeke's (2024) proposed definition[55].

I.3. Experimental human models of central sensitization

I.3.1. Techniques for inducing central sensitization

Experimental human pain models are particularly important for the development of clinical trials and are crucial for improving our knowledge of pain pathophysiology. They help bridge the gap between preclinical research

on animals and complex pain experience that patient have in a clinical context[56, 57]. Indeed, these models give researchers a safe and ethical way to study pain in human beings, enabling them to investigate the mechanisms underlying pain perception, transmission, and modulation[57]. We can find important insights into the neurobiological and psychophysics correlates of pain, by trying to replicate hypersensitivity states in an experimental setting, such as the induction of secondary hyperalgesia. Below is a brief overview of commonly utilized research models (**Table 1**).

I.3.1.1. Capsaicin-based models

Capsaicin serves as a surrogate model for studying secondary hyperalgesia in humans through a controlled experimental approach (intradermal or topical application) known as the capsaicin-induced secondary hyperalgesia model[29, 30, 39, 58]. This natural compound found in chili peppers produces a characteristic spicy and burning sensation when ingested or applied to the skin[59]. Capsaicin interacts with sensory neurons in the body, specifically the ones expressing transient receptor potential vanilloid 1 (TRPV1) triggering a cascade of physiological responses and the release of neurotransmitters such as substance P [59-61]. Intradermal injections of capsaicin are highly effective at inducing secondary hyperalgesia both in humans and animals, with short latency (few minutes) and prolonged duration (~2 hours for 50–100 µg doses)[58, 62]. However, the procedure is invasive, causes a high level of discomfort, and is technically complex, which undermines the advantages of the technique.

Due to the invasive nature of intradermal injections, topical applications are widely employed[59, 63-65]. Indeed, topical application using creams, patches, or solutions is a non-invasive procedure with a high safety profile. However, topical capsaicin requires a minimum application time of 30 minutes and induces a shorter-lasting hyperalgesic effect compared to intradermal injections. Consequently, it is often combined with heat sensitization protocols to stabilize the induction of secondary hyperalgesia[66-69].

I.3.1.2. Electrical stimulation models

Electrical stimulation is also a widely used model to induce secondary hyperalgesia both in animals and humans. The high-frequency stimulation (HFS) method involves delivering brief, repeated trains of pulses at a frequency of 100 Hz for one second, typically applied to the forearm due to its flat surface[31, 70-73]. In 2004, Klein et al. showed that after HFS the perception of electrical stimuli at the HFS site was increased compared to the control site, which could reflect homotopic and/or heterotopic LTP[72]. However, the induction of homotopic LTP with HFS remains unclear, especially in human models, since the literature is very controversial[31, 72, 74-77]. Interestingly in the same study, researchers showed that the perceived intensity of pinprick stimulation increased around the HFS site compared to the control site which is mainly due to heterotopic effects that produce secondary hyperalgesia. This finding has been consistently replicated, making increased sensitivity to mechanical pinprick stimuli in the surrounding skin the most well-documented effect of HFS conditioning in

humans[74, 77-81]. HFS is one of the most widely implemented human sensitization models due to its capacity to reliably induce secondary hyperalgesia with a short-lasting induction procedure, by activating nociceptors in an intense and sustained fashion. In the following chapters, HFS has been used for the previous reasons, but also for the fact that it is a non-invasive technique that does not require any pharmacologically active compound, and it can be administered in an operator-independent way.

Low-frequency stimulation (LFS) applied onto the skin can also be used to induce secondary hyperalgesia[82, 83]. However, although it is thought to mimic the discharge patterns of C-fibers in neuropathic conditions, it generates a smaller amount of secondary hyperalgesia compared to HFS making it less suitable to study it[72, 84]. When applied percutaneously, LFS provides a broader spread of secondary hyperalgesia, but its invasive nature and short-lasting effects make it less practical for widespread use[85, 86]. In contrast, HFS provides a non-invasive and more efficient alternative, producing longer-lasting hyperalgesia and allowing for easier application, making it the preferred choice for most experimental studies[57].

I.3.1.3. Ultraviolet models

Ultraviolet A (UVA) and Ultraviolet B (UVB) irradiation affect the skin differently. UVA penetrates deeper into the dermis and requires significantly higher doses than UVB to induce erythema. It causes immediate redness and thermal effects but does not lead to delayed hyperalgesia at moderate

intensities[87, 88]. In contrast, UVB is primarily absorbed in the epidermis and consistently induces sunburn, delayed erythema, and hyperalgesia, making it a reliable model for research[87, 89]. This distinction explains the focus on UVB in experimental studies.

UVB exposure causes keratinocyte damage and stimulates the release of inflammatory mediators, such as prostaglandins and cytokines. This leads to skin inflammation and erythema, activating immune-mediated processes that upregulate pro-inflammatory cytokines. These mediators amplify nociceptive signalling, ultimately contributing to hyperalgesia development[90]. Primary hyperalgesia seems constantly observed, whereas secondary hyperalgesia is less predictable, often displaying inconsistencies or developing after more than 10 hours post-irradiation[91-94]. While UVB techniques offer lasting effects without causing ongoing pain, they present some drawbacks, like the risk of hyperpigmentation and the challenge of reliably triggering secondary hyperalgesia[89].

I.3.1.4. Heat-injury models

Heat-injury models, which engage heat-sensitive nociceptors such as TRPV1 channels and C-fiber nociceptors, can also induce secondary hyperalgesia when thermal stimulation is applied at nonpainful temperatures (40–42°C). However, this hyperalgesia is typically brief, and similar to the capsaicin model, requires prolonged exposure to thermal stimuli for maintenance[95, 96]. This is the reason why researchers tend to use a 47°C protocol (5-7 min of application), where secondary hyperalgesia peaks after a minimum of one

hour, with long-lasting effects that vary between subjects[97-99]. A limitation of this technique is that such high temperatures may cause epidermal damage, potentially resulting in blisters or pigmentation changes[89].

I.3.1.5. Less commonly used models

Several less common models have been developed to induce secondary hyperalgesia, though they are less prevalent due to methodological constraints, inconsistent results, or low replication rates. Cold receptor activation models, such as menthol application or freeze injury, may mimic cold hypersensitivity, but they produce variable results and exhibit limited spread of secondary hyperalgesia[100-103]. TRPA1 activators (e.g., mustard oil, cinnamaldehyde) reliably induce painful sensations but inconsistently produce secondary hyperalgesia[57, 104, 105]. Incisional models effectively replicate central sensitization and consistently induce secondary hyperalgesia, but their highly invasive nature significantly limits their practical application[103, 106, 107]. Other models, such as nerve growth factor (NGF) injections, hypertonic saline, and skin irritants, have shown potential for inducing secondary hyperalgesia, but suffer from low reproducibility or restricted effects[57, 108, 109].

Category	Technique Used	Advantages	Disadvantages
Capsaicin-Based Models	**Intradermal Capsaicin**	- Induce secondary hyperalgesia effectively. - Short latency for effect (few minutes). - Long duration for doses of 50–100 µg (~2 hrs).	- Invasive. - Painful injection compared to topical capsaicin. - Difficult preparation of aqueous solutions.
	Topical Capsaicin	- Non-invasive. - Easy application. - High safety profile.	- Brief hyperalgesia duration relative to intradermal models. - Requires heat-kindling for consistent effects.
Electrical Stimulation Models	**Low-Frequency Electrical Stimulation (LFS)**	- Non-invasive when applied topically. Mimics clinical neuropathic pain. - Stable hyperalgesia.	- Invasive when applied percutaneously. - Short-lived hyperalgesia after stimulus stops.

	High-Frequency Electrical Stimulation (HFS)	- Maintenance of secondary hyperalgesia during several hours. - Non-invasive. - Inexpensive. - Operator independent.	- Unpleasant pain induction. - Limited studies on response to medication.
Ultraviolet Models	**Ultraviolet (UVB) Irradiation**	- Long-lasting effects (up to 4 days). - No spontaneous pain at onset.	- Requires calibration before use. - Risk of persistent hyperpigmentation. - High inter-individual variability.
Heat Injury Model	**Heat Stimulation**	- Rapid induction of secondary hyperalgesia. - Long-lasting hyperalgesia. - Peak effect within an hour.	- First-degree burn injury possible. - Blistering (depending on the parameters).
Cold-Induced Hyperalgesia	**Cold-Induced Hyperalgesia (e.g., Menthol application)**	- Mimics some neuropathic pain conditions. - Minimal discomfort. - No adverse effects.	- Inconsistent hyperalgesia development. - Short duration of effect. - Limited spatio-temporal amplification.

	Freeze Injury Models	- Long-lasting secondary hyperalgesia (up to 72 hrs). - Low discomfort. - No serious adverse events reported	- Hyperalgesia develops slowly (peak at ~24 hrs). - Requires more than one visit for testing.
TRPA1 Activators	**TRPA1 Activators (e.g., Mustard Oil)**	- Strong immediate pain induction. - Consistent dynamical allodynia induction.	- Highly variable hyperalgesia areas. - Limited research on this model in humans.

Table 1. Comparison of experimental pain models for inducing secondary hyperalgesia in humans

I.3.2. Techniques for measuring HFS-induced secondary hyperalgesia

The models cited above facilitate responses that can be indirectly assessed in humans through various methods, including psychophysical approaches and neurophysiological assessments, which will be detailed in the following sections.

I.3.2.1. Psychophysics

I.3.2.1.1. Mechanical pinprick stimuli

It has been demonstrated in animal studies that pain evoked by pinprick stimulation is mainly determined by the activation of Aδ fibers without significant contribution of C-fibers[110-113]. When pinprick stimuli are applied onto the skin around the area where the experimental induction of sensitization occurred, the intensity of perception increases in most cases, though it isn't always experienced as painful[113]. In 1981, van Hees and Gijbels showed that nociceptor activation can occur at lower levels of stimulation than the perception of pain (for example, stimulation with von Frey hairs up to 21 g can activate nociceptors without producing pain sensations)[114]. An integrative theory could be that during the mechanical von frey stimuli, concomitant activation of mechanoreceptors influences the signal and evokes a nonpainful pricking sensation until the nociceptor activity takes over and evokes pain[113]. Nevertheless, these findings neither fulfil Woolf's (2011) definition of central sensitization nor the term 'hyperalgesia because even though pinprick stimulations activate nociceptive fibers, it does not necessarily result in pain perception[80, 113]. On the other hand, the increase in mechanical pinprick sensitivity after sensitization cannot really be defined as “allodynia” because as previously mentioned, the pinprick stimuli activate nociceptive fibers. To ensure consistency with other authors, we will use the term "secondary mechanical hyperalgesia" to refer to the increased sensitivity to mechanical pinprick stimuli, even though we acknowledge that the term "hyperalgesia" may not be entirely accurate and might require

redefinition. Using the pinprick device, it is also possible to assess the extent of secondary mechanical hyperalgesia surrounding the point of stimulation[112, 115, 116]. The boundaries of this region are established by performing pinprick stimulations along eight, four, or two distinct paths, depending on the protocol. These paths originate well beyond the stimulated area and proceed towards it. Participants are asked to indicate when they experience a noticeable increase in perception, such as an increased pricking or burning feeling.

At that point, the boundary is marked, and measurements of each axis are taken to calculate the surface area[79, 117, 118]. A study by Cayrol et al. (2020) demonstrated, through both within-subject and between-subject designs, that the proximal-distal (longitudinal) axis is the most reliable measure for assessing secondary mechanical hyperalgesia in humans[78]. Consequently, since 2020 our measurements have exclusively focused on the proximal-distal axis.

I.3.2.1.2. Heat sensitivity

Heat sensitivity in the context of HFS-induced secondary hyperalgesia remains a topic of debate, with conflicting conclusions across studies. Some studies report increased sensitivity to heat stimuli after HFS in the area of secondary hyperalgesia when using short-duration laser stimuli. This increase in heat sensitivity is hypothesized to involve enhanced input from heat-sensitive C-fiber nociceptors[119, 120]. In contrast, other findings suggest that heat sensitivity is not affected within the area of secondary

hyperalgesia[118]. Indeed, it has been hypothesized, following contradictory results from earlier research by the same group, that changes in heat sensitivity may be confined to a smaller region near the HFS site and do not fully overlap with areas of heightened mechanical sensitivity[118, 119]. This raises questions about the differential central processing of mechanical and thermal nociceptive inputs. Despite these insights, heat sensitivity appears to be a less pronounced marker of secondary hyperalgesia compared to mechanical sensitivity, warranting further investigation into its underlying mechanisms and implications.

I.3.2.1.3. Tactile stimuli

Few studies have explored tactile stimuli in the context of HFS-induced secondary hyperalgesia. In 2006, Klein et al. demonstrated that mechanical hyperalgesia (triggered by pinprick stimuli) and dynamic mechanical allodynia (elicited by soft tactile stimuli) differ significantly in durations and response patterns following HFS[121]. Allodynia developed more variably, with only a subset of participants reporting significant pain, and a quicker return to baseline, whereas mechanical hyperalgesia showed a more consistent and longer-lasting increase in pain perception[121]. van den Broeke and Mouraux (2014) showed that HFS significantly increased the perceived intensity of mechanical pinprick stimuli but did not affect the perception elicited by vibrotactile stimulation[119]. Unpublished results presented in the review of Leone et al. (2024) showed that when tactile and pinprick stimuli of identical intensity were applied to the area of HFS-induced secondary hyperalgesia, tactile stimuli showed no increase in perceived intensity, whereas

mechanical pinprick stimuli elicited a clear enhancement in perception[116]. Given these findings, tactile stimuli do not appear to be a reliable method for assessing HFS-induced secondary hyperalgesia and will therefore not be employed in this study.

I.3.2.1.4. Electrical stimuli

Electrical stimulation has been used to evaluate both homotopic pain-LTP—referring to heightened sensitivity at the site of nociceptive stimulation (primary hyperalgesia)—and heterotopic pain-LTP, which describes increased sensitivity in the area surrounding the stimulation (secondary hyperalgesia), following HFS. Klein et al. were the first to demonstrate that after HFS application to the skin, painful electrical stimuli were perceived as more intense at the HFS site compared to a control site, highlighting a homotopic effect[72, 74]. However, they did not investigate whether the increased pain sensitivity to electrical stimuli was associated with heterotopic pain-LTP. Building on this, van den Broeke et al. (2021) examined both homotopic pain-LTP and whether the perception of electrical stimuli increased in the area surrounding the HFS site. Their results revealed that after HFS, electrical stimuli applied to the skin adjacent to the HFS site elicited a higher perceived pain intensity compared to the control site[71]. However, rather than an actual increase in perception following HFS, this effect appears to reflect a lack of habituation at the HFS site relative to the control site. In that study, the difference in pain intensity after HFS between the control site and the heterotopically conditioned site was relatively minor,

typically just one or two points. This raises the question about its clinical relevance, especially in comparison to mechanical pinprick stimuli, which elicit a more pronounced increase around the conditioned site and a clearer distinction from the control site.

I.3.2.2. Neurophysiological responses

I.3.2.2.1. Functional magnetic resonance imaging

Functional magnetic resonance imaging (fMRI) is a widely used neuroimaging technique that measures brain activity by detecting changes in blood oxygenation, offering high spatial resolution of neural processes[122]. In central sensitization research, fMRI has been widely used in identifying the brain regions involved in altered pain processing and secondary hyperalgesia. Through experimental pain models, such as those using capsaicin or UV-B irradiation, researchers have mapped the activity of key brain areas during hyperalgesic states, consistently showing increased activation in regions like the insular cortex, anterior cingulate cortex (ACC), primary (S1) and secondary (S2) somatosensory cortices, thalamus, and brainstem areas such as the periaqueductal gray (PAG)[123-125].

For instance, capsaicin-induced hyperalgesia models have shown strong activation of these regions, particularly those associated with the sensory-discriminative and affective dimensions of pain. Similarly, a recent meta-analysis by Clarke et al. (2023) has shown consistent activation patterns

across studies, particularly in the anterior insula, thalamus, and ACC, highlighting their role in processing hyperalgesia and its modulation by analgesic interventions[126].

Additionally, Lee et al. (2008) investigated how the brain's activity differs during central sensitization compared to normal states. Their findings showed that during central sensitization, the brainstem, particularly the mesencephalic pontine reticular formation, and the anterior thalamus exhibited significantly increased activity. The brainstem's activation was specifically linked to the intensity of noxious stimulation, while the thalamus showed elevated activity in both central sensitization and normal states, though to a lesser degree in normal states. Additionally, cortical areas such as the primary somatosensory cortex were found to reflect the perceived intensity of pain in both conditions. These results highlight the brainstem's central role in maintaining central sensitization and the involvement of cortical regions in processing the sensory experience of pain[123].

However, despite its value in experimental research, fMRI remains challenging to implement in routine clinical settings. This technique requires expensive equipment, and substantial time for data acquisition and analysis. Furthermore, variability in study designs and analytical approaches can complicate interpretation, making it less practical for widespread diagnostic use[127]. To address this, there is a growing demand for more practical and accessible methods to study central sensitization in clinical settings. Cost-effective, portable, and easily implementable approaches that still capture key neural dynamics could serve as alternatives to advanced imaging techniques like fMRI. Such methods have the potential to bridge the gap

between experimental research and routine clinical use, enabling a more widespread and standardized assessment of central sensitization.

I.3.2.2.2. Nociceptive flexion reflex

The nociceptive flexion reflex, known as the RIII reflex, is a polysynaptic spinal withdrawal response elicited by noxious stimuli[128]. In the context of central sensitization, the RIII reflex is particularly valuable due to its sensitivity to changes in spinal cord excitability[128, 129]. The RIII reflex, primarily used to measure the threshold for eliciting the reflex, decreases in sensitized states and has been observed following interventions such as HFS or capsaicin application to induce sensitization[130-132]. Moreover, the RIII threshold appears to correlate with subjective pain thresholds, reinforcing its validity as a proxy for spinal excitability changes during sensitization[124, 133]. However, further studies are needed to confirm and generalize these findings across different populations and experimental conditions, ensuring the robustness of this correlation. Another measure, the reflex area under the curve (AUC), quantifies the magnitude of muscle activity following stimulation. Although this parameter shows less consistent modulation across studies, it may capture dynamic changes in spinal excitability, particularly when ongoing nociceptive input is present[116, 134].

While the RIII reflex offers significant strengths, it is not without limitations. Its reliability can be affected by experimental parameters such as stimulation frequency, intensity, and electrode placement, potentially introducing variability in outcomes. These considerations underscore the importance of

standardization and meticulous methodological design in studies using the RIII reflex[116, 135].

I.3.2.2.3. N13 component

Generated by postsynaptic responses of neurons within the cervical dorsal horn, the N13 component of somatosensory evoked potentials (SEP) reflects the processing of input from large, myelinated, non-nociceptive fibers[136, 137]. This neurophysiological measure has been recognized as a promising tool for investigating central sensitization, providing a non-invasive marker to assess changes in dorsal horn dynamics[138]. Unlike other measures, such as the nociceptive flexion reflex, which require painful stimuli, the N13's reliance on non-noxious stimulation makes it more suitable for clinical settings and longitudinal pharmacological studies. Recent research has demonstrated the N13's sensitivity to experimental pain models designed to induce central sensitization. For instance, topical capsaicin application increases the N13 amplitude, suggesting enhanced dorsal horn excitability[139]. Similarly, LFS, known to induce wind-up and facilitate dorsal horn changes, has been shown to modulate N13 amplitudes in a manner distinct from HFS[140]. These findings underscore the N13 component's capacity to reflect dynamic changes in the spinal cord's excitatory and inhibitory balance, aligning well with the underlying mechanisms of central sensitization.

The study of Di Leonardo et al. (2021) showed the ability of the N13 component to track dorsal horn excitability alterations has been linked to its

responsiveness to pharmacological interventions targeting central sensitization, such as pregabalin, which prevents capsaicin-induced N13 modulation[139]. This opens avenues for its use in early-stage drug trials aimed at developing novel analgesics that target spinal mechanisms.

Despite its promise, the N13 component has limitations that must be addressed. While the N13 reflects spinal processing, its relationship with subjective pain perception and secondary hyperalgesia remains unclear, as studies have failed to find strong correlations between N13 changes and behavioural hyperalgesia scores[116, 139]. The low amplitude of the signal, particularly for ulnar nerve stimulation, presents challenges in detecting subtle changes at the individual level, potentially limiting its use as a biomarker. Furthermore, while N13 is modulated by LFS, it remains unaffected by HFS, indicating that its ability to reflect central sensitization is highly dependent on the experimental pain model used[140].

I.3.2.2.4. Electroencephalography

Electroencephalography (EEG) is a non-invasive technique commonly used to study central sensitization in humans, as it measures brain electrical activity, particularly postsynaptic activity[141-143]. With its high temporal resolution, EEG captures rapid neural dynamics by detecting real-time brain signals through surface electrodes placed on the scalp, offering valuable insights into ongoing neuronal oscillations[144]. Beyond its applications in the clinic for diagnosing neurological conditions, EEG is also used for research purposes to evaluate neurophysiological functions such as sensory and cognitive

processing[145, 146]. As pain perception is believed to arise from oscillations in brain activity, identifying objective features that reflect this process in a less operator-dependent manner is of great interest[147-149]. However, despite promising results of some pain biomarkers, most brain responses observed when a nociceptive stimulus is presented can also be observed when salient non-nociceptive (visual, auditory, tactile) stimuli are presented[44]. This underscores the need to identify biomarkers that are both highly sensitive and specific to nociception.

From this point, event-related potentials (ERPs) can offer crucial insights into the changes in brain activity that occur in response to sensory events[150, 151]. These signals manifest as brief changes in the brain electrical activity that are time-locked to specific events[152]. The idea behind ERPs is similar to EEG: when a group of neurons aligns in the same direction and suddenly become active simultaneously, this synchronized activity generates ERPs[150, 153]. Although EEG has a relatively poor spatial resolution limiting precise localization of neural sources, its high temporal resolution makes it particularly well-suited for studying event-related brain responses such as evoked potentials[151]. ERPs are measured by identifying the negative (N-wave) and positive (P-wave) peaks which reflect synchronized activation of a population of neurons[154, 155]. To detect these peaks among the ongoing electrical activity in the brain, stimuli are repeated multiple times, and responses are averaged across trials in the time-domain[156, 157]. This technique helps to get rid of the non-stimulus-evoked noise, leaving a better signal-to-noise ratio (SNR) of brain responses triggered by specific events[152].

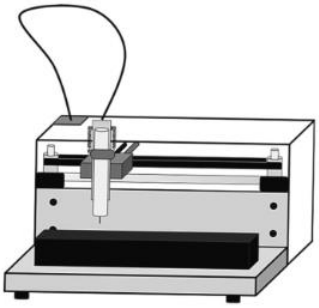
As previously mentioned, pinprick stimuli serve as a standard method for detecting secondary mechanical hyperalgesia, which is thought to be a correlate of central sensitization. The state of central sensitization induced experimentally can also be assessed through the recording of brain activity using pinprick-evoked potentials (PEPs)[141, 143, 158]. The aim of discovering more biomarkers for central sensitization is to develop a set of reliable measurements that could constitute composite biomarkers. These are believed to provide more reliable results compared to using individual markers, whether in clinical practice or experimental research settings[159, 160].

In response to brief and sharp mechanical stimulus, PEPs are defined by a biphasic complex consisting of a negative peak followed by a positive peak maximal at the vertex. The negative deflection occurs typically between 100 and 150 ms, and the positive deflection between 250 and 300 ms[141, 158, 161, 162]. During EEG recording, PEPs are induced by mechanical pinprick stimulations applied to the skin, typically performed manually but also possible using a robot-controlled device[141, 162, 163]. It has been shown that the amplitude of PEPs (the negative-positive complex) is increased after the experimental induction of central sensitization[141, 158, 162]. However, in those studies, the signal-to-noise ratio is quite low, and identifications of the peaks can then be difficult. This is probably due to the variability in the force applied manually with the pinprick to the skin, which leads to latency jitter and less synchronized responses[163]. In Iannetti's 2013 study, where PEPs were recorded before and after the induction of central sensitization through capsaicin injections, the researchers observed a significant increase in the amplitude of the negative peak post-capsaicin injections[141]. In that study, the later-latency positive peak remained unaffected. This contrasts with the

studies by van den Broeke et al. (2015, 2017), which found that only the positive peak was enhanced after the experimental induction of central sensitization by HFS[158, 161].

It is important to note that the latency of PEPs is compatible with the conduction velocity of myelinated fibers, including Aβ fibers and fast-conducting nociceptive Aδ fibers[164, 165]. Therefore, a hypothesis proposed by van den Broeke et al. (2015) states that the enhancement of the N-wave reported in the study of Iannetti in 2013 might be at least partly non-modality specific[158]. It might be related to the activity of non-nociceptive low threshold mechanoreceptors activated by a *too quick* manual application of the pinprick stimuli, which was the case in Iannetti's studies. To minimize this variability and improve the SNR of PEPs, robotic application has started to be used in this context, which seems promising regarding the high quality of the data[162, 163].

Note on the pinprick-robot functioning



The robot is equipped with three linear computer-controlled stages. The initial two stages are responsible for the horizontal (X/Y) positioning of the pinprick probe, while the third stage, which holds the pinprick probe, manages its vertical (Z) movement. Each stage can move at a rate of 25 mm/s, with a precision of 0.1 mm. The construction of the pinprick probe features a stainless-steel flat tip probe with a diameter of 0.35 mm and flat geometry, atop which a calibrated cylindrical weight is placed. This assembly is housed within an aluminium tube secured by the robot. When the probe is applied perpendicularly to the skin, it and the weight can slide freely within the tube, exerting a consistent normal force determined solely by their combined mass. A high-resistance switch within the system activates a trigger in the EEG, marking the onset of stimulation. The trigger is activated when the probe contacts the skin, reducing the impedance between the probe and an electrode positioned on the skin at the wrist. To decrease the impedance at the contact point, a thin layer of conductive gel was applied to the skin. Before each testing block began, the X/Y/Z positions of the pinprick stimulator were adjusted to ensure the probe was positioned about 5 mm above the skin, at the centre of the test area.

In addition to time-domain analysis, it is also possible to analyse the data using a time-frequency analysis[166]. Indeed, researchers have consistently

found that different types of sensory input, including nociceptive stimuli, induce noticeable changes in activity within a specific range of brain oscillations[145, 147, 167-170]. In this thesis, we will focus on gamma-band oscillations (GBOs), which occur between 30 and 100 Hz. These high-frequency oscillations have shown great promise as biomarkers for pain[148, 171, 172]. They are believed to reflect important processes in the brain related to perception, attention, and memory. Their presence in response to painful stimuli suggests they could be key indicators of pain perception[147, 173-175]. However, the exact neural origin of pain-related GBOs and their consistency across individuals remain a topic of debate[176].

I.4. Early detection, lasting relief: identifying at-risk patients for persistent postsurgical pain before surgery

Persistent postsurgical pain (PPSP) is influenced by a complex interplay of factors, including preoperative pain, acute postoperative pain, psychological and genetic factors, and even sleep quality[177-180]. One of the most consistent findings is that acute postoperative pain strongly predicts the development of chronic pain. Several studies indicates that patients experiencing severe pain immediately after surgery are more likely to develop chronic pain conditions[177, 181-184]. While the exact mechanisms driving this shift remain unclear—whether due to lasting changes in the nervous system, insufficient pain control, or other factors—it is evident that effective acute pain management is crucial.

Among the potential contributors, pronociceptive profiles have emerged as significant.

These profiles are characterized by an imbalance in pain modulation, where facilitation dominates over inhibition[185, 186]. Defined by inefficient conditioned pain modulation or exaggerated temporal summation, these profiles may predispose individuals to a heightened risk of persistent postoperative pain[187, 188]. They may also be influenced by factors interacting with central sensitization mechanisms, further amplifying pain sensitivity and facilitating the transition from acute to chronic pain[187, 189]. Preoperative psychological factors, such as anxiety and catastrophizing, have been shown to exacerbate acute and persistent pain, creating pathways that increase the likelihood of chronic pain development[190, 191]. A deeper understanding of these contributors is essential for refining predictive models of persistent pain and tailoring personalized interventions to improve patient outcomes.

I.4.1. Experimentally and surgically induced central sensitization

The relationship between experimentally induced hyperalgesia and surgically induced postoperative pain has the potential to offer valuable insights into central sensitization mechanisms, though research in this area remains limited. In 2002, Dirks et al. found a strong association between heat-induced secondary hyperalgesia and surgically induced mechanical hyperalgesia in postoperative patients, suggesting a shared mechanism of central sensitization. Their study demonstrated that both forms of hyperalgesia

were significantly associated at baseline and similarly alleviated by remifentanil, a short-acting opioid known to reduce hyperalgesia[192]. These findings reinforces the hypothesis that central sensitization contributes to both experimental and clinical pain states, particularly postoperative pain[42].

However, aside from the study mentioned above, there is limited evidence explicitly linking experimental models of hyperalgesia to clinical conditions such as persistent postoperative pain. This scarcity of research underscores the need for further research to bridge the gap between experimental models and clinical reality, allowing for a more comprehensive understanding of central sensitization and its role in postoperative pain.

I.4.2. Preoperative factors

I.4.2.1. Psychological aspects

While the literature extensively reports the role of preoperative psychological factors in pain outcomes, specific factors such as depression and anxiety are consistently identified as predictors of both acute and chronic pain[193-197]. Granot and Ferber (2005) demonstrated that pain catastrophizing and anxiety significantly predict acute postoperative pain intensity, highlighting the profound impact of psychological distress on acute pain perception[198]. Supporting this, Kornilov et al. (2016) found that patients with a HADS score above 8 experienced more hours of moderate to severe postoperative acute pain daily[199]. Additionally, Erdogan and Ozenc (2018)

identified a correlation between HADS scores and chronic neuropathic pain using the DN4 questionnaire, emphasizing the long-term implications of preoperative anxiety and depression[200].

It is widely recognized that patients who fear surgery often experience higher levels of acute postoperative pain, anxiety, and depression, which can contribute to the transition from acute to chronic pain[201, 202]. Surgical fear and pain catastrophizing are intricately linked to attentional mechanisms, which play a significant role in how pain is processed and experienced[203, 204]. It is postulated that top-down processes can modulate pain perception by shifting attention to complex cognitive tasks, thus reducing the attention on pain[205]. A recent study investigated whether performing a cognitively demanding task requiring high attention reduces the development of secondary mechanical hyperalgesia compared to an easier task with lower attentional demands[206]. No significant differences were found between the two groups in terms of intensity of mechanical pinprick sensitivity or the spatial extent of secondary mechanical hyperalgesia, which is in agreement with previous similar studies[207, 208] but in contradiction with others[83]. Thus, the precise relationship between attention and secondary mechanical hyperalgesia remains unclear, highlighting the need for further research on cognitive demands and pain modulation.

I.4.2.2. Sleep

Animal and human studies have shown that sleep deprivation can cause hyperalgesia and reduced pain threshold[209-213]. However, only one human

study has explored its effect on secondary mechanical hyperalgesia so far. In this study, Campbell et al. (2011) used the heat-capsaicin nociceptive model to investigate the relationship between self-reported habitual sleep duration and the extent of secondary hyperalgesia and skin flare[214]. They discovered that individuals who reported sleeping ≤ 6.5 hours per night experienced a larger area of secondary hyperalgesia compared to those sleeping more. Additionally, they found a correlation between habitual sleep duration and the degree of local skin flare. This suggests that, beyond its impact on nociceptive processing within the spinal cord, sleep may also influence peripheral mechanisms involved in local inflammation.

Numerous clinical studies have shown that sleep and pain have a bidirectional relationship, with sleep disturbances being stronger predictors of chronic pain than the reverse[215-219]. It has been shown that patients with lower sleep efficiency the night prior a breast surgery have significantly higher levels of acute postoperative pain[220]. A systematic review confirmed these results, by showing that preoperative sleep issues were a strong predictor of acute postoperative pain[221]. Regarding PPSP, multiple studies report similar findings to those on acute pain, indicating that poor preoperative sleep quality predicts persistent postoperative pain[222-224].

I.5. Aim of the thesis

Chronic pain is a complex and subjective experience that can have a significant impact on patients' quality of life, ability to work, and mental health[225-227]. Patients suffering from chronic pain often experience the stigma of having a disease that is not readily obvious on the outside[228]. Almost 20% of Europeans experience chronic pain in their life, and only 40% of these patients report receiving appropriate pain relief[229]. Currently, there is a lack of well-defined preventive strategies to address chronic pain, highlighting the need for both basic and clinical research. Investigating how to assess central sensitization in humans and identifying factors that influence or predict its development is therefore essential. This may contribute to the current scientific knowledge and ultimately, improve chronic pain patient care.

The aim of my thesis is to investigate methods for effectively inducing (Chapter 2), assessing (Chapter 3), and modulating (Chapter 4) central sensitization in humans through electrical skin stimulation, and to assess its potential clinical applications (Chapter 5).

Rationale for methods used to investigate central sensitization

HFS was selected as the primary method of inducing central sensitization in the following studies due to its non-invasive nature, efficiency, and reliable ability to produce long-lasting hyperalgesia[71, 72, 80].

HFS also stands out for its ease of application and absence of after-sensations, making it the preferred method in experimental studies[57]. For the assessment of secondary mechanical hyperalgesia, the pinprick device was chosen given its well-documented ability to activate nociceptive Aδ fibers involved in secondary mechanical hyperalgesia development. Unlike other available methods, the pinprick device offers reliability and consistency, as it has been consistently shown to elicit increased perception following HFS, thereby ensuring robust and reproducible results[78, 112, 118]. Finally, EEG was employed as a neurophysiological tool because of its non-invasive nature, high temporal resolution, and practicality in clinical and experimental settings.

Empirical chapters:

Chapter 2: Determining the optimal high-frequency stimulation parameters to experimentally induce central sensitization

Studying pain requires having reliable pain models to help researchers understand its mechanisms and identify factors that contribute to modulate pain perception. Secondary mechanical hyperalgesia is presumed to be a perceptual correlate of central sensitization, and it can be induced experimentally by applying HFS onto the skin[70, 73, 74, 115]. Previous studies investigating the effects of HFS on secondary mechanical hyperalgesia used fixed parameters with a frequency of 100 Hz and a pattern of burst-like pulses that were non-charge compensated[81, 117, 120]. In fact, little was known about how different HFS parameters influence the development of

secondary mechanical hyperalgesia in humans. The present chapter aims to determine the optimal HFS parameters to induce the most robust secondary mechanical hyperalgesia, which can subsequently be applied in patient studies. This chapter, composed of two complementary parts, is based on work conducted during my master's thesis and my lab-internships between 2019 and 2020. Their inclusion in the manuscript is justified by their role in supporting the overarching aims of this thesis.

Chapter 3: Cortical gamma-band oscillations as electrophysiological indicators of central sensitization

Identifying objective biomarkers of central sensitization remains a significant challenge, particularly when utilizing EEG techniques[230]. In animal studies, GBOs induced by nociceptive stimuli have been proposed as potential electrophysiological markers of central sensitization, reflecting the processing of nociceptive inputs[231]. However, no studies to date have investigated this marker in the context of central sensitization in humans. Building on this, this chapter examines the presence of scalp GBOs evoked by mechanical stimuli activating skin nociceptors in healthy volunteers, both before and after the induction of secondary mechanical hyperalgesia through HFS. This study seeks to determine whether GBOs could reliably serve as biomarkers for central sensitization in human models.

Chapter 4: The impact of negative expectations on the development of central sensitization

Negative expectations are known to influence pain perception and contribute to the persistence of pain[232-234]. A previous study demonstrated that fostering negative expectations about post-HFS mechanical pinprick sensitivity can amplify its perception[235]. Building on this, we questioned whether similar effects would occur when negative pain expectations are directed towards HFS. By exploring how the anticipation of heightened pain influences secondary mechanical hyperalgesia, this study aims to clarify the role of pain-related expectations in central sensitization.

Chapter 5: Central sensitization and persistent postoperative pain

Finding predictive markers for PPSP is crucial for tailoring preventive measures and improving patient outcomes. By identifying at-risk individuals preoperatively, clinicians can employ targeted interventions to mitigate chronic pain development[236, 237, 238-240]. Indeed, in addition to psychological factors and postoperative acute pain, detecting predictive markers prior to surgery could be more effective, enabling the implementation of preventive strategies before central sensitization has the chance to develop. Furthermore, composite biomarkers that integrate psychological and physiological factors could enhance predictive accuracy and improve patient outcomes[159]. This opens the possibility of developing a model that combines psychological factors and biomarkers to assess the likelihood of chronic pain development in patients. Therefore, the aim of this chapter is to study individual variations in the susceptibility to develop persistent postsurgical pain after a thoracotomy, with a specific focus on preoperative sensitivity to develop central sensitization.

II. Chapter 2. Determining the optimal high-frequency stimulation parameters to experimentally induce central sensitization

Part 1: Heterosynaptic facilitation of mechanical nociceptive input is dependent on the frequency of conditioning stimulation

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Abstract

Background: High frequency stimulation (HFS) applied to human skin induces an increase in mechanical pinprick sensitivity of the surrounding unconditioned skin referring to secondary mechanical hyperalgesia. High-frequency stimulation, traditionally delivered with non-charge-compensated square-wave pulses, may induce a cumulative depolarization of primary afferents and/or changes in pH at the electrode-tissue interface due to the accumulation of a net residue charge after each pulse. Both could contribute to the development of the increased pinprick sensitivity in a frequency-dependent fashion. The present study assessed the effect of frequency of conditioning stimulation on the development of this increased pinprick sensitivity in humans.

Methods: In a first experiment, we compared the increase in pinprick sensitivity induced by HFS, using monophasic non-charge-compensated pulses and biphasic charge-compensated pulses. In a second experiment, we assessed the effect of different frequencies of conditioning stimulation (5, 20, 42, and 100 Hz) using charge-compensated pulses on the development of increased pinprick sensitivity.

Results: We found no significant difference in the increase in pinprick sensitivity between HFS delivered with charge-compensated and non-charge-compensated pulses, indicating that the possible contribution of charge accumulation when non-charge-compensated pulses are used is negligible. We found that the maximal increase in pinprick sensitivity was observed at intermediate frequencies of stimulation (20 and 42 Hz).

Conclusion: In summary, our results show that the induction of increased pinprick sensitivity by repeated burst-like electrical stimulation of cutaneous nociceptors is not significantly dependent on charge accumulation within the stimulated tissues. We also found that the induced increased pinprick sensitivity is significantly dependent on the frequency of the burst stimulation, being maximal at intermediate frequencies of stimulation.

II.1. Introduction

Long-term potentiation (LTP) refers to a long-lasting increase in synaptic efficacy and was discovered after repetitive stimulation in the rabbit hippocampus[14, 241, 242]. LTP is thought to be a crucial mechanism involved in memory formation[70]. Animal studies have shown that LTP can also be induced in spinal nociceptive pathways. Indeed, it has been shown that high-frequency burst like stimulation (HFS; several bursts of 100 Hz for 1 s) of primary C-fiber nociceptors triggers LTP between the peripheral C-fiber terminals and spinal lamina I neurons projecting to the parabrachial area of the brain stem[243, 244]. Moreover, Kronschläger et al. (2016) showed that HFS also activates glial cells in the spinal cord that, via the release of D-serine and tumour necrosis factor, trigger LTP at remote or nearby C-fiber synapses[31]. LTP at synapses that were active during conditioning stimulation (homosynaptic LTP) may contribute, besides peripheral sensitization, to primary hyperalgesia, i.e., the increase in pain at the site of tissue injury or inflammation[24]. LTP at remote synapses (heterosynaptic LTP) could contribute to the phenomenon of secondary mechanical hyperalgesia, i.e., the increase in pain sensitivity that develops surrounding the site of tissue injury[18, 31]. In humans, HFS (5 trains of 100 Hz for 1 s, repeated at 10-s intervals) delivered to the skin induces a pronounced and long-lasting increase in mechanical pinprick sensitivity in the surrounding skin, a phenomenon reminiscent of secondary mechanical hyperalgesia[72, 75, 76, 117, 118]. We also showed that after HFS the perception elicited by small-spot laser stimuli selectively activating C-fiber nociceptors is enhanced when the stimuli are delivered inside the surrounding area of increased pinprick

sensitivity, although the effect of HFS on these laser stimuli was less pronounced than the effect on pinprick stimuli[120]. The effect of HFS on the perception elicited by the C-fiber laser stimuli could be a perceptual correlate of the "gliogenic" heterosynaptic LTP at C-fiber synapses identified by Kronschläger et al. (2016) in animals[31]. However, a peripheral origin cannot presently be excluded.

Previous studies using intradermal capsaicin injection to induce increased pinprick sensitivity surrounding the site of injection in humans have shown that the increase in pinprick sensitivity is mediated by A-fiber nociceptors rather than C-fibers[112]. Moreover, by recording the activity of nociceptive neurons in the primate spinal cord before and after intradermal capsaicin injection, Simone et al. (1991) showed that after the injection both high-threshold (HT) neurons in lamina I and wide-dynamic-range (WDR) neurons in lamina V respond more strongly to mechanical pinprick stimuli delivered to the skin surrounding the injection site[39]. The same group also recorded the activity of peripheral A-fiber and C-fiber nociceptors, but their activity was unchanged[58], confirming that the increase in responsive-ness of spinal neurons results from a facilitation at spinal level. Torsney (2011) found that inflammation of the hind paw of rats by complete Freund's adjuvant increases the incidence and magnitude of monosynaptic A-fiber input to lamina I neurons expressing the neurokinin-1 (NK1) receptor. It was hypothesized that this novel monosynaptic A-fiber input results from normally "silent" synapses and that this may contribute to secondary mechanical hyperalgesia[245]. It is, however, presently not known whether spinal LTP also affects A-fiber-mediated synaptic transmission[18].

Henrich et al. (2015) showed that when HFS is delivered to skin pretreated with capsaicin to induce a denervation of transient receptor potential vanilloid (TRPV) 1-expressing nociceptors, HFS does not induce any long-lasting increase in pinprick sensitivity in the surrounding skin. They also showed that both A- and C-fiber nociceptors contribute to the induction of increased pinprick sensitivity, but the contribution of C-fiber input is greater than that of A-fibers[117]. Taken together, these results suggest that mainly TRPV1-expressing C-fiber nociceptors are involved in the HFS-induced enhancement of pinprick sensitivity. It is thought that the activation of mechano-insensitive "silent" C-fiber nociceptors is crucial for the induction of secondary mechanical hyperalgesia[246, 247]. However, in pig skin this subclass of nociceptors shows conduction failure at high frequencies of stimulation, which raises the question of the extent to which this subclass of C-fibers contributes to the induction of secondary mechanical hyperalgesia by HFS[248]. However, in rats some C-fiber nociceptors are able to follow HFS [249]. Not much is known about the effect of frequency of the conditioning stimulation on the development of the increased pinprick sensitivity in humans. Xia et al. (2016) investigated the effect of three frequencies of electrical conditioning stimulation (1, 100, and 200 Hz) on the averaged magnitude of the increase in pinprick sensitivity in the surrounding skin. Although the magnitude of the increase in pinprick sensitivity was not the same between the different frequencies (100 Hz > 200 Hz > 10 Hz), no statistically significant differences were observed. In that study, the authors matched the 10-Hz and 100-Hz frequencies regarding the total number of stimuli, pulse duration, and total duration of the protocol.

Consequently, the pattern of stimulation was not the same, which makes it difficult to compare the two conditions. Indeed, whereas the 100-Hz condition consisted of five trains of 100 Hz that lasted for 1 s and were repeated in a 10-s interval, the 10-Hz condition consisted of continuous stimulation[76]. Another previous study comparing 20-Hz continuous stimulation versus 20-Hz stimulation for 1 s repeated with a 2-s intertrain interval found that continuous stimulation induces hypoalgesia to pinprick stimulation, whereas burst stimulation induces hyperalgesia[250]. Furthermore, in the study by Xia et al. (2016), they used square-wave electrical pulses. Because square-wave electrical pulses are not charge compensated, a net residue charge may accumulate after each pulse. This accumulation can be expected to be stronger when the frequency of pulse delivery is high, leading to a stronger cumulative depolarization of the membrane potential of afferent fibers and/or tissue damage or inflammation related to changes in pH at the electrode-tissue interface[251, 252]. Therefore, the present study had two aims. The first aim was to assess whether the increase in pinprick sensitivity induced by HFS is dependent on cumulative depolarization of the membrane potential and/or inflammation related to changes in pH at the electrode-tissue interface. To test this, we compared the increase in pinprick sensitivity induced by HFS delivered with non-charge-compensated versus charge-compensated electrical pulses (experiment 1). The second aim was to explore whether the development of the increase in pinprick sensitivity depends on the frequency of the conditioning stimulation (experiment 2). If indeed mechano-insensitive “silent” C-fiber nociceptors play a crucial role, one would expect a stronger increase in pinprick sensitivity induced by low frequencies of stimulation compared with high frequencies

of stimulation. In this second experiment, four frequencies were tested (5, 20, 42, and 100Hz) with charge-compensated electrical pulses, keeping constant both the total number of pulses and the stimulation pattern (1-s trains separated by a 10-s intertrain interval).

II.2. Methods

Participants

Fifteen healthy volunteers took part in experiment 1 [7 men and 8 women; aged 21–27 years old, 23.5 ± 1.6 yr (mean ± SD)]. In this experiment, participants took part in two experimental sessions separated by at least 1 week, during which they were exposed to either charge-compensated 100-Hz HFS or non-charge-compensated 100-Hz HFS. The order of the two sessions was counterbalanced across participants. Sixty participants took part in experiment 2 (31 men and 29 women; aged 18–40 years old, 23.4±4.3 years old), 15 participants per condition (5, 20, 42, and 100 Hz). For the 100-Hz group, this included the data of the seven participants of experiment 1 who had received 100-Hz charge-compensated HFS in the first experimental session. All participants were naive regarding HFS. The experiments were conducted according to the Declaration of Helsinki (except for preregistration of the trial). Approval for the experiments was obtained from the local Ethical Committee (Comité d'Ethique Hospitalo-Facultaire des Cliniques Universitaires Saint-Luc, UCLouvain) of the Université Catholique de Louvain (UCLouvain) (B403201316436). All participants gave written

informed consent and received financial compensation for their participation.

Experimental Design

In both experiments, the electrical conditioning stimulation was applied to the dominant or nondominant volar forearm, counterbalanced across participants (10 cm distal to the cubital fossa) (**Figure 1**). Handedness was assessed with the Flinders Handedness Survey[253]. Pinprick sensitivity of the skin was assessed by applying mechanical pinprick stimuli (128 mN) before applying the conditioning stimulation ("Pre") and 20 min after the end of the conditioning stimulation ("Post") to the skin surrounding the site where the conditioning stimulation was delivered ("pinprick test area") and to the corresponding skin area of the contralateral arm serving as control. In experiment 1, we compared in a crossover design the increase in pinprick sensitivity induced by 100-Hz HFS delivered with either biphasic charge-compensated pulses or monophasic non-charge-compensated pulses (**Figure 2**). In experiment 2, we compared in a between-subject design the change in pinprick sensitivity induced by 100-Hz HFS to the change in pinprick sensitivity induced by 5-, 20-, and 42-Hz conditioning stimulation. In this

second experiment, all stimuli were delivered with biphasic charge-compensated pulses.

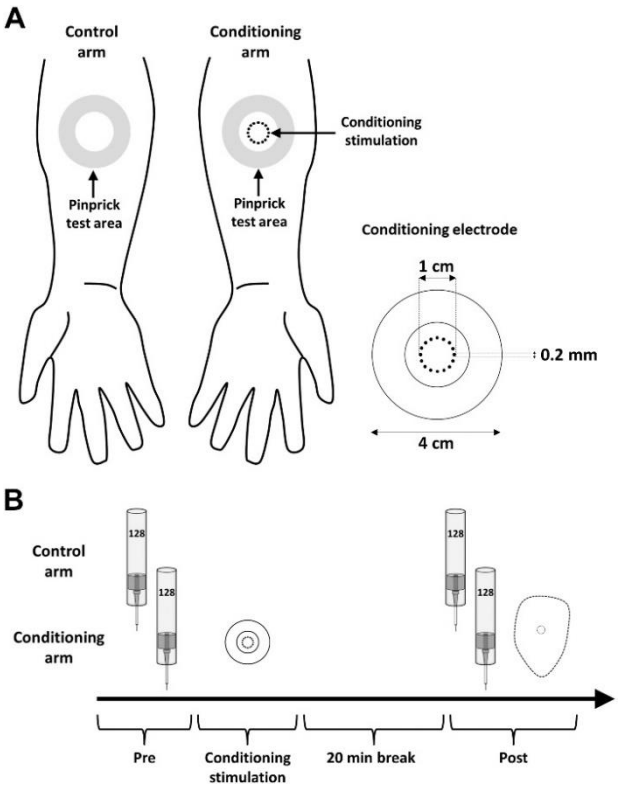

Figure 1. Experimental design. **A**, left: conditioning stimulation is applied to the dominant or nondominant volar forearm. Pinprick stimulation (128 mN) was applied to the skin surrounding the area onto which conditioning stimulation was applied ("pinprick test area") as well as to the same skin area on the contralateral control arm. Right: characteristics of the conditioning electrode. **B**: timeline of the experiment. The perceived intensity elicited by the pinprick stimulation was assessed at 2 different time points: before conditioning stimulation ("Pre") and 20 min after application of conditioning stimulation ("Post"). At the end of the experiment the area of increased pinprick sensitivity at the conditioning arm was mapped.

Conditioning stimulation

All stimuli were delivered to the forearm skin with a constant current electrical stimulator (Digitimer DS5, Welwyn Garden City, UK) and a specifically designed electrode built at the Centre for Sensory-Motor Interaction (Aalborg University, Aalborg, Denmark). The electrode consists of 16 blunt stainless-steel pins (diameter: 0.2 mm) protruding 1 mm from the base. The pins are placed in a 10-mm-diameter circle and serve as cathode. A stainless-steel anode electrode is concentrically located around the steel pins (inner diameter: 22 mm; outer diameter: 40 mm). Monophasic non-charge-compensated electrical pulses were square-wave pulses with a 2-ms pulse width (**Figure 2B**). Biphasic charge-compensated electrical pulses consisted of the same 2-ms square-wave pulse followed, after a 0.1-ms delay, by a 4-ms compensation pulse of opposite polarity with half the intensity of the first pulse (**Figure 2B**). In all conditions, intensity of the conditioning stimulation was individually adjusted to 20× the detection threshold to a single non-charge-compensated monophasic pulse (pulse width: 2 ms). The detection threshold was determined after the pre-measurements with a staircase procedure. A total of 500 electrical pulses were delivered as 1-s trains separated by a stimulation-free interval lasting 9 s. For 100-Hz HFS, 5 trains were delivered, each including 100 pulses (total duration: 50 s). For 5-Hz stimulation, a total of 100 trains were delivered, each including 5 pulses (total duration: ≈17 min). For 20-Hz stimulation, 25 trains were delivered, each including 20 pulses (total duration: ≈4 min). For 42-Hz stimulation, 12 trains were delivered, 8 including 42 pulses and 4 including 41 pulses (total duration: ≈2 min). The 42-Hz stimulation was chosen instead of 40 Hz (the double of 20) because it is able to deliver the same total number of stimuli

as the 5-Hz, 20-Hz, and 100-Hz stimulations. The electrical pulses were triggered by a National Instruments digital-analog interface (NI; National Instruments, Austin, TX) controlled by custom MATLAB code (MATLAB 2014B; MathWorks).

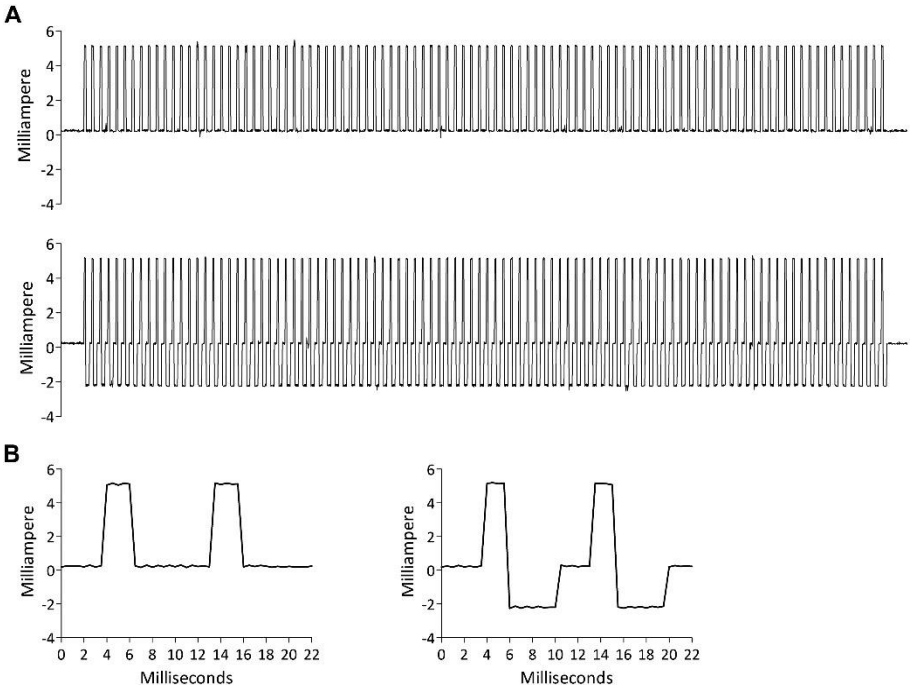

Figure 2. A: example of a train of 100 Hz (1 s) delivered with a non-charge-compensated pulse (top) and a charge-compensated pulse (bottom). Shown is the actual current delivered to the skin (stimulation intensity: 5 mA). **B**: first 2 stimuli of each train in A. Left: non-charge-compensated pulses. Right: charge-compensated pulses.

Quantifying Changes in Perceived Intensity of Mechanical Pinprick Stimuli

To assess changes in pinprick sensitivity, a calibrated pinprick stimulator exerting a normal force of 128 mN with a 0.25-mm probe (MRC Systems, Heidelberg, Germany) was applied perpendicular to the skin. Before application of the conditioning stimulation and 20 min after application of the conditioning stimulation a total of three pinprick stimuli were applied inside the pinprick test area of the conditioned arm and the contralateral control arm. The target of each pinprick stimulus was displaced after each stimulus. Participants were asked to report the intensity of perception elicited by the pinprick stimulation on a numerical rating scale ranging from 0 (no perception) to 100 (maximal pain), with 50 representing the transition from nonpainful to painful domains of sensation. For the statistical analysis, the mean of the three pinprick ratings was calculated for each arm and time point.

Mapping Area of Increased Pinprick Sensitivity

The same pinprick stimulator was used to map the area of increased mechanical pinprick sensitivity after conditioning stimulation. Mechanical pinprick stimuli were applied to the skin along eight axes, each separated by an angle of 45°. Along each axis, testing started far outside the skin showing increased pinprick sensitivity and moved towards the centre of the conditioning site in steps of 1 cm. Participants were instructed to indicate the point at which the pinprick perception changed. This point was then indicated on the skin with a marker. Then, the distance between each mark and the centre of the conditioning stimulation was measured.

Finally, the area was drawn on millimetre paper, and the surface (cm^2) was calculated with the open-source platform Fiji[254].

Statistical analysis

Statistical analyses were performed with SPSS Statistics 24 (IBM, Armonk, NY). In experiment 1, the changes in perceived pinprick intensity induced by non-charge-compensated monophasic pulses and charge-compensated biphasic pulses were compared with a repeated measures ANOVA with three within-subject factors: “time” (Pre vs. Post), “arm” (conditioned vs. control), and “condition” (charge compensated vs. non-charge compensated). Post hoc paired t-tests were performed comparing the Post minus Pre change in perception intensity at the conditioned arm versus the control arm. To compare the size of the area of increased pinprick sensitivity after charge-compensated versus non-charge-compensated HFS, we performed a paired t-test on the individual area sizes (cm2). In experiment 2, the change in intensity of pinprick perception after conditioning stimulation using four frequencies of stimulation was compared with a mixed ANOVA with two within-subject factors, “time” (Pre vs. Post) and “arm” (conditioned vs. control), and one between-subject factor, “condition” (5, 20, 42, and 100 Hz). Tukey post hoc tests were performed comparing the Post minus Pre change in pinprick intensity ratings at the conditioned arm versus the control arm. The size of the area of increased pinprick sensitivity was compared across the four frequencies of stimulation (5, 20, 42, and 100 Hz) with a one way-ANOVA. A Tukey post hoc test was performed to identify which comparisons were significantly different. Finally, to test whether the electrical detection

thresholds to a monophasic non-charge-compensated pulse differed in the two experimental sessions of experiment 1, the individual detection thresholds were compared by paired t-test. To test whether in experiment 2 the detection thresholds differed between the four different groups (5, 20, 42, and 100 Hz), the individual detection thresholds were compared with a one-way ANOVA. In all tests, the level of significance was set at $P<0.05$.

II.3. Results

Detection thresholds

The electrical detection thresholds to a single monophasic non-charge-compensated pulse in experiment 1 were 0.29 ± 0.13 mA (mean ± SD) for the non-charge-compensated condition and 0.32 ± 0.11 mA for the charge-compensated condition. The electrical detection thresholds in experiment 2 were 0.25 ± 0.09 (5 Hz), 0.27 ± 0.10 (20 Hz), 0.29 ± 0.09 (42 Hz), and 0.29 ± 0.12 (100 Hz). No significant difference in electrical detection thresholds was observed between the charge-compensated and non-charge-compensated conditions of experiment 1 and between the four groups in experiment 2.

Experiment 1

Intensity of pinprick perception

The means and SDs of the intensity of perception elicited by pinprick stimuli delivered before and after HFS at both arms (control vs. conditioned) in both conditions (charge-compensated vs. non-charge-compensated pulses) are

shown in **Figure 3A**. The repeated-measures ANOVA revealed a significant time × arm interaction [$F(1,14) = 54.684$, $P < 0.001$, $\eta^2 = 0.796$]. This means that after HFS the intensity of perception elicited by pinprick stimulation of the HFS arm was higher compared with pinprick stimulation of the control arm across the two conditions (charge-compensated and non-charge-compensated stimulation) (**Figure 3A**). No significant time × arm × condition interaction was observed [$F(1,14) = 1.392$, $P = 0.258$, $\eta^2 = 0.090$], suggesting that there was no difference in the enhancement of pinprick sensitivity after HFS delivered with charge-compensated and non-charge-compensated pulses (**Figure 3B**).

Area size

The means and SDs of the area of increased pinprick sensitivity after charge-compensated and non-charge-compensated HFS are shown in **Figure 3C**. The paired t-test comparing area sizes revealed no significant difference between charge-compensated and non-charge-compensated pulses [$t(14) = 0.738$, $P = 0.472$]. **Figure 4** shows scatterplots of the individual changes in pinprick perception and area size after HFS delivered with a charge-compensated pulse versus a non-charge-compensated pulse.

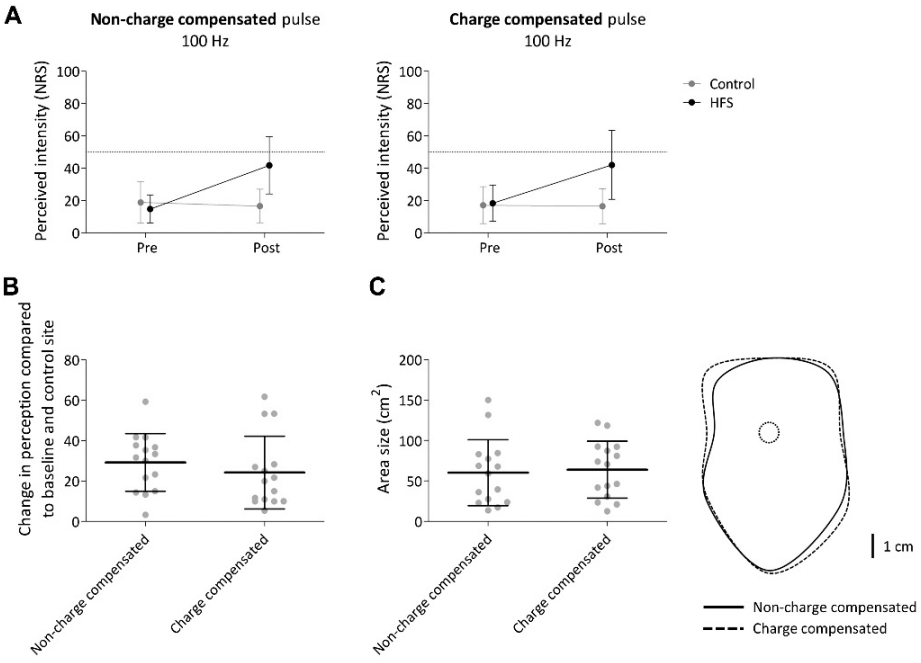

Figure 3. A: Intensity of perception elicited by the mechanical pinprick stimulation (128 mN) before and 20 min after application of high-frequency burst-like stimulation (HFS) using a monophasic non-charge-compensated pulse (left) and a biphasic charge-compensated pulse (right). Shown are the group-level average and standard deviation (SD) of the numerical rating scale scores (NRS). **B**: group-level average and SD increase in NRS compared with baseline and control site. **C**, **left**: group-level average and SD area size of the increase in pinprick sensitivity. **Right**: group-level average areas of increased pinprick sensitivity.

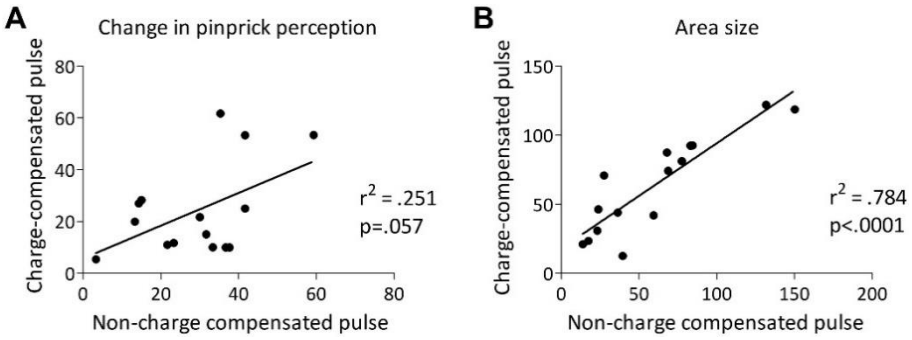

Figure 4. A: scatterplot (and linear regression line) showing the individual changes in pinprick ratings after high-frequency burst-like stimulation (HFS) (compared with baseline and control site) delivered with charge-compensated pulses (y-axis) and non-charge compensated pulses (x-axis). **B**: scatterplot (and linear regression line) showing the individual area sizes of increased pinprick sensitivity after HFS delivered with charge-compensated pulses (y-axis) and non-charge-compensated pulses (x-axis).

Experiment 2

Intensity of perception

The means and SDs of the intensity of pinprick perception before and after conditioning stimulation at both arms (conditioned vs. control) in all four groups (5, 20, 42, and 100 Hz) are shown in **Figure 5**. The mixed ANOVA revealed a significant time × arm interaction [$F(1,56) = 179.621$, $P < 0.001$, $\eta^2 = 0.762$], compatible with an increase in pinprick perception at the conditioned forearm in all four groups (5, 20, 42, and 100 Hz). Most importantly, there was a significant time × arm × condition interaction [$F(3,56) = 8.493$, $P < 0.001$ $\eta^2 = 0.313$], indicating that the strength of the increase of pinprick perception at the conditioned arm differed across the four frequencies of stimulation. To assess whether the increase in pinprick sensitivity was significant in all four groups, we then performed, for each group of participants, separate repeated-measures ANOVAs with the factors of time and arm. For all four frequencies of stimulation, there was a significant time × arm interaction [5 Hz: $F(1,14) = 26.846$, $P < 0.001$, $\eta^2 = 0.657$; 20 Hz: $F(1,14) = 58.031$, $P < 0.001$, $\eta^2 = 0.806$; 42 Hz: $F(1,14) = 86.701$, $P = 0.001$, $\eta^2 = 0.861$; 100 Hz: $F(1,14) = 20.459$, $P = 0.001$, $\eta^2 = 0.594$]. Tukey post hoc tests performed on the Post minus Pre change in pinprick perception at the conditioned arm versus the control arm revealed a significant difference between 5- and 20-Hz stimulation ($P = 0.007$), between 5- and 42-Hz stimulation ($P < 0.001$), and between 42- and 100-Hz stimulation ($P < 0.005$) (**Figure 6A**).

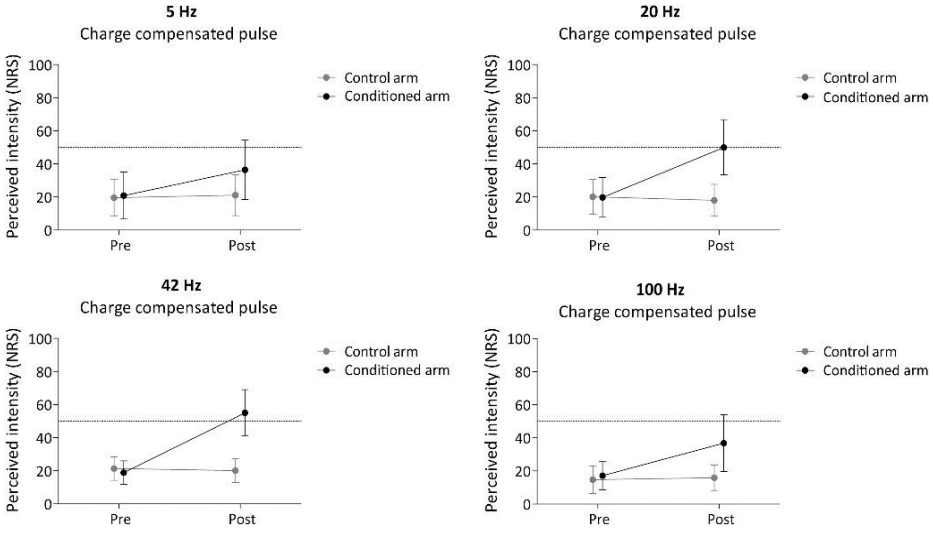

Figure 5. Intensity of perception elicited by the mechanical pinprick stimulation (128 mN) before and 20 min after application of high-frequency burst-like stimulation (HFS) using a biphasic charge-compensated pulse for all frequencies of conditioning stimulation (5, 20, 42, and 100 Hz). Shown are the group-level average and standard deviation of the numerical rating scale scores (NRS).

Area size

The means and SDs of the area of increased pinprick sensitivity after 5-, 20-, 42-, and 100-Hz conditioning stimulation are shown in **Figure 6B**. One-way ANOVA revealed a statistically significant difference between the different frequencies [$F(3,59) = 7.781$, $P < 0.001$]. Tukey post hoc tests revealed a significant difference between 5- and 42-Hz stimulation ($P < 0.001$) and between 42- and 100-Hz stimulation ($P = 0.006$) (**Figure 6B**).

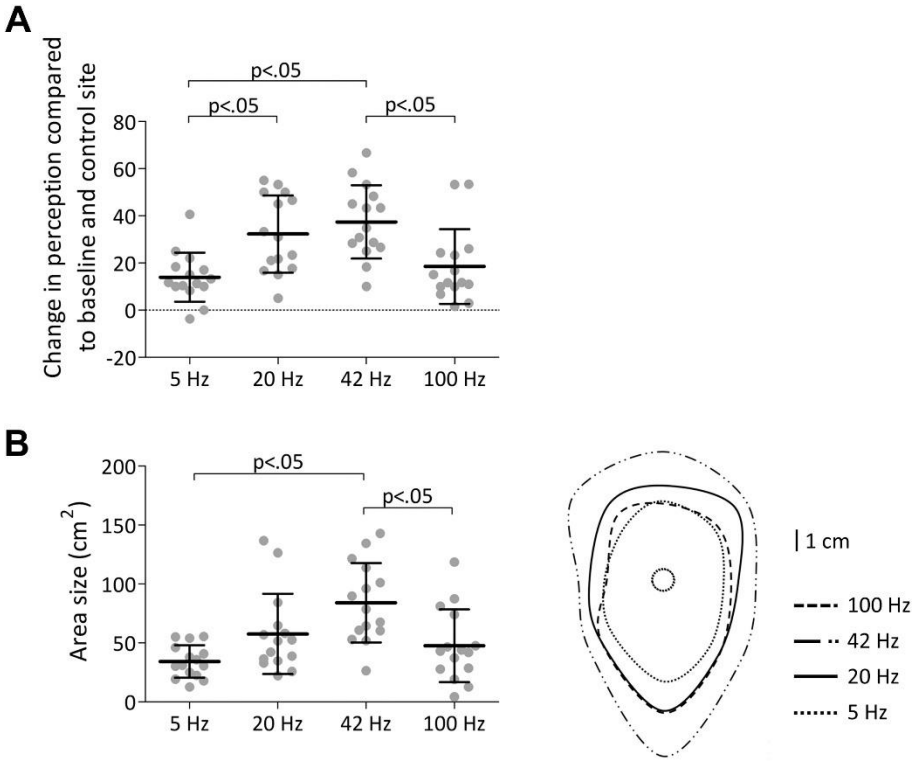

Figure 6. A: group-level average and standard deviation (SD) increase in numerical rating scale score compared with baseline and control site. **B**, left: group-level average and SD area size of the increase in pinprick sensitivity. P < 0.05 refers to the significant comparisons of the post hoc Tukey test. Right: group-level average areas of increased pinprick sensitivity.

II.4. Discussion

The present study yields two important findings. First, there is no significant difference in the intensity and area size of the increase in pinprick sensitivity induced by 100-Hz HFS delivered with charge-compensated and non-charge-compensated pulses. This result indicates that HFS is able to induce increased pinprick sensitivity even when the conditioning pulses are charge compensated and that the possible contribution of cumulative depolarization of sensory afferents and/or tissue lesion or inflammation induced by charge accumulation when non-charge-compensated pulses are used is negligible. Second, we show that the increase in pinprick sensitivity, which is thought to result from spinal heterosynaptic facilitation, is dependent on the frequency of conditioning stimulation. Indeed, with a constant number of electrical pulses delivered with the same pattern of stimulation (1-s trains delivered every 10 s), intermediate frequencies of stimulation (20 and 42 Hz) induce a stronger increase in pinprick sensitivity compared with both high-frequency stimulation (100 Hz) and low-frequency stimulation (5 Hz). At present, one can only speculate about the possible mechanism(s) underlying the frequency dependence of HFS-induced increase in pinprick sensitivity. One possibility could be that the frequency-dependent increase in pinprick sensitivity is related to spinal NK1 activation through the release of substance P following primary afferent peptidergic A- and C-fiber nociceptor stimulation. Both the release of substance P and the activation of the NK1 receptor are frequency dependent[249, 255].

Indeed, Go and Yaksh (1987) showed in cats that the release of substance P after sciatic nerve stimulation at 2, 5, 10, 20, 50, and 200 Hz was largest at 20 and 50 Hz and then decreased[255]. Furthermore, Adelson et al. (2009) showed in rats that NK1 receptor activation was maximal when C fibers were stimulated at frequencies between 30 and 100 Hz[249]. Moreover, substance P can diffuse at a considerable distance from its site of release and may be able to activate extrasynaptic NK1 receptors[74, 256]. Moreover, animal studies have shown that spinal lamina I neurons expressing the NK1 receptor play a pivotal role in central sensitization and mechanical hyperalgesia[257-260]. That high-frequency stimulation induces an increase in pinprick sensitivity similar to low-frequency stimulation is somewhat surprising, as the aforementioned studies have shown that high-frequency stimulation results in a greater release of substance P and more NK1 activation than low-frequency stimulation[249, 255]. One possibility is that HFS triggers LTP at GABAergic synapses of spinal lamina I neurons that receive monosynaptic A- or C-fiber input[261], which may influence the net output (less facilitation) of these lamina I neurons. Second, HFS may recruit more strongly descending inhibitory pathways that may interact with the development of increased pinprick sensitivity. In animals, intense nociceptive stimulation recruits diffuse inhibitory noxious controls (DNICs), which can inhibit the activity of WD neurons of the dorsal horn[262]. Simone et al. (1991) showed in primates that both HT neurons (in the superficial laminae) and WDR neurons (in deeper lamina) show increased responses to pinprick stimulation when these pinprick stimuli are applied after intradermal capsaicin injection in the surrounding skin, suggesting that WDR neurons also contribute to the increase in pinprick sensitivity, at least after capsaicin[39].

That a DNIC-like mechanism can interfere with the development of increased pinprick sensitivity has been shown recently by Xia et al. (2017)[263]. In that study they showed that 10-Hz conditioning stimulation of the forearm skin delivered just after the application of conditioned pain modulation to the foot, which is believed to recruit a DNIC-like mechanism induces a smaller increase in pinprick sensitivity compared with a control condition not preceded by conditioned pain modulation[264, 265].

II.5. Conclusion

In summary, our results show that the induction of increased pinprick sensitivity by repeated burst-like electrical stimulation of cutaneous nociceptors is not significantly dependent on charge accumulation within the stimulated tissues. We also found that the induced increased pinprick sensitivity is significantly dependent on the frequency of the burst stimulation, being maximal at intermediate frequencies of stimulation.

Chapter 2. Determining the optimal high-frequency stimulation parameters to experimentally induce central sensitization

Part 2: Burst-like conditioning electrical stimulation is more efficacious than continuous stimulation for inducing secondary hyperalgesia in humans.

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Abstract

Background: The aim of the present study was to compare the efficacy of burst-like conditioning electrical stimulation vs. continuous stimulation of cutaneous nociceptors for inducing increased pinprick sensitivity in the surrounding unstimulated skin (a phenomenon referred to as secondary mechanical hyperalgesia).

Methods: In a first experiment (n=30), we compared the increase in mechanical pinprick sensitivity induced by 50-Hz burst-like stimulation (n=15) vs. 5-Hz continuous stimulation (n=15) while maintaining constant the total number of stimuli and the total duration of stimulation. To control for the different frequency of stimulation, we compared in a second experiment (n=40) 5-Hz continuous stimulation (n=20) vs. 5-Hz burst-like stimulation (n=20), this time while keeping the total number of stimuli as well as the frequency of stimulation identical.

Results: We found a significantly greater increase in mechanical pinprick sensitivity in the surrounding unstimulated skin after 50-Hz burst-like stimulation compared with 5-Hz continuous stimulation (P=0.013, Cohen's d=0.970). Importantly, in the second experiment we found a significantly greater increase in pinprick sensitivity after 5-Hz burst-like stimulation compared with 5-Hz continuous stimulation (P=0.009, Cohen's d=0.868).

Conclusion: To conclude, our data indicate that burst-like conditioning electrical stimulation is more efficacious than continuous stimulation for inducing secondary mechanical hyperalgesia.

II.1. Introduction

Long-term potentiation (LTP) refers to a long-lasting activity-dependent increase in synaptic strength and was first demonstrated by Bliss and Lømo (1973) following brief trains of stimulation of the perforant path to dentate granule cells in the hippocampus of anaesthetized rabbits[14]. Interestingly, results seem to indicate that burst-like stimulation is more effective for inducing LTP than continuous stimulation[266]. Activity-dependent LTP can also be induced within spinal nociceptive pathways[243, 244]. For example, Ikeda et al. (2003) showed that brief trains of high frequency stimuli (HFS; 100 Hz for 1 s three times at 10-s intervals), further referred to as burst-like stimulation, applied to the rat sciatic nerve triggers homosynaptic LTP at the synapse between peripheral C fibers and spinal cord lamina I neurons projecting to the parabrachial area in the brainstem[243]. Also, low frequency continuous stimulation (2 Hz for 2 min) triggers homosynaptic LTP but at the synapse between peripheral C fibers and spinal cord lamina I neurons projecting to the periaqueductal grey[244]) Besides homosynaptic LTP, HFS also triggers heterosynaptic LTP at remote C-fibers, which is induced through the activation of spinal glial cells releasing TNF-α and D-serine[31].

In humans, HFS (100 Hz for 1 s five times at 10-s intervals) delivered to the skin to intensively activate skin nociceptors increases the perception elicited by single weak electrical stimuli delivered through the same electrode at which HFS was delivered. Moreover, it increases the perception elicited by mechanical pinprick stimuli delivered to the surrounding unstimulated skin[72, 117]. It has been suggested that the increase in perception elicited by the weak electrical stimuli after HFS is a perceptual correlate of homosynaptic LTP (also

referred to as “homotopic pain LTP”), while the increase in mechanical pinprick stimuli is a perceptual correlate of heterosynaptic LTP (also referred to as “heterotopic pain LTP”)[72, 117]. Regarding the pattern of conditioning stimulation, it is at present unclear if burst-like stimulation is more efficacious than continuous stimulation for inducing heterotopic pain LTP.

A previous study in humans compared the heterotopic pain LTP induced by 10 Hz continuous stimulation and 100 Hz burst-like stimulation (HFS), while keeping the total number of stimuli and total duration of stimulation the same for both protocols[76]. Both conditioning stimuli induced a significant increase in mechanical pinprick sensitivity of the surrounding skin. However, although the average increase in pinprick ratings was greater after burst-like stimulation (49%) as compared to continuous stimulation (27%), these differences were not statistically significant. In contrast to these results, De Col and Maihöfner (2008) found that 20 Hz continuous stimulation resulted in a decreased sensitivity to mechanical pain, i.e. increased mechanical pain thresholds in the area surrounding the stimulated skin, suggesting that continuous stimulation could induce hypoalgesia rather than hyperalgesia[250]. Finally, both Biurrun Manresa et al. (2010) and Vo and Drummond (2014) did not observe any significant changes in pinprick sensitivity after 1 Hz continuous conditioning stimulation[82, 267].

The aim of the present study was to test if continuous nociceptive conditioning stimulation is more effective than burst-like stimulation in inducing “heterotopic pain LTP”. To test this, we compared the change in mechanical pinprick sensitivity induced by 50 Hz burst-like stimulation with the change in mechanical pinprick sensitivity induced by 5 Hz continuous

stimulation (Experiment 1). Both protocols have the same total number of stimuli and the same total duration of stimulation.

Furthermore, to control for a possible effect of frequency of stimulation, we also compared the change in mechanical pinprick sensitivity induced by 5 Hz continuous stimulation with the change in mechanical pinprick sensitivity induced by 5 Hz burst-like stimulation (Experiment 2).

II.2. Methods

Participants

Thirty healthy volunteers took part in *experiment 1*. Participants were randomly assigned to either 5-Hz continuous conditioning stimulation (*n* = 15, 5 men and 10 women; age 19–27 yr, 22.4 ± 2.4 yr, mean SD) or to 50-Hz burst-like conditioning stimulation (*n* = 15, 5 men and 10 women; age 21–36 yr, 23.8 ± 4.0 yr). In *experiment 2*, 40 healthy volunteers were included (5-Hz burst-like stimulation: *n* = 20, 7 men and 13 women; age 18–40 yr, 23.0 ± 4.9 yr; 5-Hz continuous stimulation: *n* = 20, 7 men and 13 women; age 19–27 yr, 22.6 ± 2.3 yr). Parts of the data were reused from the 5-Hz continuous stimulation condition of *experiment 1* (*n* = 15) and the 5-Hz burst-like stimulation condition of our previously collected data set (*n* = 15)[80]. Comparison of these two groups of 15 participants did not reach statistical significance. However, the effect size calculated using the means and SD of the increase in pinprick sensitivity (compared with baseline and control site) was moderate (Cohen's *d* = 0.50), which could indicate that we did not have

sufficient power to detect a difference. Therefore, to reduce the risk of making a type II error, we increased the sample from 15 to 20 by collecting data from 5 new participants per group. The experiment was conducted according to the Declaration of Helsinki (except preregistration of the trial). Approval for the experiments was obtained from the local Ethical Committee (Comité d'Ethique Hospitalo-Facultaire des Cliniques Universitaires Saint-Luc UCLouvain) of UCLouvain (B403201316436). All participants signed an informed consent form and received financial compensation for their participation.

Experimental Design

In all experiments, electrical conditioning stimulation was applied to the dominant or nondominant volar forearm, counterbalanced across participants (10 cm distal to the cubital fossa; **Figure 1**). Handedness was assessed using the Flinders Handedness Survey[253]. Before (Pre) and 20 min after (Post) the end of the conditioning stimulation, pinprick sensitivity of the skin was assessed by applying mechanical pinprick stimuli (128 mN) to the skin surrounding the site where the conditioning stimulation was delivered (pinprick test area) and to the corresponding skin area of the contralateral arm serving as control.

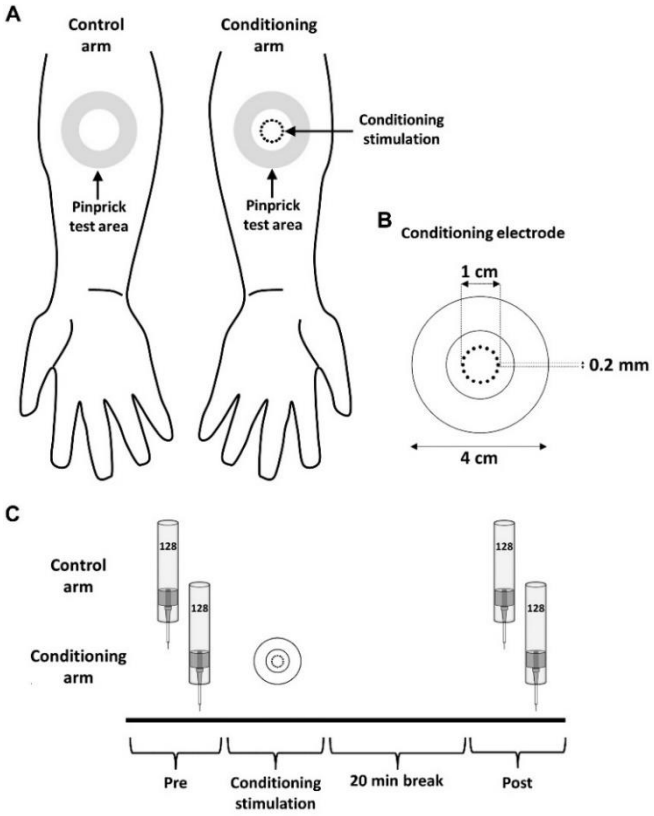

Figure 1. Experimental setup. *A*: conditioning stimulation is applied to the dominant or nondominant volar forearm. Pinprick stimulation (128 mN) was applied to the skin surrounding the area onto which conditioning stimulation was applied (pinprick test area) as well as to the same skin area on the contralateral control arm. *B*: characteristics of the conditioning electrode. *C*: timeline of the experiment. Perceived intensity elicited by the pinprick stimulation was assessed at two different time points: before conditioning stimulation (Pre) and 20 min after application of conditioning stimulation (Post).

Conditioning stimulation

In all experiments, the conditioning stimulation consisted of biphasic charge-compensated electrical pulses that were delivered to the ventral forearm using a constant-current electrical stimulator (Digitimer DS5, Welwyn Garden City, UK) and a specifically designed electrode built at the Centre for Sensory-Motor Interactin (Aalborg University, Denmark). The biphasic pulses consisted of a 2-ms square-wave pulse followed, after a 0.1 ms delay, by a 4-ms compensation pulse of opposite polarity having half the intensity of the first pulse. The electrode consists of 16 blunt stainless-steel pins (diameter: 0.2 mm) protruding 1 mm from the base (**Figure 1**). The pins are placed in a 10-mm diameter circle and serve as cathode. A stainless-steel anode electrode is concentrically located around the steel pins (inner diameter: 22 mm; outer diameter: 40 mm). The intensity of conditioning stimulation was individually adjusted to 20 times the detection threshold to a single non-charge-compensated monophasic pulse (pulse width: 2 ms). The electrical detection threshold was determined after the Pre measurement of pinprick sensitivity using a staircase procedure. In all conditions, the total number of electrical stimuli delivered during the conditioning stimulation was the same (i.e., 500). The 50-Hz burst-like stimulation consisted of 10 trains, each including 50 pulses delivered at 50 Hz. The trains lasted 1 s and were separated by a 10-s intertrain interval (total duration: 100 s). The 5-Hz continuous stimulation consisted of 1 train of 5-Hz stimulation for 100 s. Finally, the 5-Hz burst-like stimulation consisted of 100 trains, each including 5 pulses and lasting for 1 s. The trains were delivered in a 10-s intertrain interval (total duration: ≈ 17 min). The electrical pulses were triggered by a

National Instruments digital-analog interface (NI, National Instruments, Austin, TX) controlled by custom MATLAB code (MATLAB 2014B, The MathWorks, Natick, MA).

Quantifying Changes in the Perceived Intensity of Mechanical Pinprick Stimuli

To assess changes in pinprick sensitivity, we followed the same method as described in our previous study, which is summarized here[80]. A calibrated pinprick stimulator exerting a normal force of 128 mN with the use of a 0.25-mm probe (MRC Systems, Heidelberg, Germany) was applied perpendicular to the skin. Before application of the conditioning stimulation and 20 min after application of the conditioning stimulation, a total of three pinprick stimuli were applied inside the pinprick test area of the conditioned arm and the contralateral control arm. The target of each pinprick stimulus was displaced after each stimulus. Participants were asked to report the intensity of perception elicited by the pinprick stimulation on a numerical rating scale (NRS) ranging from 0 (no perception) to 100 (maximal pain), with 50 representing the transition from nonpainful to painful domains of sensation.

Statistical analyses

Statistical analyses were performed using SPSS Statistics 24 (IBM Corp., Armonk, NY). In *experiment 1*, the change in perceived pinprick intensity induced by the 5-Hz continuous stimulation and the 50-Hz burst-like stimulation was compared using a mixed two-way repeated-measures ANOVA with arm (HFS vs. control) and time (post vs. pre) as within-subject

factors and condition (5-Hz continuous vs. 50-Hz burst-like) as between-subject factor. Post hoc, an independent *t* test was used to test for differences in the increase in pinprick sensitivity (compared with baseline and control site) between the 5-Hz continuous stimulation and the 50-Hz burst-like stimulation. In experiment 2, the change in perceived pinprick intensity induced by the 5-Hz continuous stimulation and the 5-Hz burst-like stimulation was compared using a mixed two-way repeated-measures ANOVA with arm (HFS vs. control) and time (post vs. pre) as within-subject factors and condition (5-Hz continuous vs. 5-Hz burst-like) as between-subject factor. Post hoc, an independent t test was used to test for differences in the increase in pinprick sensitivity (compared with baseline and control site) between the 5-Hz continuous stimulation and the 5-Hz burst-like stimulation. In all tests, the level of significance was set at $P < 0.05$.

II.3. Results

Detection thresholds

The electrical detection thresholds to a single monophasic non-charge-compensated pulse in experiment 1 were 0.29 ± 0.08 mA (mean ± SD) for the 5-Hz continuous stimulation and 0.26 ± 0.07 mA for the 50-Hz burst-like stimulation. An independent t test revealed no statistically significant difference in electrical detection thresholds. The electrical detection thresholds in experiment 2 were 0.26 ± 0.09 mA for the 5-Hz burst-like stimulation and 0.30 ± 0.08 mA for the 5-Hz continuous stimulation. An

independent t test revealed no statistically significant difference in electrical detection thresholds.

Experiment 1: 5-Hz continuous stimulation vs. 50-Hz burst-like stimulation

Changes in Mechanical Pinprick Sensitivity

The means and SD of the intensity of perception elicited by pinprick stimuli delivered before and after conditioning stimulation at both arms (control vs. conditioned) in both conditions (5-Hz continuous stimulation vs. 50-Hz burst-like stimulation) are shown in **Figure 2**. The mixed repeated measures ANOVA revealed a significant time × arm × condition interaction [$F(1,28) = 7.062$, $P = 0.013$, $\eta^2 = 0.201$]. Separate repeated-measures ANOVAs for the 5-Hz continuous stimulation and the 50-Hz burst-like stimulation revealed a significant time × arm interaction for both protocols [5-Hz continuous: $F(1,14) = 13.883$, $P = 0.002$, $\eta^2 = 0.498$; 50-Hz burst-like stimulation: $F(1,14) = 32.859$, $P<0.001$, $\eta^2 = 0.701$]. The increase in perceived intensity was significantly greater for the 50-Hz burst-like stimulation compared with the 5-Hz continuous stimulation [$t(28) = 2.658$, $P = 0.013$, Cohen's $d = 0.970$; **Figure 2**].

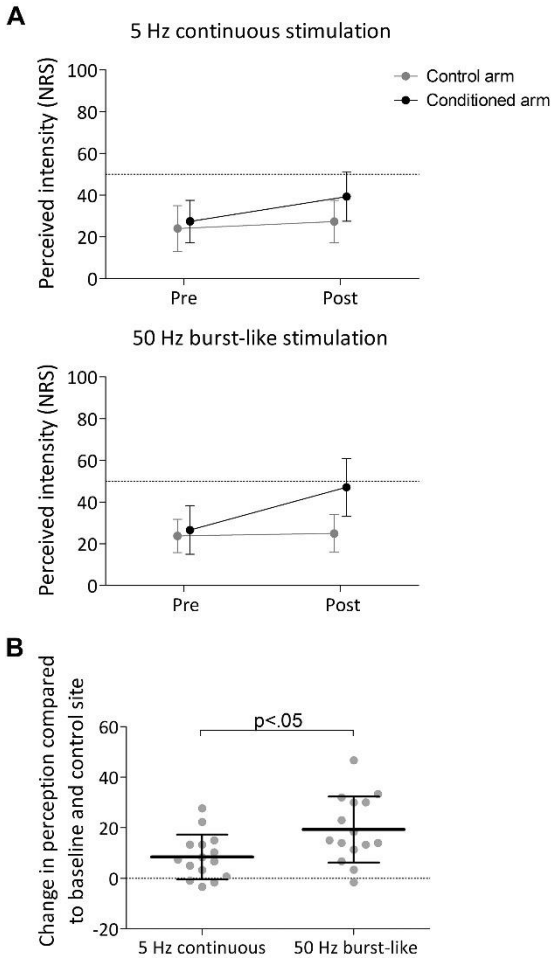

Figure 2. Experiment 1. **A**: intensity of perception elicited by the mechanical pinprick stimulation (128 mN) before (Pre) and 20 min after (Post) application of 5-Hz continuous conditioning electrical stimulation (top) or 50-Hz burst-like stimulation (bottom). Shown are the group-level average and SD of the numerical rating scale (NRS) scores. **B**: group-level average and SD increase in NRS compared with baseline and control site. P value shows the result of the independent t test on the individual changes in perception.

Experiment 2: 5-Hz continuous stimulation vs. 5-Hz burst-like stimulation

Changes in Mechanical Pinprick Sensitivity

The means and SD of the intensity of perception elicited by pinprick stimuli delivered before and after conditioning stimulation at both arms (control vs. conditioned) for both the 5-Hz burst-like stimulation and the 5-Hz continuous stimulation are shown in **Figure 3**. The mixed repeated measures ANOVA revealed a significant time × arm × condition interaction [$F(1,38) = 7.543$, $P = 0.009$, $\eta^2 = 0.166$]. Separate repeated-measures ANOVAs for the 5-Hz continuous stimulation and the 5-Hz burst-like stimulation revealed a significant time × arm interaction for both protocols [5-Hz burst-like stimulation: $F(1,19) = 37.421$, $P < 0.001$, $\eta^2 = 0.663$; 5-Hz continuous stimulation: $F(1,19) = 20.519$, $P < 0.001$, $\eta^2 = 0.519$]. The increase in perceived intensity was significantly greater for the 5-Hz burst-like stimulation compared with the 5-Hz continuous stimulation [$t(38) = 2.746$, $P = 0.009$, Cohen's $d = 0.868$; **Figure 3**].

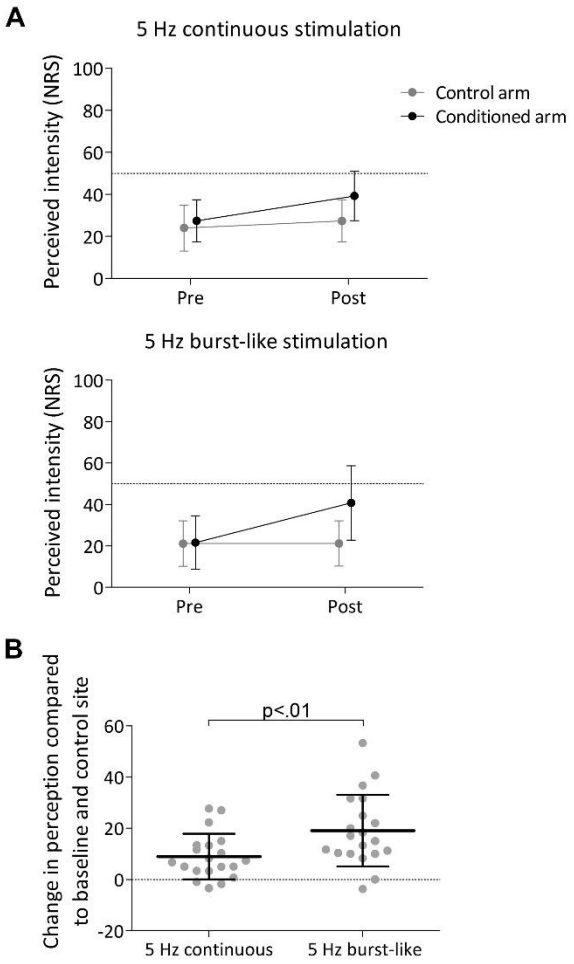

Figure 3. *Experiment 2.* A: intensity of perception elicited by the mechanical pinprick stimulation (128 mN) before (Pre) and 20 min after (Post) application of 5-Hz continuous conditioning electrical stimulation (top) or 5-Hz burst-like stimulation (bottom). Shown are the group-level average and SD of the numerical rating scale (NRS) scores. B: group-level average and SD increase in NRS compared with baseline and control site. P value shows the result of the independent t test on the individual changes in perception.

II.4. Discussion

The aim of the present study was to compare the efficacy of continuous vs. burst-like electrical stimulation of cutaneous nociceptors for the induction of heterotopic pain LTP. Our data shows that, when controlled for frequency of stimulation, burst-like conditioning stimulation induces a significantly greater increase in pinprick sensitivity than continuous stimulation. In experiment 1, we showed that the increase in mechanical pinprick sensitivity induced after 50-Hz burst-like stimulation was significantly greater compared with the increase in mechanical pain sensitivity induced after 5-Hz continuous stimulation. In this experiment, the total duration of the conditioning stimulation and the total number of applied stimuli were identical, but the stimuli were delivered at different frequencies. Hence, differences between the two conditions could have been related to differences in stimulation frequency rather than the use of burst-like vs. continuous patterns of stimulation[80]. For this reason, we conducted a second experiment in which we compared the increase in mechanical pinprick sensitivity induced by 5-Hz continuous stimulation and 5-Hz burst-like stimulation. In this experiment, frequency of stimulation and total number of stimuli were identical across conditions. Again, we found that 5-Hz burst-like stimulation induced a greater increase in pinprick sensitivity compared with 5-Hz continuous stimulation.

When studying LTP in the hippocampus, Larson and Munkácsy (2015) found that burst-like stimulation induced a relatively larger homosynaptic LTP than continuous tetanic stimulation[266].

If heterotopic pain LTP is indeed a manifestation of heterosynaptic LTP, our results suggest that burst-like stimulation is more efficacious that continuous stimulation in inducing spinal heterosynaptic LTP. Studies conducted in rodents have shown that high-frequency burst-like stimulation of the sciatic nerve can activate microglia and that activated microglia can release brain-derived neurotrophic factor (BDNF) [31, 268]. The release of BDNF is thought to contribute to central sensitization and may decrease the activity of the potassium-chloride cotransporter (KCC2), which would result in an increase in intracellular chloride concentration leading to a loss of inhibition and, as a consequence, increased excitation[268-271]. Interestingly, studies in rats have shown that unlike burst-like stimulation, continuous stimulation does not lead to the release of BDNF[272]. Xia et al. (2016) also compared changes in pinprick sensitivity induced by continuous stimulation and burst-like stimulation in humans[76]. Specifically, they compared 10-Hz continuous stimulation with 100-Hz burst-like stimulation while keeping the total number of stimuli and total duration of stimulation the same. They observed a significant increase in pinprick sensitivity after both stimulation protocols, however, and in contrast to our results, no statistically significant difference was observed, although the increases were not the same (10Hz: 27%; 100 Hz: 49%). Xia et al. used an intensity of stimulation corresponding to 10 times the detection threshold, whereas in the present study we used an intensity of 20 times the detection threshold[76]. Moreover, in the present study we compared the two conditions (5 Hz vs. 50 Hz) with respect to baseline and contralateral control arm, whereas in the study of Xia et al., they compared three conditions (10-Hz continuous, 100-Hz burst-like, and 200-Hz burst-like stimulation) with respect to baseline and a control condition in which the

electrode was attached to the skin, but no stimulation was delivered. We also show that continuous stimulation induces an increase in mechanical pinprick sensitivity. Klein et al. (2004) also observed hyperalgesia to pinprick stimulation in surrounding unstimulated skin after 1-Hz continuous conditioning stimulation, but only when the intensity of stimulation was 20 times the detection threshold[72]. The total duration of their conditioning stimulation was around 16 min, and the stimulation was ap-plied to the ventral forearm. Also, Torta et al. (2020) showed an increase in pinprick sensitivity of the skin after 2 min of 2-Hz continuous stimulation at the forearm[83]. In contrast, De Col and Maihöfner (2008) showed that 20-Hz continuous stimulation applied to the ventral forearm induced hypoalgesia rather than hyperalgesia of the skin surrounding the site at which the conditioning stimulation was delivered. However, there are differences between the present study and their study[250]. In that study, the continuous stimulation lasted for 35 min, whereas our continuous conditioning stimulation lasted 100 s only. Moreover, the electrode those authors used to deliver the conditioning stimulation is different from ours. Whereas their electrode had only two pins, our electrode has 16 pins. It is likely that our stimulation activated a larger number of afferents. Furthermore, the frequency of stimulation is different: whereas it was 20 Hz in their study, it was 5 Hz in the present study. Finally, the intensity of stimulation was different. In the study of De Col and Maihöfner, the intensity was continuously adjusted during the conditioning stimulation to a pain intensity of 5 on a numeric rating scale ranging from 0 (no pain) to 10 (worst imaginable pain), whereas in our study the intensity of stimulation was set at 20 times the detection threshold. Finally, Biurrun Manresa et al. (2010) and

Vo and Drummond (2014) also used 1-Hz continuous conditioning stimulation but did not observe any significant changes in pinprick perception in the unstimulated surrounding skin. In both studies, their conditioning stimulation was delivered at 10 times detection threshold. Moreover, in the study of Biurrun Manresa et al., they applied the conditioning stimulation to the dorsum of the foot instead of the forearm[82, 267].

II.5. Conclusion

To conclude, the present study provides evidence that burst-like conditioning stimulation is more efficacious in inducing increased pinprick sensitivity in the surrounding unstimulated skin than continuous stimulation. These results show that the pattern of peripheral nociceptive input (i.e., not only the total amount of input) is an important determinant of how much central sensitization will be induced.

III. Chapter 3. Pinprick-induced gamma-band oscillations (GBOs) are not a useful electrophysiological marker of pinprick hypersensitivity in humans

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Abstract

Background: Gamma-band oscillations (GBOs) are rhythmic fluctuations in local field potential activity recorded between 30 and 100 Hz. They can be detected using electroencephalography (EEG) or magnetoencephalography (MEG) in response to various sensory stimuli. Studies in animals suggest that GBOs induced by nociceptive stimuli may represent an electrophysiological correlate of mechanical hypersensitivity. This study aimed to investigate in healthy human's scalp GBOs induced by mechanical stimuli activating skin nociceptors before and after the induction of mechanical hypersensitivity using high-frequency electrical stimulation (HFS) of the skin.

Methods: In twenty healthy volunteers, we recorded the electroencephalogram during robot-controlled mechanical pinprick stimulation (512 mN) applied at the right ventral forearm before and after HFS.

Results: HFS induced a significant increase in mechanical pinprick sensitivity, but this increased pinprick sensitivity was, at the group level, not accompanied by a significant increase in GBOs. Visual inspection of the individual data revealed that possible cortical GBOs were present in eight out of twenty participants (40%) and the frequency of these GBOs varied substantially across participants.

Conclusion: Based on the low number of participants showing GBOs we question the (clinical) utility of mechanically induced GBOs as an electrophysiological marker of pinprick hypersensitivity in humans.

III.1. Introduction

Gamma-band oscillations (GBOs) are rhythmic fluctuations in local field potential activity (i.e. the electric potentials measured in the extracellular space around neurons) between 30 and 100 Hz. GBOs can be recorded at the scalp using electroencephalography (EEG) or magnetoencephalography (MEG) in response to stimuli of different modalities, for instance, visual and tactile[168, 169, 273]. Also, nociceptive laser stimuli, selectively activating thermal skin nociceptors, induce scalp GBOs. These GBOs (between 60 to 95 Hz) are typically present between 100 and 300 ms after the onset of the laser stimulus at central and contralateral electrodes[147, 167, 171, 274]. When recording from superficial and deep layers of the primary somatosensory cortex in animals, Yue et al. (2020) found that GBOs induced by nociceptive laser stimuli mainly originate from the activity of interneurons in the superficial layers of the primary somatosensory cortex contralateral to the site of stimulation[172]. Tan et al. (2019) recently showed in mice that paw withdrawal responses elicited by mechanical von Frey stimuli were preceded by GBOs in the primary somatosensory cortex, whereas no GBOs were observed when the same stimuli did not elicit a withdrawal response. The authors further showed that mechanical stimuli of lower stimulation intensities that were initially unable to elicit GBOs and paw withdrawal responses were able to do so after the induction of inflammation at the paw. Based on these results the authors suggested that GBOs preceding the paw withdrawal responses may represent an electrophysiological correlate of mechanical hypersensitivity[231]. If these findings could be translated to humans, the recording of GBOs might be of clinical interest for objectively establishing altered nociceptive processing. In humans, Michail et al. (2016) showed that in normal conditions

(without the presence of mechanical hypersensitivity), mechanical von Frey stimulation, applied to the skin of the hand dorsum, can also elicit GBOs. More specifically, by recording the EEG during computer-controlled mechanical punctate stimulation, exerting a force of 181 mN onto the skin, they observed GBOs between 60-80 Hz within 200 to 600 ms after the onset of the mechanical stimulus at central EEG electrodes[145]. Van den Broeke et al. (2017) investigated the presence of GBOs, elicited by manually applied mechanical pinprick stimulation, before and after the experimental induction of mechanical hypersensitivity. No GBOs of cortical origin were found either before HFS or after HFS. In that study the authors used a 64 mN mechanical pinprick stimulation intensity, which could have been too low to elicit GBOs[161]. The present study aimed to investigate in healthy human volunteers scalp GBOs elicited by strong mechanical pinprick stimulation (512 mN) before and after the induction of mechanical hypersensitivity. To this end, we recorded the EEG during robot-controlled mechanical pinprick stimulation before and after the induction of pinprick hypersensitivity using high-frequency electrical stimulation of the skin (HFS).

III.2. Methods

Participants

Twenty healthy right-handed volunteers (4 men and 16 women; aged 19 – 35 years; 23.8 ± 4.2 years [mean ± sd]), able to understand written and spoken French, were included. The exclusion criteria were: (1) suffering from acute or chronic pain, (2) having a neurological or psychiatric disease, (3)

taking medication (except contraceptive), (4) having cardiac issues (5) having a lesion on one of the forearms. The experiment was conducted according to the declaration of Helsinki. Approval for the experiment was obtained from the local Ethical Committee (Commission d'Éthique Biomédicale Hospitalo-Facultaire) of the Université Catholique de Louvain (UCL) (B403201316436). All participants signed an informed consent form and received financial compensation for their participation. The experiment was not pre-registered. All datasets are made public on the OSF repository.

Experimental design

The design of the experiment is shown in **Figures 1A** and **B**. In this within-subject study, robot-controlled mechanical pinprick stimuli were applied to the skin of the right ventral forearm ("test area") before and after applying HFS to induce pinprick hypersensitivity of the surrounding skin. During the mechanical pinprick stimulations, the electroencephalogram (EEG) was continuously recorded, and the quality of perception and perceived intensity elicited by the pinprick stimuli were collected.

High-frequency stimulation (HFS)

HFS consisted of five trains of 100 Hz electrical charge-compensated pulses. Each train lasted one second and was delivered in a 10-second interval. The intensity of HFS was set at 20 x the individual detection threshold to a single electrical non-charge compensated (rectangular) pulse (pulse duration: 2

ms). Detection thresholds were estimated using the method of limits. The electrical pulses were generated with a custom-written MATLAB code (MATLAB 2014B; MathWorks), triggered by a National Instruments digital-analog interface (NI6343, National Instruments, Austin, TX), produced by a constant current stimulator (Digitimer DS5, Welwyn Garden City, UK), and "delivered to the skin using a specifically designed electrode built at the Centre for Sensory-Motor Interaction (Aalborg University, Denmark). The cathode consists of 16 blunt stainless-steel pins with a diameter of 0.2 mm protruding 1 mm from the base. The 16 pins are placed in a circle with a diameter of 10 mm. The anode consists of a surrounding stainless-steel ring having an inner diameter of 22 mm and an outer diameter of 40 mm"[143]. Previous studies have shown that HFS induces an increase in mechanical pinprick sensitivity of the surrounding skin, which lasts several hours[81], and is reminiscent of secondary mechanical hyperalgesia which is thought to be the result of central sensitization.

Robot-controlled mechanical pinprick stimulation

A custom-built robot-controlled mechanical pinprick stimulator (blunt probe, diameter 0.35 mm, see van den Broeke et al. 2020) was used to deliver calibrated and reproducible mechanical pinprick stimuli exerting a force of 512 mN onto the skin[162]. The 512 mN stimulation intensity was chosen to make sure we activate skin nociceptors. Higher pinprick stimulation intensities may induce skin tissue damage. At each measurement (before and after HFS) a total of forty mechanical pinprick stimuli were delivered to the ventral forearm of the right arm. The pinprick stimuli were delivered

randomly within the test area (**Figure 1**). The test area consisted of a circle with a diameter of 4 cm minus an inner circle with a diameter of 1 cm. Each trial (see **Figure 1C**) started with projecting the word attention on the screen in front of the participant (approx. 60 cm). The word attention was followed by a cross in the centre of the screen. After the appearance of the cross, there was a random interval between 5 and 6 seconds before the robot started the stimulation. Each stimulation lasted 1.2 seconds, 6 including both the descending and ascending movement of the pinprick probe. The velocity of the descending and ascending movement of the probe was 33.33 mm/s. After one second, the cross disappeared and the word quality with three descriptors (pricking, touch or not detected) appeared on the screen. At that moment the participants had to indicate verbally by choosing one of the three descriptors the quality of perception elicited by the pinprick stimulus. Three seconds later the word intensity appeared on the screen together with a Numeric Rating Scale (NRS) ranging from 0 (no pinprick perception) to 100 (maximal pinprick intensity imaginable). At this moment the participants had to provide verbally a rating of the perceived pinprick intensity if the stimulus was perceived as pricking. If the stimulus was not detected or perceived as touch participants were instructed to give a score of zero. Then the pinprick probe changed its position after which the next trial started. In each block, the pinprick robot stimulated at random positions within the test area (**Figure 1A**) and never the same spot twice. During the pinprick stimulation, participants were comfortably sitting in front of a table with their right arm inside the custom-built robotic pinprick stimulator. A 48 x 57.5 cm panel with an opening for the arm was placed in front of the pinprick robot to prevent the view of the stimulated arm. Participants were instructed to wear sound-

attenuating headphones and flexible earplugs to mask any sound generated by the pinprick robot during movements. During each trial, participants were instructed to fixate their gaze on the cross on the screen. Before starting the experiment, the position of the pinprick probe relative to the skin surface was determined. The distance between the probe and the skin surface is variable across subjects and is determined by the homogeneity of the height of the skin across the tested area. The distance between the probe and the skin was approximately 0.5 cm.

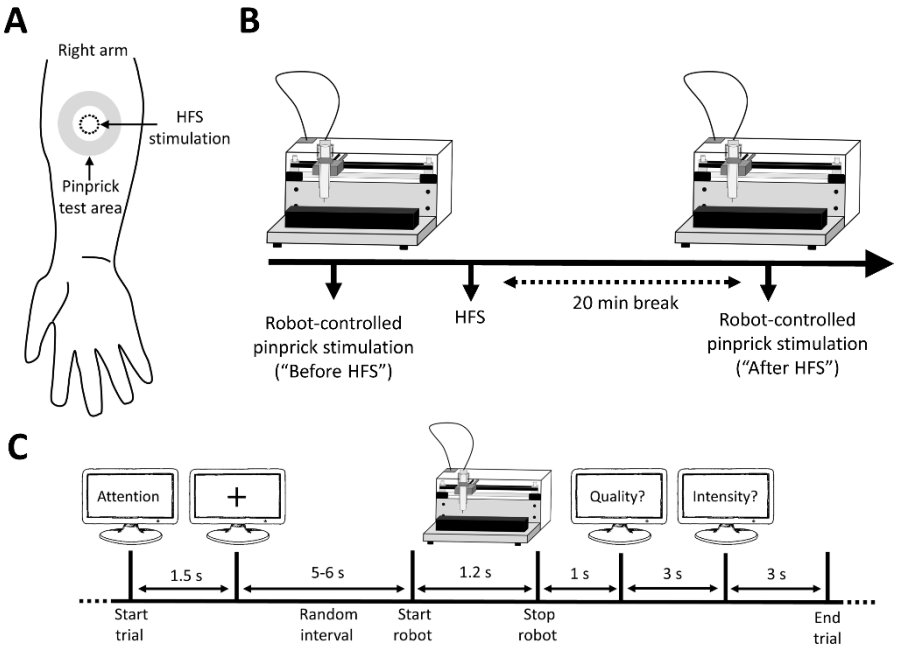

Figure 1. Experimental design. **A**. High-frequency electrical stimulation was applied to the right ventral forearm approximately 10 cm from the fossa cubital. The pinprick stimuli were randomly delivered within the test area (grey circle). **B**. Timeline of the experiment. **C**. Example of one trial.

EEG recording

The EEG was recorded using 64 Ag-AgCl electrodes mounted in an elastic cap and arranged according to the international 10-10 system (Biosemi, The Netherlands). The EEG signals were amplified and digitized using a sampling rate of 1024 Hz. The impedance of the electrodes was kept below 20 kΩ. An external electrode was placed on the nose and was used as a reference in the offline analysis of the EEG.

Data analysis

EEG analysis

The EEG was analysed offline using Letswave 6 (www.nocions.org/letswave).

Time-frequency analysis

To characterize changes in the spectral content of the EEG signal in response to mechanical pinprick stimulation we conducted the following analysis (see also van den Broeke et al. 2017)[161]. First, we re-referenced the continuous EEG to the nose electrode. After that, a 30-100 Hz band pass zero-phase Butterworth filter was applied, followed by a notch filter between 49 and 51 Hz. After this, we used Independent Component Analysis (ICA) to remove artifacts[275]. The **identification** of artifacts was based on the characteristic scalp distributions of artifacts illustrated in the online EEGlab tutorial :

https://eeglab.org/tutorials/06_RejectArtifacts/RunICA.html#inspecting-ica-components.

The reconstructed signal was segmented from -1.6 to 1.6 s relative to stimulation onset. A short-time fast Fourier transform (STFFT) with a fixed Hanning window of 200 ms was applied to the single trials to decompose the EEG signals in a time-frequency representation (TFR) of power (μV^2). Separate averaged TFRs were then computed for each participant and condition (before HFS and after HFS). Finally, a baseline correction (subtraction) was applied between -1.5 and -1.0 s to visualize changes in post-stimulus EEG power. Previous studies have shown that GBOs are present at central and contralateral electrodes[145, 167, 171, 274]. Therefore, for the identification of GBOs, we focused on electrodes Cz and C3. To evaluate the contribution of artifacts to the EEG, we re-analysed the EEG signals but without removing artifacts.

Time-domain analysis

To investigate time- and phase-locked pinprick-evoked EEG activity (pinprick-evoked brain potentials, PEPs) we analysed the EEG in the following way. First, we re-referenced the continuous EEG to the nose electrode. Then, we applied a 0.3- 30 Hz band pass zero-phase Butterworth filter. ICA was applied to correct eye movements or eye blinks.

After that, the continuous EEG was segmented into epochs extending from -1.5 to 1.5 s relative to stimulus onset. A baseline correction (-1.5 to – 1.0 s, to be consistent with the time-frequency analysis) was then applied and

epochs with amplitude values exceeding ± 75 µV were rejected as these were likely to be contaminated by artifacts. Finally, separate averaged waveforms were computed for each participant and time point (before and after HFS) and individual peak values of both the negative and positive waves of the waveforms were extracted. Based on a previous publication, we extracted the maximal value of the negative wave at electrode Cz (between 100 and 200 ms) and the maximal value of the positive wave at CPz (between 200 and 700 ms)[162].

Statistical analysis

To confirm the expected increase in perceived pinprick intensity after HFS we performed a paired t-test on the individual averaged ratings before versus after HFS. To test whether there was a significant increase in GBOs at the group level we applied a non-parametric cluster-based permutation test on the individual TFRs of the conditions before versus after HFS[161]. To test if there was a significant increase in the negative and positive wave of the PEPs after HFS we performed a paired t-test on the individual peak values before versus after HFS. All statistical analyses but one (cluster-based permutation test) were performed in SPSS Statistics 27 and the level of significance was set at P <.05 (one-sided). Effect sizes (Cohen's dz) were calculated by dividing the t-value by the square root of the total number of participants.

III.3. Results

Electrical detection thresholds

The mean (and SD) electrical detection threshold to a single electrical stimulus was 0.32 ± 0.09 mA.

Perceived intensity

The mean (and SD) perceived intensity (NRS score) elicited by the mechanical pinprick stimuli before and after HFS are shown in **Figure 2A**. The paired t-test showed a statistically significant increase in the perceived intensity elicited by the mechanical stimuli after HFS ($t(19)=5.80$, $P<.001$, Cohen's $dz=1.30$). All but one participant showed an increase in pinprick sensitivity after HFS (**Figure 2B**).

Quality of perception

Before HFS, participants described the quality of perception elicited by the mechanical pinprick stimuli as 'pinprick' in 85% of the cases, and as 'touch' in 15% (Figure 2C). After HFS, the number of cases in which the mechanical pinprick stimuli were described as 'pinprick' increased to 97.5% and only 2.5% of stimuli were qualified as 'touch'.

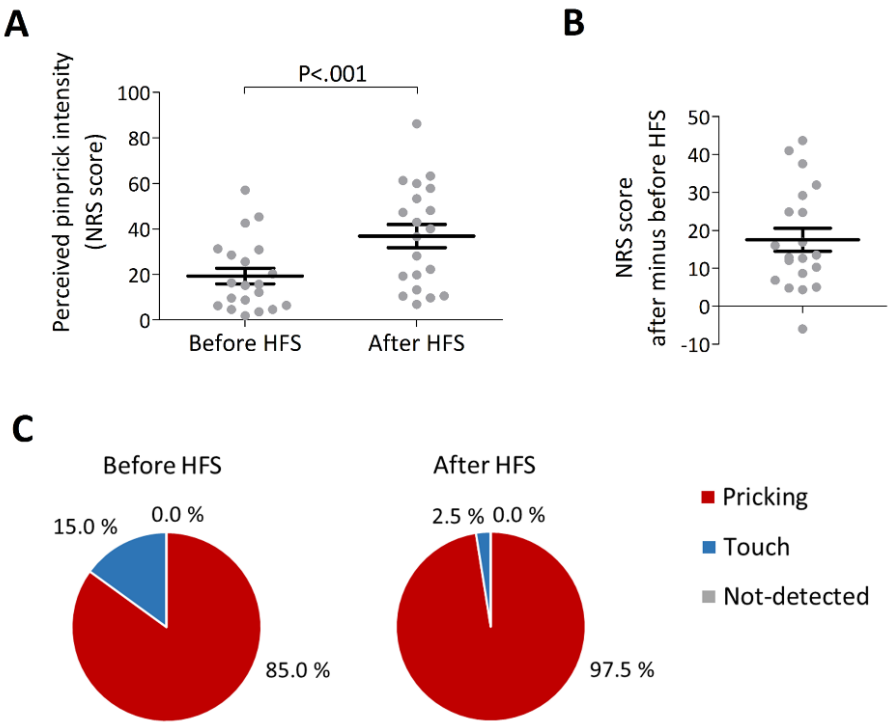

Figure 2. A. Panel A shows the group-level mean (and SD) perceived intensity elicited by the pinprick stimuli before and after HFS. Panel **B** shows the group-level mean and (SD) difference in perceived intensity (after minus before). In both panels, a dot represents a single subject. Panel **C** shows the group-level mean percentages of the quality of perception (pricking, touch or no stimulus detected) elicited by the pinprick stimuli before and after HFS.

Time-frequency analysis of the EEG

Figure 3 shows the results of the time-frequency decomposition of the artifact-free EEG signals for electrodes C3, Cz and C4, before HFS (**Figure 3A**) and after HFS (**Figure 3B**). One subject (S2) was identified as an outlier and was not included in the analysis (see supplementary **Figure S1**). A cluster-based permutation test on the individual TFRs before versus after HFS, for electrode Cz and C3 separately, revealed no cluster having a p-value smaller than the critical p-value of .025 (Bonferroni corrected for the number of electrodes). **Figure 4** shows the results of the time-frequency decomposition of the EEG signals (Cz electrode) without removing artifacts (N=20).

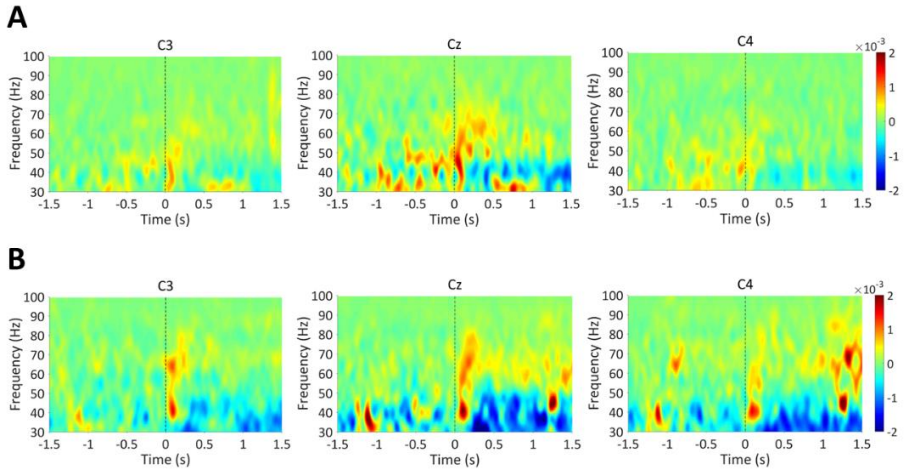

Figure 3. Group-level average time-frequency representations elicited by pinprick stimulation for electrodes C3, Cz and C4 before HFS (panel **A**) and after HFS (panel **B**). Red and blue colour denotes respectively increases and decreases in power (μV^2) compared with baseline.

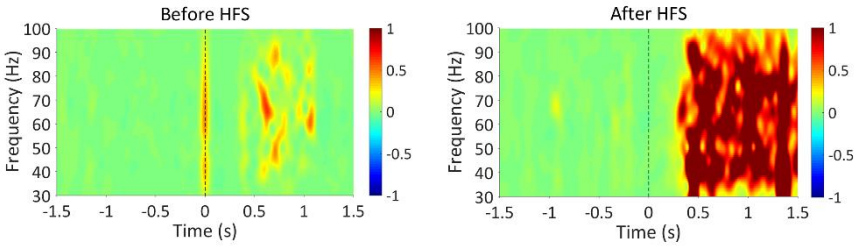

Figure 4. Group-level average time-frequency representation (Cz) elicited by pinprick stimulation applied before and after applying HFS when no artifacts are removed from the EEG signal. Red and blue colour denotes respectively increases and decreases in power (μV^2) compared with baseline.

Exploratory analyses

To better understand our data, we visually inspected the individual across-trial averaged TFRs of the artifact-free EEG. Of twenty participants, eight (S1, S3, S5, S7, S8, S11, S17, S19) showed increased post-stimulus gamma activity, compared with baseline, that had a central and/or contralateral topography, either before HFS, after HFS or in both conditions (**Supplementary Figure S1**). **Figure 5A** shows the group-level average TFRs of the before-HFS and after-HFS conditions of those eight participants. Of these eight participants, one (S7) showed a reduction in gamma activity after HFS compared with before HFS. This participant also showed a reduction in perceived pinprick intensity after HFS (see **Figure 2B**). **Figure 5B** shows the same group-level TFRs as **Figure 5A** but without the participant that did not develop pinprick hypersensitivity after HFS. **Figure 6A** shows the same group-level TFRs as **Figure 5B** but only the first 500 ms after stimulus onset. Subtraction of the

after-HFS condition from the before-HFS condition revealed a predominant increase in gamma power around 40 Hz and between 55 and 85 Hz (as shown in Figure 6B). Of note, the increase in gamma power (between 100 and 300 ms) following HFS was maximal at Cz (**Supplementary Figure S2**). A map of the distribution of univariate t-values resulting from the point-by-point paired comparisons is shown in **Figure 6C**.

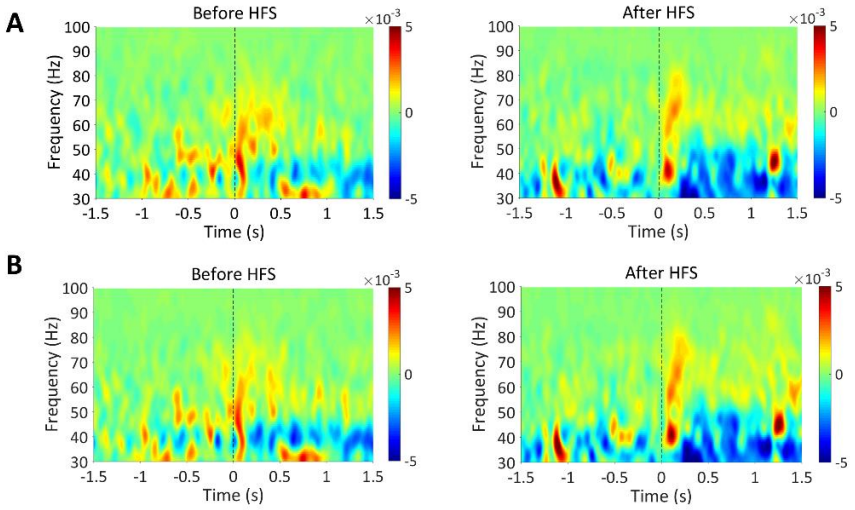

Figure 5. Panel **A** shows the group-level average time-frequency representations (Cz) of those participants showing increased (compared with baseline) post-stimulus gamma power either before HFS, after HFS or both (N=8). Panel **B** shows the same TFRs as in panel **A** but without the participant that showed no pinprick hypersensitivity following HFS. Red and blue colour denotes respectively increases and decreases in power (μV^2) compared with baseline.

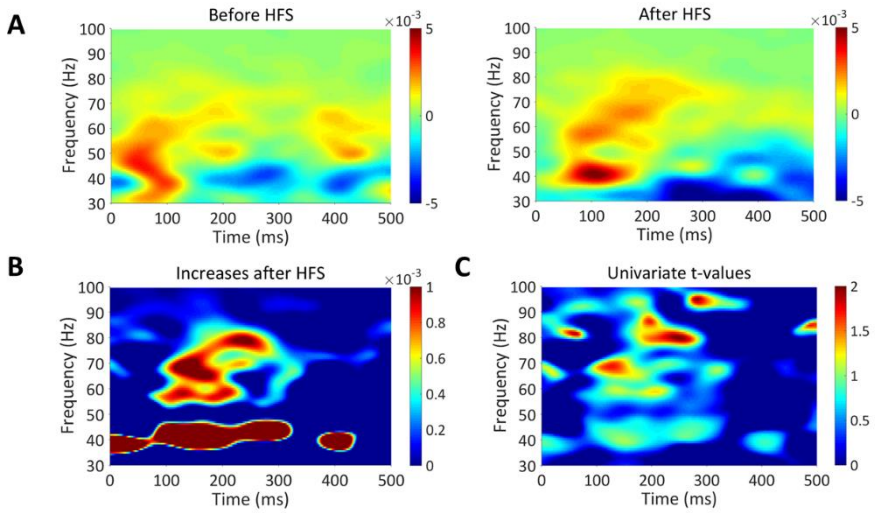

Figure 6. Panel **A** shows the TFRs of the first 500 ms after stimulus onset of those participants showing pinprick hypersensitivity following HFS and post-stimulus gamma activity either before HFS, after HFS, or both (N=7, same as **Figure 5B**). Red and blue colour denotes respectively increases and decreases in power (μV^2) compared with baseline. Panel **B** shows the increases in gamma power after HFS compared to before HFS. Panel **C** shows the distribution of univariate t-values following point-by-point paired comparisons of the before-HFS and after HFS conditions.

Supplementary Figures S1 and S2. The QR code provides a direct link to the supplementary Figures (S1 and S2) permanently hosted on GitHub: https://github.com/solenngousset/Chapter-2-Supplementary-Figures---Sgousset-thesis. These figures are presented in a separate file due to their length to avoid overloading the main body of the chapter. The figures can be accessed via the provided QR code above for ease of reference.

Time-domain analysis of the EEG

Figure 7 shows the group-level average waveforms of the PEPs elicited by the 512 mN stimulation at electrodes Cz and CPz before and after HFS. A paired t-test on the individual peak values revealed a significant increase in the magnitude of the negative peak after HFS (t(19)=2.09, P=.025, Cohen's dz=.468, **Figure 8**) at Cz and a significant increase in the magnitude of the positive peak after HFS at electrode CPz (t(19)=2.10, P=.024, Cohen's dz=.470, **Figure 8**). A significant correlation was found between the change in pinprick sensitivity after HFS and the change in negative wave of the PEPs after HFS (Pearson r= -0.52, P=.019, two-sided) but not between the change in pinprick sensitivity and the change in positive wave (Pearson r= -0.37, P=.104).

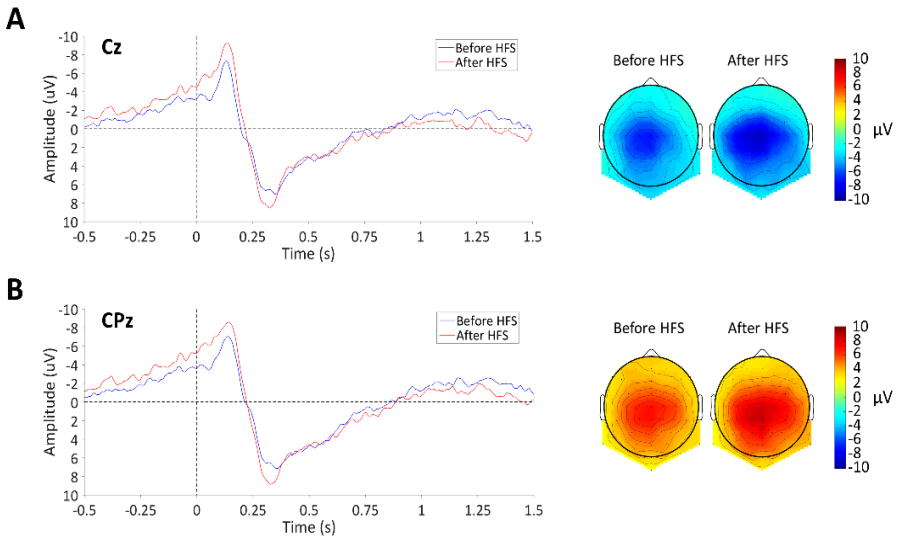

Figure 7. Panel **A** shows the group-level average pinprick-evoked brain potentials (PEPs) recorded at electrode Cz before and after applying HFS. At the right, is the

group-level topographical distribution of the voltage at the peak of the early-onset negative wave. Panel **B** shows the group-level average pinprick-evoked brain potentials (PEPs) recorded at electrode CPz before and after applying HFS. At the right, is the group-level topographical distribution of the voltage at the peak of the positive wave.

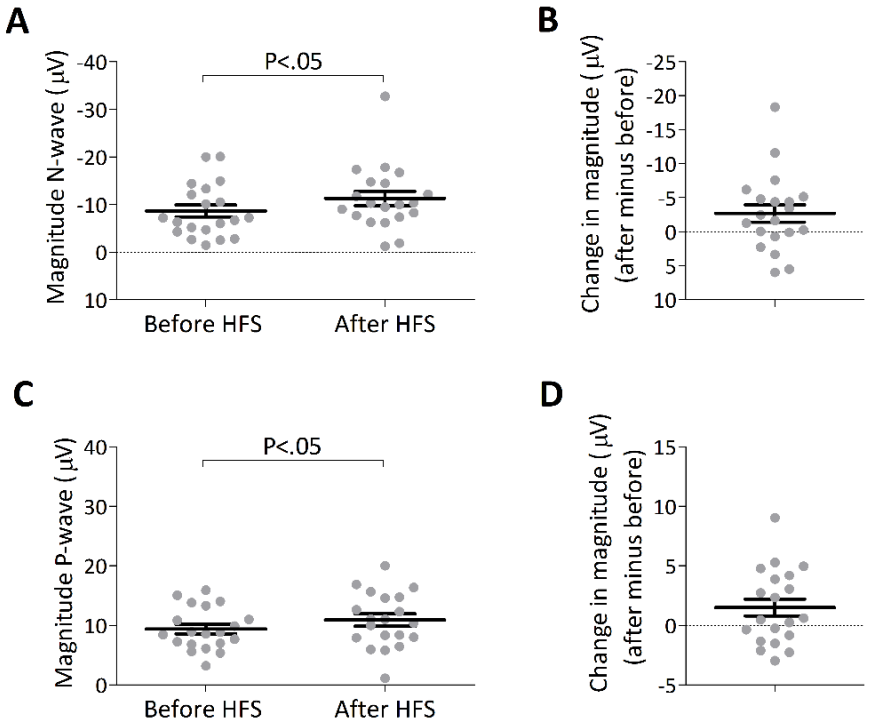

Figure 8. Panel **A** shows the mean (and SD) peak values of the PEP negativity. Each dot represents a single subject. Panel **B** shows the mean (and SD) difference in the peak value of 16 the PEP negativity (calculated as after-HFS minus before HFS). Panel **C** shows the mean (and SD) peak values of the PEP positivity. Panel **D** shows the mean (and SD) difference in peak values of the PEP positivity (calculated in the same way as for the negative peak).

III.4. Discussion

This study aimed to investigate scalp GBOs induced by robot-controlled mechanical pinprick stimuli activating skin nociceptors before and after the induction of mechanical hypersensitivity. As expected, HFS induced a significant increase in pinprick sensitivity. At the group level, no significant increase in GBOs was observed after HFS. Visual inspection of the individual data revealed that possible cortical GBOs (either before HFS, after HFS or both) were present in eight out of twenty participants (40%) and that the frequency of these GBOs varied substantially across participants. In addition to our primary aim, we also analysed the EEG responses in the time domain (PEPs). Contrary to the GBOs, we observed clear PEPs in most subjects (18 out of 20). A significant increase for both the negative and positive waves of the PEPs was found after HFS at group level. Furthermore, a significant negative correlation was found between the change in pinprick sensitivity after HFS and the change in the PEP negative wave

Scalp-recorded pinprick-evoked EEG gamma-band activity is contaminated by artifacts

The **Figure 4** shows that after applying HFS, pinprick stimulation increases post-stimulus gamma-band activity. Similar results were found in a previous publication, but we also showed that similar and even stronger activities were found in the electrooculogram, which suggests that scalp EEG is strongly contaminated by muscular eye activity[161].

Other studies have shown that scalp recorded high-frequency EEG activity is contaminated by micro saccades and muscle activity[276, 277]. The present study shows that possible cortical GBOs elicited by mechanical pinprick stimuli can be identified only when non-cortical activity is removed first (compare **Figure 4** with **Figure 3A** and **B** middle figure).

Pinprick-induced GBOs recorded in response to stimulation of non-sensitized skin

Possible cortical GBOs induced by mechanical pinprick stimuli applied to non-sensitized skin (before HFS) were observed in six out of twenty participants. In contrast, Michail et al. (2016) found GBOs in the majority of their participants[145]. However, the timing and frequency of the possible cortical GBOs in our study were, at the individual level, not always compatible with the timing and frequency of the GBOs reported in the study of Michail et al. (see rectangular in supplementary **Figure S1**). Gamma-band activity was also found after tactile stimulation by Van Ede et al. (2014) and similar to our study, they also reported that not all participants showed stimulus-induced gamma activity[168]. Moreover, the frequency of gamma activity varied substantially across participants. Why stimulus-induced gamma activity is not observed in all participants remains unknown.

Pinprick-induced GBOs recorded in response to stimulation of sensitized skin

At the group level, no significant increase in post-stimulus gamma activity was found after HFS. However, this may be the consequence of the low number of participants showing gamma activity and/or the interindividual variability in the timing and frequency of gamma activity. Of note, our data indicate that the presence of scalp GBOs does not necessarily depend on perceiving the mechanical pinprick stimulus as highly intense, as GBOs were present in individuals that reported low ratings of pinprick stimulation. GBOs were also absent in individuals rating the pinprick stimulus as highly intense.

Pinprick-evoked potentials (PEPs) revealed by time-domain analysis

Clear PEPs were observed when analysing the EEG in the time domain (**Figure 7**). At the group level the magnitude of both the negative and positive waves of the PEPs was significantly increased after HFS. This result deviates from the study of van de Broeke et al. (2015)[158]. In that study, a range of pinprick stimulation intensities (16, 32, 64, 128, 256, 512 mN) were applied to the skin of the ventral forearm to elicit PEPs before and after capsaicin injection. After the capsaicin treatment pinprick stimuli applied to the skin surrounding the site of capsaicin treatment, elicited an increase in the positive wave of the PEPs. However, this increase was the largest and only significant for the intermediate pinprick stimulation intensity (64 mN). A possible explanation of why we find a significant increase in the positive wave in the present study but not in the study of van den Broeke et al. could be the difference in statistical testing. In the study of van den Broeke et al. 2015 a non-parametric

cluster-based permutation test was used to test whether there was a significant difference between the two subtracted waveforms (after minus before HFS, two-sided), while in the present study, we compared a single value only between the two conditions (one-sided). Another possibility could be that in the study of van den Broeke et al. the pinprick stimuli were delivered to the skin manually. A robot delivers more reproducible stimuli across trials, thereby reducing the trial-to-trial variability in PEP amplitude[162]. Interestingly, we found a moderate negative correlation between the change in pinprick sensitivity following HFS and the change in magnitude of the PEP negative wave, however, this correlation should be interpreted with caution as the sample size is small.

III.5. Conclusion

The present study investigated for the first-time scalp GBOs induced by robot-controlled mechanical pinprick stimulation before and after the induction of pinprick hypersensitivity in humans. HFS successfully induced pinprick hypersensitivity, however, this was at the group level not accompanied by a significant increase in GBOs. The low number of participants showing possible cortical GBOs questions the (clinical) utility of mechanically induced GBOs as an electrophysiological marker of pinprick hypersensitivity in humans. Instead, PEPs seem to be a better outcome measure for assessing changes in the cortical processing of mechanical pinprick stimuli than GBOs.

Additional note on artifact cleaning and GBOs variability

There is a lack of consensus on how to deal with high-frequency artifacts such as EMG, and it not only decreases the reliability of experimental outcomes but also poses significant challenges for comparing findings across different studies that do not detail their visual inspection of the ICA and may have stimulus-induced EMG in their signal. One could acknowledge the complexity in describing such subjective visual analyses, however it would be useful for each investigation on the topic to make the raw data available; such transparency would allow for the re-analysis of data by others research groups, encouraging dialogue among scholars.

Now, I would like to shed light on our own analysis. To minimize the artifact contamination that may lead to a type I error, we performed an ICA based on the scalp map and the time course of the components, such as what is described on the EEGLAB tutorial :

https://eeglab.org/tutorials/06_RejectArtifacts/RunICA.html#inspecting-ica-components.

We decided to keep all the signals emerging from the electrodes of interest, namely Cz and C3 to try to keep the most possible cortical gamma band oscillations evoked by the contralateral pinprick stimulations. It means that we removed all the remaining signals from the other electrodes. The dataset was made available online.

In 2023, Jaltare et al. re-analysed the dataset and came to the same conclusion as ours[278]. Moreover, they showed that the preprocessing choices regarding when the ICA is applied, which baseline correction is used, and the baseline time window were not affecting the conclusion[278]. We recognize that the absence of a unified approach to analysing this type of data presents significant challenges. This situation underscores the need for establishing standardized protocols and/or computer-based algorithms to enhance the reliability of results and facilitate the comparisons between studies[279]. The use of ICA requires experience. Nevertheless, the pain field could benefit from a standardized pipeline to analyse these signals. For instance, Viola et al. (2009) introduced a promising method for semi-automatically identifying and clustering eye-related and heartbeat artifact components using the CORRMAP plug-in of EEGLAB[280]. This could be further developed for ICA-based muscle artifact correction. More recently, Liebisch et al. developed an algorithm designed to preserve cortical GBOs and enhance data quality, particularly for studies with significant muscle artifacts or a limited number of trials[281]. However, this is not without some limitations, such as the time-consuming nature of the process. The current version of the algorithm requires manual parameter definition due to the varied shapes and frequencies of muscle spikes in ICs. This could be improved by machine learning and faster programming languages. Moreover, with this tool, using higher-density electrode arrays (>64 electrodes) would enhance the separation of cortical and muscular sources, but this approach is less practical in experimental settings due to practical constraints. Another possible explanation for why fewer participants in our study exhibited GBOs compared to other studies is the possible interindividual

variability in expressing GBOs following salient stimuli. Numerous publications on GBOs found an effects at the group level, but the individual data are rarely reported[145, 147, 282]. In fact, the proportion of subjects within a study exhibiting GBOs can differ markedly[176, 283].

Regarding the intensity of perception, the group of Schulz (2023) found that there was no relationship between the expression of GBOs and subject's sensitivity. Such as in our study, they found participants with high perception ratings but no GBOs response and vice-versa. Indeed, although a good stability of these response across subjects, their results showed a consequent variability of GBOs between individuals[176]. The explanation of the mechanisms underlying interindividual variability of GBOs remains to be elucidated. Considering these and the previous paragraphs, the immediate clinical relevance of our observation regarding GBOs is limited.

IV. Chapter 4. Studying the effect of expectations on high-frequency electrical stimulation-induced pain and pinprick hypersensitivity

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Abstract

Background: Negative expectations about pain can amplify pain perception and its persistence, a phenomenon linked to the nocebo effect. These effects, induced by verbal suggestions and conditioning, may involve heightened spinal cord activity resembling nociceptive responses. Central sensitization is thought to contribute to persistent pain. Pinprick hypersensitivity surrounding cutaneous injury is considered as a manifestation of CS. However, the role of negative pain expectations in the development of CS in humans remains poorly understood.

Methods: This study used an inert treatment and verbal suggestions to induce expectations of increased high-frequency electrical stimulation (HFS)-induced pain and assessed their effects on pain ratings during HFS and HFS-induced pinprick hypersensitivity. Fifty healthy volunteers were randomly allocated to either a control group (N = 25) or a nocebo group (N = 25). Participants in both groups received a patch containing water on the right forearm. The nocebo group was told that the patch contained capsaicin that sensitized their skin, while the control group was told that the patch contained water that had no effect on skin sensitivity. Before and after patch attachment, single electrical stimuli were delivered to the area of the patch to measure the perceived intensity to these stimuli. After patch removal and after the participant rated expected pain and fear for HFS, HFS was delivered to the same skin site, followed by the assessment of pinprick sensitivity.

Results: The nocebo group rated the perceived intensity for the single electrical stimulus after removal of the patch as more intense compared with the control group, indicating that our manipulation worked. Yet, this effect did not transfer to expected pain for HFS, nor did it affect pain intensity ratings during HFS. HFS increased pinprick sensitivity but no group differences were found.

Conclusion: Because of the lack of differences in expected pain and pain intensity ratings for HFS between groups, no firm conclusions can be drawn regarding their effect on pinprick hypersensitivity.

IV.1. Introduction

Expectations about pain may impact pain experiences[284-286]. For instance, expectations that a treatment will produce pain relief can reduce pain, even when the treatment itself is inert (placebo effect)[287]. On the other hand, expectations that a treatment will worsen pain can increase pain (nocebo effect)[287]. Nocebo effects can be induced through verbal suggestions, but the combination of verbal suggestions and conditioning showed stronger nocebo responses on pain[288, 289]. Imaging studies have shown that negative expectations, induced by a nocebo treatment, increase spinal cord activity that overlaps with the activity triggered by nociceptive stimulation[290].

Central sensitization (CS), defined by the International Association for the Study of Pain as an "Increased responsiveness of nociceptive neurons in the central nervous system to their normal or subthreshold input,"[40] is believed to play a role in persistent pain[42, 291]. It is unclear if negative expectations about pain can promote CS.

The increase in mechanical pinprick sensitivity of the skin surrounding a cutaneous injury is considered a manifestation of CS[29, 42]. Indeed, animal studies have demonstrated that pinprick stimuli applied after capsaicin or high-frequency electrical stimulation (HFS) to adjacent skin areas, elicit increased responses of spinal nociceptive neurons without changes in the responsiveness of peripheral neurons.[29, 39, 292] If this is also the case in humans, these increased spinal responses may contribute to pinprick hypersensitivity.

To date, human studies investigating the effect of negative expectations on the development of pinprick hypersensitivity are scarce. Bedwell et al. (2022) investigated the effect of an inert treatment with verbal suggestions on HFS-induced pinprick hypersensitivity[293]. However, their manipulation was not successful, and no effect was found on pinprick hypersensitivity. More recently, Jaltare et al. (2024) used an inert pill with verbal suggestions to investigate the nocebo effect on HFS-induced pinprick hypersensitivity[294]. They found no significant effect of the manipulation on pinprick hypersensitivity. Moreover, the experimenters were not blinded. Torta et al. (2023) used observational learning to investigate the effect of nocebo on HFS-induced pinprick hypersensitivity. Participants watched either a video of an actress showing intense pain during HFS ("high pain" group) or less pain ("low pain" group) before undergoing HFS[295]. Participants in the "high pain" group reported more pain during the actual HFS compared with participants in the "low pain" group. Furthermore, HFS-induced pinprick hypersensitivity was on average higher in the "high pain" group compared with the "low pain" group. These findings suggest that expecting more HFS pain can result in experiencing more HFS pain and pinprick hypersensitivity. However, pain expectations regarding HFS after the videos and before receiving HFS were not assessed. Moreover, the experimenters were not blinded. Finally, the effects on HFS-induced pinprick hypersensitivity were weak and no effect on the spread of the area of increased pinprick sensitivity was observed.

The aim of the present study was to investigate how altering expectations related to HFS pain—specifically, expecting more pain during HFS—impacts on both HFS-evoked pain and pinprick hypersensitivity. To induce the expectation that HFS will be more painful, we used a combination of an inert

treatment and verbal suggestions. To further reinforce this expectation, we applied nonpainful electrical test stimuli with increasing stimulation intensity, with the statement that these stimuli were of the same intensity, after the inert treatment. We kept the assessor blinded and we asked participants for their pain expectations after the manipulation and before applying HFS. We expected that expectations of increased HFS pain would increase spinal cord excitability via top-down descending facilitatory pathways resulting in higher pain ratings during HFS and a larger increase and spread of HFS-induced pinprick hypersensitivity[296].

IV.2. Methods

Participants

Fifty Caucasian right-handed healthy volunteers (23 men, 27 women; ranging from 18 to 32 years old; mean age ± standard deviation (SD) = 22.74 ± 2.56) participated in the experiment. The healthy volunteers were recruited via study advertisement (flyer) *ad valvas* within UCLouvain University and via a Facebook group used to advertise experiments.

We based our sample size on the power calculated on the means and standard deviations pinprick ratings of the study of van den Broeke et al. (2013) and by considering violations of the assumption of homoscedasticity and sphericity[235]. The power analysis, based on a 2 × 2 × 2 ANalysis Of VAriance (ANOVA) (2 groups [control vs nocebo], 2 arms [control vs HFS], and 2 timepoints [pre vs post 3]), revealed an effect size of .23 and a power of .95

with N = 25 per group. For the calculation, an online software package was used (https://shiny.ieis.tue.nl/anova_power/).

Volunteers were eligible if they 1) were between 18 and 30 years old, 2) were able to understand spoken and written French, and 3) did not fulfil one or more of the following exclusion criteria: 1) evidence for a clinically significant alteration of the skin of the volar forearms; 2) pregnancy; 3) presence of a pacemaker or implanted cardiac defibrillator; 4) major neurological of psychiatric conditions; 5) taking medication (except contraception); and 6) participated in previous studies using HFS or capsaicin. Approval for the experiment was obtained from the local ethical committee (B4032021000041). All participants signed an informed consent form and received financial compensation (20€) for their participation. The study hypotheses and analysis plan was pre-registered at the Open Science Forum: https://doi.org/10.17605/OSF.IO/JZF75.

Experimental Design

In this mixed (Group [nocebo/control] × Arm [HFS/control]) experimental design (**Figure 1A**), participants received HFS onto the right forearm to induce pain and pinprick hypersensitivity[72, 292]. Before HFS, participants were randomly assigned to either a nocebo or control group and received a patch containing water on their right forearm. In the nocebo group, participants were told that the patch contained capsaicin that would sensitize their skin to electrical stimuli, while in the control group, participants were told that the patch contained water that did not affect skin sensitivity (see experimental manipulation).

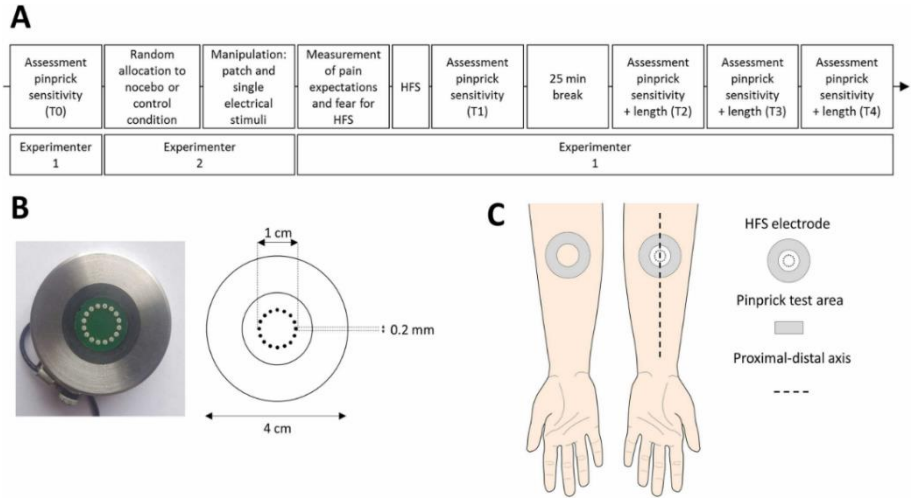

Figure 1. (**A**) Timeline of the experiment. T0 is the baseline measurement (before the manipulation and before applying HFS), T1 is the measurement directly after applying HFS, T2 is approximately at 30 minutes after HFS, T3 is 35 minutes after HFS, and T4 is 40 minutes after HFS. (**B**) Characteristics of the HFS electrode. (**C**) The area at which the pinprick stimuli were applied is shown as well as the proximal-distal axis (dotted line) for the measurement of the length of the area of increased pinprick sensitivity.

After the patch and before applying HFS, participants were asked to rate expected pain and their fear for HFS. After that, HFS was delivered to the right forearm, and participants were asked to rate their perceived pain intensity. Before (T0) and after applying HFS (T1: directly after applying HFS; T2: 30 minutes after applying HFS; T3: 35 minutes after HFS; and T4: 40 minutes after HFS), pinprick sensitivity of the skin surrounding the site of HFS and the homologous site at the contralateral arm was tested. At T2, T3, and T4, also the length of the area of increased pinprick sensitivity was measured.

Experimental induction of mechanical secondary hyperalgesia

HFS consisted of 5 trains of 100-Hz electrical charge-compensated pulses that lasted 1 second each and were delivered in a 10-second intertrain interval. The trains were programmed in Matlab 2014b (The Mathworks Inc, Natick), sent via a digital-analog interface (National Instruments, Austin, TX) to a constant-current electrical stimulator (Digitimer DS5, Welvyn Garden City, UK), and delivered to the skin of the ventral forearm approximately 10 cm from the cubital fossa via a specially designed multipin electrode (**Figure 1B**)[119]. The cathode of this electrode consisted of 16 blunt stainless-steel pins with a diameter of .2 mm, protruding 1 mm from the base and placed in a circle with a diameter of 10 mm. The anode was a stainless-steel ring with an inner diameter of 22 mm and an outer diameter of 40 mm. HFS was applied to the right volar forearm approximately 10 cm distal to the cubital fossa with a fixed intensity at 3 mA. After each train, participants were asked to rate their perceived pain intensity on a numeric rating scale (NRS), ranging from 0 ("no pain") to 100 ("worst pain imaginable").

Assessment of mechanical pinprick sensitivity

Pinprick hypersensitivity induced by HFS can be characterized by both a change in perceived intensity and area size. To assess changes in perceived intensity, at each measurement, 3 mechanical pinprick stimuli, delivered using a calibrated mechanical pinprick stimulator (128 mN, MRC Systems GmbH, Heidelberg, Germany), were applied to both forearms to the area

adjacent to the site at which HFS was delivered (within a circle between 0.5 and 2 cm from the centre of the HFS electrode) and to the homologous site of the contralateral control arm (**Figure 1C**). Each mechanical stimulus was applied perpendicular to the skin for approximately 1 second. The participants were instructed to provide an average rating of the perceived intensity of the 3 pinprick stimuli on an NRS, assessing pain, ranging from 0 ("no pain") to 100 ("worst pain imaginable"). To avoid peripheral sensitization due to repeated pinprick stimulation, the same skin site was never stimulated twice. The order of testing (control or HFS arm first) was counterbalanced across participants. The length of the area of increased pinprick sensitivity was taken as a measure for the spread of pinprick hypersensitivity. For this, repeated mechanical pinprick stimuli were applied, spaced 1 cm each along the midline axes of the forearm, from the most distal point of the forearm to the centre of the HFS-stimulated area, and from the most proximal point of the forearm to the centre of the HFS-stimulated area (**Figure 1C**). The areas to be stimulated were marked onto the skin with a pen at the beginning of the experiment. The participant was instructed to report verbally when a clear increase in pinprick sensitivity was felt between 2 points. Finally, the distance (cm) between this point and the centre of the HFS-stimulated area was measured. During the mapping, participants were instructed not to look at their arm.

Experimental manipulation of expectations

A patch was applied for 5 minutes, on the right ventral forearm at the site where HFS would be applied and consisted of a medical adhesive patch with a squared cotton attached. To prepare the patch, the cotton was sprinkled

with water, using a pipette, from one of the 2 bottles that were put in front of the participant on the table. One bottle was transparent, and the water was clearly visible. The other bottle was a brown bottle normally used for liquid drugs. At the front of the brown bottle, a sticker was attached with the name “Capsaicin” on it and the logo of the pharmacy of the *Université Catholique de Louvain (UCLouvain)*.

The patch of the participants of the nocebo group was sprinkled with the water from the brown bottle. The participants were told that they would receive a patch with capsaicin (dissolved in ethanol) that would sensitize their skin and make it more sensitive for electrical stimuli. To increase the credibility of the manipulation (application of capsaicin), the experimenter wore a white medical coat and gloves[297].

The patch of the participants of the control group was sprinkled with the water from the transparent bottle, and the participants were told that they receive a patch containing pure water, as a control for the experimental condition, which would not affect their skin sensitivity for electrical stimuli.

Before the application of the patch and directly after its removal, participants received 3 single electrical stimuli delivered through the HFS electrode at the area of the patch and where HFS would be applied. The electrical stimuli (rectangular pulse width of 2.0 ms) were triggered by a custom-written MATLAB script and generated by a constant-current electrical stimulator (Digitimer DS5, Welvyn Garden City, UK). After each electrical stimulus, participants were asked to rate the perceived intensity on an NRS ranging from 0 (“not detected”) to 100 (“maximal intensity imaginable”).

The intensity of the 3 electrical stimuli delivered before the application of the patch was fixed at .5 mA and was the same for both groups. Directly after removal of the patch, the stimulation intensity of the 3 single electrical stimuli delivered in the control group was the same as the one before application of the patch (.5 mA), while the stimulation intensity of the electrical stimuli delivered in the nocebo group was gradually increased across the 3 stimuli (.5, 1.0, and 2.0 mA). The gradual increase in stimulation intensity across the 3 single stimuli after removal of the patch, combined with the instruction that the intensity was the same as before the patch, in the nocebo group, was intended to strengthen the suggestion that the skin had become more sensitive to electrical stimuli. The single electrical stimuli were not perceived as painful.

Procedure

The experiment was conducted in a quiet laboratory room at the Institute of Neuroscience of UCLouvain, Brussels, Belgium. To keep the experimenter blinded to the condition of the participants (nocebo or control) during the assessment of mechanical pinprick sensitivity, the experiment was conducted by 2 experimenters (experimenter 1 and 2). At the beginning of the experiment, participants were informed about the purpose of the study by experimenter 1. They were told that the aim of this study was to investigate the effect of capsaicin, a substance that sensitizes peripheral cutaneous nociceptors when applied onto the skin, on pain elicited by intense electrical stimulation (HFS). They were further told that they would be assigned to either the experimental group in which they would receive

the patch with capsaicin or to a control group, in which the patch contained water that served as control for the experimental group. Then, the same experimenter performed the baseline measurement of mechanical pinprick sensitivity (T0). After that, experimenter 1 left the room and experimenter 2 entered. Experimenter 2 then randomly assigned the participant to either the nocebo group or control group and continued with the experimental manipulation of expectations.

After the manipulation, experimenter 2 explicitly instructed the participants not to inform experimenter 1 about their condition (nocebo or control) and left the room. Experimenter 1 entered again and started assessing the expected pain and fear for HFS. After that, a short explanation of the HFS protocol followed. This included the number of stimuli, the nature of the stimulation (painful electrical stimuli), and the instruction to provide a rating directly after each HFS stimulus. Then, HFS was applied to the right ventral forearm and at different timepoints (T1, T2, T3, and T4), the perceived pinprick sensitivity was measured at both arms as well as the length of the area of increased pinprick sensitivity at the HFS arm at T2, T3, and T4. At the end of the experiment, participants were verbally asked 1) what they thought would be the hypothesis of this experiment; 2) how credible the information given during the experiment was, measured on a NRS ranging from 0 (“not credible”) to 100 (“fully credible”); and 3) how honest they thought the experimenters were, ranging from 0 (“not honest”) to 100 (“fully honest”). Participants were debriefed after the experiment.

Data Analyses

Manipulation Checks

To confirm if our manipulation of the expectations of HFS painfulness was successful, we compared the scores of expected painfulness of HFS (NRS 0–100) between the 2 groups. We also compared the perceived intensity elicited by the first single electrical stimulus after removal of the patch between the 2 groups.

Primary Outcomes

The primary outcomes of this study were 1) the difference in perceived mechanical pinprick intensity (NRS), averaged across 3 post measurements (T2, T3, and T4), between the 2 arms (corrected for the baseline measurement) and between the 2 groups; and 2) the difference in proximal-distal length (cm) of the area of increased pinprick sensitivity, averaged across T2, T3, and T4, at the HFS-treated arm between the 2 groups. The reason for choosing the average of 3 post measurements (T2, T3, and T4) is to increase accuracy in the estimation of pinprick hypersensitivity.

Secondary Outcomes

The secondary outcomes in this study were 1) the perceived pain intensity (NRS 0–100) elicited by HFS, 2) the subjective fear intensity (NRS 0–100), 3) the difference in perceived mechanical pinprick intensity (NRS 0–100)

measured directly after HFS between both arms (corrected for the baseline measurement) and groups, 4) the perceived credibility of the information given during the experiment (NRS 0–100), and 5) the perceived honesty of the experimenters (NRS 0–100).

Statistical Analyses

Data were analysed using the statistical package SPSS version 28 (SPSS Inc, Chicago, IL). The critical *P* value was set at .05 (2-sided).

Manipulation Checks

Expected pain for HFS

To confirm that our manipulation of expectations was effective, we performed an independent sample t-tests on the expected painfulness of HFS scores measured before HFS.

Perceived intensity elicited by the single electrical stimuli after the patch

To test if the first rating elicited by the single electrical stimulus directly after removal of the patch was significantly different between the 2 groups, we performed an ANalysis COVAriance (ANCOVA) with the first rating after removal of the patch as a dependent variable, “Group” as independent (fixed) factor, and the average of the 3 ratings before the application of the patch (“Baseline”) as covariate.

Primary Outcomes

Perceived pinprick intensity

To test if the perceived intensity elicited by the mechanical pinprick stimuli was different after HFS between the 2 groups, we conducted a linear mixed model (LMM) (restricted maximum likelihood and Satterthwaite approximation) with the NRS ratings as a dependent variable, the factor "Arm" (control arm vs HFS arm) and "Group" (nocebo vs control) as fixed factors, the "Baseline Pinprick Ratings" as covariate, and the factor "SUBJECT" as random factor.

Length of the area of increased pinprick sensitivity

To test if there was a difference in the length of the area of increased pinprick sensitivity between the 2 groups, we performed an independent sample t-test on the total length in centimetres (averaged across T2, T3, and T4) between the 2 groups.

Secondary Outcomes

Independent sample t-tests were performed on the perceived pain intensity measured during HFS (average across the 5 trains) and the fear for HFS report measured before HFS.

To test if the first NRS rating elicited by the mechanical pinprick stimulus directly after the application of HFS was significantly different between the 2

groups, we performed a linear mixed model with the first mechanical pinprick rating after HFS as a dependent variable, “Arm” and “Group” as independent (fixed) factors, the “Baseline Pinprick Ratings” as covariate, and “Subject” as random factor.

Finally, to assess differences in the perceived credibility of the information given during the experiment and the perceived honesty of the experimenters between the 2 groups, we performed an independent sample t-test for each exit question.

The effect size in the LMM (η^2p) was calculated using the EffectSize package in the free online available statistical software R. To calculate effects sizes for independent t-tests, we used Cohen’s *d*.

IV.3. Results

Sample Demographics

The mean + SD age was 23.2 ± 2.7 for the control group (12 women and 13 men) and 22.2 ± 2.4 for the nocebo group (15 women and 10 men).

Manipulation Check

Expected Painfulness of HFS

The independent sample t-test performed on the ratings of expected painfulness of HFS revealed no significant difference (t[48] = .036, *P* = .971,

Cohen's *d* = .01) between the 2 groups (M_{nocebo} = 42.88 vs $M_{control}$ = 43.08).

Ratings Elicited by the First Single Electrical Stimulus After Removal of the Patch

The ANCOVA revealed a significant effect of "Baseline" ($F[1,47] = 65.707$, $P < .001$, $\eta^2p = .58$), meaning that the average (across the 3 stimuli) baseline ratings elicited by the single electrical stimuli affected the ratings to the single electrical stimuli delivered after removal of the patch. We found a significant effect of "Group" ($F[1,47] = 22.109$, $P < .001$, $\eta^2p = .32$), meaning that the ratings elicited by the first single electrical stimulus after removal of the patch were significantly different between the 2 groups after correcting for the baseline ratings ($M_{control}$ = 12.22 vs M_{nocebo} = 23.78, **Figure 2A**). **Figure 2B** shows the mean (+SD) ratings elicited by each single electrical stimulus before and after the patch.

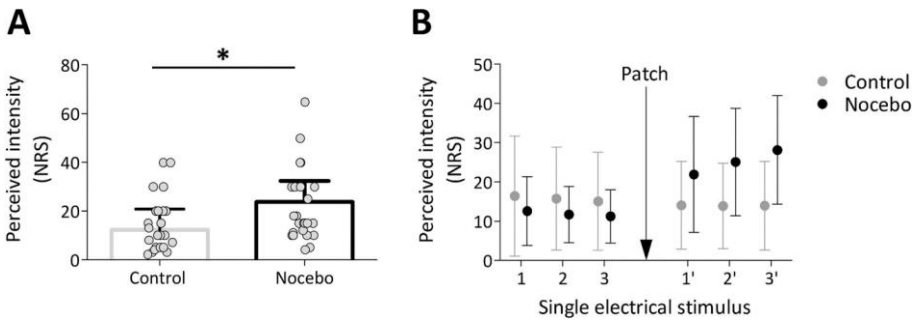

Figure 2. (**A**) Estimated marginal means (+SD) of the perceived intensity elicited by the first single electrical stimulus, delivered after removing the patch, for the control

and nocebo group. Dots are representing individual data. (**B**) Mean ratings (+SD) of each single electrical stimulus applied before and after the patch.

Primary Outcomes

Perceived pinprick intensity after HFS

The linear mixed model revealed a significant main effect of "Baseline Pinprick Rating" ($F[1,68.939] = 49.347$, $P < .001$, $\eta^2p = .42$), meaning that the pinprick ratings after HFS were influenced by the pinprick ratings before HFS. We also found a significant main effect of "Arm" ($F[1,46.043] = 53.681$, $P < .001$, $\eta^2p = .54$), meaning that the pinprick ratings after HFS across all participants differed between the 2 arms after controlling for the baseline pinprick ratings ($M_{HFS\ arm} = 22.88$ vs $M_{control\ arm} = 9.887$).

No significant main effect of "Group" ($F[1,46.437] = .252$, $P = .618$, $\eta^2p = .00$) or "Arm × Group" interaction ($F[1,46.104] = .793$, $P = .378$, $\eta^2p = .02$) was found. The estimated marginal means (+SD) of the perceived intensity elicited by the mechanical pinprick stimuli at each arm (control arm and HFS arm) in each group (control and nocebo) are shown **in Figure 3**.

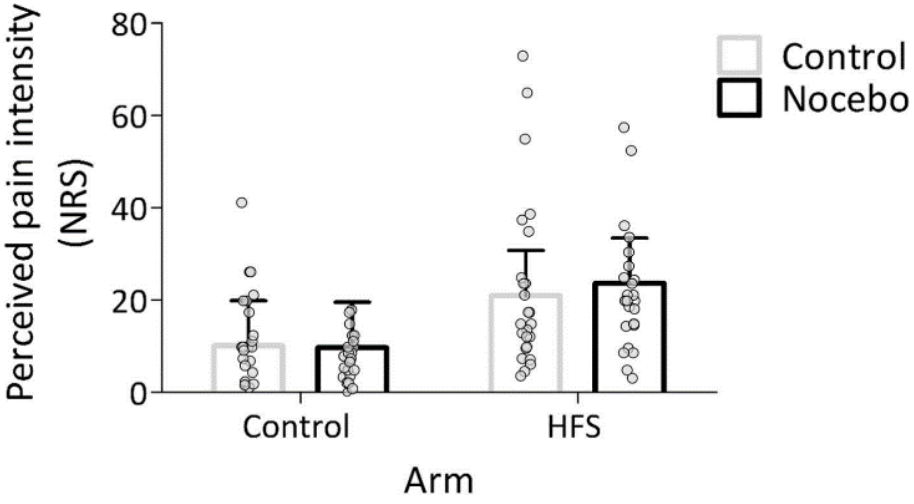

Figure 3. Estimated marginal means (+SD) perceived mechanical pinprick intensity after HFS and after controlling for baseline pinprick sensitivity for the control and HFS arm in the nocebo and control group. Dots are representing individual data.

Length of the area of increased pinprick sensitivity after HFS

An unpaired t-test performed on the individual length (averaged across timepoints T2, T3, and T4) between the 2 groups (t[48] = .068, *P* = .946, Cohen's *d* = .02) revealed no statistically significant difference (M_{nocebo} = 8.95 cm vs $M_{control}$ = 8.66 cm, **Figure 4**).

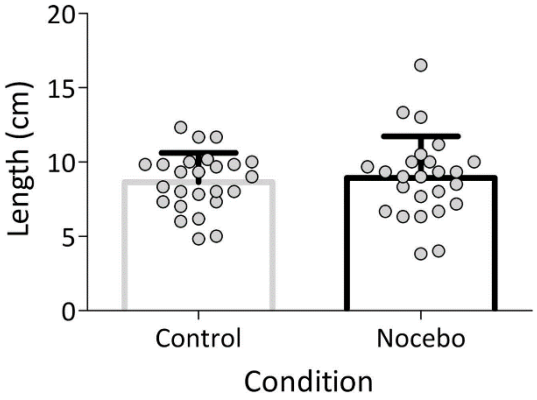

Figure 4. Mean (+SD) length (averaged across timepoints T2, T3, and T4) of the area of increased pinprick sensitivity. Length is expressed in centimetres (cm). Dots are representing individual data.

Secondary Outcomes

Experienced pain during HFS

The independent sample t-test performed on the ratings of pain elicited by HFS revealed no significant difference (t[48] = −.511, *P* = .612, Cohen's *d* = −.15) between the 2 groups (M_{nocebo} = 67.46 vs $M_{control}$ = 65.06, **Figure 5A**). **Figure 5B** shows the mean (+SD) pain ratings elicited by each HFS train for both groups.

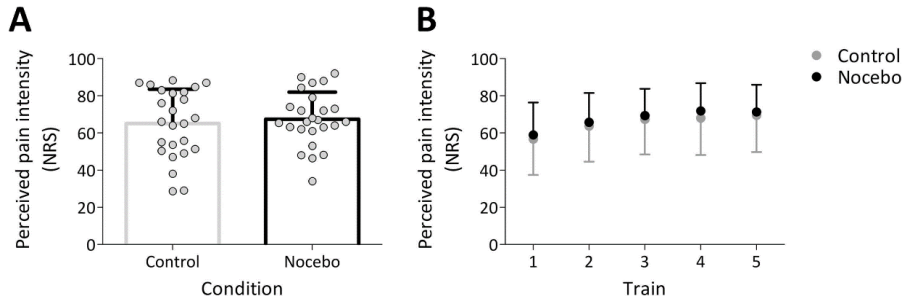

Figure 5. (**A**) Mean (+SD) of the experienced pain during HFS for the 2 groups (control and nocebo). Dots are representing individual data. (**B**) Mean (+SD) experienced pain for each train of both groups.

Fear for HFS

The independent sample t-test performed on the ratings of fear for HFS revealed no significant difference (t[48] = −.046, P = .964, Cohen's d = −.01) between the 2 groups (M_{nocebo} = 27.20 vs $M_{control}$ = 26.92).

Perceived mechanical pinprick intensity directly after HFS

The linear mixed model revealed a significant effect of "Baseline Pinprick Rating" (F[1,93.376] = 64.804, P < .001, η^2p = .41), meaning that the pinprick ratings directly after HFS are influenced by the baseline pinprick rating. We also found a significant effect of "Arm" (F[1,52.538] = 32.862, P < .001, η^2p = .38), meaning that the pinprick ratings after HFS across all participants differed between the 2 arms after controlling

for baseline pinprick ratings ($M_{Control\ arm}$ = 9.753 vs $M_{HFS\ arm}$ = 15.527). There was no significant effect of "Group" (F[1,54.354] = .798, *P* = .376, η^2p = .01) and "Arm × Group" interaction (F[1,52.627] = .259, *P* = .613, η^2p = .00).

Perceived credibility of the information

The ratings of perceived credibility of information were high in both groups (M_{nocebo} = 96.60 vs $M_{control}$ = 98.20), meaning that people in both groups believed the information they received. An independent sample t-test showed that the level of credibility was not significantly different between the 2 groups (t[48] = .646, *P* = .521, Cohen's *d* = .19).

Perceived honesty of the experimenters

The ratings of perceived honesty of the experimenters were high in both groups (M_{nocebo} = 96.60 vs $M_{control}$ = 96.80), meaning that the participants perceived the experimenters as honest. An independent sample t-test showed that the level of honesty was not significantly different between the 2 groups (t[48] = .073, *P* = .942, Cohen's *d* = .02).

Exploratory Analysis

A significant and moderately strong correlation (Pearson r = .58, $P < .01$) was found between the expected painfulness of HFS and the actual reported pain during HFS (averaged across the 5 trains) across all participants (**Figure 6**).

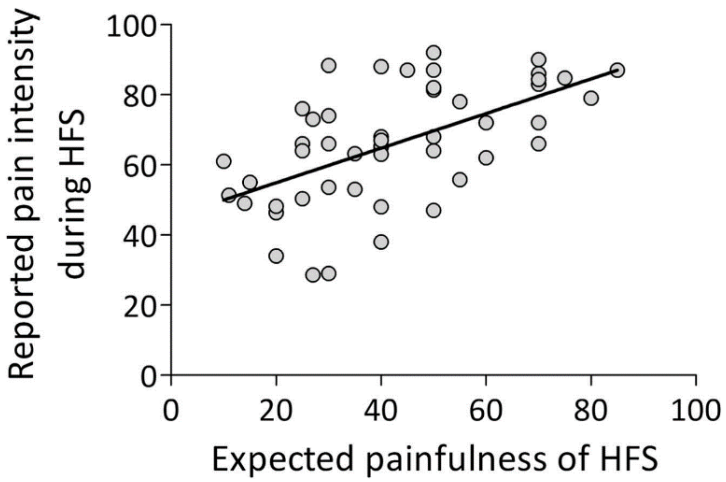

Figure 6. Correlation between the expected painfulness of HFS and the actual reported pain during HFS across all participants. Dots are representing individual data.

IV.4. Discussion

The present study aimed to investigate if expecting more pain increases HFS-induced pain and pinprick hypersensitivity in healthy volunteers. We hypothesized that expecting more pain for HFS would increase pain ratings

during HFS and would promote the development of pinprick hypersensitivity, resulting in a larger spread and more intense pinprick hypersensitivity.

During the manipulation, we found that the nocebo group reported a higher intensity rating to the first single electrical stimulus after removing the patch compared with the control group. Since the stimulation intensity of this electrical stimulus was the same for both groups, this finding indicates that the participants in the nocebo group felt the stimulus more intense and/or were convinced that their skin indeed had become more sensitive after the patch. Surprisingly, the nocebo group did not provide higher ratings for the expected painfulness of HFS compared with the control group and did not rate the pain during HFS as more intense compared with the control group. Hence, the expectation about the single stimulus did not transfer to the HFS stimuli, despite both being electrical stimuli.

Nevertheless, exploratory analyses showed a significant and moderate correlation between the expected painfulness of HFS, and the actual pain intensity reported during HFS, suggesting that expectations may influence pain experiences[284, 286].

The threat that an intervention will increase pain may induce fear of pain and may facilitate the nocebo response[298]. In the present study, no differences were found in the reported fear for HFS between the 2 groups, indicating that the manipulation in the nocebo group did not induce more fear for HFS compared with the control group. A recent study found a mediating role of fear in nocebo hyperalgesia but only if high pain is experienced and not when it is merely anticipated[299]. The participants in the present study were naive

regarding HFS and did not experience HFS before the manipulation. Future studies may include an example HFS train at the beginning of the experiment.

On the other hand, the participants in the study of Torta et al. (2023) also did not experience HFS before, but participants in the "high pain" group reported significantly higher pain ratings during HFS compared with the "low pain" group[295]. Moreover, no significant difference in the self-reported fear between the 2 groups was found.

Fear generalization is when fear acquired to one stimulus transfers to another stimulus. Fear conditioning experiments have shown that this is the case for stimuli that are perceptually similar[300]. One possibility is that the single electrical stimuli applied after the patch are perceptually too different compared with the HFS, and therefore the acquired fear during the manipulation may not have transferred to the high-frequency stimuli.

Another possibility could be that expectations created for *nonpainful* electrical stimuli do not generalize to expectations for *painful* electrical stimuli, because of the different qualitative nature of the stimuli. This is supported by a recent study showing that nocebo effects on cowhage-evoked itch generalized to mechanically evoked itch but not to mechanically evoked touch[301]. Another study, conducted by the same group, showed that nocebo effects on pain may generalize within but not across stimulus modalities[302]. It would be interesting to repeat this experiment but using painful single electrical stimuli.

A recent study found that the effect of a cue has less effect on pain when the prediction error is large[303]. The expectation of HFS pain was on average 40

on the NRS, but the averaged pain intensity during HFS around 60. Any effect of the electrode (cue), through which the single electrical stimuli were delivered before and after the patch, could have been mitigated by the (large) difference between expected pain for HFS and the actual pain experienced during HFS.

Importantly, the perceived credibility of the information as well as the perceived honesty of the experimenters were on average very high and not different between the 2 groups, indicating that the participants trusted the information given during the experiment and the experimenters. On the other hand, these ratings were asked verbally and therefore it cannot be ruled out that they were affected by the experimenter demand effect.

Because we were not able to manipulate the expectations of HFS pain differently between the 2 groups, it is difficult to interpret the lack of changes in the HFS-induced spread and intensity of increased pinprick sensitivity.

Previous studies have shown an average increase in pinprick sensitivity after HFS of 20 points on the NRS when using an HFS stimulation intensity corresponding to 20× the detection threshold to a single electrical stimulus[80, 118]. The average detection threshold for this type of electrode is usually around .3 mA[80, 118]. The HFS stimulation intensity in the present study was 3 mA that corresponds to an intensity of 10× the individual detection threshold to a single electrical stimulus. In the present study, the increase in pinprick ratings after HFS was on average 10 points on the NRS (in the control group), which is half of the increase compared with HFS studies that used a 20× detection threshold HFS intensity. Based on this, we believe that the lack

of difference in pinprick hypersensitivity after HFS between the 2 groups is most likely not due to a ceiling effect in the increase in pinprick ratings.

A previous study found that expectations of pain reduction directed to 1 body part did not transfer to other body parts, suggesting that placebo effects are somatotopic-specific[304]. In the present study, pinprick hypersensitivity was tested adjacent to the site at which expectations of pain increase were directed and could explain a lack of effect. However, it is unclear if somatotopic specificity is present for nocebo effects and how narrow the spatial specificity is.

When performing exploratory post hoc analyses, no associations between the averaged pain during HFS and the length or intensity of increased pinprick sensitivity were found. In contrast, Torta et al. (2023) found a significant correlation between HFS pain and the intensity of increased pinprick sensitivity. It is unclear why ratings of the painfulness of HFS are sometimes correlated and sometimes not to the increased pinprick sensitivity. They also also observed a higher average pain elicited by HFS in their experimental group compared with their control group, but no changes were observed in the length of the area of HFS-induced increased pinprick sensitivity[295]. This latter suggests that experiencing more pain as a consequence of expecting more pain does not necessarily result in a larger spread of increased pinprick sensitivity. However, they did find a significant 3-way interaction between time, side, and group for the pinprick ratings, suggesting that the increase in mechanical pinprick sensitivity after HFS at the HFS-treated arm was larger in the “high pain” group compared with the “low pain” group. However, no follow-up comparisons were statistically

significant, and the effect size was rather small; therefore, an alternative explanation could be that the significant 3-way interaction is a chance finding.

IV.5. Conclusion

To conclude, despite the finding that participants in the nocebo group reported a higher perceived intensity to the single electrical stimulus after the patch compared with the control group, they did not expect HFS to be more painful and did not report more pain during HFS compared with the control group. Because the expectations about the HFS painfulness were not significantly different between the 2 groups, and no differences were observed in the pain ratings during HFS, it is difficult to interpret the lack of differences in HFS-induced pinprick hypersensitivity between the 2 groups. However, our data do provide evidence for an association between expectations and subsequent pain experiences.

V. Chapter 5. Preoperative susceptibility to developing secondary hyperalgesia is associated with post-thoracotomy pain at two months

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Abstract

Background: Persistent pain is frequent after thoracotomy. Identifying the subset of patients at risk for persistent post-thoracotomy pain preoperatively is clinically important, as they could benefit from targeted prevention measures. In this prospective cohort study, we investigated if the preoperatively assessment of individual susceptibility to developing experimentally induced secondary mechanical hyperalgesia predicts post-thoracotomy pain at two months.

Methods: Patients scheduled to undergo a posterolateral thoracotomy were recruited before surgery and followed prospectively for two months. Of the forty-one patients that were recruited only twenty could be included. The day before surgery, we experimentally induced secondary mechanical hyperalgesia at one of the two forearms and measured the change of perception to mechanical pinprick stimuli and the spread of hyperalgesia. On postoperative day 4, day 15 and at the 2-month follow-up, patients were asked about their pain intensity at rest and during coughing and the area of secondary mechanical hyperalgesia around the scar as well as the change in perception to mechanical pinprick stimuli was measured.

Results: Forty percent reported pain at the two months follow-up. All of them reported cough-evoked pain and ten percent also reported pain at rest. A binary logistic regression model with both the magnitude and extent of experimentally induced secondary mechanical hyperalgesia was statistically significant (Chi2=12.439, P=.002, McFadden R2 = .462) and showed excellent discriminative power (AUC=.938) for the presence or absence of cough-evoked pain at the two-month follow-up.

Conclusion: Our findings indicate that the individual susceptibility to developing experimentally induced secondary mechanical hyperalgesia preoperatively may identify patients who are potentially vulnerable to develop persistent post-thoracotomy pain.

V.1. Introduction

Persistent pain is frequent after thoracotomy, with a reported prevalence between 30% and 60%[180, 305]. Identifying the subset of patients at risk for persistent post-thoracotomy pain is clinically important, as they could benefit from targeted pre-emptive measures.

It is believed that central sensitization, defined by the International Association for the Study of Pain (IASP) as "increased responsiveness of nociceptive neurons in the central nervous system to their normal or subthreshold afferent input", contributes to persistent pain[42, 306-309]. Secondary mechanical hyperalgesia, the increased mechanical pinprick sensitivity of the skin surrounding cutaneous tissue injury, is considered a perceptual correlate of central sensitization[29, 42, 310].

Using a prospective design, Martinez et al. (2012) showed that patients who suffered from postsurgical neuropathic pain at three months had larger areas of incision-induced secondary mechanical hyperalgesia in the immediate postoperative period[311]. Moreover, clinical studies have shown that analgesic protocols that reduce the area of incision-induced secondary mechanical hyperalgesia also reduce pain at three and six months[312-315]. While other prospective studies did not find a relationship between the area of incision-induced secondary hyperalgesia and pain at six months, Momeni et al. reported that significantly more patients with pain at six months had mechanical hyperalgesia at postoperative day 5[316, 317]. Taken together, the relationship between the size of the area of incision-induced secondary

mechanical hyperalgesia and the development of persistent postsurgical pain remains unclear[318].

Importantly, the aforementioned studies focused on incision-induced secondary hyperalgesia, which can be affected by several factors (e.g., extent of wound surgery, wound complications, analgesic treatment, etc.). Moreover, one would ideally need a preoperative measure to identify at-risk patients as early as possible.

Secondary hyperalgesia can be induced experimentally, and a previous study found that the areas of experimentally heat-induced secondary hyperalgesia and incision-induced secondary hyperalgesia after gynaecology surgery moderately correlated[57, 192]. This raises the intriguing question of whether interindividual variations in the preoperative susceptibility to develop experimentally induced secondary hyperalgesia may also predict who will develop persistent postsurgical pain. To the best of our knowledge, no clinical studies have investigated this yet.

We have shown that noxious electrical cutaneous stimulation can induce secondary mechanical hyperalgesia[80, 118, 119]. Electrical stimulation has the advantage that it can be applied in a highly standardized and well-controlled manner. By comparing different frequencies of electrical stimulation, we found that middle-frequency stimulation (MFS) induced maximal secondary mechanical hyperalgesia in healthy human volunteers[80]. A follow-up reliability study further showed that the area of MFS-induced secondary mechanical hyperalgesia is highly reliable[78].

The aim of this prospective cohort study was to investigate, in patients scheduled to undergo thoracotomy for the treatment of lung cancer, if the extent of MFS-induced secondary mechanical hyperalgesia before surgery predicted the presence of pain at two months. The two-month endpoint was chosen to avoid potential confounding of adjuvant treatments (e.g., chemotherapy) to the development of persistent pain[319]. We hypothesized that individuals who develop greater experimentally induced secondary mechanical hyperalgesia before surgery are more likely to have pain two months after surgery.

V.2. Methods

Patients

The study was approved by the Comité d'Éthique Hospitalo-Facultaire Saint-Luc, UCLouvain (B403201940443) and conducted according to the Declaration of Helsinki. The study was pre-registered on clinicaltrials.gov (NCT04220697). We estimated that a total of 70 patients would be needed. The sample size calculation was based on a logistic regression analysis of a binary response variable (presence/absence of pain at two months) and a continuous, normally distributed variable (perceived pinprick intensity). Patients were recruited between January 2020 and December 2023 at the Department of Cardiovascular and Thoracic Surgery of the Cliniques Universitaires Saint-Luc in Brussels, Belgium.

Inclusion criteria were: 1) being between 18 and 80 years old, 2) being scheduled for a pulmonary anatomical lung resection by posterolateral thoracotomy to treat primary lung cancer, and 3) being able to provide written informed consent. Exclusion criteria were: 1) evidence for a clinically significant alteration of the skin of the volar forearms, 2) being pregnant, and 3) having a pacemaker or implanted cardiac defibrillator.

Figure 1 details the patient selection. At the end of the study period, forty-one eligible patients were included. All patients signed an informed consent. Of those forty-one patients, we had to exclude nine patients because of problems with data collection. Furthermore, seven patients were excluded because they did not tolerate the pain induced by the MFS stimulation and requested to withdraw. One patient was excluded because his surgery was rescheduled some months after the preoperative assessment. Finally, we were not able to do the two-month follow-up in four patients. Therefore, the final analysis included 20 patients (12 males/8 females, mean (±SD) age: 65.6 years ± 12.5, ranging from 81 to 39 years).

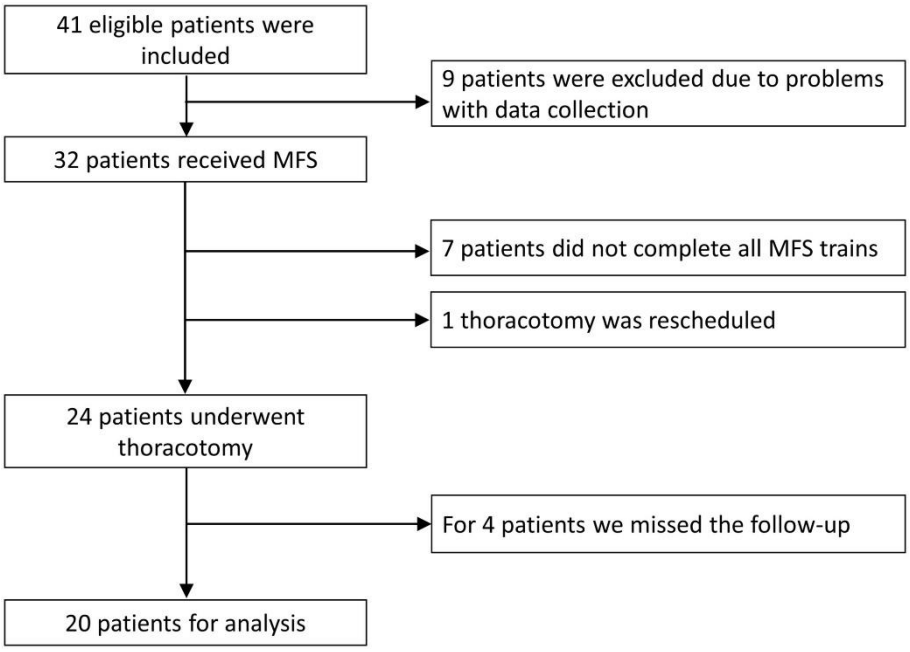
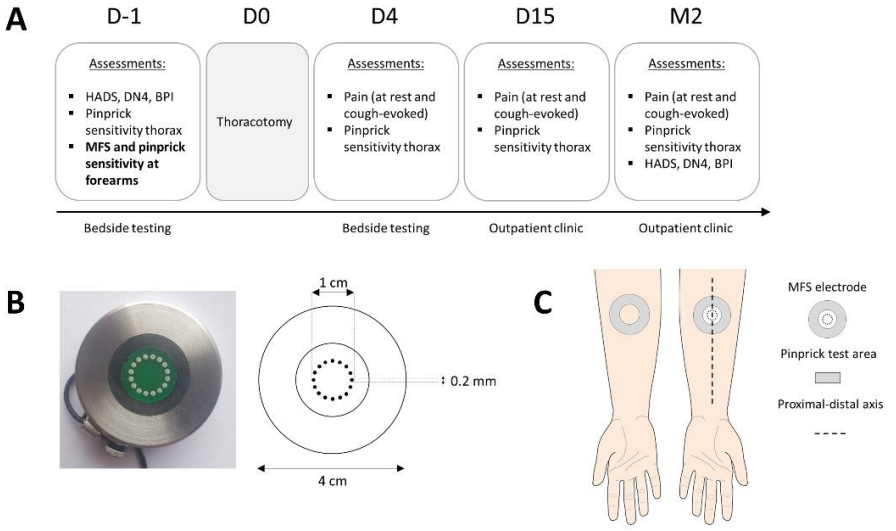


Figure 1. Flowchart of patient selection (see text).

Experimental design

Figure 2A shows the study design. On the day prior to their surgery (D-1), patients were asked to fill out a set of questionnaires about the presence and impact of pain (Brief Pain Inventory), whether this pain present some neuropathic components (DN4) and about anxiety and depression (Hospital Anxiety and Depression Scale). After that, the mechanical pinprick sensitivity of the chest skin of the side that had to be operated was assessed. Then, the mechanical pinprick sensitivity of the skin at both forearms was assessed before and after MFS was applied to induce secondary mechanical hyperalgesia at one of the two forearms. The next day (D0) patients

underwent surgery (thoracotomy). Four days after their surgery (D4), patients were asked about the intensity of their pain at rest and during coughing. Furthermore, the mechanical pinprick sensitivity around the scar was assessed (intensity of perception and area of secondary mechanical hyperalgesia). Those same assessments were repeated at fifteen days (D15) and two months (M2) after surgery. At the 2-month follow-up, patients were also asked to fill out the same questionnaires as the ones on the day before



surgery (D-1).

Figure 2. A. Design of the study (see text). D-1 = day before surgery, D0 = day of surgery, D4 = day 4 after surgery, D15 = day 15 after surgery, M2 = two months after surgery. MFS = middle-frequency electrical stimulation to induce secondary mechanical hyperalgesia. HADS = Hospitality Anxiety and Depression Scale, DN4 = Douleur Neuropathique 4 Questionnaire, BPI = Brief Pain Inventory. **B**. Characteristics of the MFS electrode. **C**. MFS was applied through the MFS electrode. Changes in pinprick sensitivity were assessed before and after MFS within the grey

areas. The length of the area of increased pinprick sensitivity was assessed along the proximal-distal axes at the arm that received MFS.

Conditioning stimulation

MFS was used to induce secondary mechanical hyperalgesia on the skin of the volar forearm skin. MFS consisted of twelve trains of 42 Hz biphasic charge-compensated electrical pulses[80]. Each biphasic pulse consisted of a 2-ms square-wave pulse followed, after a 0.1-ms delay, by a 4-ms compensation pulse of opposite polarity having half the intensity of the first pulse. Each train lasted one second and was delivered in a 10-second interval. The total duration of the stimulation protocol was two minutes.

MFS was applied to one of the two forearms approximately 10 cm from the cubital fossa. The electrical pulses were triggered by a digital-analog interface (National Instruments, Austin, USA) controlled by MATLAB 2014B (The MathWorks Inc., Natick, USA) and delivered using a constant current electrical stimulator (Digitimer DS5, Digitimer, UK) via a specially designed electrode (**Figure 2B**). The electrode consists of 16 blunt stainless-steel pins with a diameter of 0.2 mm protruding 1 mm from the base. The 16 pins are placed inside a circle with a diameter of 10 mm and serve as the cathode. A stainless-steel reference electrode that serves as the anode is concentrically located and has an inner diameter of 22 mm and an outer diameter of 40 mm. The intensity of MFS was individually adjusted to twenty times the detection threshold to a single non-charge-compensated monophasic pulse (pulse width: 2 ms). The detection threshold was determined using the method of limits. During the MFS stimulation, patients were instructed to

rate each train on a Numeric Rating Scale (NRS) that ranged from 0 (“No pain”) to 100 ("Maximum pain imaginable").

Assessment of MFS-induced secondary hyperalgesia

The assessment of MFS-induced secondary mechanical hyperalgesia was performed as described in Cayrol et al. (2020), using a 128 mN calibrated mechanical pinprick stimulator (MRC Systems, 200 Heidelberg, Germany)[78]. Mechanical pinprick sensitivity was evaluated before and 30, 35, and 40 minutes after applying MFS. At each time-point a total of three stimuli were applied to the forearm receiving MFS within a circle of 1.5 cm outside the cathode and to the homologous skin of the contralateral control arm (**Figure 2C**). The pinprick stimuli were delivered perpendicular to the skin and were never delivered twice at the exact same location, to avoid sensitizing the skin. After each pinprick stimulus patients were asked to rate the perceived intensity on a Numeric Rating Scale (NRS) ranging from 0 (“No detection”) to 100 (“Maximal pain imaginable”), with 50 representing the transition from nonpainful to painful domains of sensation. At all three post-MFS measurements, after collecting the perceived intensity ratings, we also estimated the length of the area of secondary mechanical hyperalgesia at the MFS-stimulated arm along the proximal-distal axis of the volar forearm (**Figure 2C**). For this, we used the same pinprick stimulator to stimulate the skin along the proximal-distal axis originating from the centre of the area at which MFS was delivered. Mechanical pinprick stimulation started close to the cubital fossa and just before the wrist. Each stimulus was separated by steps of 1 cm, at a pace of 1-second stimulation and 1-second interval, in the

direction of the MFS site[78]. During the mapping, patients were instructed to keep their eyes closed and to say “now” when they felt a clear increase of pinprick sensitivity. Then, the pinprick stimulation was delivered in steps of 0.5 cm before and after this point to confirm the border.

Assessment of postsurgical pain

For evaluating the intensity of clinical postsurgical pain, it is recommended to differentiate between pain at rest and movement-evoked pain[320]. At day 4 (D4), day 15 (D15) and 2-months (M2), patients were asked to evaluate their pain intensity at rest and during coughing using a NRS ranging from 0 (“No pain”) to 100 (“Maximal pain imaginable”). For cough-evoked pain, patients were instructed to cough while sitting upright. Pain at rest was always asked before cough-evoked pain.

Thoracic surgery

Patients underwent a posterolateral thoracotomy (5-6th intercostal space) for anatomical resection of lung cancer. All thoracotomies were limited, muscle-sparing (Serratus muscle) and performed by the same surgeon (VL).

Anaesthesia and postoperative analgesic treatment

Patients were premeditated with alprazolam 0.5 mg or 1 mg. General anaesthesia was induced with sufentanil (max 0.2 mcg/kg), propofol (1-2 mg/kg), rocuronium or atracurium (0.5 mg/kg, adjusted according to

neuromuscular monitoring), and ketorolac (0.5 mg/kg and max 30 mg). General anaesthesia was maintained with a continuous administration of propofol or sevoflurane, titrated based on the intraoperative EEG. An epidural catheter was inserted before induction of general anaesthesia in all patients, unless contraindicated or technically impossible. Intraoperative analgesia was achieved with a levobupivacaine 0.5% and sufentanil bolus, followed by a continuous infusion or iterative boluses every 50 minutes (levobupivacaine 0.25%). Postoperative analgesia was provided by patient-controlled epidural analgesia (PCEA, levobupivacaine). Patients without epidural received intraoperative ketamine (0.5 mg/kg followed by 0.25 mg/kg/h) and postoperative patient-controlled intravenous analgesia (PCIA, morphine or piritramide).

Assessment of mechanical pinprick sensitivity around the scar

At the day before surgery (D-1) the mechanical pinprick sensitivity of the skin of the side to be operated was assessed using the same mechanical pinprick stimulator as for the assessment of pinprick sensitivity before and after MFS. Patients were asked to lay on the non-operated side and a total of three pinprick stimuli were delivered in the region of the future surgical incision. After each stimulus, patients were asked to rate the perceived intensity on a NRS ranging from 0 (“No pain”) to 100 (“Maximal pain imaginable”).

At day 4 (D4), day 15 (D15) and 2-month (M2) follow-up, mechanical pinprick sensitivity of the skin surrounding the scar was assessed using the same pinprick stimulator. First, the area of secondary mechanical hyperalgesia was

determined. For this, pinprick stimulation was applied along 14 radial lines. For each line, the stimulation started far outside the scar and was delivered in steps of 0.5 cm towards the scar until the patient reported a clear increase in pinprick sensitivity, which indicated the border and was marked on the skin. The distance between this point and the scar was measured and noted. The stimulation stopped approximately 0.5 cm before the scar.

Then, three pinprick stimuli were delivered inside the area of secondary mechanical hyperalgesia (at least 0.5 cm from the scar), or if not present, 1 cm around the scar. After each stimulus, patients were asked to rate the perceived intensity on the same NRS as the one used for assessing changes in pinprick sensitivity at the chest at the day before surgery (D-1).

The area size of secondary mechanical hyperalgesia was estimated using a cubic spline interpolation ('interpclosed' function using cubic pchip interpolation; Santiago Benito 2021 MATLAB) across all fourteen points.

Questionnaires

The Hospital Anxiety and Depression Scale (HADS) was designed to detect states of depression and anxiety in the setting of a hospital or a medical outpatient clinic[321]. The scale consists of 14 items. Seven relate to anxiety and seven to depression. Each item is scored on a scale from 0 (absence of symptom) to 3 (maximum symptom severity).

The Douleur Neuropathique 4 questions (DN4) questionnaire was used to screen for a possible presence of neuropathic components. The DN4 consists

of 10 questions assessing sensory descriptors and signs associated with neuropathic pain[322]. A score ≥ 4 is used as a cutoff for possible neuropathic pain.

The Brief Pain Inventory (BPI) was used to evaluate the intensity of pain and its interference in daily activities. It was designed to assess the severity and impact of pain experienced primarily, but not exclusively, by cancer patients[323]. It is divided into two parts: one part enquires about pain intensity (sensory dimension) and the other part about pain interference (reactive dimension). Items are scored on a 0-10 scale, where 0 indicates no pain or interference and 10 indicates the highest imaginable pain or complete interference[323].

Statistical analysis

All statistical analyses were performed in JASP v. 17 (www.jasp-stats.org).

Primary outcome

To answer the question of whether MFS-induced secondary mechanical hyperalgesia predicts the likelihood of having pain (yes or no) two months after surgery, we conducted two univariate logistic regression analyses for each of the variables separately (model 1: MFS-induced change in pinprick intensity; model 2: length of the MFS-induced area of secondary mechanical hyperalgesia) and a multivariable logistic regression analysis with both variables (model 3). The goodness-of-fit of each model was assessed using the chi-square statistic, associated p-value, and the McFadden R-squared,

this latter being considered a measure of "predictive power"[324]. The classification performance of the models was evaluated with the area under the Receiver Operating Characteristic (ROC) curve, which quantifies how well the model can distinguish between positive and negative cases, and the Akaike Information Criterion (AIC) which estimates the prediction error.

Secondary outcomes

We calculated a Pearson correlation to investigate if there was a relationship between the length of the area of MFS-induced secondary mechanical hyperalgesia and the spatial extent of the area of incision-induced secondary mechanical hyperalgesia at D4 after surgery. The area of incision-induced mechanical secondary hyperalgesia was normalized to the length of the scar (which was different across patients) and expressed as its square root.

To assess if incision-induced secondary hyperalgesia at D4 is predictive for pain at two months we conducted two univariate logistic regression analyses: one for the change in perceived intensity (model 1) and one for the extent of the area (model 2), and a multivariate analysis with both variables were included (model 3).

We also investigated whether the reported pain intensity at D4 predicted the presence of pain at two months. For this, we ran two univariate logistic regression analyses: one with the spontaneous pain at D4 (model 1) and one with the evoked pain at D4 (model 2), and a multivariate analysis where we included both variables in the same model (model 3).

V.3. Results

Incidence of pain at two months (M2)

We found that eight patients (40%) reported pain at the two-month follow-up. All these patients reported cough-evoked pain; two of them (10%) reported pain at rest (**Figure 3**). Since cough-evoked pain was more prevalent than pain at rest, we focused our analysis on this type of pain. Table 1 compares pre-, peri-, and postoperative variables between patients with and without cough-evoked pain at two months.

Time	Variables	Without pain at M2 (n=12)	With pain at M2 (n=8)	*P*
Preoperative				
	Gender (M:F)	7:5	5:3	ns
	Age (years)	69.5 (46.0-77.8)	66.0 (50.0-72.3)	ns
	BMI	25.1 (23.9-29.4)	24.0 (22.9-27.6)	ns
	Hypertension (n)	4 (33.3%)	4 (50.0%)	ns
	HADS-Anxiety scale	7.0 (5.0-11.0)	7.0 (4.5-10.5)	ns
	HADS-Depression scale	5.0 (4.0-6.8)	2.5 (2.0-5.8)	ns
	DN4 (n)[1]	0	0	-
	Pre-existing pain (n, %)	2[2] (16.7%)	3[3] (37.5%)	ns
	Pain at this moment[4] (n, %)	0 (0%)	3 (37.5%)	ns
	Pinprick thorax (NRS)	5.8 (2.3-10.9)	18.3 (4.3-27.8)	ns

Perioperative				
	Premedication (n, %)	4 (33.3%)	3 (37.5%)	ns
	Epidural (n, %)	9 (75%)	7 (87.5%)	ns
	Levobupivacaine (mg)	25 (20-25)	25 (25-25)	ns
	Sufentanil 10 µg (n, %)	4 (33.3%)	7 (87.5%)	ns
	Maintenance (ml)	12.5 (8.8-27.5)	12.5 (12.5-15.6)	ns
	Duration surgery (min)	141 (106-177)	148 (123-181)	ns
	Type of method			
	(lob:bilob:wed:seg:lymp)[5]	7:1:2:2:0	3:1:1:1:2	ns
	Complications	4	0	ns
Postoperative				
D0-4	Mode of administration	4:7	1:7	ns

	analgesia (PCIA:PCEA)[6]			
D4	Length of the scar (cm)	10.0 (9.1-13.4)	11.8 (9.8-12.8)	ns
	Pain – at rest (NRS)	2.0 (0.0-10.0)[7]	35.0 (13.8-50.0)	0.0071
	Pain – cough-evoked (NRS)	35.0 (10.0-50.0)[7]	70.0 (46.3-77.5)	0.0145
	Pinprick thorax (NRS)	11.7 (1.3-28.3)[7]	31.7 (11.3-49.2)	ns
	Normalized SH area (cm^2)	14.6 (0.4-39.1)[7]	46.1 (29.2-88.6)	0.0093
D15	Pain – at rest (NRS)	0.0 (0.0-21.3)	22.5 (10.0-40.0)	0.0388
	Pain – cough-evoked (NRS)	20.0 (0.0-36.3)	55.0 (35.0-71.3)	0.0187
	Pinprick thorax (NRS)	13.3 (3.3-33.3)[7]	20.0 (15.0-33.3)[8]	ns
	Normalized SH area (cm^2)	18.3 (12.3-52.5)[7]	36.9 (13.4-46.3)[8]	ns

M2	Pain – at rest (NRS)	0.0 (0.0-0.0)	0.0 (0.0-22.5)	-
	Pain – cough-evoked (NRS)	0.0 (0.0-0.0)	15.0 (11.3-50.0)	-
	Pinprick thorax (NRS)	3.3 (0.0-19.6)[9]	15.8 (10.4-27.1)	ns
	Normalized SH area (cm^2)	5.8 (0.0-31.5)[9]	23.7 (15.5-34.6)	ns
	HADS – Anxiety scale	5.5 (3.3-8.8)	1.5 (0.3-6.0)	ns
	HADS – Depression scale	4.0 (2.3-6.8)	3.0 (0.5-4.8)	ns
	DN4 (n)[1]	0	2	ns
	BPI-Mean Pain Severity	0.0 (0.0-0.2)	1.0 (0.0-2.1)	0.0452
	BPI-Interference	0.0 (0.0-0.0)	0.8 (0.0-2.3)	0.0293

Table 1. Patient characteristics. Number of patients (n) with percentages (%) or medians with interquartile ranges are shown. P = p-value from either a Chi-square test in the case of frequencies or a non-parametric Mann-Whitney U test. BMI = body mass index, HADS = Hospital Anxiety and Depression Scale, DN4 = Douleur Neuropathique 4 Questions, BPI = Brief Pain Inventory, NRS = Numeric Rating Scale.

ns = not significant, - = not possible to run the Mann-Witney U test because of all zeros in the without pain group. D0-4 = Day 0 to 4 after surgery, D4 = day 4 after surgery, D15 = day 15 after surgery, M2 = 2-month follow-up.

[1] : Number of patients that scored ≥4.

[2] : One patient had low back pain, and one patient had shoulder pain.

[3] One patient had low back pain, one patient had pain in the post-nephrectomy belt and one patient had shoulder pain (probably related to a previous thoracotomy).

[4] The number reported here refers to the number of patients reporting pain at that moment and was based on the item BPI-Now.

[5] Refers to Lobectomy: Bi-lobectomy: Wedge resection: Segmentectomy: lymph node dissection.

[6] PCIA = Patient-Controlled Intravenous Analgesia, PCEA = Patient Controlled Epidural Analgesia

[7] N=11

[8] N=7

[9] N=8

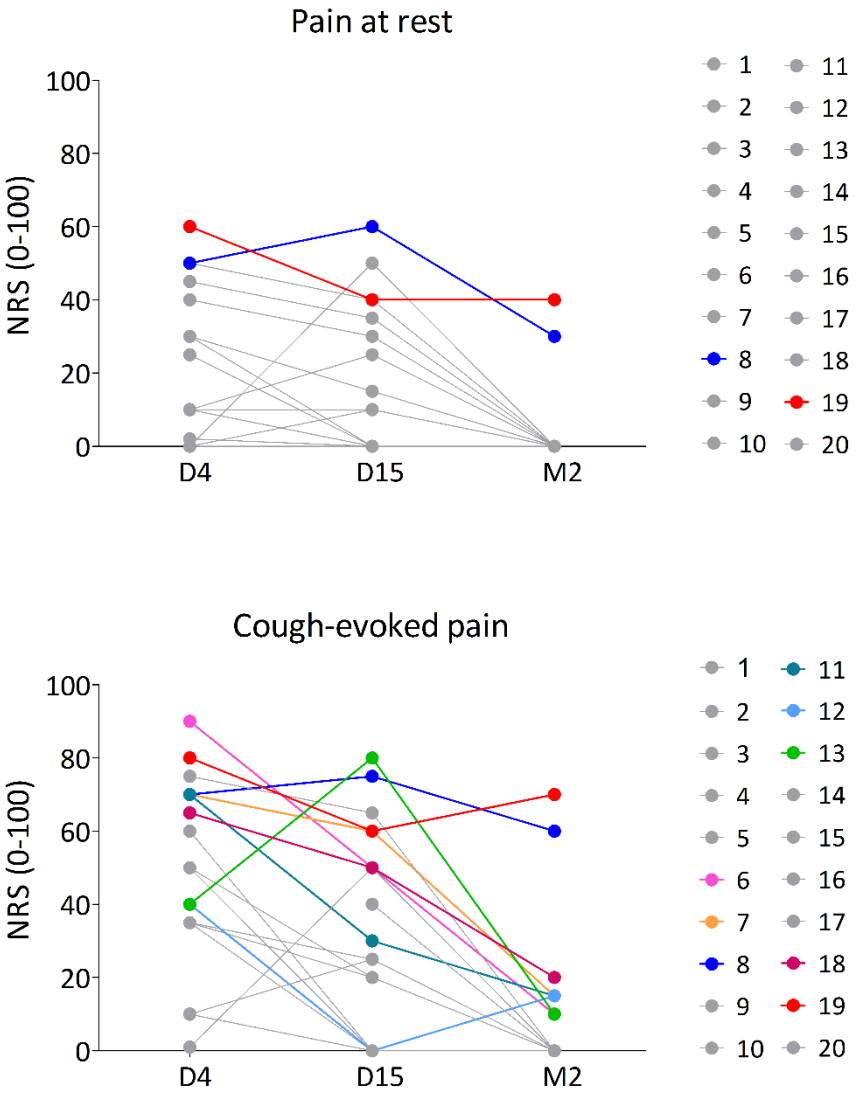


Figure 3. Pain scores for pain at rest (top) and during coughing (below) at D4, D15, and M2 for each patient (numbers on the right). Coloured lines are those patients reporting pain at the 2- month follow-up. NRS = Numeric Ratings Scale.

MFS-induced pain and secondary **mechanical** hyperalgesia

The mean (and SD) electrical detection threshold determined before applying MFS was 0.25 (±.08) mA across all patients. The electrical detection threshold was not significantly different between the patients with and without pain (Mann-Whitney U test: U=29.5, P=.162). The median (and interquartile range) threshold was 0.26 (0.25-.030) mA for the patients without pain and 0.20 (0.12-0.30) mA for the patients with pain.

Figure 4A shows the pain intensity elicited by MFS (averaged across the twelve trains) for both groups (with and without cough-evoked pain at two months). **Figure 4B** shows the pain intensity for each train. **Figure 4C** shows the increase (compared to baseline and control site) in perceived pinprick intensity induced by MFS. **Figure 4D** shows the length of the area of secondary mechanical hyperalgesia.

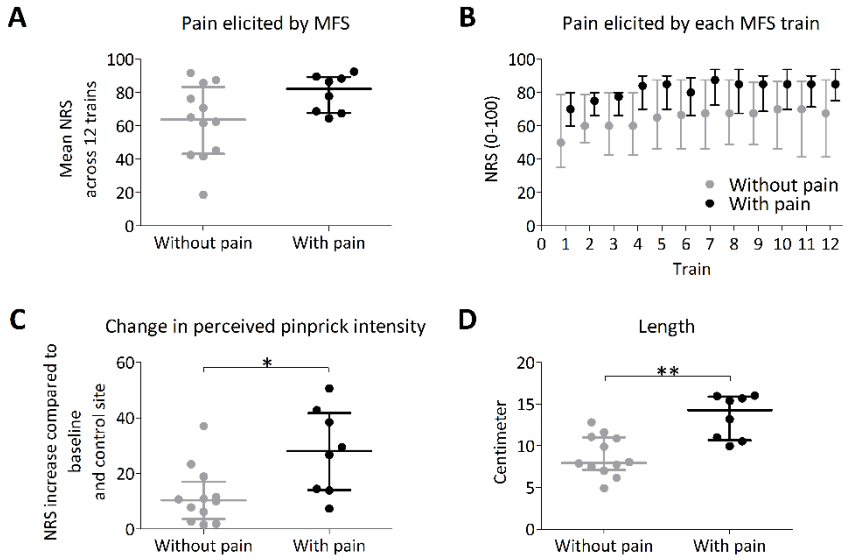


Figure 4. MFS-induced pain and secondary mechanical hyperalgesia compared between patients with and without cough-evoked pain at the two-month follow-up. A. Median pain elicited by MFS (averaged across all twelve trains). B. Median pain induced by MFS for each train. C. Median change in perceived pinprick intensity. D. Median length of the area. Shown are the median and interquartile ranges. Each dot represents a single patient. * = P<.05, ** = P<.01. Significance refers to the Mann-Whitney U test.

Primary outcome

First, we inspected the data for outliers and multicollinearity. We found no values larger than 1 at the Cook's Distance test and no values larger than 3 for the Standardized Residuals. We found that the length of hyperalgesia and change in perceived intensity were correlated (Pearson's r=.605, P=.005), but less than .8 (critical threshold). The Variation Inflation Factor (VIF) was 1.173 (threshold for multicollinearity is at 4).

The logistic regression results are shown in Table 2. All three models were statistically significant, indicating that they all provide a significantly better fit to the data than a model without predictors. The McFadden R-squared is larger for models 2 and 3 compared to model 1, indicating that these models have more predictive power. According to the area under the ROC curve (AUC), models 1 and 2 performed excellently (AUC >.8, Table 2), and model 3 outstandingly (AUC > .9). The AIC of models 2 vs. 3 were comparable (model 2: 18.95 and model 3: 20.48) using the criterion of a difference of less than two points. However, the AIC of model 1 was larger (24.28) compared to model 3.

The univariate logistic regression analyses (models 1 and 2) revealed a significant positive coefficient for both the change in perceived intensity and the length of the area (Table 3). A positive coefficient means that an increase in the unit of the predictor is associated with an increase in the probability of having pain two months after surgery. For example, in model 2 the odds ratio (OR) is 2.070, meaning that for each additional centimetre there is a 107% increase in the odds of having pain at two months after surgery. In the multivariate analysis (model 3) however, neither predictor was statistically significant.

Model	Factors	Chi^2 (df)	*P-value*	McFadden R^2	AUC
1	Change in perceived intensity	6.639 (18)	.010	.247	.833
2	Length of the area	11.968 (18)	.0005	.445	.885
3	Change in perceived intensity **AND** Length of the area	12.439 (17)	.002	.462	.938

Table 2. Logistic regression model summary for the prediction of cough-evoked pain two months after surgery. For each model the Chi-squared, its p-value, the McFadden R2 and area under the Receiver Operating Curve (AUC) are shown.

Model	Factors	Coefficient	Error	OR	95% CI	Wald	*P-value*
1	Change in perceived intensity	.098	.046	1.103	1.008-1.206	4.555	.033
2	Length of the area	.728	.324	2.070	1.098-3.904	5.052	.025
3	Change in perceived intensity **AND** Length of the area	.039 .612	.058 .342	1.040 1.843	0.928-1.166 0.943-3.602	0.453 3.202	.501 .074

Table 3. Logistic regression coefficients for the prediction of cough-evoked pain two months after surgery. For each model, the regression coefficient with its error term, the Odds Ratio (OR) with the 95% confidence interval, and the Wald statistic with its p-value are shown.

Secondary outcomes

We found a significant and positive correlation (r=.533, P=.019) between the length of the area of MFS-induced secondary mechanical hyperalgesia and the spatial extent of the area of incision-induced secondary mechanical hyperalgesia on day 4 normalized for scar length.

The logistic regression analysis with the incision-induced secondary mechanical hyperalgesia at day 4 showed that the model with the change in perceived pinprick intensity (model 1) was not statistically significant (Chi-squared = 1.299, P = .250, McFadden R-squared = .048). In contrast, the model with the spatial extent (model 2) was statistically significant (Chi-squared = 8.200, P = .004, McFadden R-squared = .317). The spatial extent was a significant positive predictor (Coefficient=.620, SE=0.294, Wald=4.456, P=.035, OR=1.859, 95% CI=1.045-3.305). Also model 3 (with both the change in perceived intensity and spatial extent) was statistically significant (Chi-squared = 8.642, P = .013, McFadden R-squared = .334). The AUC of model 2 (AUC=.864) and model 3 (AUC=.852) were comparable. The AIC of model 2 (21.66) and model 3 (23.22) were also comparable. Thus, model 3 did not perform better than model 2.

The logistic regression analysis with pain at rest at D4 and cough-evoked pain at D4 revealed that all three models were statistically significant and performed well (Table S1). Furthermore, the AIC was comparable between model 1 (21.951) or model 2 (22.141) vs. model 3 (23.142). The two types of pain (at rest and evoked) were significantly correlated (Pearson's r=.787, P<.001), but the VIF was 1.876. Table S2 shows the logistic regression coefficients.

Model	Factors	Chi^2 (df)	*P-value*	McFadden R^2	AUC
1	Spontaneous pain	7.913 (17)	.005	.306	.869
2	Evoked pain	7.723 (17)	.005	.299	.841
3	Spontaneous pain **AND** Evoked pain	8.722 (16)	.013	.337	.898

Table S1. Logistic regression model summary for the prediction of cough-evoked pain two months after surgery. For each model the Chi-squared, its p-value, the McFadden R2 and area under the Receiver Operating Curve (AUC) are shown.

Model	Factors	Coefficient	Error	OR	95% CI	Wald	*P-value*
1	Spontaneous pain	.080	.035	1.083	1.012-1.159	5.249	.022
2	Evoked pain	.074	.035	1.076	1.006-1.152	4.488	.034
3	Spontaneous pain	.047	.049	1.048	0.953-1.153	0.931	.335
	AND						
	Evoked pain	.039	.046	1.040	0.951-1.138	0.733	.392

Table S2. Logistic regression coefficients for the prediction of cough-evoked pain two months after surgery. For each model, the regression coefficient

with its error term, the Odds ratio with the 95% confidence interval, and the Wald statistic with its p-value are shown.

V.4. Discussion

This study aimed to investigate whether the individual susceptibility to developing MFS-induced secondary mechanical hyperalgesia preoperatively predicted the presence of post-thoracotomy pain at two months. We found that 40% of the included patients reported cough-evoked pain at the two-month follow-up. We show, for the first time, that the magnitude (intensity and extent) of preoperatively assessed MFS-induced secondary mechanical hyperalgesia displays excellent discriminative power (AUC >. 9) for the presence or absence of cough-evoked pain two months after thoracotomy. These results support the hypothesis that a heightened individual susceptibility to develop experimentally induced secondary mechanical hyperalgesia may identify patients who are vulnerable to the development of persistent post-thoracotomy pain.

Interestingly, the extent of the area of MFS-induced secondary mechanical hyperalgesia and the extent of the area of incision-induced secondary mechanical hyperalgesia were correlated. This has already been demonstrated with experimental heat-induced secondary hyperalgesia and incision-induced secondary hyperalgesia after gynaecologic surgery[192]. These findings suggest that the individual susceptibility for spreading hyperalgesia may be similar across different pain-inducing events.

In a recent study, Patel et al. (2024) recorded in rats, before and after HFS, the responses of spinal wide-dynamic range neurons elicited by mechanical pinprick stimuli delivered to the glabrous skin of the paw[292]. They found increased WDR neuron responses after HFS when the mechanical stimuli were applied to the HFS-treated area and the adjacent area. They also found that when HFS was applied to the receptive field, an expansion of receptive field size for mechanical pinprick stimuli was observed but when HFS was delivered adjacent to the receptive field, no expansion of the receptive field was observed. These findings suggest that the increased WDR neuron responses elicited by mechanical pinprick stimuli applied at distant sites might involve a mechanism different than the one underlying the receptive field expansion. A possible candidate mechanism could be descending facilitation[292, 325, 326]. Similar effects are probably observed after MFS and the questions arises whether the variability in the area size of MFS-induced secondary mechanical hyperalgesia observed in patients the day before surgery could have been (partly) the result of individual differences in descending facilitation. Similar findings with respect to WDR neuron activity have been observed after incision of the paw[327-329]. A posterolateral thoracotomy activates both somatic and visceral nociceptive afferents and induces peripheral and central sensitization[330, 331].

Given that the extent of the area of MFS-induced secondary mechanical hyperalgesia and the extent of the area of incision-induced secondary mechanical hyperalgesia were correlated in our patients, and that the extent of the area of MFS-induced secondary mechanical hyperalgesia predicted cough-evoked pain at two months, it may not be surprising that the extent

of postoperative hyperalgesia also predicted cough-evoked pain at two months.

We also found that the reported pain intensity on day 4 predicted cough-evoked pain at two months. This is in line with the known literature, as the intensity of postoperative has long been recognized as a predictive factor for the development of persistent postsurgical pain in several surgical models, including thoracotomy[180]. Besides postoperative pain, younger age, female sex, hypertension, preoperative pain, open thoracotomy, more extensive procedures (bilobectomy, pneumonectomy, lobectomy plus wedge resection and pleurectomy) and wound complications were also found to be predictors for persistent post-thoracotomy pain[180].

Some studies also reported anxiety and depression as risk factors for post-thoracotomy pain[180, 193, 198]. Indeed, a recent meta-analysis showed that state anxiety has a significant association with persistent postsurgical pain[332]. In our study we found no significant differences in the HADS-anxiety score or HADS-depression score between patients with and without cough-evoked pain. A possibility is that our sample size was too small to detect differences or that the way we tested for anxiety (HADS questionnaire) may not be sensitive enough.

Our study sample size is small. Due to the COVID pandemic and its impact on clinical activity and access to patients for clinical research, we were able to recruit fewer patients in this period (2020-2021) of the study. Moreover, during the last period of our study, other clinical studies recruited patients of the same population, which probably reduced the number of patients available for our study as well. Finally, a significant number of recruited

patients (N=9, 22%) did not tolerate the entire MFS procedure and, therefore, dropped out. To reduce the drop-out rate as a result of MFS, one might consider lowering the stimulation intensity or using the high-frequency stimulation (HFS) protocol, in which only five trains are delivered[80]. It would also be interesting to investigate whether the same result will be obtained if MFS-induced hyperalgesia is assessed not on the day before surgery but, for example, a week before. This would reduce the discomfort patients may experience from MFS on the day before surgery. The small sample size limited the number of predictors we could include in the regression models. In the literature, the rule of ten events per variable is often advised, although there seems to be no statistical justification for this recommendation[333, 334]. Despite the small sample size, we found the extent of MFS-induced secondary mechanical hyperalgesia to be a statistically significant predictor. Nevertheless, these results need to be validated in a larger sample.

V.5. Conclusion

Our findings indicate that the individual susceptibility to developing experimentally-induced secondary mechanical hyperalgesia preoperatively may identify patients who are potentially vulnerable to the development of persistent post-thoracotomy pain. The ability to preoperatively identify patients at risk for developing persistent post-thoracotomy pain using a simple assessment of secondary mechanical hyperalgesia would be clinically helpful. It would allow targeted prevention measures that may reduce the incidence of persistent post-thoracotomy pain.

VI. Chapter 6. Discussion and perspectives

This thesis explored four empirical chapters, each investigating a different facet of central sensitization. The objective was to investigate methods for effectively inducing (Chapter 2), assessing (Chapter 3), and modulating (Chapter 4) central sensitization in humans through electrical skin stimulation, as well as to evaluate its potential clinical applications (Chapter 5).

VI.1. Induction of central sensitization through electrical stimulation

The objective of Chapter 2 was to investigate ways to improve the HFS protocol to experimentally induce central sensitization in experimental and clinical studies.

In the first experiment, we investigated whether the increase in secondary mechanical hyperalgesia depended on non-charge-compensated pulses (monophasic), which could lead to cumulative depolarization of the membrane potential. In this experiment, we found no differences between non-charge- compensated and charge compensated (biphasic) HFS pulses in the development of secondary mechanical hyperalgesia.

In the second experiment, we investigated whether HFS frequency influences the development of secondary mechanical hyperalgesia. We found that depending on the frequency of stimulation, electrical stimulation induces a stronger or weaker secondary mechanical hyperalgesia.

Medium-frequency stimulation (42 Hz) induced the most robust secondary mechanical hyperalgesia compared to lower (5 Hz, 20 Hz) and higher (100 Hz) frequencies.

In the third experiment of this Chapter, we compared the effects of burst-like and continuous stimulation patterns on the development of secondary mechanical hyperalgesia. The findings indicate that burst-like stimulation induces more efficiently secondary hyperalgesia.

VI.1.1. Conditioning stimulation delivered using charge-compensated vs non-charge-compensated pulses

Monophasic electrical pulses deliver electrical current in a single direction, leading to a continuous flow of ions in one direction across the tissue, which can cause accumulation of charge at the electrode-tissue interface. This can lead to cumulative depolarization of the cell membrane that may exceed the physiological threshold, leading to excessive calcium influx, activation of destructive enzymes, and potential cell death. The toxic substances released can damage surrounding tissues[251]. In contrast, biphasic pulses alternate the direction of current flow, which reduce the likelihood of charge accumulation and electrolysis[335]. Indeed, by rapidly reversing the current direction, biphasic pulses minimize the amplitude of depolarization, helping to maintain the physiological integrity of cells[335, 336].

The lack of significant differences in the increase of pinprick sensitivity between charge-compensated and non-charge-compensated pulses

suggests that cumulative depolarization is not the primary driver of experimentally induced secondary hyperalgesia. Studies in animals have demonstrated that for stimulations using a single pulse with identical current intensity, the average amplitude of the evoked population spike (OPS) generated by a monophasic pulse is not significantly different from that produced by a biphasic pulse[336]. However, precise quantification of this phenomenon remains notably challenging, especially in human populations.

To break it down, for studies involving humans we recommend charge-compensated electrical pulses to induce central sensitization. This method is safer as it minimizes the risk of tissue damage. Furthermore, when comparing different stimulation frequencies, minimizing cumulative effects from monophasic high- frequency stimulation (with short inter-pulse intervals) is essential to ensure that any observed increase in pinprick hypersensitivity at higher frequencies is not solely attributable to this cumulative phenomenon.

VI.1.2. Frequency and pattern of conditioning stimulation

In the 2000s, Chul Han et al. demonstrated that stimulation frequencies around 10 Hz closely mimic the firing patterns observed in conditions of inflammatory and neuropathic pain in rodents. Their findings suggested a strong correlation between the intensity of ectopic discharges and behavioral outcomes in a rat model of neuropathic pain[337]. Another study showed that in the context of inflammatory pain *in vitro*, C-fibers showed ectopic discharges with a mean discharge rate of 0.38 Hz[338].

However, a fixed frequency of C-fibers discharge was not confirmed by Serra et al. (2012), who observed in both patients and rodents that nociceptors exhibit spontaneous activity that often appears sporadic, leading to "saw-tooth" shaped latency profiles that are irregular. Moreover, they were unable to identify a relationship between clinical pain levels and spontaneous activity[339]. A comparable conclusion was made by Xiao and Bennett (2009), who observed distinctly irregular firing patterns in a rodent model of neuropathic pain[340]. Given these different findings across studies, it is challenging to replicate the exact discharge frequency of C-fibers in healthy volunteer models using HFS. The sporadic and irregular nature of C-fiber activity observed in pathological conditions is difficult to mimic in controlled experimental settings. Moreover, this is not clear if this spontaneous activity is either continuous or a burst-like pattern, or a combination of both[341, 342].

However, one known feature that can be reliably induced with HFS in these models is secondary mechanical hyperalgesia, which has consistently served as a proxy for investigating central sensitization in controlled experimental conditions. Importantly, our study did not aim to replicate the neuropathic firing rates of nociceptive fibers. Instead, we focused on using electrical stimulation to induce central sensitization as a model to study the potential underlying mechanisms of persistent pain. This approach allows for a focused assessment of central sensitization independently of the complex and variable patterns of nociceptor activity observed in pathological states, providing a controlled framework for exploring its contribution to chronic pain.

The frequencies selected (5, 20, 42, and 100 Hz) were based on the findings of Go and Yaksh (1986), who demonstrated that substance P release peaks between 20 and 50 Hz, compared to both lower frequencies (e.g., 2, 5, and 10 Hz) and higher frequencies (e.g., 200 Hz)[255] . Our objective was to evaluate a range of frequencies under standardized conditions, using the same stimulation pattern (burst-like stimulations of 1 second separated by 9 seconds) and the same total number of pulses (500). Instead of including 2 Hz as a low-frequency condition, we chose 5 Hz because achieving 500 pulses at 2 Hz burst-like stimulation would have required a much longer protocol, with approximately 42 minutes of stimulation including 9-seconds intervals. With 5 Hz burst-like stimulation, the total duration was reduced to 17 minutes, making it more practical while maintaining the study's objectives. For the medium frequency, we selected 42 Hz rather than 50 Hz because, although substance P release peaks within the 20–50 Hz range, the data suggested that release might plateau or even decrease around 50 Hz[255].

Nevertheless, we acknowledge that the range of frequencies tested in this study is limited. To gain a more precise characterization of the frequency-response relationship and the development of secondary mechanical hyperalgesia, a broader range of stimulation frequencies should be explored in future studies.

Unpublished experimental observations using 42 Hz in healthy volunteers revealed that the pain during the twelve MFS trains was much higher than with the other frequencies tested. Consequently, we opted for the standard 100 Hz protocol in Chapter 5. The goal in this chapter was not to delve into the amount of secondary mechanical hyperalgesia, but to explore the brain's

evoked responses following the experimental induction of secondary mechanical hyperalgesia. Based on our observations and related literature, it is unlikely that the frequency would not have significantly altered our results.

The clinical relevance in Chapter 5 of using MFS at 42 Hz was to induce a strong nociceptive input leading to the maximal amplification of secondary mechanical hyperalgesia, to closely mimic the intense character of the nerve stimulation experienced during surgery.

In experiments that manipulate experimental conditions, selecting the 'optimum' stimulation frequency is highly dependent on the specific protocol and the outcomes being measured. For instance, when investigating the placebo effect on central sensitization induced by electrical stimulation in a double-blind randomized design, it is important to allow for a margin by using a frequency that elicits a significant increase in pinprick sensitivity in the control group, thereby avoiding a floor effect in the placebo group. Conversely, when studying the nocebo effect, a frequency that induces a significant increase in pinprick sensitivity should be chosen, but it should not be too high, otherwise, it may result in a ceiling effect.

VI.2. Assessing central sensitization through gamma-band oscillations

The objective of Chapter 3 was to assess the changes in GBOs induced by robot-controlled pinprick stimuli, after the experimental induction of central sensitization with HFS. Such as in the study of van den Broeke et al. (2017),

we found that mechanical pinprick stimulation induced a strong post-stimulus increase in high-frequency activity within the signal. After removing artifacts as described earlier, we did not find an increase of cortical GBOs post-stimulus after HFS. Visual inspection of the data showed that cortical GBOs were present in only eight out of twenty participants. It seems likely that the high-frequency activity observed after HFS (without artifact cleaning) reflects non-cortical sources.

Our findings diverge from several studies that focused on the detection of GBOs related to various stimuli, such as nociceptive, visual or tactile[147-149, 171]. Such studies, which identify GBOs following a salient stimulus, suggest a significant relationship between GBOs and the perception of the stimulus.

While cortical GBOs initially held promise as a potential biomarker candidate for nociception, their inherent variability across individuals and the challenges associated with their analysis make it unsuitable for a widespread use[176, 283]. As stated in a paper by Eldabe et al. (2022) : “For biomarkers to be clinically useful, they need to be specific, accessible, and also scalable”[160]. Consequently, there arises a pressing need to develop a biomarker that is more stable across individuals, comparatively easier to analyse, and in a latest step, readily accessible to practitioners.

Prior to our study, van den Broeke and colleagues investigated the presence of GBOs, elicited by manually applied mechanical pinprick stimulation, before and after the experimental induction of mechanical hypersensitivity. Such as in the present study, they did not find consistent GBOs across participants neither an increase after HFS.

Conversely, they observed an increased activity in the electro-oculogram, indicating that the EEG recordings from the scalp could be significantly contaminated by muscle activity associated with eye movements[161]. In our study, part of our analysis revealed that pinprick-evoked artifacts, which could be related to muscular activity, exhibited greater potential as a biomarker of central sensitization compared to GBOs, as they showed a consequent increase post- stimulus following HFS.

The Facial Action Coding System (FACS) has shown that certain facial expressions (brow lowering, cheek raising, lid tightening, nose wrinkling and upper lip raising) are associated with experimental and clinical pain[343, 344]. More recently, two studies used electromyography (EMG) in this context, and their results were in line with the previous studies using the FACS, by showing that muscle activity around the eyes are also associated with pain[345, 346]. However, FACS is limited to observing visible muscle movements and does not account for subtle changes in muscle tone. Additionally, it is a time-consuming method that relies heavily on the subjective interpretation of the assessor[347]. In contrast, EMG offers the advantage of objectively detecting subtle facial muscle activity that may not be visible to an observer[348, 349]. To date, no study has investigated facial muscle activity measured via EMG as a potential marker of central sensitization. Based on our previous findings, it would be of interest to investigate facial EMG activity during robot-controlled pinprick stimulations following the experimental induction of central sensitization in healthy human volunteers.

VI.3. Modulating central sensitization through descending pathways

Chapter 4 aimed to determine whether negative expectations influence the development of secondary mechanical hyperalgesia. Our results revealed no significant differences between the nocebo group and the control group in the development of secondary mechanical hyperalgesia, expected pain from HFS, fear of HFS, or pain during HFS. This absence of effect could partly reflect the experimental setting, which may not fully replicate the level of anxiety typically observed in clinical contexts. Laboratory participants, often students, are generally less anxious as they voluntarily consent to participate, are aware of the controlled nature of the procedures, and are informed of the absence of significant risks. These conditions may make it more challenging to induce strong negative expectations or fear compared to clinical situations, where heightened anxiety is common due to uncertainty, vulnerability, or prior negative experiences with pain or surgery. While this remains speculative, one could question the ecological validity of laboratory paradigms for studying the nocebo effect in relation to central sensitization.

Although the present study did not observe significant differences in HFS-induced pain or secondary mechanical hyperalgesia between groups, the exploratory findings suggest a subtle association between expectations and pain perception. This is in line with evidence demonstrating that cognitive processes, such as attention and expectations, can modulate pain perception in healthy volunteers and in patients[285, 350-353]. Neuroimaging studies suggest that attention can influence pain-modulatory regions, which are involved to descending pain control[354, 355]. However, direct evidence linking attention to changes in the development of central sensitization is limited, with only one

study supporting this possibility[356]. Furthermore, studies using withdrawal reflexes as proxies for spinal processing have reported inconsistent findings, highlighting the difficulty of establishing attention's role at the spinal level[357, 358]. In 2022, Della Porta et al. found no significant differences in secondary mechanical hyperalgesia between the arm participants were instructed to focus their attention on (attended arm) and the arm they were instructed to ignore (unattended arm) during bilateral HFS, questioning whether selective attention can modulate secondary mechanical hyperalgesia[359]. Building on this, their 2024 study investigated the effects of cognitive load, and consistent with previous findings by Meyers et al. (2023) they found no evidence that engaging participants in a high-demand working memory task influenced the development of HFS-induced secondary mechanical hyperalgesia[206-208]. These results do not support earlier results which suggested that cognitive engagement or attentional focus might have a modulatory effect on central sensitization[83, 360]. Taken together, these observations emphasize the need for further research to better understand if cognitive factors interact with descending systems in modulating central sensitization.

VI.4. Translational potential of preoperative experimentally-induced central sensitization

This last chapter primarily aimed to evaluate an experimental model of central sensitization to predict surgery-induced central sensitization, specifically focusing on persistent postsurgical pain (PPSP). We examined

whether preoperative MFS- induced hyperalgesia could predict PPSP two months after surgery. Our logistic regression analysis supports this hypothesis. We also confirmed that surgically induced hyperalgesia was a predictor of PPSP and correlated with MFS-induced hyperalgesia. Moreover, while this phenomenon is recognized and has been demonstrated in previous studies, our findings confirm that the severity of acute postoperative pain also predicts PPSP.

VI.4.1. Shared mechanisms between experimentally- and surgically-induced central sensitization

The correlation between experimentally- and surgically- induced hyperalgesia suggest that central sensitization, whether induced experimentally or through a surgical procedure, likely shares common underlying mechanisms. In animal studies, the activation of NMDA receptors has been shown to play an important role in the development and maintenance of central sensitization, applicable in both experimental models and postsurgical contexts[26, 31, 42, 243]. Indeed, intense neural input triggers significant NMDA-mediated increase in intracellular calcium (Ca^{2+}) within both second-order neurons and astrocytes. This calcium elevation provokes the release of BDNF and activates purinergic receptors on glial cells, facilitating various neurobiological responses responsible of central sensitization[24, 269]. In humans, we cannot directly establish the link between experimentally- and surgery-induced central sensitization, however Stubhaug et al. (1997) demonstrated that a low dose of ketamine, an NMDA

receptor antagonist, effectively reduces secondary mechanical hyperalgesia after surgery[310]. Taken together, it is reasonable to hypothesize that NMDA activation and the subsequent phosphorylation cascades represent common mechanisms underlying both experimentally- and surgically-induced central sensitization.

Recognizing these shared mechanisms, researchers have turned to clinical tools to assess central sensitization in patient. The Central Sensitization Inventory (CSI) score has been evaluated to determine its potential as a predictor of PPSP[361]. However, consensus on its predictive value remains elusive, because despite various studies exploring the relationship between CSI scores and psychophysical measures of central sensitization, the findings have been inconsistent, leading to ongoing debate within the scientific community regarding its efficacy as a reliable predictive tool[362-365]. Further research is needed to clarify this relationship and establish standardized guidelines for using the CSI in monitoring psychological factors associated with central sensitization.

VI.4.2. Practical strategies for preventing PPSP

To mitigate the risk of PPSP, it is crucial to prioritize effective pain management strategies, especially for patients identified as "high-risk"[236, 237]. More and more research indicate that a multimodal approach can be particularly beneficial for these patients[366, 367]. Techniques such as nerve blocks, the administration of appropriate postoperative medications, addressing psychosocial factors, and incorporating physical rehabilitation

have all shown significant promise in reducing the incidence of PPSP[238-240]. By implementing diverse interventions, healthcare providers can better support their patients in achieving optimal postoperative outcomes. However, multimodal interventions are costly and time-consuming, making it difficult in clinical practice to provide them to all the patients operated[361]. Preventing a disease should be approached with targeted strategies, similar to those used for other well-known medical conditions. For instance, individuals at high risk for osteoporosis, can benefit from specific preventive treatments like calcium and vitamin D supplements or bisphosphonate medications[368]. Similarly, for pain it is impractical to provide these treatments to everyone, that is why it is essential to stratify patients to identify those who would most benefit from such preventive measures. Our findings, when replicated, may have important clinical implications because experimentally-induced central sensitization could help identify patients at risk for developing high-intensity postoperative pain. Patient stratification may also be important in allocating treatment resources to patients at risk and in identifying relevant groups of individuals to be included in trials of new analgesics[369, 370]. A study by Cook et al. (2014) showed that Phase II projects often failed due to a lack of confidence in selecting the correct patient population, in opposition to projects with well-defined patient stratification plans, that showed a higher likelihood of success[371]. Therefore, in clinical trials where patients are selected based on specific biomarkers, the probability of a positive outcome increases, as the treatment is more likely to target the underlying biological mechanisms of the disease in these patients[372]. More studies are required to evaluate the effectiveness of early postoperative multimodal interventions targeting patients who are identified as high risk for developing PPSP.

VII. General conclusion

This thesis contributes to the edifice of research on central sensitization in humans, with notable contributions to the methodology of induction and the understanding of potential biomarkers. First, we developed an improved protocol for HFS that more effectively induces central sensitization in humans. Secondly, our investigation into GBOs revealed that they do not appear to be a suitable marker for central sensitization. This finding directs future research away from GBOs towards other potential biomarkers, such as pinprick-evoked muscular activity. Thirdly, inducing negative expectations in a controlled laboratory framework is challenging, as participants typically lack anxiety and vulnerability seen in clinical settings. This highlights the need for refined methodologies to better mimic real-world conditions and improve the ecological validity of laboratory studies. Finally, our clinical study has identified the preoperative assessment of MFS-induced secondary hyperalgesia as a promising predictive biomarker for persistent postsurgical pain. This discovery opens the research for better diagnostic tools and personalized treatment plans for patients at risk of developing chronic pain. More research is needed to bridge the gap between human and animal studies to further elucidate the underlying mechanisms of central sensitization and ultimately, improve the care of patients suffering from chronic pain.

VIII. Bibliography

1. Raja SN, Carr DB, Cohen M, Finnerup NB, Flor H, Gibson S, et al. The revised International Association for the Study of Pain definition of pain: concepts, challenges, and compromises. Pain. 2020;161(9):1976-82.
2. Grichnik KP, Ferrante FM. The difference between acute and chronic pain. Mt Sinai J Med. 1991;58(3):217-20.
3. Treede R-D, Rief W, Barke A, Aziz Q, Bennett MI, Benoliel R, et al. A classification of chronic pain for ICD-11. PAIN. 2015;156(6):1003-7.
4. Merskey H, Bogduk N. Classification of Chronic Pain. IASP Press, Seattle.1994.
5. Elliott TE, Renier CM, Palcher JA. Chronic pain, depression, and quality of life: correlations and predictive value of the SF-36. Pain Med. 2003;4(4):331-9.
6. Kawai K, Kawai AT, Wollan P, Yawn BP. Adverse impacts of chronic pain on health-related quality of life, work productivity, depression and anxiety in a community-based study. Fam Pract. 2017;34(6):656-61.
7. Wallman LJ. Diagnosis and Management of Pain Syndromes. JAMA. 1969;209(7):1091-.
8. Gatchel RJ, Peng YB, Peters ML, Fuchs PN, Turk DC. The biopsychosocial approach to chronic pain: scientific advances and future directions. Psychol Bull. 2007;133(4):581-624.
9. Meints SM, Edwards RR. Evaluating psychosocial contributions to chronic pain outcomes. Prog Neuropsychopharmacol Biol Psychiatry. 2018;87(Pt B):168-82.
10. Engel GL. The need for a new medical model: a challenge for biomedicine. Science. 1977;196(4286):129-36.
11. Ghaemi SN. The rise and fall of the biopsychosocial model. British Journal of Psychiatry. 2009;195(1):3-4.
12. Lawn T, Sendel M, Baron R, Vollert J. Beyond biopsychosocial: The keystone mechanism theory of pain. Brain, Behavior, and Immunity. 2023;114:187-92.
13. Bolton D, Gillett G. The Biopsychosocial Model of Health and Disease: New Philosophical and Scientific Developments. Palgrave MacMillan. 2019.
14. Bliss TVP, Lømo T. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. The Journal of Physiology. 1973;232(2):331-56.

15. Lomo T. Potentiation of monosynaptic EPSPs in the perforant path-dentate granule cell synapse. Exp Brain Res. 1971;12(1):46-63.
16. Lomo T. Patterns of activation in a monosynaptic cortical pathway: the perforant path input to the dentate area of the hippocampal formation. Exp Brain Res. 1971;12(1):18-45.
17. Cooke SF, Bliss TVP. Plasticity in the human central nervous system. Brain. 2006;129(7):1659-73.
18. Ruscheweyh R, Wilder-Smith O, Drdla R, Liu X-G, Sandkühler J. Long-Term Potentiation in Spinal Nociceptive Pathways as a Novel Target for Pain Therapy. Molecular Pain. 2011;7:1744-8069-7-20.
19. Pogatzki-Zahn E, Segelcke D, Zahn P. Mechanisms of acute and chronic pain after surgery: update from findings in experimental animal models. Current Opinion in Anesthesiology. 2018;31(5):575-85.
20. Julius D, Basbaum AI. Molecular mechanisms of nociception. Nature. 2001;413(6852):203-10.
21. Lewin GR, Lu Y, Park TJ. A plethora of painful molecules. Curr Opin Neurobiol. 2004;14(4):443-9.
22. Costigan M, Woolf CJ. Pain: Molecular mechanisms. The Journal of Pain. 2000;1(3, Supplement):35-44.
23. Liu S, Kelliher L. Physiology of pain—a narrative review on the pain pathway and its application in the pain management. Digestive Medicine Research. 2022;5.
24. Sandkühler J, Gruber-Schoffnegger D. Hyperalgesia by synaptic long-term potentiation (LTP): an update. Curr Opin Pharmacol. 2012;12(1):18-27.
25. Yam MF, Loh YC, Tan CS, Khadijah Adam S, Abdul Manan N, Basir R. General Pathways of Pain Sensation and the Major Neurotransmitters Involved in Pain Regulation. Int J Mol Sci. 2018;19(8).
26. Latremoliere A, Woolf CJ. Central sensitization: a generator of pain hypersensitivity by central neural plasticity. The journal of pain : official journal of the American Pain Society. 2009;10(9):895-926.
27. Raja SN, Campbell JN, Meyer RA. Evidence for different mechanisms of primary and secondary hyperalgesia following heat injury to the glabrous skin. Brain. 1984;107 (Pt 4):1179-88.
28. Ali Z, Meyer RA, Campbell JN. Secondary hyperalgesia to mechanical but not heat stimuli following a capsaicin injection in hairy skin. Pain. 1996;68(2-3):401-11.
29. LaMotte RH, Shain CN, Simone DA, Tsai EF. Neurogenic hyperalgesia: psychophysical studies of underlying mechanisms. J Neurophysiol. 1991;66(1):190-211.

30. Simone DA, Baumann TK, LaMotte RH. Dose-dependent pain and mechanical hyperalgesia in humans after intradermal injection of capsaicin. Pain. 1989;38(1):99-107.
31. Kronschläger MT, Drdla-Schutting R, Gassner M, Honsek SD, Teuchmann HL, Sandkühler J. Gliogenic LTP spreads widely in nociceptive pathways. Science. 2016;354(6316):1144-8.
32. Donnelly CR, Andriessen AS, Chen G, Wang K, Jiang C, Maixner W, et al. Central Nervous System Targets: Glial Cell Mechanisms in Chronic Pain. Neurotherapeutics. 2020;17(3):846-60.
33. Ji RR, Berta T, Nedergaard M. Glia and pain: is chronic pain a gliopathy? Pain. 2013;154 Suppl 1(0 1):S10-28.
34. Lewis T. Nocifensor tenderness. Pain. New York, NY, US: MacMillan Co; 1942. p. 68-83.
35. Hardy JD, Wolff HG, Goodell H. Experimental evidence on the nature of cutaneous hyperalgesia. J Clin Invest. 1950;29(1):115-40.
36. Woolf CJ, Fitzgerald M. The properties of neurones recorded in the superficial dorsal horn of the rat spinal cord. J Comp Neurol. 1983;221(3):313-28.
37. Woolf CJ, Fitzgerald M. Somatotopic organization of cutaneous afferent terminals and dorsal horn neuronal receptive fields in the superficial and deep laminae of the rat lumbar spinal cord. J Comp Neurol. 1986;251(4):517-31.
38. Woolf CJ, Thompson SW, King AE. Prolonged primary afferent induced alterations in dorsal horn neurones, an intracellular analysis in vivo and in vitro. J Physiol (Paris). 1988;83(3):255-66.
39. Simone DA, Sorkin LS, Oh U, Chung JM, Owens C, LaMotte RH, et al. Neurogenic hyperalgesia: central neural correlates in responses of spinothalamic tract neurons. Journal of Neurophysiology. 1991;66(1):228-46.
40. (IASP) IAftSoP. IASP terminology 2008 [Available from: https://www.iasp-pain.org/resources/terminology/.
41. Pain IAftSo. IASP terminology. 2017.
42. Woolf CJ. Central sensitization: implications for the diagnosis and treatment of pain. Pain. 2011;152(3 Suppl):S2-S15.
43. van den Broeke EN. Central sensitization and pain hypersensitivity: Some critical considerations. F1000Res. 2018;7:1325.
44. Legrain V, Iannetti GD, Plaghki L, Mouraux A. The pain matrix reloaded: a salience detection system for the body. Prog Neurobiol. 2011;93(1):111-24.

45. Velasco E, Flores-Cortés M, Guerra-Armas J, Flix-Díez L, Gurdiel-Álvarez F, Donado-Bermejo A, et al. Is chronic pain caused by central sensitization? A review and critical point of view. Neuroscience & Biobehavioral Reviews. 2024;167:105886.
46. Cayrol T, Draper-Rodi J, Fabre L, Pitance L, Broeke ENvd. Stuck in the Middle With You: Why a Broad-Brush Approach to Defining Central Sensitization Does Not Help Clinicians and Patients. Journal of Orthopaedic & Sports Physical Therapy. 2021;51(5):204-6.
47. den Boer C, Dries L, Terluin B, van der Wouden JC, Blankenstein AH, van Wilgen CP, et al. Central sensitization in chronic pain and medically unexplained symptom research: A systematic review of definitions, operationalizations and measurement instruments. J Psychosom Res. 2019;117:32-40.
48. van den Broeke EN, Van den Bergh O. Central sensitization in humans: Popular phrase or useful concept? J Psychosom Res. 2019;119:51-2.
49. Adams LM, Turk DC. Central sensitization and the biopsychosocial approach to understanding pain. Journal of Applied Biobehavioral Research. 2018;23(2):e12125.
50. Pitance L, Piraux E, Lannoy B, Meeus M, Berquin A, Eeckhout C, et al. Cross cultural adaptation, reliability and validity of the French version of the central sensitization inventory. Manual Therapy. 2016;25:e83-e4.
51. Nijs J, Huysmans E. Clinimetrics: The Central Sensitisation Inventory: a useful screening tool for clinicians, but not the gold standard. J Physiother. 2022;68(3):207.
52. Mayer TG, Neblett R, Cohen H, Howard KJ, Choi YH, Williams MJ, et al. The Development and Psychometric Validation of the Central Sensitization Inventory. Pain Practice. 2012;12(4):276-85.
53. Nijs J, George SZ, Clauw DJ, Fernández-de-las-Peñas C, Kosek E, Ickmans K, et al. Central sensitisation in chronic pain conditions: latest discoveries and their potential for precision medicine. The Lancet Rheumatology. 2021;3(5):e383-e92.
54. Schug SA, Bruce J. Risk stratification for the development of chronic postsurgical pain. PAIN Reports. 2017;2(6):e627.
55. Van den Broeke E, Crombez G, Vlaeyen J. Reconceptualizing sensitization in pain: back to basics. PAIN Reports. 2024;9:e1125.
56. Arendt-Nielsen L, Curatolo M, Drewes A. Human experimental pain models in drug development: translational pain research. Curr Opin Investig Drugs. 2007;8(1):41-53.

57. Quesada C, Kostenko A, Ho I, Leone C, Nochi Z, Stouffs A, et al. Human surrogate models of central sensitization: A critical review and practical guide. Eur J Pain. 2021;25(7):1389-428.
58. Baumann TK, Simone DA, Shain CN, LaMotte RH. Neurogenic hyperalgesia: the search for the primary cutaneous afferent fibers that contribute to capsaicin-induced pain and hyperalgesia. Journal of Neurophysiology. 1991;66(1):212-27.
59. Luo XJ, Peng J, Li YJ. Recent advances in the study on capsaicinoids and capsinoids. Eur J Pharmacol. 2011;650(1):1-7.
60. Schmelz M, Schmid R, Handwerker HO, Torebjörk HE. Encoding of burning pain from capsaicin-treated human skin in two categories of unmyelinated nerve fibres. Brain. 2000;123 Pt 3:560-71.
61. Purkiss J, Welch M, Doward S, Foster K. Capsaicin-stimulated release of substance P from cultured dorsal root ganglion neurons: involvement of two distinct mechanisms. Biochem Pharmacol. 2000;59(11):1403-6.
62. Torebjörk HE, Lundberg LE, LaMotte RH. Central changes in processing of mechanoreceptive input in capsaicin-induced secondary hyperalgesia in humans. J Physiol. 1992;448:765-80.
63. Linde LD, Srbely JZ. The Acute Effect of Skin Preheating on Capsaicin-Induced Central Sensitization in Humans. Pain Pract. 2019;19(8):811-20.
64. Petersen KL, Jones B, Segredo V, Dahl JB, Rowbotham MC. Effect of remifentanil on pain and secondary hyperalgesia associated with the heat--capsaicin sensitization model in healthy volunteers. Anesthesiology. 2001;94(1):15-20.
65. Cavallone LF, Frey K, Montana MC, Joyal J, Regina KJ, Petersen KL, et al. Reproducibility of the heat/capsaicin skin sensitization model in healthy volunteers. J Pain Res. 2013;6:771-84.
66. Dirks J, Petersen KL, Dahl JB. The heat/capsaicin sensitization model: A methodologic study. The Journal of Pain. 2003;4(3):122-8.
67. Dirks J, Petersen Karin L, Rowbotham Michael C, Dahl Jørgen B. Gabapentin Suppresses Cutaneous Hyperalgesia following Heat-Capsaicin Sensitization. Anesthesiology. 2002;97(1):102-7.
68. Petersen KL, Rowbotham MC. A new human experimental pain model: the heat/capsaicin sensitization model. Neuroreport. 1999;10(7):1511-6.
69. Srbely JZ, Dickey JP, Bent LR, Lee D, Lowerison M. Capsaicin-Induced Central Sensitization Evokes Segmental Increases in Trigger Point Sensitivity in Humans. The Journal of Pain. 2010;11(7):636-43.

70. Bliss TVP, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. Nature. 1993;361(6407):31-9.
71. van den Broeke EN, Urdí M, Mouraux A, Biurrun Manresa JA, Torta DME. High-frequency electrical stimulation of cutaneous nociceptors differentially affects pain perception elicited by homotopic and heterotopic electrical stimuli. J Neurophysiol. 2021;126(4):1038-44.
72. Klein T, Magerl W, Hopf H-C, Sandkühler J, Treede R-D. Perceptual Correlates of Nociceptive Long-Term Potentiation and Long-Term Depression in Humans. The Journal of Neuroscience. 2004;24(4):964.
73. Xiao WH, Bennett GJ. Persistent low-frequency spontaneous discharge in A-fiber and C-fiber primary afferent neurons during an inflammatory pain condition. Anesthesiology. 2007;107(5):813-21.
74. Klein T, Stahn S, Magerl W, Treede R-D. The role of heterosynaptic facilitation in long-term potentiation (LTP) of human pain sensation. PAIN. 2008;139(3):507-19.
75. Vo L, Drummond PD. High frequency electrical stimulation concurrently induces central sensitization and ipsilateral inhibitory pain modulation. Eur J Pain. 2013;17(3):357-68.
76. Xia W, Mørch CD, Andersen OK. Exploration of the conditioning electrical stimulation frequencies for induction of long-term potentiation-like pain amplification in humans. Exp Brain Res. 2016;234(9):2479-89.
77. van den Broeke EN, Vanmaele T, Mouraux A, Stouffs A, Biurrun-Manresa J, Torta DM. Perceptual correlates of homosynaptic long-term potentiation in human nociceptive pathways: a replication study. R Soc Open Sci. 2021;8(1):200830.
78. Cayrol T, Lebleu J, Mouraux A, Roussel N, Pitance L, van den Broeke EN. Within- and between-session reliability of secondary hyperalgesia induced by electrical high-frequency stimulation. European Journal of Pain. 2020;24(8):1585-97.
79. Gousset S, Mouraux A, van den Broeke EN. Burst-like conditioning electrical stimulation is more efficacious than continuous stimulation for inducing secondary hyperalgesia in humans. Journal of Neurophysiology. 2019;123(1):323-8.
80. van den Broeke EN, Gousset S, Bouvy J, Stouffs A, Lebrun L, van Neerven SGA, et al. Heterosynaptic facilitation of mechanical nociceptive input is dependent on the frequency of conditioning stimulation. Journal of Neurophysiology. 2019;122(3):994-1001.
81. Pfau DB, Klein T, Putzer D, Pogatzki-Zahn EM, Treede RD, Magerl W. Analysis of hyperalgesia time courses in humans after painful electrical high-

frequency stimulation identifies a possible transition from early to late LTP-like pain plasticity. Pain. 2011;152(7):1532-9.
82. Manresa JAB, Mørch CD, Andersen OK. Long-term facilitation of nociceptive withdrawal reflexes following low-frequency conditioning electrical stimulation: A new model for central sensitization in humans. European Journal of Pain. 2010;14(8):822-31.
83. Torta DM, De Laurentis M, Eichin KN, von Leupoldt A, van den Broeke EN, Vlaeyen JWS. A highly cognitive demanding working memory task may prevent the development of nociceptive hypersensitivity. PAIN. 2020;161(7):1459-69.
84. Han HC, Lee DH, Chung JM. Characteristics of ectopic discharges in a rat neuropathic pain model. Pain. 2000;84(2-3):253-61.
85. Koppert W, Dern Sara K, Sittl R, Albrecht S, Schüttler J, Schmelz M. A New Model of Electrically Evoked Pain and Hyperalgesia in Human Skin: The Effects of Intravenous Alfentanil, S(+)-ketamine, and Lidocaine. Anesthesiology. 2001;95(2):395-402.
86. Geber C, Fondel R, Krämer HH, Rolke R, Treede RD, Sommer C, et al. Psychophysics, flare, and neurosecretory function in human pain models: capsaicin versus electrically evoked pain. J Pain. 2007;8(6):503-14.
87. Hoffmann RT, Schmelz M. Time course of UVA- and UVB-induced inflammation and hyperalgesia in human skin. Eur J Pain. 1999;3(2):131-9.
88. Kaidbey KH, Kligman AM. The acute effects of long-wave ultraviolet radiation on human skin. J Invest Dermatol. 1979;72(5):253-6.
89. Siebenga PS, van Amerongen G, Klaassen ES, de Kam ML, Rissmann R, Groeneveld GJ. The ultraviolet B inflammation model: Postinflammatory hyperpigmentation and validation of a reduced UVB exposure paradigm for inducing hyperalgesia in healthy subjects. Eur J Pain. 2019;23(5):874-83.
90. Saadé NE, Nasr IW, Massaad CA, Safieh-Garabedian B, Jabbur SJ, Kanaan SA. Modulation of ultraviolet-induced hyperalgesia and cytokine upregulation by interleukins 10 and 13. Br J Pharmacol. 2000;131(7):1317-24.
91. Lorenzini KI, Besson M, Daali Y, Salomon D, Dayer P, Desmeules J. Validation of the Simplified UVB Model to Assess the Pharmacodynamics of Analgesics in Healthy Human Volunteers. CHIMIA. 2012;66(5):296.
92. Modir JG, Wallace MS. Human Experimental Pain Models 1: The Ultraviolet Light UV-B Pain Model. In: Szallasi A, editor. Analgesia: Methods and Protocols. Totowa, NJ: Humana Press; 2010. p. 159-64.
93. O'Neill J, Sikandar S, McMahon SB, Dickenson AH. Human psychophysics and rodent spinal neurones exhibit peripheral and central

mechanisms of inflammatory pain in the UVB and UVB heat rekindling models. J Physiol. 2015;593(17):4029-42.
94. Bishop T, Ballard A, Holmes H, Young AR, McMahon SB. Ultraviolet-B induced inflammation of human skin: characterisation and comparison with traditional models of hyperalgesia. Eur J Pain. 2009;13(5):524-32.
95. Cervero F, Gilbert R, Hammond RGE, Tanner J. Development of secondary hyperalgesia following non-painful thermal stimulation of the skin: a psychophysical study in man. PAIN. 1993;54(2):181-9.
96. Schifftner C, Schulteis G, Wallace MS. Effect of Intravenous Alfentanil on Nonpainful Thermally Induced Hyperalgesia in Healthy Volunteers. J Clin Pharmacol. 2017;57(9):1207-14.
97. Werner MU, Lassen B, Kehlet H. Analgesic effects of dexamethasone in burn injury. Reg Anesth Pain Med. 2002;27(3):254-60.
98. Yucel A, Miyazawa A, Andersen OK, Arendt-Nielsen L. The effect of heat conditioning of the primary area before and after induction of hyperalgesia by topical/intradermal capsaicin or by controlled heat injury. Somatosens Mot Res. 2001;18(4):295-302.
99. Yucel A, Miyazawa A, Andersen OK, Arendt-Nielsen L. Comparison of hyperalgesia induced by capsaicin injection and controlled heat injury: effect on temporal summation. Somatosens Mot Res. 2004;21(1):15-24.
100. Binder A, Stengel M, Klebe O, Wasner G, Baron R. Topical high-concentration (40%) menthol-somatosensory profile of a human surrogate pain model. J Pain. 2011;12(7):764-73.
101. Chassaing C, Schmidt J, Eschalier A, Cardot JM, Dubray C. Hyperalgesia induced by cutaneous freeze injury for testing analgesics in healthy volunteers. Br J Clin Pharmacol. 2006;61(4):389-97.
102. Mahn F, Hüllemann P, Wasner G, Baron R, Binder A. Topical high-concentration menthol: reproducibility of a human surrogate pain model. Eur J Pain. 2014;18(9):1248-58.
103. Namer B, Seifert F, Handwerker HO, Maihöfner C. TRPA1 and TRPM8 activation in humans: effects of cinnamaldehyde and menthol. Neuroreport. 2005;16(9):955-9.
104. Sjölund KF, Segerdahl M, Sollevi A. Adenosine reduces secondary hyperalgesia in two human models of cutaneous inflammatory pain. Anesth Analg. 1999;88(3):605-10.
105. Bandell M, Story GM, Hwang SW, Viswanath V, Eid SR, Petrus MJ, et al. Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. Neuron. 2004;41(6):849-57.

106. Koltzenburg M, Torebjörk HE, Wahren LK. Nociceptor modulated central sensitization causes mechanical hyperalgesia in acute chemogenic and chronic neuropathic pain. Brain. 1994;117 (Pt 3):579-91.
107. Andersen HH, Gazerani P, Arendt-Nielsen L. High-Concentration L-Menthol Exhibits Counter-Irritancy to Neurogenic Inflammation, Thermal and Mechanical Hyperalgesia Caused by Trans-cinnamaldehyde. J Pain. 2016;17(8):919-29.
108. Dyck PJ, Peroutka S, Rask C, Burton E, Baker MK, Lehman KA, et al. Intradermal recombinant human nerve growth factor induces pressure allodynia and lowered heat-pain threshold in humans. Neurology. 1997;48(2):501-5.
109. Ernberg M, Castrillon EE, Ghafouri B, Larsson B, Gerdle B, List T, et al. Experimental myalgia induced by repeated infusion of acidic saline into the human masseter muscle does not cause the release of algesic substances. Eur J Pain. 2013;17(4):539-50.
110. Slugg RM, Meyer RA, Campbell JN. Response of Cutaneous A- and C-Fiber Nociceptors in the Monkey to Controlled-Force Stimuli. Journal of Neurophysiology. 2000;83(4):2179-91.
111. Slugg RM, Campbell JN, Meyer RA. The Population Response of A- and C-Fiber Nociceptors in Monkey Encodes High-Intensity Mechanical Stimuli. The Journal of Neuroscience. 2004;24(19):4649-56.
112. Ziegler EA, Magerl W, Meyer RA, Treede RD. Secondary hyperalgesia to punctate mechanical stimuli: Central sensitization to A-fibre nociceptor input. Brain. 1999;122(12):2245-57.
113. Greenspan JD, McGillis SLB. Stimulus Features Relevant to the Perception of Sharpness and Mechanically Evoked Cutaneous Pain. Somatosensory & Motor Research. 1991;8(2):137-47.
114. Van Hees J, Gybels J. C nociceptor activity in human nerve during painful and non painful skin stimulation. J Neurol Neurosurg Psychiatry. 1981;44(7):600-7.
115. Meyer RA, Campbell JN. Myelinated nociceptive afferents account for the hyperalgesia that follows a burn to the hand. Science. 1981;213(4515):1527-9.
116. Leone CM, Lenoir C, van den Broeke EN. Assessing signs of central sensitization: A critical review of physiological measures in experimentally induced secondary hyperalgesia. European Journal of Pain. 2024.
117. Henrich F, Magerl W, Klein T, Greffrath W, Treede RD. Capsaicin-sensitive C- and A-fibre nociceptors control long-term potentiation-like pain amplification in humans. Brain. 2015;138(Pt 9):2505-20.

118. van den Broeke EN, Lenoir C, Mouraux A. Secondary hyperalgesia is mediated by heat-insensitive A-fibre nociceptors. The Journal of Physiology. 2016;594(22):6767-76.
119. van den Broeke EN, Mouraux A. High-frequency electrical stimulation of the human skin induces heterotopical mechanical hyperalgesia, heat hyperalgesia, and enhanced responses to nonnociceptive vibrotactile input. J Neurophysiol. 2014;111(8):1564-73.
120. Lenoir C, Plaghki L, Mouraux A, van den Broeke EN. Quickly responding C-fibre nociceptors contribute to heat hypersensitivity in the area of secondary hyperalgesia. The Journal of Physiology. 2018;596(18):4443-55.
121. Klein T, Magerl W, Treede R-D. Perceptual Correlate of Nociceptive Long-Term Potentiation (LTP) in Humans Shares the Time Course of Early-LTP. Journal of Neurophysiology. 2006;96(6):3551-5.
122. Buxton RB. The physics of functional magnetic resonance imaging (fMRI). Reports on Progress in Physics. 2013;76.
123. Lee MC, Zambreanu L, Menon DK, Tracey I. Identifying brain activity specifically related to the maintenance and perceptual consequence of central sensitization in humans. J Neurosci. 2008;28(45):11642-9.
124. Zambreanu L, Wise RG, Brooks JCW, Iannetti GD, Tracey I. A role for the brainstem in central sensitisation in humans. Evidence from functional magnetic resonance imaging. PAIN. 2005;114(3):397-407.
125. Mainero C, Zhang WT, Kumar A, Rosen BR, Sorensen AG. Mapping the spinal and supraspinal pathways of dynamic mechanical allodynia in the human trigeminal system using cardiac-gated fMRI. Neuroimage. 2007;35(3):1201-10.
126. Clarke S, Rogers R, Wanigasekera V, Fardo F, Pia H, Nochi Z, et al. Systematic review and co-ordinate based meta-analysis to summarize the utilization of functional brain imaging in conjunction with human models of peripheral and central sensitization. European Journal of Pain. 2024;28(7):1069-94.
127. Specht K. Current Challenges in Translational and Clinical fMRI and Future Directions. Frontiers in Psychiatry. 2020;10.
128. Sandrini G, Serrao M, Rossi P, Romaniello A, Cruccu G, Willer JC. The lower limb flexion reflex in humans. Progress in Neurobiology. 2005;77(6):353-95.
129. Lim EC, Sterling M, Pedler A, Coombes BK, Vicenzino B. Evidence of spinal cord hyperexcitability as measured with nociceptive flexion reflex (NFR) threshold in chronic lateral epicondylalgia with or without a positive neurodynamic test. J Pain. 2012;13(7):676-84.

130. Leone C, Di Lionardo A, Di Pietro G, Di Stefano G, Falco P, Blockeel AJ, et al. How different experimental models of secondary hyperalgesia change the nociceptive flexion reflex. Clin Neurophysiol. 2021;132(12):2989-95.
131. Biurrun Manresa JA, Finnerup NS, Johannesen IL, Biering-Sørensen F, Jensen TS, Arendt-Nielsen L, et al. Central sensitization in spinal cord injured humans assessed by reflex receptive fields. Clin Neurophysiol. 2014;125(2):352-62.
132. Linde LD, Bent LR, Dickey JP, Kumbhare DA, Srbely JZ. Exploring the effect of capsaicin-induced central sensitization on the upper limb nociceptive withdrawal reflex threshold. Exp Brain Res. 2021;239(11):3405-15.
133. Willer JC. Comparative study of perceived pain and nociceptive flexion reflex in man. Pain. 1977;3(1):69-80.
134. Franz S, Heutehaus L, Tappe-Theodor A, Weidner N, Treede RD, Schuh-Hofer S. Noxious radiant heat evokes bi-component nociceptive withdrawal reflexes in spinal cord injured humans-A clinical tool to study neuroplastic changes of spinal neural circuits. Front Hum Neurosci. 2023;17:1141690.
135. Guekos A, Grata AC, Hubli M, Schubert M, Schweinhardt P. Are changes in nociceptive withdrawal reflex magnitude a viable central sensitization proxy? Implications of a replication attempt. Clinical Neurophysiology. 2023;145:139-50.
136. Desmedt JE, Cheron G. Somatosensory evoked potentials in man: subcortical and cortical components and their neural basis. Ann N Y Acad Sci. 1982;388:388-411.
137. Mauguière F, Courjon J. The origins of short-latency somatosensory evoked potentials in humans. Annals of Neurology. 1981;9(6):607-11.
138. Mauguière F. Anatomic Origin of the Cervical N13 Potential Evoked by Upper Extremity Stimulation. Journal of Clinical Neurophysiology. 2000;17(3):236-45.
139. Di Lionardo A, Di Stefano G, Leone C, Di Pietro G, Sgro E, Malara E, et al. Modulation of the N13 component of the somatosensory evoked potentials in an experimental model of central sensitization in humans. Scientific Reports. 2021;11(1):20838.
140. Leone C, Di Pietro G, Salman Y, Galosi E, Di Stefano G, Caspani O, et al. Modulation of the spinal N13 SEP component by high- and low-frequency electrical stimulation. Experimental pain models matter. Clin Neurophysiol. 2023;156:28-37.

141. Iannetti GD, Baumgärtner U, Tracey I, Treede RD, Magerl W. Pinprick-evoked brain potentials: a novel tool to assess central sensitization of nociceptive pathways in humans. J Neurophysiol. 2013;110(5):1107-16.
142. Scheuren PS, Rosner J, Curt A, Hubli M. Pain-autonomic interaction: A surrogate marker of central sensitization. Eur J Pain. 2020;24(10):2015-26.
143. van den Broeke EN, Lambert J, Huang G, Mouraux A. Central Sensitization of Mechanical Nociceptive Pathways Is Associated with a Long-Lasting Increase of Pinprick-Evoked Brain Potentials. Front Hum Neurosci. 2016;10:531.
144. Biasiucci A, Franceschiello B, Murray MM. Electroencephalography. Current Biology. 2019;29(3):R80-R5.
145. Michail G, Dresel C, Witkovský V, Stankewitz A, Schulz E. Neuronal Oscillations in Various Frequency Bands Differ between Pain and Touch. Frontiers in Human Neuroscience. 2016;10.
146. Li W, Wang H, Zhuang L. GCNs–FSMI: EEG recognition of mental illness based on fine-grained signal features and graph mutual information maximization. Expert Systems with Applications. 2023;228:120227.
147. Gross J, Schnitzler A, Timmermann L, Ploner M. Gamma Oscillations in Human Primary Somatosensory Cortex Reflect Pain Perception. PLOS Biology. 2007;5(5):e133.
148. Zhang ZG, Hu L, Hung YS, Mouraux A, Iannetti GD. Gamma-band oscillations in the primary somatosensory cortex--a direct and obligatory correlate of subjective pain intensity. J Neurosci. 2012;32(22):7429-38.
149. May ES, Nickel MM, Ta Dinh S, Tiemann L, Heitmann H, Voth I, et al. Prefrontal gamma oscillations reflect ongoing pain intensity in chronic back pain patients. Human Brain Mapping. 2019;40(1):293-305.
150. Lu X, Hu L. Electroencephalography, Evoked Potentials, and Event-Related Potentials. 2019. p. 23-42.
151. Nunez PL, Srinivasan R. Electric Fields of the Brain: The neurophysics of EEG: Oxford University Press; 2006 01 May 2009.
152. Mouraux A, Iannetti GD. Across-trial averaging of event-related EEG responses and beyond. Magn Reson Imaging. 2008;26(7):1041-54.
153. Pfurtscheller G, Lopes da Silva FH. Event-related EEG/MEG synchronization and desynchronization: basic principles. Clinical Neurophysiology. 1999;110(11):1842-57.
154. Avitan L, Teicher M, Abeles M. EEG Generator—A Model of Potentials in a Volume Conductor. Journal of Neurophysiology. 2009;102(5):3046-59.

155. Holmes GL, Khazipov R. Basic Neurophysiology and the Cortical Basis of EEG. In: Blum AS, Rutkove SB, editors. The Clinical Neurophysiology Primer. Totowa, NJ: Humana Press; 2007. p. 19-33.
156. Dawson GD. A summation technique for detecting small signals in a large irregular background. J Physiol. 1951;115(1):2p-3p.
157. Dawson GD. A summation technique for the detection of small evoked potentials. Electroencephalography and Clinical Neurophysiology. 1954;6:65-84.
158. van den Broeke EN, Mouraux A, Groneberg AH, Pfau DB, Treede RD, Klein T. Characterizing pinprick-evoked brain potentials before and after experimentally induced secondary hyperalgesia. J Neurophysiol. 2015;114(5):2672-81.
159. Tracey I, Woolf CJ, Andrews NA. Composite Pain Biomarker Signatures for Objective Assessment and Effective Treatment. Neuron. 2019;101(5):783-800.
160. Eldabe S, Obara I, Panwar C, Caraway D. Biomarkers for Chronic Pain: Significance and Summary of Recent Advances. Pain Research and Management. 2022;2022(1):1940906.
161. van den Broeke EN, de Vries B, Lambert J, Torta DM, Mouraux A. Phase-locked and non-phase-locked EEG responses to pinprick stimulation before and after experimentally-induced secondary hyperalgesia. Clinical Neurophysiology. 2017;128(8):1445-56.
162. van den Broeke EN, de Hemptinne P, Mercken M, Torta DM, Lambert J, Mouraux A. Central sensitization of nociceptive pathways demonstrated by robot-controlled pinprick-evoked brain potentials. Clinical Neurophysiology. 2020;131(10):2491-8.
163. Valentini E, Schulz E. Automatised application of pinprick-evoked potentials improves investigation of central sensitisation in humans. Clin Neurophysiol. 2020;131(10):2482-3.
164. Djouhri L, Lawson SN. Abeta-fiber nociceptive primary afferent neurons: a review of incidence and properties in relation to other afferent A-fiber neurons in mammals. Brain Res Brain Res Rev. 2004;46(2):131-45.
165. Treede R-D, Meyer RA, Campbell JN. Myelinated Mechanically Insensitive Afferents From Monkey Hairy Skin: Heat-Response Properties. Journal of Neurophysiology. 1998;80(3):1082-93.
166. Zhang Z. Spectral and Time-Frequency Analysis. 2019. p. 89-116.
167. Heid C, Mouraux A, Treede RD, Schuh-Hofer S, Rupp A, Baumgärtner U. Early gamma-oscillations as correlate of localized nociceptive processing

in primary sensorimotor cortex. Journal of Neurophysiology. 2020;123(5):1711-26.
168. van Ede F, Szebényi S, Maris E. Attentional modulations of somatosensory alpha, beta and gamma oscillations dissociate between anticipation and stimulus processing. NeuroImage. 2014;97:134-41.
169. Bauer M, Oostenveld R, Peeters M, Fries P. Tactile Spatial Attention Enhances Gamma-Band Activity in Somatosensory Cortex and Reduces Low-Frequency Activity in Parieto-Occipital Areas. The Journal of Neuroscience. 2006;26(2):490.
170. Kim JA, Davis KD. Neural Oscillations: Understanding a Neural Code of Pain. The Neuroscientist. 2021;27(5):544-70.
171. Hu L, Iannetti GD. Neural indicators of perceptual variability of pain across species. Proceedings of the National Academy of Sciences. 2019;116(5):1782-91.
172. Yue LP, Iannetti GD, Hu L. The neural origin of nociceptive-induced gamma band oscillations. The Journal of Neuroscience. 2020:JN-RM-0255-20.
173. Engel AK, Singer W. Temporal binding and the neural correlates of sensory awareness. Trends Cogn Sci. 2001;5(1):16-25.
174. Tallon-Baudry C, Bertrand O, Hénaff MA, Isnard J, Fischer C. Attention modulates gamma-band oscillations differently in the human lateral occipital cortex and fusiform gyrus. Cereb Cortex. 2005;15(5):654-62.
175. Herrmann CS, Kaiser J. EEG γ-band responses reflect human behavior: an overview. Int J Psychophysiol. 2011;79(1):1-2.
176. Valentini E, Shindy A, Witkovsky V, Stankewitz A, Schulz E. Interindividual variability and individual stability of pain- and touch-related neuronal gamma oscillations. J Neurophysiol. 2023;129(6):1400-13.
177. Kehlet H, Jensen TS, Woolf CJ. Persistent postsurgical pain: risk factors and prevention. The Lancet. 2006;367(9522):1618-25.
178. Caumo W, Schmidt AP, Schneider CN, Bergmann J, Iwamoto CW, Adamatti LC, et al. Preoperative predictors of moderate to intense acute postoperative pain in patients undergoing abdominal surgery. Acta Anaesthesiologica Scandinavica. 2002;46(10):1265-71.
179. Gotoda Y, Kambara N Fau - Sakai T, Sakai T Fau - Kishi Y, Kishi Y Fau - Kodama K, Kodama K Fau - Koyama T, Koyama T. The morbidity, time course and predictive factors for persistent post-thoracotomy pain. Eur J Pain. 2001;5(1):89–96.

180. Lim J, Chen D, McNicol E, Sharma L, Varaday G, Sharma A, et al. Risk factors for persistent pain after breast and thoracic surgeries: a systematic literature review and meta-analysis. Pain. 2022;163(1):3-20.
181. Katz J, Jackson M, Kavanagh BP, Sandler AN. Acute pain after thoracic surgery predicts long-term post-thoracotomy pain. Clin J Pain. 1996;12(1):50-5.
182. Daoust R, Paquet J, Cournoyer A, Piette É, Morris J, Lessard J, et al. Relationship between acute pain trajectories after an emergency department visit and chronic pain: a Canadian prospective cohort study. BMJ Open. 2020;10(12):e040390.
183. Poleshuck EL, Katz J, Andrus CH, Hogan LA, Jung BF, Kulick DI, et al. Risk factors for chronic pain following breast cancer surgery: a prospective study. J Pain. 2006;7(9):626-34.
184. Nikolajsen L, Sørensen HC, Jensen TS, Kehlet H. Chronic pain following Caesarean section. Acta Anaesthesiologica Scandinavica. 2004;48(1):111-6.
185. Owens MA, Parker R, Rainey RL, Gonzalez CE, White DM, Ata AE, et al. Enhanced facilitation and diminished inhibition characterizes the pronociceptive endogenous pain modulatory balance of persons living with HIV and chronic pain. Journal of NeuroVirology. 2019;25(1):57-71.
186. Bossmann T, Brauner T, Horstmann T. Differences in Pain Intensity in Anti- and Pro-Nociceptive Pain Profile Subgroups in Patients with Knee Osteoarthritis. Pain Management. 2018;8(1):27-36.
187. Yarnitsky D, Granot M, Granovsky Y. Pain modulation profile and pain therapy: between pro- and antinociception. Pain. 2014;155(4):663-5.
188. Wilder-Smith OH, Schreyer T, Scheffer GJ, Arendt-Nielsen L. Patients with chronic pain after abdominal surgery show less preoperative endogenous pain inhibition and more postoperative hyperalgesia: a pilot study. J Pain Palliat Care Pharmacother. 2010;24(2):119-28.
189. Nahman-Averbuch H, Yarnitsky D, Granovsky Y, Sprecher E, Steiner M, Tzuk-Shina T, et al. Pronociceptive Pain Modulation in Patients with Painful Chemotherapy-Induced Polyneuropathy. Journal of Pain and Symptom Management. 2011;42(2):229-38.
190. Riecke J, Zerth SF, Schubert A-K, Wiesmann T, Dinges H-C, Wulf H, et al. Risk factors and protective factors of acute postoperative pain: an observational study at a German university hospital with cross-sectional and longitudinal inpatient data. BMJ Open. 2023;13(5):e069977.

191. Springborg AH, Visby L, Kehlet H, Foss NB. Psychological predictors of acute postoperative pain after total knee and hip arthroplasty: A systematic review. Acta Anaesthesiologica Scandinavica. 2023;67(10):1322-37.
192. Dirks J, Møiniche S, Hilsted KL, Dahl JB. Mechanisms of postoperative pain: clinical indications for a contribution of central neuronal sensitization. Anesthesiology. 2002;97(6):1591-6.
193. Katz J, Poleshuck EL, Andrus CH, Hogan LA, Jung BF, Kulick DI, et al. Risk factors for acute pain and its persistence following breast cancer surgery. Pain. 2005;119(1-3):16-25.
194. McCowat M, Fleming L, Vibholm J, Dixon D. The Psychological Predictors of Acute and Chronic Pain in Women Following Breast Cancer Surgery: A Systematic Review. Clin J Pain. 2019;35(3):261-71.
195. Schreiber KL, Kehlet H, Belfer I, Edwards RR. Predicting, Preventing and Managing Persistent Pain After Breast Cancer Surgery: The Importance of Psychosocial Factors. Pain Management. 2014;4(6):445-59.
196. Schreiber KL, Zinboonyahgoon N, Xu X, Spivey T, King T, Dominici L, et al. Preoperative Psychosocial and Psychophysical Phenotypes as Predictors of Acute Pain Outcomes After Breast Surgery. J Pain. 2019;20(5):540-56.
197. Giusti EM, Lacerenza M, Gabrielli S, Manzoni GM, Manna C, D'Amario F, et al. Psychological factors and trajectories of post-surgical pain: A longitudinal prospective study. Pain Practice. 2022;22(2):159-70.
198. Granot M, Ferber SG. The roles of pain catastrophizing and anxiety in the prediction of postoperative pain intensity: a prospective study. he Clinical journal of pain. 2005;21(5):439-45.
199. Kornilov N, Lindberg MF, Gay C, Saraev A, Kuliaba T, Rosseland LA, et al. Factors Related to Postoperative Pain Trajectories following Total Knee Arthroplasty: A Longitudinal Study of Patients Admitted to a Russian Orthopaedic Clinic. Pain Research and Treatment. 2016;2016(1):3710312.
200. Erdogan E, Ozenc E. Factors associated with acute and chronic pain after inguinal herniorraphy. Rom J Anaesth Intensive Care. 2018;25(1):31-5.
201. Peters ML, Sommer M, de Rijke JM, Kessels F, Heineman E, Patijn J, et al. Somatic and Psychologic Predictors of Long-term Unfavorable Outcome After Surgical Intervention. Annals of Surgery. 2007;245(3):487-94.
202. Hoofwijk DMN, Fiddelers AAA, Peters ML, Stessel B, Kessels AGH, Joosten EA, et al. Prevalence and Predictive Factors of Chronic Postsurgical Pain and Poor Global Recovery 1 Year After Outpatient Surgery. The Clinical Journal of Pain. 2015;31(12):1017-25.

203. Crombez G, Van Damme S, Eccleston C. Hypervigilance to pain: An experimental and clinical analysis. PAIN. 2005;116(1):4-7.
204. Hasenbring M. Attentional control of pain and the process of chronification. Progress in Brain Research. 129: Elsevier; 2000. p. 525-34.
205. Legrain V, Damme SV, Eccleston C, Davis KD, Seminowicz DA, Crombez G. A neurocognitive model of attention to pain: Behavioral and neuroimaging evidence. PAIN. 2009;144(3):230-2.
206. Della Porta D, Scheirman E, Legrain V. Top-down attention does not modulate mechanical hypersensitivity consecutive to central sensitization: insights from an experimental analysis. PAIN. 2024;165(9):2098-110.
207. Meyers E, Alves M, Teugels A, Torta DM. No Evidence That Working Memory Modulates the Plasticity of the Nociceptive System, as Measured by Secondary Mechanical Hypersensitivity. J Pain. 2023;24(11):1931-45.
208. Meyers E, Vlaeyen JWS, van den Broeke EN, von Leupoldt A, Palmer AJ, Torta DM. The effect of high versus low cognitive load on the development of nociceptive hypersensitivity: The roles of sympathetic arousal, sex and pain-related fear. Eur J Pain. 2023;27(6):682-98.
209. Lautenbacher S, Kundermann B, Krieg J-C. Sleep deprivation and pain perception. Sleep Medicine Reviews. 2006;10(5):357-69.
210. Lentz MJ, Landis CA, Rothermel J, Shaver JL. Effects of selective slow wave sleep disruption on musculoskeletal pain and fatigue in middle aged women. J Rheumatol. 1999;26(7):1586-92.
211. Moldofsky H, Scarisbrick P. Induction of neurasthenic musculoskeletal pain syndrome by selective sleep stage deprivation. Psychosom Med. 1976;38(1):35-44.
212. Kundermann B, Spernal J, Huber MT, Krieg J-C, Lautenbacher S. Sleep Deprivation Affects Thermal Pain Thresholds but Not Somatosensory Thresholds in Healthy Volunteers. Biopsychosocial Science and Medicine. 2004;66(6):932-7.
213. Hakki Onen S, Alloui A, Jourdan D, Eschalier A, Dubray C. Effects of rapid eye movement (REM) sleep deprivation on pain sensitivity in the rat. Brain Res. 2001;900(2):261-7.
214. Campbell CM, Bounds SC, Simango MB, Witmer KR, Campbell JN, Edwards RR, et al. Self-reported sleep duration associated with distraction analgesia, hyperemia, and secondary hyperalgesia in the heat-capsaicin nociceptive model. Eur J Pain. 2011;15(6):561-7.
215. Jansson-Fröjmark M, Boersma K. Bidirectionality between pain and insomnia symptoms: A prospective study. British Journal of Health Psychology. 2012;17(2):420-31.

216. Frohnhofen H. Pain and sleep : A bidirectional relationship. Z Gerontol Geriatr. 2018;51(8):871-4.
217. Koffel E, Kroenke K, Bair MJ, Leverty D, Polusny MA, Krebs EE. The bidirectional relationship between sleep complaints and pain: Analysis of data from a randomized trial. Health Psychol. 2016;35(1):41-9.
218. Tang NK, Goodchild CE, Sanborn AN, Howard J, Salkovskis PM. Deciphering the temporal link between pain and sleep in a heterogeneous chronic pain patient sample: a multilevel daily process study. Sleep. 2012;35(5):675-87a.
219. Smith MT, Haythornthwaite JA. How do sleep disturbance and chronic pain inter-relate? Insights from the longitudinal and cognitive-behavioral clinical trials literature. Sleep Med Rev. 2004;8(2):119-32.
220. Wright CE, Bovbjerg DH, Montgomery GH, Weltz C, Goldfarb A, Pace B, et al. Disrupted sleep the night before breast surgery is associated with increased postoperative pain. J Pain Symptom Manage. 2009;37(3):352-62.
221. Yang MMH, Hartley RL, Leung AA, Ronksley PE, Jetté N, Casha S, et al. Preoperative predictors of poor acute postoperative pain control: a systematic review and meta-analysis. BMJ Open. 2019;9(4):e025091.
222. Schreiber KL, Zinboonyahgoon N, Flowers KM, Hruschak V, Fields KG, Patton ME, et al. Prediction of Persistent Pain Severity and Impact 12 Months After Breast Surgery Using Comprehensive Preoperative Assessment of Biopsychosocial Pain Modulators. Ann Surg Oncol. 2021;28(9):5015-38.
223. Mustonen L, Aho T, Harno H, Sipilä R, Meretoja T, Kalso E. What makes surgical nerve injury painful? A 4-year to 9-year follow-up of patients with intercostobrachial nerve resection in women treated for breast cancer. Pain. 2019;160(1):246-56.
224. Boye Larsen D, Laursen M, Simonsen O, Arendt-Nielsen L, Petersen KK. The association between sleep quality, preoperative risk factors for chronic postoperative pain and postoperative pain intensity 12 months after knee and hip arthroplasty. British Journal of Pain. 2021;15(4):486-96.
225. Hadi MA, McHugh GA, Closs SJ. Impact of Chronic Pain on Patients' Quality of Life: A Comparative Mixed-Methods Study. J Patient Exp. 2019;6(2):133-41.
226. Fine PG. Long-Term Consequences of Chronic Pain: Mounting Evidence for Pain as a Neurological Disease and Parallels with Other Chronic Disease States. Pain Medicine. 2011;12(7):996-1004.
227. Patel AS, Farquharson R, Carroll D, Moore A, Phillips CJ, Taylor RS, et al. The impact and burden of chronic pain in the workplace: a qualitative systematic review. Pain Pract. 2012;12(7):578-89.

228. Perugino F, De Angelis V, Pompili M, Martelletti P. Stigma and Chronic Pain. Pain Ther. 2022;11(4):1085-94.
229. Breivik H, Collett B, Ventafridda V, Cohen R, Gallacher D. Survey of chronic pain in Europe: prevalence, impact on daily life, and treatment. Eur J Pain. 2006;10(4):287-333.
230. Zhang LB, Chen YX, Li ZJ, Geng XY, Zhao XY, Zhang FR, et al. Advances and challenges in neuroimaging-based pain biomarkers. Cell Rep Med. 2024;5(10):101784.
231. Tan LL, Oswald MJ, Heinl C, Retana Romero OA, Kaushalya SK, Monyer H, et al. Gamma oscillations in somatosensory cortex recruit prefrontal and descending serotonergic pathways in aversion and nociception. Nature Communications. 2019;10(1):983.
232. Wang F, Shen X, Xu S, Liu Y, Ma L, Zhao Q, et al. Negative words on surgical wards result in therapeutic failure of patient-controlled analgesia and further release of cortisol after abdominal surgeries. Minerva Anestesiol. 2008;74(7-8):353-65.
233. Blasini M, Corsi N, Klinger R, Colloca L. Nocebo and pain: an overview of the psychoneurobiological mechanisms. PAIN Reports. 2017;2(2):e585.
234. Boersma K, Linton SJ. Expectancy, fear and pain in the prediction of chronic pain and disability: a prospective analysis. Eur J Pain. 2006;10(6):551-7.
235. van den Broeke EN, Geene N, van Rijn CM, Wilder-Smith OH, Oosterman J. Negative expectations facilitate mechanical hyperalgesia after high-frequency electrical stimulation of human skin. Eur J Pain. 2013;18(1):86-91.
236. Mills S, Torrance N, Smith BH. Identification and Management of Chronic Pain in Primary Care: a Review. Current Psychiatry Reports. 2016;18(2):22.
237. Kim BR, Yoon SH, Lee HJ. Practical strategies for the prevention and management of chronic postsurgical pain. Korean J Pain. 2023;36(2):149-62.
238. Yuksel SS, Chappell AG, Jackson BT, Wescott AB, Ellis MF. “Post Mastectomy Pain Syndrome: A Systematic Review of Prevention Modalities”. JPRAS Open. 2022;31:32-49.
239. Horn A, Kaneshiro K, Tsui BCH. Preemptive and Preventive Pain Psychoeducation and Its Potential Application as a Multimodal Perioperative Pain Control Option: A Systematic Review. Anesth Analg. 2020;130(3):559-73.

240. Kairaluoma PM, Bachmann MS, Rosenberg PH, Pere PJ. Preincisional paravertebral block reduces the prevalence of chronic pain after breast surgery. Anesth Analg. 2006;103(3):703-8.
241. Bliss TV, Gardner-Medwin AR. Long-lasting potentiation of synaptic transmission in the dentate area of the unanaestetized rabbit following stimulation of the perforant path. J Physiol. 1973;232(2):357-74.
242. Lømo T. The discovery of long-term potentiation. Philos Trans R Soc Lond B Biol Sci. 2003;358(1432):617-20.
243. Ikeda H, Heinke B, Ruscheweyh R, Sandkühler J. Synaptic plasticity in spinal lamina I projection neurons that mediate hyperalgesia. Science. 2003;299(5610):1237-40.
244. Ikeda H, Stark J, Fischer H, Wagner M, Drdla R, Jäger T, et al. Synaptic amplifier of inflammatory pain in the spinal dorsal horn. Science. 2006;312(5780):1659-62.
245. Torsney C. Inflammatory Pain Unmasks Heterosynaptic Facilitation in Lamina I Neurokinin 1 Receptor-Expressing Neurons in Rat Spinal Cord. The Journal of Neuroscience. 2011;31(13):5158.
246. Sauerstein K, Liebelt J, Namer B, Schmidt R, Rukwied R, Schmelz M. Low-Frequency Stimulation of Silent Nociceptors Induces Secondary Mechanical Hyperalgesia in Human Skin. Neuroscience. 2018;387:4-12.
247. Schmelz M, Schmid R, Handwerker HO, Torebjörk HE. Encoding of burning pain from capsaicin-treated human skin in two categories of unmyelinated nerve fibres. Brain. 2000;123(3):560-71.
248. Werland F, Hirth M, Rukwied R, Ringkamp M, Turnquist B, Jorum E, et al. Maximum axonal following frequency separates classes of cutaneous unmyelinated nociceptors in the pig. The Journal of Physiology. 2021;599(5):1595-610.
249. Adelson D, Lao L, Zhang G, Kim W, Marvizon JC. Substance P release and neurokinin 1 receptor activation in the rat spinal cord increase with the firing frequency of C-fibers. Neuroscience. 2009;161(2):538-53.
250. De Col R, Maihöfner C. Centrally mediated sensory decline induced by differential C-fiber stimulation. PAIN. 2008;138(3):556-64.
251. Piallat B, Chabardès S, Devergnas A, Torres N, Allain M, Barrat E, et al. MONOPHASIC BUT NOT BIPHASIC PULSES INDUCE BRAIN TISSUE DAMAGE DURING MONOPOLAR HIGH-FREQUENCY DEEP BRAIN STIMULATION. Neurosurgery. 2009;64(1).
252. Grover LM, Kim E, Cooke JD, Holmes WR. LTP in hippocampal area CA1 is induced by burst stimulation over a broad frequency range centered around delta. Learning & Memory. 2009;16(1):69-81.

253. Nicholls MER, Thomas NA, Loetscher T, Grimshaw GM. The Flinders Handedness survey (FLANDERS): A brief measure of skilled hand preference. Cortex. 2013;49(10):2914-26.
254. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, et al. Fiji: an open-source platform for biological-image analysis. Nature Methods. 2012;9(7):676-82.
255. Go VL, Yaksh TL. Release of substance P from the cat spinal cord. The Journal of Physiology. 1987;391(1):141-67.
256. Liu H, Brown JL, Jasmin L, Maggio JE, Vigna SR, Mantyh PW, et al. Synaptic relationship between substance P and the substance P receptor: light and electron microscopic characterization of the mismatch between neuropeptides and their receptors. Proceedings of the National Academy of Sciences of the United States of America. 1994;91(3):1009-13.
257. Abbadie C, Trafton J, Liu H, Mantyh PW, Basbaum AI. Inflammation Increases the Distribution of Dorsal Horn Neurons That Internalize the Neurokinin-1 Receptor in Response to Noxious and Non-Noxious Stimulation. The Journal of Neuroscience. 1997;17(20):8049.
258. Khasabov SG, Rogers SD, Ghilardi JR, Peters CM, Mantyh PW, Simone DA. Spinal Neurons that Possess the Substance P Receptor Are Required for the Development of Central Sensitization. The Journal of Neuroscience. 2002;22(20):9086.
259. Mantyh PW, Rogers SD, Honore P, Allen BJ, Ghilardi JR, Li J, et al. Inhibition of Hyperalgesia by Ablation of Lamina I Spinal Neurons Expressing the Substance P Receptor. Science. 1997;278(5336):275-9.
260. Nichols ML, Allen BJ, Rogers SD, Ghilardi JR, Honore P, Luger NM, et al. Transmission of Chronic Nociception by Spinal Neurons Expressing the Substance P Receptor. Science. 1999;286(5444):1558-61.
261. Fenselau H, Heinke B, Sandkühler J. Heterosynaptic Long-Term Potentiation at GABAergic Synapses of Spinal Lamina I Neurons. The Journal of Neuroscience. 2011;31(48):17383.
262. Le Bars D. The whole body receptive field of dorsal horn multireceptive neurones. Brain Research Reviews. 2002;40(1):29-44.
263. Xia W, Mørch CD, Matre D, Andersen OK. Exploration of conditioned pain modulation effect on long-term potentiation-like pain amplification in humans. European Journal of Pain. 2017;21(4):645-57.
264. Bannister K, Dickenson AH. The plasticity of descending controls in pain: translational probing. J Physiol. 2017;595(13):4159-66.

265. Yarnitsky D. Conditioned pain modulation (the diffuse noxious inhibitory control-like effect): its relevance for acute and chronic pain states. Current Opinion in Anesthesiology. 2010;23(5).
266. Larson J, Munkácsy E. Theta-burst LTP. Brain Research. 2015;1621:38-50.
267. Vo L, Drummond PD. Analgesia to pressure–pain develops in the ipsilateral forehead after high- and low-frequency electrical stimulation of the forearm. Experimental Brain Research. 2014;232(2):685-93.
268. Zhou L-J, Peng J, Xu Y-N, Zeng W-J, Zhang J, Wei X, et al. Microglia Are Indispensable for Synaptic Plasticity in the Spinal Dorsal Horn and Chronic Pain. Cell Reports. 2019;27(13):3844-59.e6.
269. Retamal J, Reyes A, Ramirez P, Bravo D, Hernandez A, Pelissier T, et al. Burst-Like Subcutaneous Electrical Stimulation Induces BDNF-Mediated, Cyclotraxin B-Sensitive Central Sensitization in Rat Spinal Cord. Frontiers in Pharmacology. 2018;9.
270. Coull JAM, Boudreau D, Bachand K, Prescott SA, Nault F, Sík A, et al. Trans-synaptic shift in anion gradient in spinal lamina I neurons as a mechanism of neuropathic pain. Nature. 2003;424(6951):938-42.
271. Dedek A, Xu J, Kandegedara CM, Lorenzo L-É, Godin AG, De Koninck Y, et al. Loss of STEP61 couples disinhibition to N-methyl-d-aspartate receptor potentiation in rodent and human spinal pain processing. Brain. 2019;142(6):1535-46.
272. Lever IJ, Bradbury EJ, Cunningham JR, Adelson DW, Jones MG, McMahon SB, et al. Brain-Derived Neurotrophic Factor Is Released in the Dorsal Horn by Distinctive Patterns of Afferent Fiber Stimulation. The Journal of Neuroscience. 2001;21(12):4469.
273. Tallon-Baudry C, Bertrand O, Delpuech C, Pernier J. Stimulus Specificity of Phase-Locked and Non-Phase-Locked 40 Hz Visual Responses in Human. The Journal of Neuroscience. 1996;16(13):4240.
274. Linde LD, Ortiz O, Choles CM, Kramer JLK. Pain-related gamma band activity is dependent on the features of nociceptive stimuli: a comparison of laser and contact heat. J Neurophysiol. 2023;129(1):262-70.
275. Jung TP, Makeig S, Humphries C, Lee TW, McKeown MJ, Iragui V, et al. Removing electroencephalographic artifacts by blind source separation. Psychophysiology. 2000;37(2):163-78.
276. Yuval-Greenberg S, Tomer O, Keren AS, Nelken I, Deouell LY. Transient induced gamma-band response in EEG as a manifestation of miniature saccades. Neuron. 2008;58(3):429-41.

277. Muthukumaraswamy S. High-frequency brain activity and muscle artifacts in MEG/EEG: A review and recommendations. Frontiers in Human Neuroscience. 2013;7.
278. Jaltare K, Torta D, van den Broeke E. Preprocessing choices do not affect the chances of observing increased scalp recorded gamma-band oscillations in the context of mechanical hypersensitivity. 2023.
279. Olbrich S, Jödicke J, Sander C, Himmerich H, Hegerl U. ICA-based muscle artefact correction of EEG data: What is muscle and what is brain?: Comment on McMenamin et al. NeuroImage. 2011;54(1):1-3.
280. Campos Viola F, Thorne J, Edmonds B, Schneider T, Eichele T, Debener S. Semi-automatic identification of independent components representing EEG artifact. Clinical Neurophysiology. 2009;120(5):868-77.
281. Liebisch AP, Eggert T, Shindy A, Valentini E, Irving S, Stankewitz A, et al. A novel tool for the removal of muscle artefacts from EEG: Improving data quality in the gamma frequency range. Journal of Neuroscience Methods. 2021;358:109217.
282. Nickel MM, May ES, Tiemann L, Schmidt P, Postorino M, Ta Dinh S, et al. Brain oscillations differentially encode noxious stimulus intensity and pain intensity. Neuroimage. 2017;148:141-7.
283. Gousset S, Torta DM, Mouraux A, Lambert J, van den Broeke EN. Pinprick-induced gamma-band oscillations are not a useful electrophysiological marker of pinprick hypersensitivity in humans. Clinical Neurophysiology. 2023;153:102-10.
284. Büchel C. The role of expectations, control and reward in the development of pain persistence based on a unified model. eLife. 2023;12:e81795.
285. Fields HL. How expectations influence pain. PAIN. 2018;159:S3-S10.
286. Peerdeman KJ, Laarhoven AIMv, Peters ML, Evers AWM. An Integrative Review of the Influence of Expectancies on Pain. Frontiers in Psychology. 2016;7.
287. Atlas LY, Wager TD. How expectations shape pain. Neuroscience Letters. 2012;520(2):140-8.
288. Petersen GL, Finnerup NB, Colloca L, Amanzio M, Price DD, Jensen TS, et al. The magnitude of nocebo effects in pain: A meta-analysis. PAIN®. 2014;155(8):1426-34.
289. Thomaidou MA, Blythe JS, Peerdeman KJ, van Laarhoven AIM, Van Schothorst MME, Veldhuijzen DS, et al. Learned Nocebo Effects on Cutaneous Sensations of Pain and Itch: A Systematic Review and Meta-

analysis of Experimental Behavioral Studies on Healthy Humans. Psychosomatic Medicine. 2023;85(4).
290. Geuter S, Büchel C. Facilitation of pain in the human spinal cord by nocebo treatment. Journal of Neuroscience. 2013;33(34):13784-90.
291. Arendt-Nielsen L, Morlion B, Perrot S, Dahan A, Dickenson A, Kress HG, et al. Assessment and manifestation of central sensitisation across different chronic pain conditions. European Journal of Pain. 2018;22(2):216-41.
292. Patel R, Taylor JL, Dickenson AH, McMahon SB, Bannister K. A back-translational study of descending interactions with the induction of hyperalgesia by high-frequency electrical stimulation in rat and human. Pain. 2024.
293. Bedwell GJ, Louw C, Parker R, van den Broeke E, Vlaeyen JW, Moseley GL, et al. The influence of a manipulation of threat on experimentally-induced secondary hyperalgesia. PeerJ. 2022;10:e13512.
294. Jaltare KP, Meyers E, Torta DM. The Role of Pain Expectations in the Development of Secondary Pinprick Hypersensitivity: Behavioral-Neurophysiological Evidence and the Role of Pain-Related Fear. The Journal of Pain. 2024;25(9):104567.
295. Torta DM, Meyers E, Polleunis K, De Wolf S, Meulders A, van den Broeke EN. The Effect of Observing High or Low Pain on the Development of Central Sensitization. The Journal of Pain. 2023;24(1):167-77.
296. Blasini M, Corsi N, Klinger R, Colloca L. Nocebo and pain: An overview of the psychoneurobiological mechanisms. Pain Reports. 2017;2(2).
297. Bernstein MH, Locher C, Kube T, Buergler S, Stewart-Ferrer S, Blease C. Putting the 'Art' Into the 'Art of Medicine': The Under-Explored Role of Artifacts in Placebo Studies. Frontiers in Psychology. 2020;11.
298. Aslaksen PM, Lyby PS. Fear of pain potentiates nocebo hyperalgesia. J Pain Res. 2015(1178-7090 (Print)).
299. Thomaidou MA, Veldhuijzen DS, Meulders A, Evers AWM. An experimental investigation into the mediating role of pain-related fear in boosting nocebo hyperalgesia. PAIN. 2021;162(1).
300. Dunsmoor JE, Paz R. Fear Generalization and Anxiety: Behavioral and Neural Mechanisms. Biological Psychiatry. 2015;78(5):336-43.
301. Weng L, van Laarhoven AIM, Peerdeman KJ, Evers AWM. Induction and generalization of nocebo effects on itch. Experimental Dermatology. 2022;31(6):878-89.

302. Weng L, Peerdeman KJ, Della Porta D, van Laarhoven AIM, Evers AWM. Can placebo and nocebo effects generalize within pain modalities and across somatosensory sensations? PAIN. 2022;163(3):548-59.
303. Hird EJ, Charalambous C, El-Deredy W, Jones AK, Talmi D. Boundary effects of expectation in human pain perception. Scientific Reports. 2019;9(1).
304. Benedetti F, Arduino C, Amanzio M. Somatotopic Activation of Opioid Systems by Target-Directed Expectations of Analgesia. The Journal of Neuroscience. 1999;19(9):3639-48.
305. Bayman EO, Brennan TJ. Incidence and severity of chronic pain at 3 and 6 months after thoracotomy: meta-analysis. J Pain. 2014;15(9):887-97.
306. Lavand'homme P. The progression from acute to chronic pain. Curr Opin Anaesthesiol. 2011;24(5):545-50.
307. van Helmond N, Steegers MA, Filippini-de Moor GP, Vissers KC, Wilder-Smith OH. Hyperalgesia and Persistent Pain after Breast Cancer Surgery: A Prospective Randomized Controlled Trial with Perioperative COX-2 Inhibition. PLoS One. 2016;11(12):e0166601.
308. Woolf CJ. Pain amplification—A perspective on the how, why, when, and where of central sensitization. Journal of Applied Biobehavioral Research. 2018;23(2):e12124.
309. Pain IAftSo. Terminology. 2024.
310. Stubhaug A, Breivik H, Eide PK, Kreunen M, Foss A. Mapping of punctuate hyperalgesia around a surgical incision demonstrates that ketamine is a powerful suppressor of central sensitization to pain following surgery. Acta Anaesthesiol Scand. 1997;41(9):1124-32.
311. Martinez V, Ammar SB, Judet T, Bouhassira D, Chauvin M, Fletcher D. Risk factors predictive of chronic postsurgical neuropathic pain: the value of the iliac crest bone harvest model. Pain. 2012;153(7):1478-83.
312. De Kock M, Lavand'homme P, Waterloos H. 'Balanced analgesia' in the perioperative period: is there a place for ketamine? Pain. 2001;92(3):373-80.
313. De Kock M, Lavand'homme P, Waterloos H. The short-lasting analgesia and long-term antihyperalgesic effect of intrathecal clonidine in patients undergoing colonic surgery. Anesth Analg. 2005;101(2):566-72.
314. Lavand'homme P, De Kock M, Waterloos H. Intraoperative epidural analgesia combined with ketamine provides effective preventive analgesia in patients undergoing major digestive surgery. Anesthesiology. 2005;103(4):813-20.

315. Salengros JC, Huybrechts I, Ducart A, Faraoni D, Marsala C, Barvais L, et al. Different anesthetic techniques associated with different incidences of chronic post-thoracotomy pain: low-dose remifentanil plus presurgical epidural analgesia is preferable to high-dose remifentanil with postsurgical epidural analgesia. J Cardiothorac Vasc Anesth. 2010;24(4):608-16.
316. Momeni M, Kock M, Lavandhomme P, Watremez C, Van Dyck M, Baele P. Abnormal sensations evoked over the chest and persistent peri-incisional chest pain after cardiac surgery. Acta anaesthesiologica Belgica. 2010;61:55-62.
317. Setälä P, Kalliomäki ML, Järvelä K, Huhtala H, Sisto T, Puolakka P. Postoperative hyperalgesia does not predict persistent post-sternotomy pain; observational study based on clinical examination. Acta Anaesthesiol Scand. 2016;60(4):520-8.
318. Brennan TJ, Kehlet H. Preventive analgesia to reduce wound hyperalgesia and persistent postsurgical pain: not an easy path. Anesthesiology. 2005;103(4):681-3.
319. Yoon S, Hong WP, Joo H, Kim H, Park S, Bahk JH, et al. Long-term incidence of chronic postsurgical pain after thoracic surgery for lung cancer: a 10-year single-center retrospective study. Reg Anesth Pain Med. 2020;45(5):331-6.
320. Gilron I, Lao N, Carley M, Camiré D, Kehlet H, Brennan TJ, et al. Movement-evoked Pain versus Pain at Rest in Postsurgical Clinical Trials and in Meta-analyses: An Updated Systematic Review. Anesthesiology. 2024;140(3):442-9.
321. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta Psychiatr Scand. 1983;67(6):361-70.
322. Bouhassira D, Attal N, Alchaar H, Boureau F, Brochet B, Bruxelle J, et al. Comparison of pain syndromes associated with nervous or somatic lesions and development of a new neuropathic pain diagnostic questionnaire (DN4). Pain. 2005;114(1-2):29-36.
323. Keller S, Bann CM, Dodd SL, Schein J, Mendoza TR, Cleeland CS. Validity of the brief pain inventory for use in documenting the outcomes of patients with noncancer pain. Clin J Pain. 2004;20(5):309-18.
324. Hughes R, Gao F. Pain control for thoracotomy. Continuing Education in Anaesthesia Critical Care & Pain. 2005;5(2):56-60.
325. Pertovaara A. A neuronal correlate of secondary hyperalgesia in the rat spinal dorsal horn is submodality selective and facilitated by supraspinal influence. Exp Neurol. 1998;149(1):193-202.

326. Urban MO, Gebhart GF. Supraspinal contributions to hyperalgesia. Proc Natl Acad Sci U S A. 1999;96(14):7687-92.
327. Brennan TJ, Vandermeulen EP, Gebhart GF. Characterization of a rat model of incisional pain. Pain. 1996;64(3):493-502.
328. Vandermeulen EP, Brennan TJ. Alterations in ascending dorsal horn neurons by a surgical incision in the rat foot. Anesthesiology. 2000;93(5):1294-302; discussion 6A.
329. Zahn PK, Brennan TJ. Incision-induced changes in receptive field properties of rat dorsal horn neurons. Anesthesiology. 1999;91(3):772-85.
330. Hughes G, Choudhury RA, McRoberts N. Summary Measures of Predictive Power Associated with Logistic Regression Models of Disease Risk. Phytopathology. 2019;109(5):712-5.
331. Mesbah A, Yeung J, Gao F. Pain after thoracotomy. BJA Education. 2016;16(1):1-7.
332. Giusti EM, Lacerenza M, Manzoni GM, Castelnuovo G. Psychological and psychosocial predictors of chronic postsurgical pain: a systematic review and meta-analysis. Pain. 2021;162(1):10-30.
333. Ranganathan P, Pramesh CS, Aggarwal R. Common pitfalls in statistical analysis: Logistic regression. Perspect Clin Res. 2017;8(3):148-51.
334. van Smeden M, de Groot JA, Moons KG, Collins GS, Altman DG, Eijkemans MJ, et al. No rationale for 1 variable per 10 events criterion for binary logistic regression analysis. BMC Med Res Methodol. 2016;16(1):163.
335. Merrill DR, Bikson M, Jefferys JGR. Electrical stimulation of excitable tissue: design of efficacious and safe protocols. Journal of Neuroscience Methods. 2005;141(2):171-98.
336. Yuan Y, Zheng L, Feng Z, Yang G. Different effects of monophasic pulses and biphasic pulses applied by a bipolar stimulation electrode in the rat hippocampal CA1 region. BioMedical Engineering OnLine. 2021;20(1):25.
337. Chul Han H, Hyun Lee D, Mo Chung J. Characteristics of ectopic discharges in a rat neuropathic pain model. PAIN. 2000;84(2).
338. Bernal L, Lopez-Garcia JA, Roza C. Spontaneous activity in C-fibres after partial damage to the saphenous nerve in mice: Effects of retigabine. European Journal of Pain. 2016;20(8):1335-45.
339. Serra J, Bostock H, Solà R, Aleu J, García E, Cokic B, et al. Microneurographic identification of spontaneous activity in C-nociceptors in neuropathic pain states in humans and rats. PAIN. 2012;153(1).
340. Xiao WH, Bennett GJ. Chemotherapy-evoked neuropathic pain: Abnormal spontaneous discharge in A-fiber and C-fiber primary afferent neurons and its suppression by acetyl-L-carnitine. Pain. 2008;135(3):262-70.

341. Serra J, Campero M, Ochoa J, Bostock H. Activity-dependent slowing of conduction differentiates functional subtypes of C fibres innervating human skin. J Physiol. 1999;515 (Pt 3)(Pt 3):799-811.
342. Michaelis M, Liu X, Jänig W. Axotomized and intact muscle afferents but no skin afferents develop ongoing discharges of dorsal root ganglion origin after peripheral nerve lesion. J Neurosci. 2000;20(7):2742-8.
343. Prkachin KM. The consistency of facial expressions of pain: a comparison across modalities. Pain. 1992;51(3):297-306.
344. Prkachin KM, Solomon PE. The structure, reliability and validity of pain expression: Evidence from patients with shoulder pain. PAIN. 2008;139(2):267-74.
345. Wolf K, Raedler T, Henke K, Kiefer F, Mass R, Quante M, et al. The face of pain--a pilot study to validate the measurement of facial pain expression with an improved electromyogram method. Pain Res Manag. 2005;10(1):15-9.
346. Mieronkoski R, Syrjälä E, Jiang M, Rahmani A, Pahikkala T, Liljeberg P, et al. Developing a pain intensity prediction model using facial expression: A feasibility study with electromyography. PLoS One. 2020;15(7):e0235545.
347. Sathyanarayana S, Satzoda RK, Sathyanarayana S, Thambipillai S. Vision-based patient monitoring: a comprehensive review of algorithms and technologies. Journal of Ambient Intelligence and Humanized Computing. 2018;9(2):225-51.
348. Boxtel A. Facial EMG as a tool for inferring affective states2010.
349. Wolf K. Measuring facial expression of emotion. Dialogues Clin Neurosci. 2015;17(4):457-62.
350. Blasini M, Corsi N, Klinger R, Colloca L. Nocebo and pain: An overview of the psychoneurobiological mechanisms. Pain Rep. 2017;2(2).
351. Wiech K, Seymour B, Kalisch R, Enno Stephan K, Koltzenburg M, Driver J, et al. Modulation of pain processing in hyperalgesia by cognitive demand. NeuroImage. 2005;27(1):59-69.
352. Henderson LA, Di Pietro F, Youssef AM, Lee S, Tam S, Akhter R, et al. Effect of Expectation on Pain Processing: A Psychophysics and Functional MRI Analysis. Front Neurosci. 2020;14:6.
353. Salomons TV, Moayedi M, Erpelding N, Davis KD. A brief cognitive-behavioural intervention for pain reduces secondary hyperalgesia. Pain. 2014;155(8):1446-52.
354. Valet M, Sprenger T, Boecker H, Willoch F, Rummeny E, Conrad B, et al. Distraction modulates connectivity of the cingulo-frontal cortex and the midbrain during pain--an fMRI analysis. Pain. 2004;109(3):399-408.

355. Tracey I, Ploghaus A, Gati JS, Clare S, Smith S, Menon RS, et al. Imaging attentional modulation of pain in the periaqueductal gray in humans. J Neurosci. 2002;22(7):2748-52.
356. Sprenger C, Eippert F, Finsterbusch J, Bingel U, Rose M, Büchel C. Attention Modulates Spinal Cord Responses to Pain. Current Biology. 2012;22(11):1019-22.
357. Henrich MC, Frahm KS, Coghill RC, Andersen OK. Spinal Nociception is Facilitated during Cognitive Distraction. Neuroscience. 2022;491:134-45.
358. Deldar Z, Blanchette I, Piché M. Reduction of Pain and Spinal Nociceptive Transmission by Working Memory is Load Dependant. J Pain. 2021;22(7):797-805.
359. Della Porta D, Vilz M-L, Kuzminova A, Filbrich L, Mouraux A, Legrain V. No evidence for an effect of selective spatial attention on the development of secondary hyperalgesia: A replication study. Frontiers in Human Neuroscience. 2022;16.
360. Filbrich L, van den Broeke EN, Legrain V, Mouraux A. The focus of spatial attention during the induction of central sensitization can modulate the subsequent development of secondary hyperalgesia. Cortex. 2020;124:193-203.
361. Manfuku M, Nishigami T, Mibu A, Yamashita H, Imai R, Kanamori H, et al. Predictors of persistent post-surgical pain intensity and interference at 1 year after breast cancer surgery: assessing central sensitization, central sensitivity symptoms, and psychological factors. Breast Cancer. 2023;30(2):271-81.
362. Gervais-Hupé J, Pollice J, Sadi J, Carlesso LC. Validity of the central sensitization inventory with measures of sensitization in people with knee osteoarthritis. Clin Rheumatol. 2018;37(11):3125-32.
363. Kregel J, Schumacher C, Dolphens M, Malfliet A, Goubert D, Lenoir D, et al. Convergent Validity of the Dutch Central Sensitization Inventory: Associations with Psychophysical Pain Measures, Quality of Life, Disability, and Pain Cognitions in Patients with Chronic Spinal Pain. Pain Pract. 2018;18(6):777-87.
364. Proença JdS, Baad-Hansen L, Braido GVdV, Mercante FG, Campi LB, Gonçalves DAdG. Lack of correlation between central sensitization inventory and psychophysical measures of central sensitization in individuals with painful temporomandibular disorder. Archives of Oral Biology. 2021;124:105063.
365. Adams GR, Gandhi W, Harrison R, van Reekum CM, Wood-Anderson D, Gilron I, et al. Do "central sensitization" questionnaires reflect measures

of nociceptive sensitization or psychological constructs? A systematic review and meta-analyses. Pain. 2023;164(6):1222-39.
366. Clarke H, Poon M, Weinrib A, Katznelson R, Wentlandt K, Katz J. Preventive Analgesia and Novel Strategies for the Prevention of Chronic Post-Surgical Pain. Drugs. 2015;75(4):339-51.
367. Thapa P, Euasobhon P. Chronic postsurgical pain: current evidence for prevention and management. Korean J Pain. 2018;31(3):155-73.
368. Zaheer S, LeBoff MS. Osteoporosis: Prevention and Treatment. In: Feingold KR, Anawalt B, Blackman MR, Boyce A, Chrousos G, Corpas E, et al., editors. Endotext. South Dartmouth (MA): MDText.com, Inc.Copyright © 2000-2024, MDText.com, Inc.; 2000.

369. Soliman N, Kersebaum D, Lawn T, Sachau J, Sendel M, Vollert J. Improving neuropathic pain treatment – by rigorous stratification from bench to bedside. Journal of Neurochemistry. 2023;n/a(n/a).
370. Smith SM, Dworkin RH, Turk DC, Baron R, Polydefkis M, Tracey I, et al. The Potential Role of Sensory Testing, Skin Biopsy, and Functional Brain Imaging as Biomarkers in Chronic Pain Clinical Trials: IMMPACT Considerations. J Pain. 2017;18(7):757-77.
371. Cook D, Brown D, Alexander R, March R, Morgan P, Satterthwaite G, et al. Lessons learned from the fate of AstraZeneca's drug pipeline: a five-dimensional framework. Nature Reviews Drug Discovery. 2014;13(6):419-31.
372. Simon R, Maitournam A. Evaluating the Efficiency of Targeted Designs for Randomized Clinical Trials. Clinical Cancer Research. 2004;10(20):6759-63.