How negative experiences influence the brain in pain: neuroimaging and biobehavioral insights

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Chapter 8.

Summary and general discussion

Summary

This thesis adds to a growing literature that has been challenging antiquated understandings of pain as a bottom-up process. In this project, we conducted a series of biobehavioral studies to further our understanding of how bottom-up pain signaling can be influenced by the top-down processing that may often be involved in pain. We employed diverse methodologies, such as a large-scale meta-analysis, a comprehensive review, behavioral experimental studies, as well as experiments utilizing imaging techniques such as fMRI, EEG, and EMG. We examined the types of experiences, such as receiving negative information or experiencing a negative effect first-hand, that may lead to stronger or more persistent nocebo effects on pain. We furthermore aimed to unravel underlying biobehavioral components of such learned pain responses. Behavioral paradigms were used to model real-life pain experiences, through validated experimental pain induction methods, novel experimental learning manipulations, as well as a close examination of emotional correlates such as fear. Concurrently, diverse, innovative neuroscientific methods –including a pharmacological manipulation– were used to examine the biobehavioral underpinnings of nocebo hyperalgesic responses. Our findings add to the growing knowledgebase from the field of nocebo hyperalgesia, demonstrating that learning by experience can decisively influence the processing and perception of noxious stimuli.

In **chapter 2**, a systematic review and meta-analysis indicated that learning by experience is a potent mechanism that can influence the perception and persistence of pain. Building upon the past two decades of proliferation in nocebo research, this comprehensive meta-analysis delivers novel insights into the currently known behavioral correlates and pain outcomes under nocebo hyperalgesic conditions. Classical

conditioning was found to be more powerful than verbally delivered negative information, showing that experienced adversity may be more powerful in inducing negative expectations, as compared to verbal suggestions. In examining what moderates effect sizes of nocebo responses between different studies, however, we found no significant moderating factors within our data. It should be noted that several factors were not systematically measured, such as fear of the pain stimulations or specific contextual factors, which may potentially account for some of the variability in nocebo magnitudes. More systematic and comparable studies on these aspects are needed. **Chapter 2** thus leaves little doubt regarding the potency of learned effects on pain perception, but raises a number of questions and points to knowledge-gaps regarding the potential cognitive-emotional and biobehavioral moderating and mediating factors in nocebo hyperalgesia.

Chapter 2 also highlighted nocebo effects as being present across the different sensations and types of pain, which led us, in **chapter 3**, to dive deeper into the diverse literature on the neurobiological correlates of nocebo hyperalgesia. To summarize and further utilize current knowledge, a comprehensive review of the neurobiological nocebo literature on pain was conducted. Twenty-two studies were included based on exhaustive database searches. A narrative review of these experiments highlighted the nocebo effect as a top-down phenomenon based on learned effects. Nocebo effects were shown to be influenced by basic nociceptive signal conduction in the spinal cord, as well as by higher cognitive functions such as emotional processing and expectations. Importantly, a marked inconsistency in methods used and results yielded between nocebo studies, led to a motivation for using consistent and comparable methods in the experimental work of this PhD project. We suggest that the field as whole attempts reproduction and replication of experimental methods, in order to reach a robust and reliable knowledge base for nocebo effects. Finally, with this review of the literature, the central question emerged which exact learning

mechanisms may give rise to nocebo responses and how this relates to pain outside of the laboratory and in real-world settings.

Chapter 4 presents a first experimental study that aimed to demonstrate whether nocebo effects can be induced –and how they may persist– when based on inconsistent and variable learning, more akin to what patients may experience within clinical settings. We compared a typical conditioning paradigm to one with variable reinforcement of the nocebo association between pain and an inert treatment. We also attempted to attenuate the induced nocebo effect to examine the dynamics of different learning schedules over time. While it was unsurprising to find that a more ambiguous learning method led to smaller –albeit significant– nocebo effects, we observed that, interestingly, these smaller effects were more persistent over time, and resisted counterconditioning. This study addressed treatment resistance and chronification of pain relevant to potential experiences in clinical settings and highlighted a role of different types of learning in nocebo hyperalgesia, thereby addressing some of the questions left open in chapters 2 and 3. However, this study did not address the impact of important emotional correlates such as fear, a factor that was not consistently reported in the studies analyzed in chapter 2, but may be implicated in nocebo hyperalgesia.

Therefore, in **chapter 5**, we designed a follow-up experimental study to examine the role of fear in learned pain responses. Despite its known involvement in pain and other clinical outcomes, fear was mostly overlooked by the nocebo field, and our study was the first to manipulate and measure the involvement of different types of fear in nocebo hyperalgesia. Here, we also imaged fear responses, by measuring startle reactions via EMG during a nocebo paradigm. While we retained a typical nocebo induction group as a control, we additionally created one group which would receive higher pain stimulations overall, and another group that received frightful information regarding a potential

bad outcome. These two groups, as expected, reported overall higher levels of fear of pain, and the higher-pain group also responded with significantly larger nocebo hyperalgesia. The results further indicated that more research is needed to unravel the intricacies of central pain integration with cognitive-emotional factors. The value of utilizing known imaging markers to measure fear of pain on a biobehavioral level, led to the novel approach of using electrophysiological biomarkers to further understand pain integration and processing under nocebo hyperalgesic conditions. In the next chapter, we thus applied imaging of the brain by use of EEG, in order to better understand how neurocognitive processing affects pain experiences under hyperalgesic conditions.

Chapter 6 examines the electrophysiology of learned pain responses through the lens of the currently known markers of pain and of emotional processing. We utilized sophisticated EEG biomarkers to characterize complex electrophysiological patterns during baseline pain perception, learning, and then evocation of nocebo responses. We additionally measured and computed brain electrophysiology at rest, before and after the experimental paradigm, to explore baseline characteristics that may modulate the acquisition of hyperalgesic effects and to examine the changes from before to after nocebo acquisition. Indeed, we found that individuals who exhibit higher complexity of neuronal patterns of oscillations at baseline showed larger nocebo responses. At the same time, differences were also found in how the brain processes increased pain stimulation at baseline versus a nocebo-augmented perceived pain increase. EEG provided several novel insights into the neurophysiological phenotype of nocebo hyperalgesia, enabling us to paint an initial broad picture of a complex neural signature of nocebo hyperalgesia. Questions were also raised by this study, as EEG methods encounter limitations in terms of localizing effects in the brain, as well as in measuring specific functional contributions of

different brain structures. A follow-up study utilizing fMRI, in chapter 7, attempts to address such limitations.

In **chapter 7** a novel pharmacological fMRI study examined closely the specific contribution of distinct brain regions and the NMDA receptors that occupy them and facilitate learning. While still utilizing consistent experimental nocebo induction methods for purposes of comparability and reliability within the field, in this study we attempted a pharmacological manipulation of learning during nocebo induction in the MR scanner. We used D-cycloserine, a medication known for its potential to enhance learning through NMDA receptor agonism, to examine whether a group with augmented learning ability would show a larger nocebo response than a group of participants receiving placebo. We found that, despite the pharmacological manipulation not showing any significant behavioral effects, brain regions previously implicated in associative types of learning differentiate nocebo stimuli from control trials. This final neuroimaging study also confirmed results found in chapters 3, 5, and 6 on the emotional correlates of nocebo hyperalgesia, thereby opening the door for future research to focus on district brain plasticity mechanisms as potential driving factors of learned effects on pain.

In the general discussion of this thesis, we integrate and interpret the findings of this PhD project in relation to each other and to the broader literature on learned effects on experimental and clinical pain. There are two central findings that arise from the work of this dissertation, both related to the intricate dynamics between nociceptive processing and cognitive-emotional experiential factors. The most central finding, that specific modes of learning shape pain processing in the brain, is discussed as the chief cognitive driver of nocebo hyperalgesia. We discuss how learning is able to alter future pain experiences based on past experience and negative expectations. The second critical finding of this project, that fear-learning may play a mediating role in nocebo

induction and persistence, is discussed in relation to gaps in the literature and our general understanding of negativity bias and emotional memory. We further discuss the limitations of this work and of this model-based scientific field as a whole, and we propose future directions in nocebo research and for clinical practice. We conclude that nocebo hyperalgesia decidedly influences pain, and that such learned effects rely on the brain's tendency to learn, adapt, and integrate cognitive and emotional information, especially in relation to prior negative experiences.

General discussion

Nocebo hyperalgesia has been researched as a negative pain outcome for over three decades. The work on reviewing this literature, conducted as part of this PhD project, resynthesized current knowledge and investigated common themes such as the central role of behavioral conditioning, as well as a focus of the field on emotions such as anxiety and stress. In the sections that follow, we start by discussing *lessons from the literature and the impact of methodological focus in understanding nocebo hyperalgesia*. Next, we discuss this project in relation to the overarching concepts and wider implications of two central conclusions derived from our findings. First, we *identify cognitive mechanisms under the umbrella of associative learning*, beyond the more general established correlate of associative learning. Second, we discuss *a potential cooperation of cognitive and fear-specific learning mechanisms*. Limitations in the research are addressed and theoretical considerations as well as future directions for the field are also discussed.

Lessons from the literature: impact of methodological focus in understanding nocebo hyperalgesia

On the consistent measurement of relevant covariates

As **chapter 2** concluded, learning by experience, for example via classical conditioning, influences how pain is ultimately perceived, but which biobehavioral processes underlie this indirect outcome remained an open question. Our primary findings indicated that classical conditioning was more powerful and reliable in inducing nocebo effects, as compared to mere verbal suggestion of a negative outcome. As corroborated by our study in **chapter 5**, this indicates that when a negative effect is practically experienced, nocebo effects are stronger than when a negative outcome is only verbally communicated. While this may seem intuitive, it is valuable to produce an evidence-based verification, from studies across the board, that associative learning (the cognitive mechanism underlying classical conditioning [2–4]) is the most powerful means for inducing nocebo effects on pain. In **chapter 2** we also highlighted how multiple types of pain are influenced by negative learned associations, indicating that under nocebo hyperalgesic conditions, pain processing can lead to amplified pain responses regardless of the nature of the noxious stimulus. The finding that across different experimental paradigms, contexts, and types of pain, nocebo effects are consistently induced, is in line with novel perspectives of pain as a subjective and ever-changing experience. Nevertheless, our meta-analysis was unable to fully rely on current published research to address some crucial questions of interest on nocebo hyperalgesia, mainly due to methodological and logistical limitations. For example, the nocebo literature may face research challenges such as publication bias for significant findings, or the content and ecological validity of experimental models built to induce nocebo effects. Further on, we discuss the limitations posed by unpublished null or underwhelming results that are inaccessible to our literature review efforts, and we

expand on concerns emerging from **chapter 2** regarding experimental modeling approaches.

Other wider methodological considerations arising from this project concern the choice of measures and paradigms in pain research. In our meta-analysis, overall magnitudes of nocebo responding could not be explained based on any of the measures that we collected from the experimental studies included. It appears that, no matter the number of learning trials, the type of sensation, or any other obtainable factor, nocebo effects up to 2.5 points magnitude (out of 10) can be obtained, with no one factor moderating this variability. This finding opened questions for future research relating to the variables that we were not able to obtain from previous studies. For example, while some important factors that influence nocebo have only incidentally been studied (see for example a study by Tinnerman and colleagues [5]), using more consistent methods in experimental models, as well as consistent in- and exclusion criteria, may provide a more stable platform on which nocebo magnitudes can be assessed and compared between studies. Additionally, measuring fear levels and reporting in detail the intensities of administered pain may point us towards potentially stronger moderators of nocebo magnitudes.

Implications for biobehavioral nocebo research

Methodological challenges may be of particular importance in biobehavioral and neuroimaging research into nocebo hyperalgesia. The neurobiological foundations of nocebo hyperalgesia are characterized by an apparent intricacy and consistency as well as replicability are central in understanding and tackling negative learned effects. **Chapter 3** presents a comprehensive review of the neurobiological underpinnings of nocebo hyperalgesia, with a focus on neuroimaging. Much of what we know about pain perception is based on self-reported pain levels.

Complex sensory phenomena such as nocebo hyperalgesia, that may implicate diverse cognitive processes, are thus very difficult to investigate reliably based on influenceable and volatile scores obtained through self-report. While self-report is the most accurate measure of subjective pain experiences that we currently have, in order to gain a comprehensive picture of learned effects on pain, there is a need for directly measuring biobehavioral factors under nocebo hyperalgesic conditions. This closer look into the neurobiology of nocebo effects is of high importance given the convolution, subjectivity, and potential inter- and intra-individual variability of experienced pain.

Despite the important takeaways provided by our comprehensive summary of the neurobiological nocebo literature, widespread inconsistencies in findings are also shown and we discuss this as a worrying trend to be addressed in future research. The utilization of distinct learning paradigms for inducing nocebo hyperalgesia may influence neurobiological findings. In other fields of research, such as in the domains of learning and memory, different types of learning have been shown to employ different brain processes, with complex architectures underlying distinct learning systems [6–9]. Concurrently, differences in emotional load, frightfulness of negative suggestions [10,11], or even the magnitude of induced hyperalgesia [12], may influence the neurobiological processes that are involved in nocebo responses. For these reasons, it is important for the nocebo field to begin employing more consistent methods and pursue replication of studies, in order to achieve reliable and meaningful findings. In the experimental parts of this project, we conformed with this recommendation, using validated and consistent experimental models for nocebo induction, while also implementing novel aspects.

Identifying cognitive mechanisms under the umbrella of associative learning

Learning as a non-unitary phenomenon

While it is apparent that nocebo effects involve a vast array of brain structures and processes [13,14], upon a systematic and detailed inspection of research to date, in **chapter 3**, we were able to synthesize a complete summary of those reproducible findings that paint a more concise and accurate picture of nocebo neurobiology. Our comprehensive review of the neuroscientific nocebo literature highlights a small number of consistent neuroimaging findings that tend to implicate specific cognitive correlates in the processing of nocebo pain. When discounting for known pain processing and sensory discrimination areas such as the somatosensory cortices and thalamus, the brain structures consistently implicated in nocebo hyperalgesia indicate a central role of learning by experience and cognitive pain modulation. When different types of learning and pain integration become involved in this process, evident by imaging findings –including our own– nocebo hyperalgesia can broadly be seen as a complex cognitive-sensory mechanism that arises through the integration of negative association learning and nociception.

While learning was shown to broadly underlie nocebo responses on pain in **chapter 3**, learning is not a unitary phenomenon, but rather it is shown to rely on distinct and often competing mechanisms [8,15,16]. For instance, even in basic non-conscious systems such as polymer networks and magnetic spins in solids, learning networks have been shown to memorize associative patterns from their environment based on specific learning modes that depend on particular contextual and stimulus-specific factors [17]. Higher order systems such as the human brain have been shown to learn and retrieve information based on distinct and often cooperating neural systems [18,19]. In our experimental studies we set out to examine specific learning mechanisms and their unique

contributions to learned nocebo effects. As discussed below, our findings add some level of detail to the existing literature, by focusing in on specific cognitive and emotional mechanisms, beyond the usual focus on the broader concept of associative learning.

To understand some of the features of nocebo hyperalgesia that the current behavioral and neuroimaging literature does not tackle, we designed and carried out a series of experimental studies on learned nocebo effects. **Chapter 4** indicates that, when replicating a clinically relevant context on ambiguous and inconsistent learning, nocebo effects can still be induced. In the continuous reinforcement group of this study we used a typical nocebo paradigm, comparable to many previous studies [109–111][20–24]. But using a second group, we also set out to reproduce results from a prior study [24] that utilized partially reinforced learning. Our objective was achieved; we showed that next to a typical nocebo paradigm (that is shown to dependably induce a nocebo effect in **chapter 2**), a more ambiguous and ecologically valid learning method is still able to induce a hyperalgesic effect, at least to some extent.

Not only is this realistic type of learning sufficient to alter the experience of pain, but ambiguity may add strength to learning so that nocebo effects can withstand attenuation over time. This was an important building block in our understanding of pain chronification from the lens of nocebo hyperalgesia. Our **chapter 4** results were in line with some initial studies that have indicated that nocebo effects may rely upon especially durable learned associations that resist attenuation [23–25]. When attempting to attenuate the induced effects, we observed that continuously reinforced, reliable nocebo associations were easier to reverse, whereas ambiguous, partially reinforced learning led to significant resistance to attenuation. We confirmed that ambiguous and variable learning can lead to hyperalgesic effects, and additionally showed that these variable associations persist over time, even after active countering of such a negative association. It appears that negative

and aversive experience prevails over newly learned positive information, and the uncertainty that comes from this variability of possible outcomes seems to reinforce negative pain expectations [24]. This serves an important realistic indicator for learning under specific real-world conditions, where, according to nocebo research, patients are thought to acquire hyperalgesic effects on their symptoms due to a variable mixture of contextual, communicative, and experiential factors [1,26–29].

In attenuating nocebo effects in **Chapter 4**, we compared a typical extinction paradigm, where learning of nocebo associations is simply discontinued, to counterconditioning. In counterconditioning, we reversed the learned associations by pairing the nocebo treatment with a positive, instead of a negative pain outcome. During both attenuation methods, new learning takes place. But our novel counterconditioning method taught participants that instead of increased pain, they would experience reduced pain when a nocebo treatment was applied. Essentially representing a placebo paradigm [21], this attenuation method showed for the first time that counterconditioning is a more potent method than extinction for the attenuation of nocebo hyperalgesia. This finding indicated that new, positive learning may effectively overwrite negative pain expectations, which may open new directions for behavioral treatments for pain symptoms that may be aggravated as a result of prior negative experiences [11].

Neuroimaging evidence of multifaceted learning processes

Building on this research and on the few existing nocebo neuroimaging studies summarized in **chapter 3**, in **chapter 6** we report an EEG experiment that expands our knowledge of the neurophysiological characteristics of learning in nocebo hyperalgesia. In **chapter 3** we described results from EEG studies that are not yet replicated, with each

study using vastly diverging methods. Our study partly overlapped with two previous nocebo experiments [4,20], but additionally to the resting state measurements we were reproducing from those existing studies, we endeavored for the first time to image the brain's electrophysiology during the learning and evocation of negative pain associations. Thanks to the rigorous analytical power of established EEG biomarkers, we were able to image complex neurophysiological patterns that are markers of specific learning patterns that engage complex cortical and subcortical learning processes.

Our most important findings in **chapter 6** were based on detrended fluctuation analysis, a sophisticated analytical method that reveals the patterns of long-range temporal correlations in the brain, during rest or within a specific task, such as nocebo induction. Our findings added to what we saw in **chapter 4**: complex learning dynamics –translated in **chapter 6** into enhanced complexity in neural dynamics– were associated with larger nocebo magnitudes. Long-range neural networks have been associated with integrative processes in the brain and when thought of in relation to a pain learning task, may mark a process of consolidating information via cooperating memory and sensory processing systems in the brain. In line with this interpretation, connectivity findings in fMRI and also EEG results in **chapter 3** provided evidence of cognitive-sensory integration in nocebo hyperalgesia, for instance by highlighting a role of connectivity between memory regions and the ACC. Taken together, these findings suggest that individuals whose neural patterns of activation are characterized by complex dynamics at rest may engage in increased cognitive integration between past and current pain experiences, in turn being potentially more susceptible to learning nocebo associations.

Past pain experiences have been shown to form differential expectations that influence pain processing [4,20,24,26,30]. In **chapter 6** we reported significant increases in alpha-band power in nocebo responders during

nocebo-augmented pain compared to a baseline pain stimulus. In line with the literature, this finding reflects the role of alpha-band oscillations in the formation of expectations [31,32] and in the cognitive regulation of pain through the integration of past experiences in pain processing [32,33]. Taken together, our EEG findings went beyond merely implicating associative learning in nocebo, by providing a more detailed neurophysiological characterization of a potential cortical integration between learned effects and the processing of noxious stimuli. Findings that point towards long-range temporal correlations in neural dynamics as feature of learning negative associations are crucial because they suggest a potential involvement of integrative learning in nocebo hyperalgesia.

In **chapter 7** we reported an fMRI study designed to examine more precise implications of brain plasticity in pain processing, utilizing a targeted pharmacological manipulation of NMDA-dependent learning. We used induction methods consistent with our previous experiments in **chapters 4** to **6** and comparable to some existing fundamental nocebo fMRI studies [5,34–36]. The results supported findings of an integration of learned associations with sensory inputs under nocebo hyperalgesic conditions. Particularly, results that implicated regions such as the ACC and insula in learning nocebo associations, which are generally in line with the literature as reviewed in **chapter 3**, suggest that the most prominent difference between nocebo and control cues can be seen in brain areas that are thought to synthesize sensory perception based on beliefs and expectations [37]. Activity in the ACC has been related to the graded encoding of pain based on the magnitude of expected pain [37,38]. Brain mechanisms that involve the insula and ACC may thus reflect the meaning of learned negative cues [39]. This type of meaning-related processing of pain through learned expectations could be critical for preparing the sensory system to optimally process noxious information. [37]".

Facilitatory mechanisms are able to amplify the pain experience [39] through a long-range integrative process involving specific aspects of learning that encode and consolidate beliefs and expectations about previously experienced stimuli. Yet, different forms of biobehavioral modulation can influence pain via distinct systems [40] and many variables related to cognitive and emotional factors may further influence nocebo effects. It is noteworthy that in our nocebo meta-analysis presented in **chapter 2**, the studies examined did not generally report exact measures of certain key learning characteristics. For example, measures of baseline learning ability in distinct domains, such as the verbal or visual learning measures we obtained in **chapter 7**, can be helpful in pinpointing sub-processes of learning that are crucial for nocebo responding. Accordingly, direct physiological and behavioral measures of fear, when measured across studies, may hold the potential of better explaining under which conditions learned nocebo responses are augmented. While experimental studies most often measure anxiety levels, in **chapter 3** we showed that anxiety cannot reliably be shown to impact nocebo responses, as measured neurochemically and via imaging techniques. It is thus possible that, in accordance with our results in **chapters 5**, **6**, and **7**, integrative cognitive learning mechanisms function in collaboration with affective learning, despite these latter emotional factors being somewhat neglected in nocebo studies. More precise measures of learning and memory could indeed show a moderating effect on nocebo magnitudes and help explain these effects across the nocebo literature – an important objective for future research.

A potential cooperation of cognitive and fear-specific learning mechanisms

Fear seems to play a significant role in nocebo hyperalgesia, and our work has added to the understanding of how affective learning may

influence the formation of negative associations. In **chapter 3**, limbic structures such as the hippocampus and amygdala point towards a processing of fear in the brain under nocebo conditions [34,35,41]. Our threat manipulations in **chapter 5** support the notion that fear can amplify nocebo responses. At the same time, our EEG results in the gamma-band lead us to speculate that nocebo hyperalgesia potentially involves emotional processes such as fear, that have been shown to engage similar patterns of gamma coupling in the amygdala [42]. This aligned with our fMRI results that also implicated the amygdala in nocebo hyperalgesia. Both behavioral and brain imaging evidence thus suggests that fear is involved in nocebo, and our project attempted to pinpoint precise mechanisms by which fear of pain may affect pain endurance and chronification.

Nocebo attenuation and the challenge of negativity bias

Our behavioral study presented in **chapter 4** was one of the first studies to show an endurance effect of nocebo, and such a resistance to attenuation aligns well with earlier literature in fear conditioning [9,10]. In line with this literature, the resistance effects observed in **chapter 4** may be at least partly attributable to negativity bias (i.e., the tendency to attend to and remember negative experiences over neutral or positive ones [45–47]). A long line of research indicates that negativity bias is a potent attentional effect that can significantly impact our perception [45–47]. When provided with mixed positive and negative information regarding a given stimulus, individuals are more likely to retain negative knowledge [48]. In our study, such a negativity bias may have taken place in the ambiguous learning group that was exposed to a wider range of negative and positive suggestions and associations. In line with previous literature about this type of negativity bias [48], this effect may be of important clinical relevance in pain chronification after exposure to

inconsistent, mixed information and experiences in the clinical setting. Studies indicate that the amygdala is directly involved in coding not only fear but also ambiguity and uncertainty, and amygdala reactivity has previously been linked to classical conditioning under uncertain conditions [49]. Moreover, what we observed in our **chapter 2** meta-analysis was that, when compared to meta-analyses on placebo effects, learned effect on pain that rely on negative rather than positive associations appear to be larger in magnitude –albeit we were not able to systematically compare nocebo and placebo effects in the same set of studies. A potential stronger potency of negative, as compared to positive associations may in part be explained by enhanced learning under negative conditions, such as in experiments where participants learn to expect pain worsening rather than pain relief. We thus observe that during negative pain experiences a potent process of associative learning may interact with fear processing subcortically in the limbic system to create negative expectations and exert an important and enduring effect of the brain and its processing of pain.

Increased negativity: the role of fear

A long line of research has indicated that negative emotions, experiences, and negatively framed information are given more importance and learned more firmly by the brain [50–54], something thought to have an evolutionary explanation in the significance of negative information in avoiding threat [55]. In line with earlier work on fear [3,50,55], our experiment in **chapter 5** indicated that during conditioning, fear resulting from intense pain experiences adds to negative learning, but when the higher pain is never experienced but only anticipated, learning remains mostly unaffected. **Chapter 5** thus in part suggests that a concrete negative experience such as increased pain leads to worse pain responses than a mere anticipated negative experience,

and this effect was fully mediated by pain-related fear. For the first time in a nocebo study, we manipulated and measured fear levels directly and precisely (see also **chapter 2**), by obtaining self-reported levels as well as imaging startle responses via EMG. Startle responses are thought to represent a more direct biobehavioral fear response, as compared to self-reported fear [56,57]. Our results may thus add to a more complete picture of nocebo responses, that may be shaped through a process of learning pain associations by experience, in combination with the cooccurrence of adverse emotional factors such as fear.

This involvement of emotional factors in pain perception highlights the top-down features of pain processing. However, our research shows that nocebo effects do not always involve fear processing and the amygdala. Rather, it seems that only when a stimulus such as pain is identified as emotive to some level, meaning that it may be especially negative or frightening, brain regions concerned with the emotional and cognitive components of pain, such as the amygdala, hippocampus, insula, and ACC become involved [39]. Indeed, this seems to be the case in patients with chronic pain, who may have formed emotive associations with pain and for whom often it is fear of pain that is particularly disabling [58]. A recent study comparing young chronic pain patients and healthy peers indicated that in patients only, increased pain catastrophizing was associated with enhanced threat-safety learning and found resting-state functional connectivity alterations between the amygdala and the inferior parietal lobe, including the insula [59]. These findings are aligned with our fMRI results implicating the amygdala and insula in pain that is aggravated through learning. Insular activity is indeed not only involved in subjective pain experiences, but is also associated with fear processing, and conditions such as irritable bowel syndrome, chronic fatigue, and persisting or insufficiently explained pain symptoms [39,60,61]. Thus, based on our current understanding of the physiological underpinnings of emotional elements that can influence pain processing, learning often seems to take place on two levels. On one hand, a cortical-

level associative learning mechanism may be at the core of acquiring learned effects on pain. On the other hand, it appears that fear-related learning, that may take place in subcortical loops, mediates pain worsening, and may be associated to pain chronification.

In our research, overall, learning through the integration of experiences and pain processing may be differentiated from fear-learning under nocebo hyperalgesic conditions. In **chapters 3**, **4**, and **5** we discussed a mediating role of uncertainty and fear in nocebo hyperalgesia. However, in **chapter 7**, D-cycloserine not having any detectable effect on nocebo hyperalgesia is discussed from the perspective of subcortical NMDA receptor modulation. Because D-cycloserine seems to sometimes yield results in research on learned fear responses [62–66] but not always on other types of non-affective learning [67]. It is thus possible to speculate that D-cycloserine may be more effective in modulating subcortical NMDA circuits engaged in paradigms with a heavier fear load [68]. As such, our pharmacological experiment led us to speculate that the fear component reflected through findings in **chapters 3**, **5**, and **7**, could potentially be a secondary affective component that could modulate –but may not primarily underlie– nocebo hyperalgesic responses. While this is merely one speculation, further research specifically measuring fear levels is needed in order to understand the role of NMDA-dependent learning in nocebo hyperalgesia. Understanding the exact vulnerabilities caused by cooccurring affective and sensory processing is highly relevant for unravelling the etiology of persisting pain symptoms that are not fully explained by physical damage [59,61,69].

The challenge of persisting pain symptoms lies in the multidimensional character of pain processing, influenced by previous experiences, beliefs, pain cognitions, as well as emotional factors, additionally to neurobiological factors directly related to sensory input [70,71]. And while the cognitive and emotional literature on pain has yielded abundant evidence for their role in pain aggravation and chronification [61,72–75], the

current understanding of the precise mechanisms that underlie established biobehavioral correlates of nocebo hyperalgesia is still in its infancy. But as growing evidence, discussed in the current dissertation, builds on an explanatory framework for pain aggravation and chronification, the cooperation between negative experiences, cognitive and emotional learning, and sensory integration becomes increasingly relevant for experimental and clinical pain research. Maladaptive learning and emotional factors provide a clinical relevance to the currently known biobehavioral correlates of nocebo hyperalgesia, and have led some to hypothesize that targeted treatments could influence and even reverse the relevant neurobiological aberrances, by addressing learning and emotional dynamics [61]. Targeting central components such as aversive learning and fear of pain in patients may help normalize specific brain alterations that underlie learned pain responses. Still, issues of generalizability and ecological validity, as well as a lack in replication of findings within the field, may pose limitations in nocebo research and interpretation.

Limitations in the project and the field

A central limitation in the neuroscientific nocebo literature, as initially found in **chapters 2** and **3**, is the widespread inconsistency in methods used and results yielded by experimental research. In this project in particular, while **chapter 6** generally confirmed the involvement of intricate learning dynamics in the top-down, cognitive processing of pain signals, it did not replicate specific results of two previous EEG nocebo studies [4,20]. Three studies to date, including our own, that have examined the involvement of alpha oscillations in nocebo effects, have found divergent results. Alpha-band neuronal activity has long been implicated in internal cognitive states with low external informational loads [76–79]. It is thus likely that different phases and contexts of nocebo

experiments engage internal cognitive processing differentially and should be examined with precision within and between studies. We underscore a limitation within the nocebo field to replicate precise findings, as also discussed in detail in **chapter 3**, which can be overcome by sharing study protocols between researchers and a collaborative consideration of experimental study designs –an important objective for open and reliable science.

A lack of consistency and specificity in the research and reproduction of findings in the nocebo literature is an unsurprising feature of a young field of research. Biobehavioral nocebo studies have been striving to contribute novel findings to the knowledge base of learned pain responses, attempting new experimental models, methods, and manipulations each time. Our work in **chapters 2** and **3**, however, suggests that as the literature is growing, there is a pressing need for confirmatory research, of the kind that will at least keep one eye on comparability and replication of existing studies in the field. Through our systematic and comprehensive reviews in this dissertation, we found many novel paradigms and results, with novelty supposed as a golden standard in scientific publishing, as though an objective in itself. In pain research, however, novelty is not inherently equated to the successful furthering of our understanding of nocebo effects and pain. Yet, grant subsidies for research are mostly awarded for novel research and ethical dilemmas may arise when focusing on replication alone, which complicates the issue of replicating previous findings. While we strived, in this PhD project, to maintain consistent methods throughout our experiments and the existing literature, we also fell short of conducting direct and precise study replications.

Indeed, in a field of science that is still in its infancy, **chapters 4** to **7** contributed a mixture of reproduced findings (such as that of partial reinforcement in **chapter 4**), cutting-edge novel methods (such as the application of EEG biomarkers on the imaged experience of nocebo-

augmented pain), and novel biobehavioral manipulations and results. There is a known bias in publishing unique ideas that create novel scientific work. What our reviews (**chapters 2** and **3**) have inherently and inevitably discounted, is the unsuccessful attempts to induce or manipulate nocebo effects on pain. Our knowledge base for nocebo hyperalgesia thus has a blind spot, in that we cannot factor in those variables and outcomes that were never published in peer-reviewed scientific journals –an explicit inclusion criterion in **chapter 2**. With vast estimated numbers of "unexciting" unpublished scientific research in the social sciences, it is imperative for our scientific community to make an active effort in creating fairer and more accessible publication routes for those null results that add to our genuine understanding of complex and potentially detrimental biobehavioral effects on pain.

Further limitations relate to the experimental work of this thesis and concern the methods used, as well as the reproducibility and clinical significance of findings. What is the significance of findings in young, educated, healthy participants that experienced short-lived experimentally induced pain, fear, and nocebo effects? Ours is not the only field of biobehavioral science that largely relies on psychophysiological modelling approaches [80] in order to induce and quantify phenomena such as nocebo hyperalgesia. But in the construction of experimental models of nocebo hyperalgesia, less attention is paid to their clinical validity and more to creating the strongest, most reliable, or most reproducible laboratory models. Some studies have paid particular attention to the accuracy of modeling clinical pain, by inducing realistic visceral pain symptoms [29,81,82], which is an important step towards the real-world applicability of experimental conclusions. In our studies, we carefully considered the different types of experimental models that we could possibly build to represent the putative clinical phenomenon of nocebo hyperalgesia. We opted for idealized and exploratory models [83], in which a deliberate simplification of hypothesized mechanisms and processes was able to keep other

variables constant while exploring specific learned effects on pain. In **chapter 3** we discussed in detail that these types of models are necessary in studying nocebo effects, due to the multifaceted and convoluted nature of pain. But in utilizing experimental models, we and much of the field at large neglect to scrutinize the imbalances between epistemic accessibility to specific variables, and the ability to draw conclusions regarding a realistic and clinically relevant target system or process [83] such as nocebo hyperalgesia. There are lessons to be learned from decades of academic research into the modeling of hypothetical phenomena [83] for every branch of biobehavioral science. Such lessons may indicate that the field of nocebo research should progressively shift away from fundamental science –notwithstanding the invaluable scientific contribution of early fundamental research in any given field– and graduate to more ecologically valid research with a focus on clinical nocebo phenomena.

Future directions and recommendations

Considerations of the nature and content of our experimental models open new avenues for nocebo research as a model-based science. Currently, nocebo experiments typically induce hyperalgesia in healthy individuals, often building representational, idealized models of nocebo hyperalgesic effects, from acquisition to extinction. A vast array of scientific models are representational, in that they represent a selected aspect of the world, which is thus the model's target system [83]. Examples include the Bohr model of the atom, models of predator–prey interaction, the scale model of a bridge, and learned nocebo effects on experimentally induced pain. Different types of models could represent different aspects of the target system, or even distort the system or processes itself, raising the question what it means for an experimental model to represent a select part of a real or hypothetical phenomenon.

These are important questions for the field of pain and nocebo research to address and bear in mind while building experimental nocebo models. Idealized and exploratory models are a crucial means for pain research to cope with systems that are as difficult to study in their full complexity as pain [84]. But the shortcomings of experimental modeling need to be moved to the foreground if we are to attempt improving the ecological validity and representational powers of experimental nocebo research. For example, a consensus could be achieved between researchers and clinicians regarding which models best and most accurately represent nocebo hyperalgesia, and these models can provide a basis on which nocebo effects are researched, as is largely the case for example for animal models of schizophrenia [85,86].

Another solution for the distance between experimental nocebo models and real-world pain phenomena could be to utilize validation and calibration techniques based on clinical knowledge. Models play an important role in science, as vehicles for learning about the phenomena observed in the world that are out of reach or intensely convoluted, such as chronic pain. Experimental models of nocebo effects allow for 'surrogative reasoning', a mode of scientific investigation in which features and outcomes of a system are examined by studying a model, rather than reality itself [87]. But this type of model-based reasoning, with its limitations as discussed above, should be based on active evaluation and adaptation of models [88,89] if we are to best represent real phenomena in patient populations. Bach and colleagues have proposed a valuable method to assess face validity and the fit of an experimental model, called retrodictive validity since the aim is to 'retroactively predict' the experimentally induced value of a given biobehavioral attribute [80,90] such as a nocebo effect on pain. In experimental research on such attributes, hypothetical true scores can be influenced by experimental manipulations, and this allows us to apply metrological calibrations. Bach and colleagues propose that an influenced value representing the true score in such a calibration experiment can provide a retrodictive

validity criterion to assess the accuracy of a model [90]. A comprehensive validation of an experimental nocebo model should thus rely on some understanding of "true" or clinical nocebo scores and their correlates within their natural systems, such as in clinical practice and based on, for example, specific clusters and characteristics of chronic pain symptoms.

For experimental models to evolve and improve, and for the consistency that we missed in **chapters 2** and **3** to be achieved in the field, there is thus a need to obtain clinical markers that can provide a basis for model validations. This is not to say that nocebo experiments should necessarily be performed on clinical populations, but rather, clinical pain scores and nocebo markers can serve to optimize the valuable models on which we can examine nocebo effects with accuracy and precision [89,91]. In other words, focusing on the symptomatology of pain patients that are thought to present with negative learned effects on their pain may provide researchers with more accurate representations of potential nocebo magnitudes and impacts outside the laboratory. Potentially unrealistic assumptions are inevitable features of experimental modeling [91], especially so of hypothetical biobehavioral phenomena, but clinical measurements can provide promising avenues forward for building more clinically applicable representations of nocebo hyperalgesia. While few studies have attempted to measure nocebo susceptibility and responding in patient populations [28,29,92], it is imperative to start building on this work. Validating the assumptions of our idealized experimental models in a way that can be applicable across experimental settings holds the potential of combating the inconsistencies and lack of replicability in results, while concurrently encouraging a more valid and accurate platform for understanding nocebo effects and their biobehavioral moderators.

Clinical practice is proposed here as a powerful reflective tool for experimental research, a tool to inspect and improve our modeling and understanding of nocebo effects, learned associations, and top-down

pain processing. Yet, three decades of research into nocebo hyperalgesia have also provided us with important and clinically relevant insights into the detrimental effect of learning on pain experiences. The early knowledge that nocebo research has generated for clinical practice should not be underestimated. It is consistently shown that contextual experiences and communication of negative outcomes can shape the way in which individuals experience pain [12,92–96]. In this dissertation, we additionally showed that fear of pain as well as individuals' physiological learning patterns and baseline brain dynamics can further facilitate negative pain associations resulting in increased pain sensitivity. The phenomenon of pain can thus be seen as a system that, prior to conscious pain perception, engages in the top-down cognitive and often emotional processing of ascending noxious stimuli, giving rise to an inherently subjective pain experience. This PhD project supported and expanded upon previous work on nocebo effects, showing that learning and fear play key roles in this top-down processing of pain. In the clinic, these findings could be applied to defuse those factors that we now know to reinforce negative associations, such as negative suggestions by healthcare professionals, contextual triggers of negative associations, traumatic pain experiences, uncertainty, and fear. From the clinical perspective our finding that counterconditioning was more effective than extinction in minimizing nocebo responses may also open avenues for behavioral treatments for pain symptoms that may be aggravated by learning.

Conclusions

Negative experiences influence the brain and can decidedly alter the experience of pain. Past experience appears to shape future experience. This PhD dissertation focused on enriching our understanding of negative learned effects on pain by investigating nocebo hyperalgesic

effects and the factors that characterize biobehavioral aspects of pain processing. We carried out systematic and comprehensive reviews and a meta-analysis of existing studies, as well as a series of experimental studies utilizing a resourceful mixture of classic and innovative biobehavioral methods, including classical conditioning, EMG, EEG, and fMRI. Our findings emerging from this work support the understanding of learning as an intricate, multifaceted, and powerful process, able to detrimentally influence sensory perception, altering the way in which individuals perceive pain. Our knowledge from nocebo research highlights the vast variability of sensory perception and conscious experience in humans. The results of the present PhD project further support the notion that negative inputs from the environment become encoded in our plastic brains, producing measurable adverse effects on pain. If we are to utilize research to improve pain management and outcomes, there is a pressing need for scientific research to translate this growing understanding of learned pain responses beyond the laboratory and into clinical practice.

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